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The Utility of Concurrent TBS/fNIRS for Antidepressant Treatment Optimization: A Protocol

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Abstract:

Introduction: Repetitive transcranial magnetic stimulation (rTMS) with theta bursts (i.e. TBS) of the dorsolateral prefrontal cortex (DLPFC) is an innovative treatment for major depressive disorder (MDD). However, fewer than 50% of patients show sufficient response to this treatment; markers for response prediction are urgently needed. Research shows considerable individual variability in the brain responses to rTMS. However, whether differences in individual DLPFC modulation by rTMS can be utilized as a predictive marker for treatment response remains to be investigated. Here, we present a research program that will exploit the combination of functional near-infrared spectroscopy (fNIRS) with brain stimulation. Concurrent TBS/fNIRS will allow us to systematically investigate TBS-induced modulation of blood oxygenation as a proxy for induced brain activity changes. The findings from this study will (1) elucidate the immediate effects of excitatory and inhibitory TBS on prefrontal activity in TBS treatment-naïve patients with MDD and (2) validate the potential utility of TBS-induced brain modulation at baseline for the prediction of antidepressant response to four weeks of daily TBS treatment.

Methods and analysis: Open-label, parallel-group experiment consisting of two parts. In part one, 43 patients and 37 healthy controls will be subjected to concurrent TBS/fNIRS. Intermittent TBS (iTBS) and continuous TBS (cTBS) will be applied on the left and right DLPFC, respectively. fNIRS data will be acquired before, during and several minutes after stimulation. In part two, patients who participated in part one will receive a 4-week iTBS treatment of the left DLPFC, performed daily for 5 days per week. Psychometric evaluation will be performed periodically and at 1 month treatment follow up. Statistical analysis will include a conventional, as well as a machine learning approach.

Ethics and dissemination: Ethics approval was obtained from the Institutional Review Board. Findings will be disseminated through scientific journals, conferences, and university courses.

Registration: clinicaltrials.gov Identifier: 15100120

Keywords: Theta-burst stimulation, major depression, treatment prediction, functional NIRS, concurrent TBS/fNIRS

Strengths and limitations of this study

- Concurrent application of TMS and fNIRS
- Investigation of the immediate effects of excitatory and inhibitory TBS on prefrontal activity in major depression
- Exploration of the utility of TBS-induced brain modulation at baseline for the prediction of the antidepressant response to four weeks of daily TBS treatment
- Concurrent TBS/fNIRS bears technical challenges that need to be remediated
- The NIRS probe used in our study covers only a small area underneath the coil, limiting the analysis of stimulation effects to a small region of interest

Introduction

Stratified medicine is still an unmet need for biological psychiatry. Despite major efforts by others and us in utilizing neuroimaging tools to uncover diagnostic and predictive markers (e.g.,[1]), psychiatrists are still lacking such indicators with clinical utility.[2] The urgency for developing biomarkers for psychiatric disorders such as MDD is demonstrated by the fact that mental disorders are the leading global burden in terms of years lived with disability.[3] Moreover, mental disorders are associated with economic costs that are higher than cardiovascular disorders, cancer, and diabetes combined.[4] In light of the high percentage of treatment refractoriness, a particular need for psychiatry is to uncover markers that predict the outcome of treatments before or at an early stage after treatment start.

TBS, a special form of patterned rTMS, has finally found its way into clinical practice for the treatment of MDD. TBS is safe, effective in depressed patients that are refractory to standard pharmacological treatments, and has the advantage of increased efficiency over standard rTMS. However, response rates for rTMS as well as TBS, while promising enough to offer this treatment (with only minor side effects) to patients with MDD, are still achieved in only about 50% of patients.[5] Several attempts to predict antidepressant response were made in recent years but they only succeeded at a group level, whereas markers that are sufficiently accurate to guide decisions on an individual level are still absent.[6] For example, baseline functional connectivity between subgenual anterior cingulate cortex and DLPFC has been proposed as a biomarker for the individualization of the stimulation target to optimize treatment response.[7 8] Yet, when functional connectivity-based target selection is implemented, response rates still do not exceed the 50% mark.[9] Other attempts to predict response rates include measurements of cortical thickness.[10] or

corticospinal excitability,[11] as well as many other patient-related, illness-related, and stimulation procedure-related factors, for a review, see [12 13].

Concurrent neuroimaging with TMS may be especially fruitful to probe diagnostic and predictive neuroimaging markers as it aims to uncover the immediate modulatory effects of stimulation. Indeed, the prevailing view on therapeutic brain stimulation is that modulation of prefrontal excitability mediates its antidepressant effect. Hence, direct modulatory effects of prefrontal excitability during and immediately after rTMS likely forecast long-lasting changes in cortical excitability by promoting synaptic plasticity, which, according to current theory, should accompany rTMS treatment response.[11] Technological advances within the last decade allowed for the application of concurrent brain measurements with TMS using functional magnetic resonance imaging (fMRI) or electroencephalogram (EEG).[14] Authors observed prefrontal activation upon 1Hz rTMS with BOLD responses correlating with increasing stimulation strength. Activations were observed during the 10-second stimulation blocks and lasting 4-6 seconds after the last stimulation.[15] However, although highly promising for future research, it is questionable whether such a sophisticated combination of TMS and fMRI will eventually translate into a routinely used clinical test. Moreover, simultaneous image acquisitions during the application of a stimulation-burst or train of high frequency (HF) rTMS is impossible in an fMRI setting.

Concurrent TMS/fNIRS may be clinically superior to TMS/fMRI in order to probe the direct modulatory effect of prefrontal excitability during and immediately after stimulation. fNIRS is cheap, easy and harmless to apply and a widely available method to measure superficial brain

activity and connectivity by means of changes in blood hemoglobin concentrations. Indeed, a recent study from Boston University attempted to predict the antidepressant response to rTMS by utilizing fNIRS (NCT01192685). Unfortunately, the study had to be terminated due to a technical failure of the neuronavigation system. However, more research is needed along this line because there is evidence indicating that cortical activity modulations before treatment commencement may be able to predict antidepressant treatment response. In a recent study published by Oliveira-Maia and colleagues, they measured stimulation-induced changes in motor evoked potentials (MEP) at baseline and found that modulations of corticospinal excitability predicted the antidepressant response of a two-week daily HF rTMS treatment.[11] The results of this study indicated that immediate modulatory effects of rTMS of the motor cortex forecast synaptic plasticity and associated antidepressant treatment effects. However, the motor cortex is not considered a key brain area in the neuropathology of MDD and predictions based on modulations of MEPs have been only moderately successful.[11] Therefore, research is needed that probes cortical modulations directly in the DLPFC; that is, the site where therapeutic brain stimulation is applied.

Noteworthy, studies measuring cortical and corticospinal activity reveal a substantial degree of individual variability in TMS-induced modulations.[11 16-19] For example, Maeda et al.[16] investigated the effects of 1 Hz, 10 Hz, 15 Hz and 20 Hz rTMS on MEP shortly after stimulation. Although responses were on average lowest at 1Hz and highest at 20 Hz, authors detected a high degree of variability, with some individuals even showing the opposite pattern, i.e., stronger excitatory responses at 1 Hz compared to 20 Hz. A high degree of variation in cortical excitability was also reported in Oliveira-Maia et al.[11] and in a recent TMS/fMRI study.[19] The aim of the current proposal

is to utilize such individual patterns of DLPFC modulations for personalized medicine in MDD.

There are two objectives in our proposal: The first objective is to provide mechanistic evidence for the direct effects of TBS of the healthy and presumed neuropathological prefrontal cortex. Specifically, we aim to assess excitability modulations of prefrontal HbO by applying TBS on DLPFC in patients with MDD compared to HC. The second objective is to evaluate the relationship between immediate excitability modulations of the DLPFC and treatment response and thus provide a novel biomarker for individual patient selection. Given previous evidence and based on our line of reasoning given above, we will test the following operational hypotheses regarding our first objective: 1. We hypothesize an average increase in prefrontal HbO upon iTBS and an average decrease in HbO upon cTBS in MDD and HC;[20 21] 2. We hypothesize average changes in prefrontal HbO will occur during stimulation, compared to baseline;[20 22] 3. We hypothesize significantly increased variance in HbO responses during and after stimulation compared to baseline;[11 16-19] 4. We hypothesize that patients with MDD have lower variability in TBS-induced HbO modulations compared to HC.[11 23 24] The following operational hypothesis will be tested regarding our second objective: 5. We hypothesize that individual TBS-induced HbO modulations predict the antidepressant response after treatment.[23 24]

Methods and analysis

Study design

The study is designed as an open-label, parallel-group experiment and has two parts (see Figure 1). In part one, patients with MDD and HC will be subjected to concurrent TBS/fNIRS. iTBS will be

applied on the left DLPFC, whereas cTBS will be applied on the right DLPFC. Each participant will receive iTBS first, followed by cTBS after a delay of 1 hour to exclude possible interaction effects.[25] fNIRS data acquisition will include a baseline measurement of a few minutes, will continue during the stimulation period and last for several minutes post-stimulation period. The length of the post-stimulation period will be optimized to cover the entire duration of anticipated facilitatory effects of iTBS.[25] The TMS operator, as well as the researchers performing data analyses will be blinded regarding group membership. In part two, patients who participated in part one will receive a 4-week brain stimulation treatment trial with iTBS of the left DLPFC, performed daily for 5 days per week. Patients and doctors prescribing and evaluating the treatment as well as TMS operators administering the treatment are kept blind to fNIRS results. Psychometric evaluation will be performed on the day of TBS/fNIRS measurements and at the day of treatment start, as well as after 2 and after 4 weeks of treatment. A follow-up assessment will be performed 1 month after treatment ends.

Participants

Forty-three patients diagnosed with MDD in a current major depressive episode will be included. Key inclusion criteria are: MDD (DSM-5), 18≤age≤60, Hamilton depression rating scale (HAMD-17) ≥18, approval for TBS treatment by the physician in charge, stable antidepressive medication 4 weeks before treatment. Key exclusion criteria are: a history of brain surgery, head injury, stroke or neurodegenerative disorder, diagnosis of personality disorder, psychotic features, active suicidal intent, severe somatic comorbidities, cardiac pacemakers, deep brain stimulation, intracranial metallic particles, history of seizures, antiepileptics and benzodiazepines corresponding to a dose of

>1 mg lorazepam/d, substance dependence or abuse, if it is the primary clinical problem. The sample size was determined based on previous studies demonstrating that motor cortex excitability modulation significantly predicts antidepressant response of a two-week rTMS treatment.[11] A sample size of 37 was determined using an effect size of r=0.43,[11] a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (Point biseral correlation model, two-tailed). Given an expected dropout rate of 15%, a conservative sample size was set to 43 participants. In addition, we will recruit 47 HC to participate in the TBS/fNIRS measurement. The sample is based on the comparison of MEP facilitation obtained in MDD (8 ± 49%)[11] and HC (37.9 ± 53.6%),[16] determined using an effect size of d=0.58, a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (unpaired two sample t-test, two-tailed).

Theta-burst stimulation (TBS)

TBS comprises 3-pulse 50-Hz bursts, applied every 200 ms (at 5 Hz) as described previously.[25] iTBS consists of 2-second trains with an inter-train-interval of 8 seconds. We will repeat trains (30 pulses; 10 bursts) for 20 times to reach a total number of 600 pulses (3x10x20). cTBS will comprise uninterrupted bursts to reach a total number of 600 pulses.[26] Concurrent TBS/fNIRS stimulation will be applied over the left (iTBS) and right (cTBS) DLPFC at an intensity of 90% resting motor threshold (RMT), this corresponds to ~110% of the active motor threshold, an intensity that also elicited robust DLPFC activation in a recent concurrent TMS/fMRI study.[15] Stimulation at 90% RMT will also ensure compliance, reduced sensory discomfort, and minimize dropout rates during the concurrent TBS/fNIRS experiment. Still, scalp discomfort will be recorded directly after the stimulation. We refrained from choosing an intensity of 120% RMT (which will be applied during

antidepressant treatment) for the concurrent TBS/fNIRS experiment because such intensity would unlikely be tolerated by all patients as they are stimulation-naïve at the time of the experiment. The stimulation site over the DLPFC will be determined using the international 10-20 system and corresponds to the F3 label, determined using the optimized method by Beam et al.[27] TBS of the prefrontal cortex (PFC) is generally well tolerated, even at higher stimulation intensities. Antidepressant treatment comprises daily sessions of iTBS of the left DLPFC, five times a week for four weeks. Stimulation intensity will be 120% RMT (titration to full therapeutic dose over the first three days), as approved by the FDA in the U.S.[5] The stimulation site will be the same as in the concurrent TBS/fNIRS stimulation. Treatment will be performed at the TMS treatment centers of the participating local clinics (Department of Psychiatry, The University of Hong Kong and The Chinese University of Hong Kong, and at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University).

Functional near-infrared spectroscopy (fNIRS)

We will make use of the fNIRS system ImagentTM from ISS Inc., Champaign, IL, US (http://www.iss.com/biomedical/instruments/imagent.html) to determine changes in hemoglobin concentrations before, during and after TBS. ImagentTM uses a sensor that is embedded in a rectangular rubber pad with prisms inside so that optical fibers are rested tangentially instead of perpendicularly on the head surface. This arrangement allows the placement of the sensor directly underneath the TMS coil in close proximity to the stimulation site.[28] Concurrent TBS/fNIRS poses technical and conceptual challenges. Although NIRS has the advantage of being relatively insensitive to motion artifacts, pilot data from our lab show significant stimulation-related artifacts

if sensors and the TMS coil touch each other. Hence, a minimum distance of a few millimeters between probes and the TMS coil will be ensured before the start of measurements. The lowest fNIRS probes will match the Fp1-Fp2 line in order to cover most of the prefrontal cortex extending to the temporal lobes (DLPFC, ventrolateral, frontopolar and superior temporal regions). The device measures changes in HbO, Hb and total Hb using two wavelengths of infrared light (695 and 830 nm). With a source-detector spacing of 3cm, changes in Hb can be measured at a depth of 2-3cm corresponding to the cerebral cortex. fNIRS is not a perfect measure of brain activity and there are several sources of artifacts that need to be carefully considered. We will primarily focus on HbO since it may closer reflect BOLD changes as measured by fMRI[29] The primary imaging endpoint will be the mean HbO amplitude of left DLPFC during the first-minute post iTBS. Secondary endpoints include HbO amplitudes on right DLPFC during and after iTBS and cTBS, global and local amplitude maxima, duration of increased HbO values, the steepness in the decline and ascent of HbO values, as well as variability measures of HbO during and after stimulation. During concurrent TBS/fNIRS, participants will comfortably sit in a chair. Participants will be carefully instructed about the nature of the experiment prior to the TBS/fNIRS run.

Psychiatric assessment

Psychiatric assessment includes a range of clinical scales, administered at baseline on the day of the TBS/fNIRS measurement, after 4 weeks of treatment, as well as at follow-up 1 month after treatment ends. Primary clinical outcome measure: Response rate after treatment (Montgomery-Asberg depression rating scale, MADRS≤50% of baseline). Secondary endpoints: Remission rate after treatment (MADRS≤8). Reduction of mean MADRS, HAMD17, Inventory of depression

symptomatology-clinician rated (IDS-C30), response and remission rates defined using HAMD17, as well as mentioned clinical outcomes at follow-up. Adverse events (AE) will be assessed according to good clinical practice (ICH/GCP) using an AE-questionnaire to detect unwanted side effects related to the treatment. Suicidality will be evaluated on each treatment day. Similarly, depression severity will be evaluated at each treatment day using Beck's depression inventory-II (BDI-II). Patients will be discontinued if they experience worsening in depression, defined as an increase in BDI from baseline of more than 25% during two consecutive assessments, or development of active suicidal intent or attempted suicide. Potentially occurring serious AEs (SAEs) will be recorded.

Data processing and statistical analysis

fNIRS data analysis will follow the standard processing steps. This includes spatial registration (recording of standard cranial landmarks nasion, inion, left and right ear, and the 3D locations of the fNIRS probes); transformation to MNI space; band-pass filtering for motion artifact removal; and estimation of the hemodynamic response function using GLM, as implemented in the NIRS Toolbox for MATLAB. Comparisons between HbO values at baseline and during/after stimulation will reveal TBS-related de/activations. A t-test between pre- and post-stimulation will be performed to test hypothesis 1. Hypothesis 1 will be supported if there is a significant increase and decrease in prefrontal HbO after iTBS and cTBS, respectively. A t-test between pre-stimulation and during stimulation will be performed to test hypothesis 2. Hypothesis 2 will be supported if there is a significant change in prefrontal HbO during stimulation. An F-test for the comparison of the variance in HbO values before versus after stimulation will be performed to test hypothesis 3.

Hypothesis 3 will be supported if the F-test is significant. Similarly, an F-test for the comparison of the variance in HbO values in MDD versus HC will be performed to test hypothesis 4. Hypothesis 4 will be supported if the F-test is significant. Analyses will be performed using the IBM SPSS software (http://www-01.ibm.com/software/analytics/spss/). The alpha level will be set at 0.05, adjusted for multiple comparisons using the Bonferroni-Holm procedure. For hypothesis 5, we will pursue two predictive modeling approaches, a conventional statistical analysis approach, as well as a machine learning approach.[30] First, a logistic regression analysis will be performed to define significant predictors of treatment response (defined as MADRS≤50% of baseline, see above). Logistic regression will be calculated as implemented in the generalized linear model function 'glm' of the statistical software 'R' (https://www.r-project.org/). Predictors will include imaging endpoints as given above, as well as sociodemographic and psychosocial variables (including the classification of patients as pharmacologically treatment resistant, TRD, defined by a failed treatment response after two or more consecutive antidepressants of adequate duration and dosage). Hierarchical multiple linear regression models will also be calculated to determine the relationship between MADRS reductions and secondary imaging endpoints as potential response predictors. Second, we will use machine learning algorithms for the classification of patients. We will test different algorithms since there is no established rule for the choice of an optimal machine learning approach. We will start with a dichotomous classification using the RandomForest (RF) package for R (ran.r-project.org/) and determine the most useful predictors for distinguishing responders from non-responders.[31] RF is an ensemble tree classification tool that randomly selects subsamples of observations and builds a decision tree for the optimal splitting of these observations according to an outcome variable by a combination of predictors. For each split, the best performing predictor

out of a random selection is applied. RF has the advantage of being straightforward and less susceptible to overfitting compared to other machine learning classifiers. To measure the predictive power of our classification model, we will use a five-fold cross-validation design. This allows for optimal validation in the absence of an independent test set.[32] There is no established method of power calculation for RF. However, we will restrict HbO measurements to few prefrontal channels in order to keep the number of features for classification below the number of observations, thereby preventing the problem of hyperdimensionality. Receiver operating characteristics will be plotted using the ROCR package for the R-software.

Ethics and dissemination

Ethics approval was obtained from the Institutional Review Board of the Hong Kong Polytechnic University (reference numbers HSEARS20200120005, CRESC202009), as well as from the Institutional Review Boards of participating hospitals. An information sheet will be provided to participants before the experiment and a consent form will be signed by both PI and participant to protect the right of both parties. Participants will receive reimbursement for their participation. The data will be stored in an encrypted way and the accessibility is restricted to the researcher team.

The findings of this study will be disseminated through scientific journals, academic conferences, and university courses.

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.

Footnotes:

Contributions: GSK conceived the study and analysis. GSK and RLDK drafted the protocol and will conduct the data analysis. RLDK, BBBZ and KNKF will collect the data. ADPM, SKWC and WCL will recruit participants and perform the stimulation treatment. All the authors reviewed the protocol and agree to the final version being submitted.

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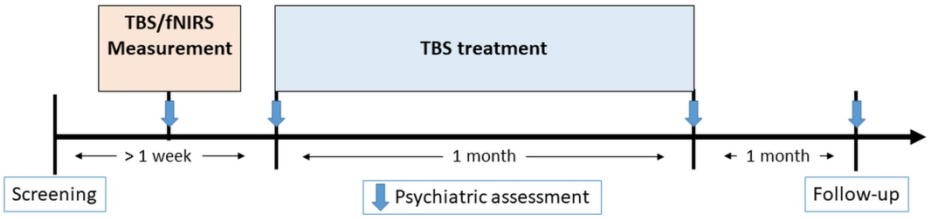
Figure legends

Figure 1. Study design



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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	Yes (available in clinicaltrials.gov Identifier: 15100120)
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 14
	5b	Name and contact information for the trial sponsor	N/A
	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
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	7
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, noninferiority, exploratory)	7, 8
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	10
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)	8, 9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-11
	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)	12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 10, 12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8

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	11, 12
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)	7, 8, 19
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	8, 9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target size	14
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	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	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	10-12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
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	14
Statistical methods	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	12-14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13, 14
	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)	N/A
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	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
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	12
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
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	14
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)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	14
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	14

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	14
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

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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The Utility of Concurrent TBS/fNIRS for Antidepressant Treatment Optimization: A Protocol

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The Utility of Concurrent TBS/fNIRS for Antidepressant Treatment Optimization: A Protocol

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Abstract:

Introduction: Repetitive transcranial magnetic stimulation (rTMS) with theta bursts (i.e. TBS) of the dorsolateral prefrontal cortex (DLPFC) is an innovative treatment for major depressive disorder (MDD). However, fewer than 50% of patients show sufficient response to this treatment; markers for response prediction are urgently needed. Research shows considerable individual variability in the brain responses to rTMS. However, whether differences in individual DLPFC modulation by rTMS can be utilized as a predictive marker for treatment response remains to be investigated. Here, we present a research program that will exploit the combination of functional near-infrared spectroscopy (fNIRS) with brain stimulation. Concurrent TBS/fNIRS will allow us to systematically investigate TBS-induced modulation of blood oxygenation as a proxy for induced brain activity changes. The findings from this study will (1) elucidate the immediate effects of excitatory and inhibitory TBS on prefrontal activity in TBS treatment-naïve patients with MDD and (2) validate the potential utility of TBS-induced brain modulation at baseline for the prediction of antidepressant response to four weeks of daily TBS treatment.

Methods and analysis: Open-label, parallel-group experiment consisting of two parts. In part one, 70 patients and 37 healthy controls will be subjected to concurrent TBS/fNIRS. Intermittent TBS (iTBS) and continuous TBS (cTBS) will be applied on the left and right DLPFC, respectively. fNIRS data will be acquired before, during and several minutes after stimulation. In part two, patients who participated in part one will receive a 4-week iTBS treatment of the left DLPFC, performed daily for 5 days per week. Psychometric evaluation will be performed periodically and at 1 month treatment follow up. Statistical analysis will include a conventional, as well as a machine learning approach.

Ethics and dissemination: Ethics approval was obtained from the Institutional Review Board. Findings will be disseminated through scientific journals, conferences, and university courses.

Registration: clinicaltrials.gov Identifier: NCT04526002

Keywords: Theta-burst stimulation, major depression, treatment prediction, functional NIRS, concurrent TBS/fNIRS

Strengths and limitations of this study

- Concurrent application of TMS and fNIRS
- Investigation of the immediate effects of excitatory and inhibitory TBS on prefrontal activity in major depression
- Exploration of the utility of TBS-induced brain modulation at baseline for the prediction of the antidepressant response to four weeks of daily TBS treatment
- Concurrent TBS/fNIRS bears technical challenges that need to be remediated
- The NIRS probe used in our study covers only a small area underneath the coil, limiting the analysis of stimulation effects to a small region of interest

Introduction

Stratified medicine is still an unmet need for biological psychiatry. Despite major efforts by others and us in utilizing neuroimaging tools to uncover diagnostic and predictive markers (e.g.,[1]), psychiatrists are still lacking such indicators with clinical utility.[2] The urgency for developing biomarkers for psychiatric disorders such as major depressive disorder (MDD) is demonstrated by the fact that mental disorders are the leading global burden in terms of years lived with disability.[3] Moreover, mental disorders are associated with economic costs that are higher than cardiovascular disorders, cancer, and diabetes combined.[4] In light of the high percentage of treatment refractoriness, a particular need for psychiatry is to uncover markers that predict the outcome of treatments before or at an early stage after treatment start.

Theta burst stimulation (TBS), a special form of patterned repetitive transcranial magnetic stimulation (rTMS), has finally found its way into clinical practice for the treatment of MDD. TBS is safe, effective in depressed patients that are refractory to standard pharmacological treatments, and has the advantage of increased efficiency over standard rTMS. However, response rates for rTMS as well as TBS, while promising enough to offer this treatment (with only minor side effects) to patients with MDD, are still achieved in only about 50% of patients.[5] Several attempts to predict antidepressant response were made in recent years but they only succeeded at a group level, whereas markers that are sufficiently accurate to guide decisions on an individual level are still absent. For example, baseline functional connectivity between subgenual anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) has been proposed as a biomarker for the individualization of the stimulation target to optimize treatment response.[6 7] Yet, when functional connectivity-based target selection is implemented, response rates still do not exceed the 50% mark.[8] Other attempts to

predict response rates include measurements of cortical thickness.[9] or corticospinal excitability,[10] as well as many other patient-related, illness-related, and stimulation procedure-related factors, for a review, see [11 12].

Concurrent neuroimaging with TMS may be especially fruitful to probe diagnostic and predictive neuroimaging markers as it aims to uncover the immediate modulatory effects of stimulation. Indeed, the prevailing view on therapeutic brain stimulation is that modulation of prefrontal excitability mediates its antidepressant effect. Hence, direct modulatory effects of prefrontal excitability during and immediately after rTMS likely forecast long-lasting changes in cortical excitability by promoting synaptic plasticity, which, according to current theory, should accompany rTMS treatment response.[10] Technological advances within the last decade allowed for the application of concurrent brain measurements with TMS using functional magnetic resonance imaging (fMRI) or electroencephalogram (EEG).[13] Authors observed prefrontal activation upon 1Hz rTMS with BOLD responses correlating with increasing stimulation strength. Activations were observed during the 10-second stimulation blocks and lasting 4-6 seconds after the last stimulation.[14] However, although highly promising for future research, it is questionable whether such a sophisticated combination of TMS and fMRI will eventually translate into a routinely used clinical test. Moreover, simultaneous image acquisitions during the application of a stimulation-burst or train of high frequency (HF) rTMS is impossible in an fMRI setting.

Functional near-infrared spectroscopy (fNIRS) is cheap, easy and harmless to apply and a widely available method to measure superficial brain activity and connectivity by means of changes in

blood hemoglobin concentrations. Concurrent TMS/fNIRS may be clinically superior to TMS/fMRI in order to probe the direct modulatory effect of prefrontal excitability during and immediately after stimulation. Indeed, a recent study from Boston University attempted to predict the antidepressant response to rTMS by utilizing fNIRS (NCT01192685). Unfortunately, the study had to be terminated due to a technical failure of the neuronavigation system. However, more research is needed along this line because there is evidence indicating that cortical activity modulations before treatment commencement may be able to predict antidepressant treatment response. In a recent study published by Oliveira-Maia and colleagues, they measured stimulation-induced changes in motor evoked potentials (MEP) at baseline and found that modulations of corticospinal excitability predicted the antidepressant response of a two-week daily HF rTMS treatment.[10] The results of this study indicated that immediate modulatory effects of rTMS of the motor cortex forecast synaptic plasticity and associated antidepressant treatment effects. However, the motor cortex is not considered a key brain area in the neuropathology of MDD and predictions based on modulations of MEPs have been only moderately successful.[10] Therefore, research is needed that probes cortical modulations directly in the DLPFC; that is, the site where therapeutic brain stimulation is applied.

Noteworthy, studies measuring cortical and corticospinal activity reveal a substantial degree of individual variability in TMS-induced modulations.[10 15-18] For example, Maeda et al.[15] investigated the effects of 1 Hz, 10 Hz, 15 Hz and 20 Hz rTMS on MEP shortly after stimulation. Although responses were on average lowest at 1Hz and highest at 20 Hz, authors detected a high degree of variability, with some individuals even showing the opposite pattern, i.e., stronger excitatory responses at 1 Hz compared to 20 Hz. A high degree of variation in cortical excitability was also

reported in Oliveira-Maia et al.[10] and in a recent TMS/fMRI study.[18] The aim of the current proposal is to utilize such individual patterns of DLPFC modulations for personalized medicine in MDD.

There are two objectives in our proposal: The first objective is to provide mechanistic evidence for the direct effects of TBS of the healthy and presumed neuropathological prefrontal cortex. Specifically, we aim to assess excitability modulations of prefrontal oxyhemoglobin (HbO) by applying TBS on DLPFC in patients with MDD compared to healthy controls (HC). The second objective is to evaluate the relationship between immediate excitability modulations of the DLPFC and treatment response and thus provide a novel biomarker for individual patient selection. Given previous evidence and based on our line of reasoning given above, we will test the following operational hypotheses regarding our first objective: 1. We hypothesize an average (over all participants) increase in prefrontal HbO upon intermittent TBS (iTBS) and an average decrease in HbO upon continuous TBS (cTBS) in MDD and HC.[19 20] 2. We hypothesize average changes in prefrontal HbO will occur during stimulation, compared to baseline.[19 21] 3. We hypothesize significantly increased variance in HbO responses during and after stimulation compared to baseline.[10 15-18] 4. We hypothesize that patients with MDD have lower variability in TBS-induced HbO modulations compared to HC.[10 22 23] The following operational hypothesis will be tested regarding our second objective: 5. We hypothesize that individual TBS-induced HbO modulations predict the antidepressant response after treatment.[22 23]

Methods and analysis

Study design

The study is designed as an open-label, parallel-group experiment and has two parts (see Figure 1). In part one, patients with MDD and HC will be subjected to concurrent TBS/fNIRS. iTBS will be applied on the left DLPFC, whereas cTBS will be applied on the right DLPFC. Each participant will receive iTBS first, followed by cTBS after a delay of 1 hour to exclude possible interaction effects.[24] fNIRS data acquisition will include a baseline measurement of a few minutes, will continue during the stimulation period and last for several minutes post-stimulation period. The length of the post-stimulation period will be optimized to cover the entire duration of anticipated facilitatory effects of iTBS.[24] The TMS operator, as well as the researchers performing data analyses will be blinded regarding group membership. In part two, patients who participated in part one will receive a 4-week brain stimulation treatment trial with iTBS of the left DLPFC, performed daily for 5 days per week. Patients and doctors prescribing and evaluating the treatment as well as TMS operators administering the treatment are kept blind to fNIRS results. Psychometric evaluation will be performed on the day of TBS/fNIRS measurements and at the day of treatment start, as well as after 2 and after 4 weeks of treatment. A follow-up assessment will be performed 1 month after treatment ends.

Participants

Seventy patients diagnosed with MDD in a current major depressive episode will be included. Key inclusion criteria are: MDD (DSM-5), 18≤age≤60, Hamilton depression rating scale (HAMD-17) ≥18, approval for TBS treatment by the physician in charge, stable antidepressive medication 4 weeks before treatment. Key exclusion criteria are: a history of brain surgery, head injury, stroke or neurodegenerative disorder, diagnosis of personality disorder, psychotic features, active suicidal

intent, severe somatic comorbidities, cardiac pacemakers, deep brain stimulation, intracranial metallic particles, history of seizures, antiepileptics and benzodiazepines corresponding to a dose of >1 mg lorazepam/d, substance dependence or abuse, if it is the primary clinical problem. For the HC group, key inclusion criteria are: age between 18 and 60, right-handedness. Key exclusion criteria are: a current or previous diagnosis of a psychiatric, neurological disorder or severe internal illness, common contraindications to rTMS,[25] and a psychiatric disorder in their first-degree relatives.

The sample size was determined based on previous studies demonstrating that motor cortex excitability modulation significantly predicts antidepressant response of a two-week rTMS treatment.[10] A minimum sample size of 37 was determined using an effect size of r=0.43,[10] a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (Point biseral correlation model, two-tailed). Given an expected dropout rate of 15%, conservative sample size was set to 43 participants. However, in order to ensure adequate power and the ability to have reliable estimates and replicable findings, we aim to include a sample size of 70 or above. In addition, we will recruit 47 HC to participate in the TBS/fNIRS measurement. The sample is based on the comparison of MEP facilitation obtained in MDD (8 ± 49%)[10] and HC (37.9 ± 53.6%),[15] determined using an effect size of d=0.58, a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (unpaired two sample t-test, two-tailed). Four sites will be involved in patient recruitment: (1) the Department of Psychiatry, Chinese University of Hong Kong and its associated hospital, the Prince of Wales Hospital (Dr. Arthur Mak, Co-I); (2) the OT outpatient clinic at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University (Dr. Kenneth Fong, Co-I); (3) the Department of Psychiatry, Kowloon hospital (Dr. Wai Ching Yan, Dr. Athena K.Y. Chan) and (4) the Department of

Psychiatry, The University of Hong Kong and its associated hospital, the Queen Mary Hospital (Dr. Sherry K.W. Chan, Co-I). Healthy control participants will be recruited via posters and leaflets displayed at billboards on the University campus and community centers, community websites and social media. Participants will be screened by an experienced psychiatrist and the study including all study-related procedures will be explained to participants in oral and written form prior inclusion. The study will be performed in accordance with the Declaration of Helsinki,[26] including current revisions. All participants will be asked for written informed consent prior to inclusion in the study. Participants can decide to withdraw from the study at any time. The investigator may remove participants from the trial if exclusion criteria have been met or ending the participation is in the interest of the participant or study.

Theta-burst stimulation (TBS)

TBS comprises 3-pulse 50-Hz bursts, applied every 200 ms (at 5 Hz) as described previously.[24] iTBS consists of 2-second trains with an inter-train-interval of 8 seconds. We will repeat trains (30 pulses; 10 bursts) for 20 times to reach a total number of 600 pulses (3x10x20). cTBS will comprise uninterrupted bursts to reach a total number of 600 pulses.[27] Concurrent TBS/fNIRS stimulation will be applied over the left (iTBS) and right (cTBS) DLPFC at an intensity of 90% resting motor threshold (RMT), this corresponds to ~110% of the active motor threshold, an intensity that also elicited robust DLPFC activation in a recent concurrent TMS/fMRI study.[14] Stimulation at 90% RMT will also ensure compliance, reduce sensory discomfort, and minimize dropout rates during the concurrent TBS/fNIRS experiment. Still, scalp discomfort will be recorded directly after the stimulation. We refrained from choosing an intensity of 120% RMT (which will be applied during

antidepressant treatment) for the concurrent TBS/fNIRS experiment because such intensity would unlikely be tolerated by all patients as they are stimulation-naïve at the time of the experiment. The stimulation site over the DLPFC will be determined using the international 10-20 system and corresponds to the F3 label, determined using the optimized method by Beam et al.[28] TBS of the prefrontal cortex (PFC) is generally well tolerated, even at higher stimulation intensities. Antidepressant treatment comprises daily sessions of iTBS of the left DLPFC, five times a week for four weeks. Stimulation intensity will be 120% RMT (titration to full therapeutic dose over the first three days), as approved by the FDA in the U.S.[5] The stimulation site will be the same as in the concurrent TBS/fNIRS stimulation. Treatment will be performed at the TMS treatment centers of the participating local clinics (Department of Psychiatry, The University of Hong Kong and The Chinese University of Hong Kong, and at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University).

Functional near-infrared spectroscopy (fNIRS)

We will make use of the fNIRS system ImagentTM from ISS Inc., Champaign, IL, US (http://www.iss.com/biomedical/instruments/imagent.html) to determine changes in hemoglobin concentrations before, during and after TBS. ImagentTM uses a sensor that is embedded in a rectangular rubber pad with prisms inside so that optical fibers are rested tangentially instead of perpendicularly on the head surface. This arrangement allows the placement of the sensor directly underneath the TMS coil in close proximity to the stimulation site. Concurrent TBS/fNIRS poses technical and conceptual challenges. Although NIRS has the advantage of being relatively insensitive to motion artifacts, pilot data from our lab show significant stimulation-related artifacts

if sensors and the TMS coil touch each other. Hence, a minimum distance of a few millimeters between probes and the TMS coil will be ensured before the start of measurements. The lowest fNIRS probes will match the Fp1-Fp2 line in order to cover most of the prefrontal cortex extending to the temporal lobes (DLPFC, ventrolateral, frontopolar and superior temporal regions). The device measures changes in HbO, deoxy-hemoglobin (HbR) and total hemoglobin (HbT) using two wavelengths of infrared light (695 and 830 nm). With a source-detector spacing of 3cm, changes in Hb can be measured at a depth of 2-3cm corresponding to the cerebral cortex. fNIRS is not a perfect measure of brain activity and there are several sources of artifacts that need to be carefully considered. We will primarily focus on HbO since it may closer reflect BOLD changes as measured by fMRI.[29] The primary imaging endpoint will be the mean HbO amplitude of left and right DLPFC during and after the TBS stimulation. Secondary endpoints include mean Hb amplitudes during and after iTBS and cTBS, the steepness in the decline of Hb and ascent of HbO values, as well as the area under curve of HbO and Hb values during stimulation.. During concurrent TBS/fNIRS, participants will comfortably sit in a chair. Participants will be carefully instructed about the nature of the experiment prior to the TBS/fNIRS run.

Psychiatric assessment

HAMD17 is a standard instrument used in most clinical trials to screen for major depressive disorder. We will use a baseline score of HAMD17≥18 to apply a generally accepted definition of depression severity as inclusion criteria and to ensure comparability with other clinical trials. However, we will use the Montgomery-Asberg depression rating scale (MADRS) as the primary outcome measure because this symptom rating scale is more sensitive to changes over time.[30 31] In addition, we will

also use the Patient Health Questionnaire 9 (PHQ-9) as self-report questionnaire to assess subjective treatment effects over time. The PHQ-9 is widely used in psychiatric research. Therefore, we use different instruments for inclusion criteria and measurement of treatment response including both rater assessment and self-report inventories, a common practice in many clinical trials.

All psychometric scales used in this study are available in Chinese,[32-34] and show comparable psychometric qualities compared to the original scales. For example, the inter-rater reliability of the Chinese version of HAMD was r=0.94, as was the sensitivity (0.79) and specifictity (0.80).[32] The Chinese version of MADRS and QIDS-C showe a high correlation with the HAMD (0.853 and 0.75, respectively).[33] The Chinese version of all scales have been used in several previous clinical trials which involved Hong Kong populations.[35 36]

Psychiatric assessment includes a range of clinical scales, administered at baseline on the day of the TBS/fNIRS measurement, after 2 and 4 weeks of treatment, as well as at follow-up 1 month after treatment ends. The primary clinical outcome measure will be the response rate after treatment (defined by a C-MADRS reduction ≥50% of baseline). Secondary endpoints will be the remission rate after treatment (defined by a C-MADRS≤7), cutoff scores for the C-MADRS are based on Liu et al 2014.[33]. Further, secondary endpoints include the absolute reduction of mean C-HAMD17 and C-IDS-C after 2 and 4 weeks of treatment and at 1 month follow-up, as well as the response (C-IDS-C30 and C-HAMD17 ≥50% of baseline) and remission rates (C-IDS-C30≤12, C-HAMD17≤7) of patients after 4 weeks of treatment,[37 38] a reduction of 50% on a depression symptom rating scale is the most common response criterium in depression trials. Adverse events (AE) will be assessed according to good clinical practice (ICH/GCP) using an AE-questionnaire to detect unwanted side effects related to the treatment. Suicidality will be evaluated on each treatment day. Similarly,

depression severity will be evaluated at each treatment day using the Chinese version of the PHQ-9 (C-PHQ-9). Patients will be discontinued if they experience worsening in depression, defined as an increase in C-PHQ-9 from baseline of more than 25% during two consecutive assessments, or development of active suicidal intent or attempted suicide. Potentially occurring serious AEs (SAEs) will be recorded.

Data processing and statistical analysis

fNIRS data analysis will follow the standard processing steps. This includes spatial registration (recording of standard cranial landmarks nasion, inion, left and right ear, and the 3D locations of the fNIRS probes); transformation to MNI space; band-pass filtering for motion artifact removal; and estimation of the hemodynamic response function using GLM, as implemented in the NIRS Toolbox for MATLAB. Comparisons between HbO values at baseline and during/after stimulation will reveal TBS-related de/activations. A t-test between pre- and post-stimulation will be performed to test hypothesis 1. Hypothesis 1 will be supported if there is a significant increase and decrease in prefrontal HbO after iTBS and cTBS, respectively. A t-test between pre-stimulation and during stimulation will be performed to test hypothesis 2. Hypothesis 2 will be supported if there is a significant change in prefrontal HbO during stimulation. An F-test for the comparison of the variance in HbO values before versus after stimulation will be performed to test hypothesis 3. Hypothesis 3 will be supported if the F-test is significant. Similarly, an F-test for the comparison of the variance in HbO values in MDD versus HC will be performed to test hypothesis 4. Hypothesis 4 will be supported if the F-test is significant. Analyses will be performed using the IBM SPSS software (http://www-01.ibm.com/software/analytics/spss/). The alpha level will be set at 0.05,

adjusted for multiple comparisons using the Bonferroni-Holm procedure. For hypothesis 5, we will pursue two predictive modeling approaches, a conventional statistical analysis approach, as well as a machine learning approach.[39] First, a logistic regression analysis will be performed to define significant predictors of treatment response (defined as MADRS reduction ≥50% of baseline, see above). Logistic regression will be calculated as implemented in the generalized linear model function 'glm' of the statistical software 'R' (https://www.r-project.org/). Predictors will include imaging endpoints as given above, as well as sociodemographic and psychosocial variables (including the classification of patients as pharmacologically treatment resistant, TRD, defined by a failed treatment response after two or more consecutive antidepressants of adequate duration and dosage). Hierarchical multiple linear regression models will also be calculated to determine the relationship between MADRS reductions and secondary imaging endpoints as potential response predictors. Second, we will use machine learning algorithms for the classification of patients. We will test different algorithms since there is no established rule for the choice of an optimal machine learning approach. We will start with a dichotomous classification using the RandomForest (RF) package for R (ran.r-project.org/) and determine the most useful predictors for distinguishing responders from non-responders. RF is an ensemble tree classification tool that randomly selects subsamples of observations and builds a decision tree for the optimal splitting of these observations according to an outcome variable by a combination of predictors. For each split, the best performing predictor out of a random selection is applied. RF has the advantage of being straightforward and less susceptible to overfitting compared to other machine learning classifiers. To measure the predictive power of our classification model, we will use a five-fold cross-validation design. This allows for optimal validation in the absence of an independent test set.[40] There is no established

method of power calculation for RF. However, we will restrict HbO measurements to few prefrontal channels in order to keep the number of features for classification below the number of observations, thereby preventing the problem of hyperdimensionality. Receiver operating characteristics will be plotted using the ROCR package for the R-software.

Ethics and dissemination

Ethics approval was obtained from the Institutional Review Board of the Hong Kong Polytechnic University (reference numbers HSEARS20200120005, CRESC202009), as well as from the Institutional Review Boards of participating hospitals. An information sheet will be provided to participants before the experiment and a consent form will be signed by both PI and participant to protect the right of both parties. Participants will receive reimbursement for their participation. The data will be stored in an encrypted way and the accessibility is restricted to the researcher team. The study will start in January 2022 and is expected to be completed in December 2023.

The findings of this study will be disseminated through scientific journals, academic conferences, and university courses.

Challenges and potential limitations of this study

Although fNIRS has the advantage of being relatively insensitive to motion artefacts, we expect stimulation related artifacts caused by muscle contractions on the scalp given that sensors are in close proximity to the TMS coil. Furthermore, stimulation may have direct effects on superficial microvasculature. A challenge of this study will therefore be to minimize such artifacts. A variety

of techniques have been proposed to resolve these and other issues related to TMS-fNIRS integration.[41] Moreover, utilizing NIRS in this study will limit the interpretation of our results due to the inherent limitations of the technique of fNIRS. This includes a restriction to mesurements of shallow cortical regions (compared to fMRI) and restrictions in temporal resolution (as compared to EEG). Finally, the NIRS probe proposed to be used in our study only covers a small cortical area underneath the coil, which limits the analysis of stimulation effects to a small region of interest.

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.

Footnotes:

Contributions: GSK conceived the study and analysis. GSK and RLDK drafted the protocol and will conduct the data analysis. RLDK, BBBZ and KNKF will collect the data. ADPM and SKWC will recruit participants and perform the stimulation treatment. All the authors reviewed the protocol and agree to the final version being submitted.

Abbreviations list: rTMS: repetitive transcranial magnetic stimulation, DLPFC: dorsolateral prefrontal cortex, MDD: major depressive disorder, fNIRS: functional near-infrared spectroscopy, TBS: theta burst stimulation, iTBS: intermittent TBS, cTBS: continuous TBS, fMRI: functional magnetic resonance imaging, EEG: electroencephalogram, HF: high frequency, MEP: motor evoked potentials, HC: healthy controls, HbO: oxy-hemoglobin, HbR: deoxy-hemoglobin, HbT: total hemoglobin, C-HAMD-17: Chinese version of Hamilton depression rating scale, RMT: resting motor threshold, PFC: prefrontal cortex, C-MADRS: Chinese version of Montgomery-Asberg depression rating scale, C-IDS-C30: Chinese version of Inventory of depression symptomatology-clinician, AE: adverse events, C-PHQ-9: Chinese version of Patient Health Questionnaire, RF: RandomForest.

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Competing interests: None declared.

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Figure legends

Figure 1. Study design

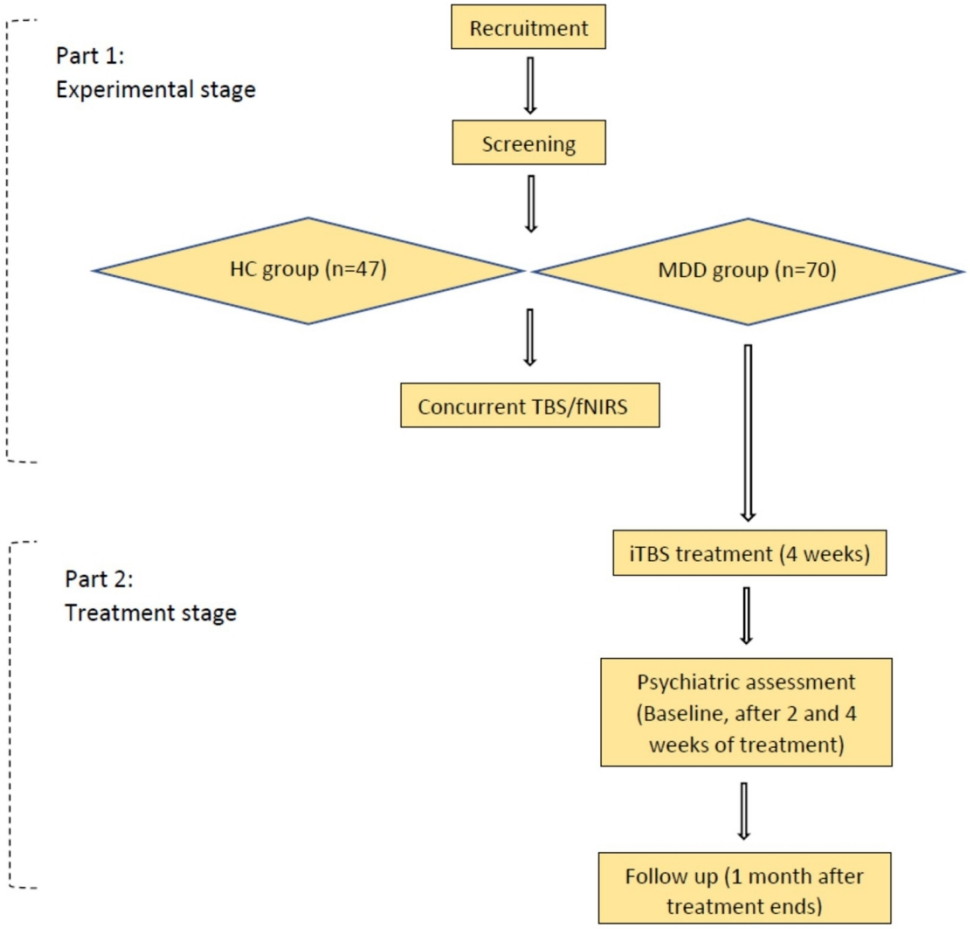

Figure 1. Study design

140x132mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	Yes (available in clinicaltrials.gov Identifier: NCT04526002)
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 9, 17
	5b	Name and contact information for the trial sponsor	N/A
	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
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	7
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, noninferiority, exploratory)	7, 8
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	9
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)	8, 9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
	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)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11-13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8

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	12, 13
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)	8, 13, 16
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target size	9, 10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	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	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16
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	16
Statistical methods	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	14-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15, 16
	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)	N/A
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	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
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	14
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
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
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)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10, 16
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	16

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	10, 16
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

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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The Utility of Concurrent TBS/fNIRS for Antidepressant Treatment Optimization: A Protocol

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The Utility of Concurrent TBS/fNIRS for Antidepressant Treatment Optimization: A Protocol

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Abstract:

Introduction: Repetitive transcranial magnetic stimulation (rTMS) with theta bursts (i.e. TBS) of the dorsolateral prefrontal cortex (DLPFC) is an innovative treatment for major depressive disorder (MDD). However, fewer than 50% of patients show sufficient response to this treatment; markers for response prediction are urgently needed. Research shows considerable individual variability in the brain responses to rTMS. However, whether differences in individual DLPFC modulation by rTMS can be utilized as a predictive marker for treatment response remains to be investigated. Here, we present a research program that will exploit the combination of functional near-infrared spectroscopy (fNIRS) with brain stimulation. Concurrent TBS/fNIRS will allow us to systematically investigate TBS-induced modulation of blood oxygenation as a proxy for induced brain activity changes. The findings from this study will (1) elucidate the immediate effects of excitatory and inhibitory TBS on prefrontal activity in TBS treatment-naïve patients with MDD and (2) validate the potential utility of TBS-induced brain modulation at baseline for the prediction of antidepressant response to four weeks of daily TBS treatment.

Methods and analysis: Open-label, parallel-group experiment consisting of two parts. In part one, 70 patients and 37 healthy controls will be subjected to concurrent TBS/fNIRS. Intermittent TBS (iTBS) and continuous TBS (cTBS) will be applied on the left and right DLPFC, respectively. fNIRS data will be acquired before, during and several minutes after stimulation. In part two, patients who participated in part one will receive a 4-week iTBS treatment of the left DLPFC, performed daily for 5 days per week. Psychometric evaluation will be performed periodically and at 1 month treatment follow up. Statistical analysis will include a conventional, as well as a machine learning approach.

Ethics and dissemination: Ethics approval was obtained from the Institutional Review Board. Findings will be disseminated through scientific journals, conferences, and university courses.

Registration: clinicaltrials.gov Identifier: NCT04526002

Keywords: Theta-burst stimulation, major depression, treatment prediction, functional NIRS, concurrent TBS/fNIRS

Strengths and limitations of this study

- Concurrent application of TMS and fNIRS
- Investigation of the immediate effects of excitatory and inhibitory TBS on prefrontal activity in major depression
- Exploration of the utility of TBS-induced brain modulation at baseline for the prediction of the antidepressant response to four weeks of daily TBS treatment
- Concurrent TBS/fNIRS bears technical challenges that need to be remediated
- The NIRS probe used in our study covers only a small area underneath the coil, limiting the analysis of stimulation effects to a small region of interest

Introduction

Stratified medicine is still an unmet need for biological psychiatry. Despite major efforts by others and us in utilizing neuroimaging tools to uncover diagnostic and predictive markers (e.g.,[1]), psychiatrists are still lacking such indicators with clinical utility.[2] The urgency for developing biomarkers for psychiatric disorders such as major depressive disorder (MDD) is demonstrated by the fact that mental disorders are the leading global burden in terms of years lived with disability.[3] Moreover, mental disorders are associated with economic costs that are higher than cardiovascular disorders, cancer, and diabetes combined.[4] In light of the high percentage of treatment refractoriness, a particular need for psychiatry is to uncover markers that predict the outcome of treatments before or at an early stage after treatment start.

Theta burst stimulation (TBS), a special form of patterned repetitive transcranial magnetic stimulation (rTMS), has finally found its way into clinical practice for the treatment of MDD. TBS is safe, effective in depressed patients that are refractory to standard pharmacological treatments, and has the advantage of increased efficiency over standard rTMS. However, response rates for rTMS as well as TBS, while promising enough to offer this treatment (with only minor side effects) to patients with MDD, are still achieved in only about 50% of patients.[5] Several attempts to predict antidepressant response were made in recent years but they only succeeded at a group level, whereas markers that are sufficiently accurate to guide decisions on an individual level are still absent. For example, baseline functional connectivity between subgenual anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) has been proposed as a biomarker for the individualization of the stimulation target to optimize treatment response.[6 7] Yet, when functional connectivity-based target selection is implemented, response rates still do not exceed the 50% mark.[8] Other attempts to

predict response rates include measurements of cortical thickness.[9] or corticospinal excitability,[10] as well as many other patient-related, illness-related, and stimulation procedure-related factors, for a review, see [11 12].

Concurrent neuroimaging with TMS may be especially fruitful to probe diagnostic and predictive neuroimaging markers as it aims to uncover the immediate modulatory effects of stimulation. Indeed, the prevailing view on therapeutic brain stimulation is that modulation of prefrontal excitability mediates its antidepressant effect. Hence, direct modulatory effects of prefrontal excitability during and immediately after rTMS likely forecast long-lasting changes in cortical excitability by promoting synaptic plasticity, which, according to current theory, should accompany rTMS treatment response.[10] Technological advances within the last decade allowed for the application of concurrent brain measurements with TMS using functional magnetic resonance imaging (fMRI) or electroencephalogram (EEG).[13] Authors observed prefrontal activation upon 1Hz rTMS with BOLD responses correlating with increasing stimulation strength. Activations were observed during the 10-second stimulation blocks and lasting 4-6 seconds after the last stimulation.[14] However, although highly promising for future research, it is questionable whether such a sophisticated combination of TMS and fMRI will eventually translate into a routinely used clinical test. Moreover, simultaneous image acquisitions during the application of a stimulation-burst or train of high frequency (HF) rTMS is impossible in an fMRI setting.

Functional near-infrared spectroscopy (fNIRS) is cheap, easy and harmless to apply and a widely available method to measure superficial brain activity and connectivity by means of changes in

blood hemoglobin concentrations. Concurrent TMS/fNIRS may be clinically superior to TMS/fMRI in order to probe the direct modulatory effect of prefrontal excitability during and immediately after stimulation. Indeed, a recent study from Boston University attempted to predict the antidepressant response to rTMS by utilizing fNIRS (NCT01192685). Unfortunately, the study had to be terminated due to a technical failure of the neuronavigation system. However, more research is needed along this line because there is evidence indicating that cortical activity modulations before treatment commencement may be able to predict antidepressant treatment response. In a recent study published by Oliveira-Maia and colleagues, they measured stimulation-induced changes in motor evoked potentials (MEP) at baseline and found that modulations of corticospinal excitability predicted the antidepressant response of a two-week daily HF rTMS treatment.[10] The results of this study indicated that immediate modulatory effects of rTMS of the motor cortex forecast synaptic plasticity and associated antidepressant treatment effects. However, the motor cortex is not considered a key brain area in the neuropathology of MDD and predictions based on modulations of MEPs have been only moderately successful.[10] Therefore, research is needed that probes cortical modulations directly in the DLPFC; that is, the site where therapeutic brain stimulation is applied.

Noteworthy, studies measuring cortical and corticospinal activity reveal a substantial degree of individual variability in TMS-induced modulations.[10 15-18] For example, Maeda et al.[15] investigated the effects of 1 Hz, 10 Hz, 15 Hz and 20 Hz rTMS on MEP shortly after stimulation. Although responses were on average lowest at 1Hz and highest at 20 Hz, authors detected a high degree of variability, with some individuals even showing the opposite pattern, i.e., stronger excitatory responses at 1 Hz compared to 20 Hz. A high degree of variation in cortical excitability was also

reported in Oliveira-Maia et al.[10] and in a recent TMS/fMRI study.[18] The aim of the current proposal is to utilize such individual patterns of DLPFC modulations for personalized medicine in MDD.

There are two objectives in our proposal: The first objective is to provide mechanistic evidence for the direct effects of TBS of the healthy and presumed neuropathological prefrontal cortex. Specifically, we aim to assess excitability modulations of prefrontal oxyhemoglobin (HbO) by applying TBS on DLPFC in patients with MDD compared to healthy controls (HC). The second objective is to evaluate the relationship between immediate excitability modulations of the DLPFC and treatment response and thus provide a novel biomarker for individual patient selection. Given previous evidence and based on our line of reasoning given above, we will test the following operational hypotheses regarding our first objective: 1. We hypothesize an average (over all participants) increase in prefrontal HbO upon intermittent TBS (iTBS) and an average decrease in HbO upon continuous TBS (cTBS) in MDD and HC.[19 20] 2. We hypothesize average changes in prefrontal HbO will occur during stimulation, compared to baseline.[19 21] 3. We hypothesize significantly increased variance in HbO responses during and after stimulation compared to baseline.[10 15-18] 4. We hypothesize that patients with MDD have lower variability in TBS-induced HbO modulations compared to HC.[10 22 23] The following operational hypothesis will be tested regarding our second objective: 5. We hypothesize that individual TBS-induced HbO modulations predict the antidepressant response after treatment.[22 23]

Methods and analysis

Study design

The study is designed as an open-label, parallel-group experiment and has two parts (see Figure 1). In part one, patients with MDD and HC will be subjected to concurrent TBS/fNIRS. iTBS will be applied on the left DLPFC, whereas cTBS will be applied on the right DLPFC. Each participant will receive iTBS first, followed by cTBS after a delay of 1 hour to exclude possible interaction effects.[24] fNIRS data acquisition will include a baseline measurement of a few minutes, will continue during the stimulation period and last for several minutes post-stimulation period. The length of the post-stimulation period will be optimized to cover the entire duration of anticipated facilitatory effects of iTBS.[24] The TMS operator, as well as the researchers performing data analyses will be blinded regarding group membership. In part two, patients who participated in part one will receive a 4-week brain stimulation treatment trial with iTBS of the left DLPFC, performed daily for 5 days per week. Patients and doctors prescribing and evaluating the treatment as well as TMS operators administering the treatment are kept blind to fNIRS results. Psychometric evaluation will be performed on the day of TBS/fNIRS measurements and at the day of treatment start, as well as after 2 and after 4 weeks of treatment. A follow-up assessment will be performed 1 month after treatment ends.

Participants

Seventy patients diagnosed with MDD in a current major depressive episode will be included. Key inclusion criteria are: MDD (DSM-5), 18≤age≤60, Hamilton depression rating scale (HAMD-17) ≥18, approval for TBS treatment by the physician in charge, stable antidepressive medication 4 weeks before treatment. Key exclusion criteria are: a history of brain surgery, head injury, stroke or neurodegenerative disorder, diagnosis of personality disorder, psychotic features, active suicidal

intent, severe somatic comorbidities, cardiac pacemakers, deep brain stimulation, intracranial metallic particles, history of seizures, antiepileptics and benzodiazepines corresponding to a dose of >1 mg lorazepam/d, substance dependence or abuse, if it is the primary clinical problem. For the HC group, key inclusion criteria are: age between 18 and 60, right-handedness. Key exclusion criteria are: a current or previous diagnosis of a psychiatric, neurological disorder or severe internal illness, common contraindications to rTMS,[25] and a psychiatric disorder in their first-degree relatives.

The sample size was determined based on previous studies demonstrating that motor cortex excitability modulation significantly predicts antidepressant response of a two-week rTMS treatment.[10] A minimum sample size of 37 was determined using an effect size of r=0.43,[10] a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (Point biseral correlation model, two-tailed). Given an expected dropout rate of 15%, conservative sample size was set to 43 participants. However, in order to ensure adequate power and the ability to have reliable estimates and replicable findings, we aim to include a sample size of 70 or above. In addition, we will recruit 47 HC to participate in the TBS/fNIRS measurement. The sample is based on the comparison of MEP facilitation obtained in MDD (8 ± 49%)[10] and HC (37.9 ± 53.6%),[15] determined using an effect size of d=0.58, a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (unpaired two sample t-test, two-tailed). Four sites will be involved in patient recruitment: (1) the Department of Psychiatry, Chinese University of Hong Kong and its associated hospital, the Prince of Wales Hospital (Dr. Arthur Mak, Co-I); (2) the OT outpatient clinic at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University (Dr. Kenneth Fong, Co-I); (3) the Department of Psychiatry, Kowloon hospital (Dr. Wai Ching Yan, Dr. Athena K.Y. Chan) and (4) the Department of

Psychiatry, The University of Hong Kong and its associated hospital, the Queen Mary Hospital (Dr. Sherry K.W. Chan, Co-I). Healthy control participants will be recruited via posters and leaflets displayed at billboards on the University campus and community centers, community websites and social media. Participants will be screened by an experienced psychiatrist and the study including all study-related procedures will be explained to participants in oral and written form prior inclusion. The study will be performed in accordance with the Declaration of Helsinki,[26] including current revisions. All participants will be asked for written informed consent prior to inclusion in the study. Participants can decide to withdraw from the study at any time. The investigator may remove participants from the trial if exclusion criteria have been met or ending the participation is in the interest of the participant or study.

Theta-burst stimulation (TBS)

TBS comprises 3-pulse 50-Hz bursts, applied every 200 ms (at 5 Hz) as described previously.[24] iTBS consists of 2-second trains with an inter-train-interval of 8 seconds. We will repeat trains (30 pulses; 10 bursts) for 20 times to reach a total number of 600 pulses (3x10x20). cTBS will comprise uninterrupted bursts to reach a total number of 600 pulses.[27] Concurrent TBS/fNIRS stimulation will be applied over the left (iTBS) and right (cTBS) DLPFC at an intensity of 90% resting motor threshold (RMT), this corresponds to ~110% of the active motor threshold, an intensity that also elicited robust DLPFC activation in a recent concurrent TMS/fMRI study.[14] Stimulation at 90% RMT will also ensure compliance, reduce sensory discomfort, and minimize dropout rates during the concurrent TBS/fNIRS experiment. Still, scalp discomfort will be recorded directly after the stimulation. We refrained from choosing an intensity of 120% RMT (which will be applied during

antidepressant treatment) for the concurrent TBS/fNIRS experiment because such intensity would unlikely be tolerated by all patients as they are stimulation-naïve at the time of the experiment. The stimulation site over the DLPFC will be determined using the international 10-20 system and corresponds to the F3 label, determined using the optimized method by Beam et al.[28] TBS of the prefrontal cortex (PFC) is generally well tolerated, even at higher stimulation intensities. Antidepressant treatment comprises daily sessions of iTBS of the left DLPFC, five times a week for four weeks. Stimulation intensity will be 120% RMT (titration to full therapeutic dose over the first three days), as approved by the FDA in the U.S.[5] The stimulation site will be the same as in the concurrent TBS/fNIRS stimulation. Treatment will be performed at the TMS treatment centers of the participating local clinics (Department of Psychiatry, The University of Hong Kong and The Chinese University of Hong Kong, and at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University).

Functional near-infrared spectroscopy (fNIRS)

We will make use of the fNIRS system ImagentTM from ISS Inc., Champaign, IL, US (http://www.iss.com/biomedical/instruments/imagent.html) to determine changes in hemoglobin concentrations before, during and after TBS. ImagentTM uses a sensor that is embedded in a rectangular rubber pad with prisms inside so that optical fibers are rested tangentially instead of perpendicularly on the head surface. This arrangement allows the placement of the sensor directly underneath the TMS coil in close proximity to the stimulation site. Concurrent TBS/fNIRS poses technical and conceptual challenges. Although NIRS has the advantage of being relatively insensitive to motion artifacts, pilot data from our lab show significant stimulation-related artifacts

if sensors and the TMS coil touch each other. Hence, a minimum distance of a few millimeters between probes and the TMS coil will be ensured before the start of measurements. The lowest fNIRS probes will match the Fp1-Fp2 line in order to cover most of the prefrontal cortex extending to the temporal lobes (DLPFC, ventrolateral, frontopolar and superior temporal regions). The device measures changes in HbO, deoxy-hemoglobin (HbR) and total hemoglobin (HbT) using two wavelengths of infrared light (695 and 830 nm). With a source-detector spacing of 3cm, changes in Hb can be measured at a depth of 2-3cm corresponding to the cerebral cortex. fNIRS is not a perfect measure of brain activity and there are several sources of artifacts that need to be carefully considered. We will primarily focus on HbO since it may closer reflect BOLD changes as measured by fMRI.[29] The primary imaging endpoint will be the mean HbO amplitude of left and right DLPFC during and after the TBS stimulation. Secondary endpoints include mean Hb amplitudes during and after iTBS and cTBS, the steepness in the decline of Hb and ascent of HbO values, as well as the area under curve of HbO and Hb values during stimulation.. During concurrent TBS/fNIRS, participants will comfortably sit in a chair. Participants will be carefully instructed about the nature of the experiment prior to the TBS/fNIRS run.

Psychiatric assessment

HAMD17 is a standard instrument used in most clinical trials to screen for major depressive disorder. We will use a baseline score of HAMD17≥18 to apply a generally accepted definition of depression severity as inclusion criteria and to ensure comparability with other clinical trials. However, we will use the Montgomery-Asberg depression rating scale (MADRS) as the primary outcome measure because this symptom rating scale is more sensitive to changes over time.[30 31] In addition, we will

also use the Patient Health Questionnaire 9 (PHQ-9) as self-report questionnaire to assess subjective treatment effects over time. The PHQ-9 is widely used in psychiatric research. Therefore, we use different instruments for inclusion criteria and measurement of treatment response including both rater assessment and self-report inventories, a common practice in many clinical trials.

All psychometric scales used in this study are available in Chinese,[32-34] and show comparable psychometric qualities compared to the original scales. For example, the inter-rater reliability of the Chinese version of HAMD was r=0.94, as was the sensitivity (0.79) and specifictity (0.80).[32] The Chinese version of MADRS and QIDS-C showe a high correlation with the HAMD (0.853 and 0.75, respectively).[33] The Chinese version of all scales have been used in several previous clinical trials which involved Hong Kong populations.[35 36]

Psychiatric assessment includes a range of clinical scales, administered at baseline on the day of the TBS/fNIRS measurement, after 2 and 4 weeks of treatment, as well as at follow-up 1 month after treatment ends. The primary clinical outcome measure will be the response rate after treatment (defined by a C-MADRS reduction ≥50% of baseline). Secondary endpoints will be the remission rate after treatment (defined by a C-MADRS≤7), cutoff scores for the C-MADRS are based on Liu et al 2014.[33]. Further, secondary endpoints include the absolute reduction of mean C-HAMD17 and C-IDS-C after 2 and 4 weeks of treatment and at 1 month follow-up, as well as the response (C-IDS-C30 and C-HAMD17 ≥50% of baseline) and remission rates (C-IDS-C30≤12, C-HAMD17≤7) of patients after 4 weeks of treatment,[37 38] a reduction of 50% on a depression symptom rating scale is the most common response criterium in depression trials. Adverse events (AE) will be assessed according to good clinical practice (ICH/GCP) using an AE-questionnaire to detect unwanted side effects related to the treatment. Suicidality will be evaluated on each treatment day. Similarly,

depression severity will be evaluated at each treatment day using the Chinese version of the PHQ-9 (C-PHQ-9). Patients will be discontinued if they experience worsening in depression, defined as an increase in C-PHQ-9 from baseline of more than 25% during two consecutive assessments, or development of active suicidal intent or attempted suicide. Potentially occurring serious AEs (SAEs) will be recorded.

Data processing and statistical analysis

fNIRS data analysis will follow the standard processing steps. This includes spatial registration (recording of standard cranial landmarks nasion, inion, left and right ear, and the 3D locations of the fNIRS probes); transformation to MNI space; band-pass filtering for motion artifact removal; and estimation of the hemodynamic response function using GLM, as implemented in the NIRS Toolbox for MATLAB. Comparisons between HbO values at baseline and during/after stimulation will reveal TBS-related de/activations. A t-test between pre- and post-stimulation will be performed to test hypothesis 1. Hypothesis 1 will be supported if there is a significant increase and decrease in prefrontal HbO after iTBS and cTBS, respectively. A t-test between pre-stimulation and during stimulation will be performed to test hypothesis 2. Hypothesis 2 will be supported if there is a significant change in prefrontal HbO during stimulation. An F-test for the comparison of the variance in HbO values before versus after stimulation will be performed to test hypothesis 3. Hypothesis 3 will be supported if the F-test is significant. Similarly, an F-test for the comparison of the variance in HbO values in MDD versus HC will be performed to test hypothesis 4. Hypothesis 4 will be supported if the F-test is significant. Analyses will be performed using the IBM SPSS software (http://www-01.ibm.com/software/analytics/spss/). The alpha level will be set at 0.05,

adjusted for multiple comparisons using the Bonferroni-Holm procedure. For hypothesis 5, we will pursue two predictive modeling approaches, a conventional statistical analysis approach, as well as a machine learning approach.[39] First, a logistic regression analysis will be performed to define significant predictors of treatment response (defined as MADRS reduction ≥50% of baseline, see above). Logistic regression will be calculated as implemented in the generalized linear model function 'glm' of the statistical software 'R' (https://www.r-project.org/). Predictors will include imaging endpoints as given above, as well as sociodemographic and psychosocial variables (including the classification of patients as pharmacologically treatment resistant, TRD, defined by a failed treatment response after two or more consecutive antidepressants of adequate duration and dosage). Hierarchical multiple linear regression models will also be calculated to determine the relationship between MADRS reductions and secondary imaging endpoints as potential response predictors. Second, we will use machine learning algorithms for the classification of patients. We will test different algorithms since there is no established rule for the choice of an optimal machine learning approach. We will start with a dichotomous classification using the RandomForest (RF) package for R (ran.r-project.org/) and determine the most useful predictors for distinguishing responders from non-responders. RF is an ensemble tree classification tool that randomly selects subsamples of observations and builds a decision tree for the optimal splitting of these observations according to an outcome variable by a combination of predictors. For each split, the best performing predictor out of a random selection is applied. RF has the advantage of being straightforward and less susceptible to overfitting compared to other machine learning classifiers. To measure the predictive power of our classification model, we will use a five-fold cross-validation design. This allows for optimal validation in the absence of an independent test set.[40] There is no established

method of power calculation for RF. However, we will restrict HbO measurements to few prefrontal channels in order to keep the number of features for classification below the number of observations, thereby preventing the problem of hyperdimensionality. Receiver operating characteristics will be plotted using the ROCR package for the R-software.

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.

Ethics and dissemination

Ethics approval was obtained from the Institutional Review Board of the Hong Kong Polytechnic University (reference numbers HSEARS20200120005, CRESC202009), as well as from the Institutional Review Boards of participating hospitals. An information sheet will be provided to participants before the experiment and a consent form will be signed by both PI and participant to protect the right of both parties. Participants will receive reimbursement for their participation. The data will be stored in an encrypted way and the accessibility is restricted to the researcher team. The study will start in January 2022 and is expected to be completed in December 2023.

The findings of this study will be disseminated through scientific journals, academic conferences, and university courses.

Challenges and potential limitations of this study

Although fNIRS has the advantage of being relatively insensitive to motion artefacts, we expect

stimulation related artifacts caused by muscle contractions on the scalp given that sensors are in close proximity to the TMS coil. Furthermore, stimulation may have direct effects on superficial microvasculature. A challenge of this study will therefore be to minimize such artifacts. A variety of techniques have been proposed to resolve these and other issues related to TMS-fNIRS integration.[41] Moreover, utilizing NIRS in this study will limit the interpretation of our results due to the inherent limitations of the technique of fNIRS. This includes a restriction to mesurements of shallow cortical regions (compared to fMRI) and restrictions in temporal resolution (as compared to EEG). Finally, the NIRS probe proposed to be used in our study only covers a small cortical area underneath the coil, which limits the analysis of stimulation effects to a small region of interest.

Footnotes:

Contributions: GSK conceived the study and analysis. GSK and RLDK drafted the protocol and will conduct the data analysis. RLDK, BBBZ and KNKF will collect the data. ADPM and SKWC will recruit participants and perform the stimulation treatment. All the authors reviewed the protocol and agree to the final version being submitted.

Abbreviations list: rTMS: repetitive transcranial magnetic stimulation, DLPFC: dorsolateral prefrontal cortex, MDD: major depressive disorder, fNIRS: functional near-infrared spectroscopy, TBS: theta burst stimulation, iTBS: intermittent TBS, cTBS: continuous TBS, fMRI: functional magnetic resonance imaging, EEG: electroencephalogram, HF: high frequency, MEP: motor evoked potentials, HC: healthy controls, HbO: oxy-hemoglobin, HbR: deoxy-hemoglobin, HbT: total hemoglobin, C-HAMD-17: Chinese version of Hamilton depression rating scale, RMT: resting motor threshold, PFC: prefrontal cortex, C-MADRS: Chinese version of Montgomery-Asberg depression rating scale, C-IDS-C30: Chinese version of Inventory of depression symptomatology-clinician, AE: adverse events, C-PHQ-9: Chinese version of Patient Health Questionnaire, RF: RandomForest.

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Competing interests: None declared.

Patient consent for publication: Not required.

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Figure legends

Figure 1. Study design

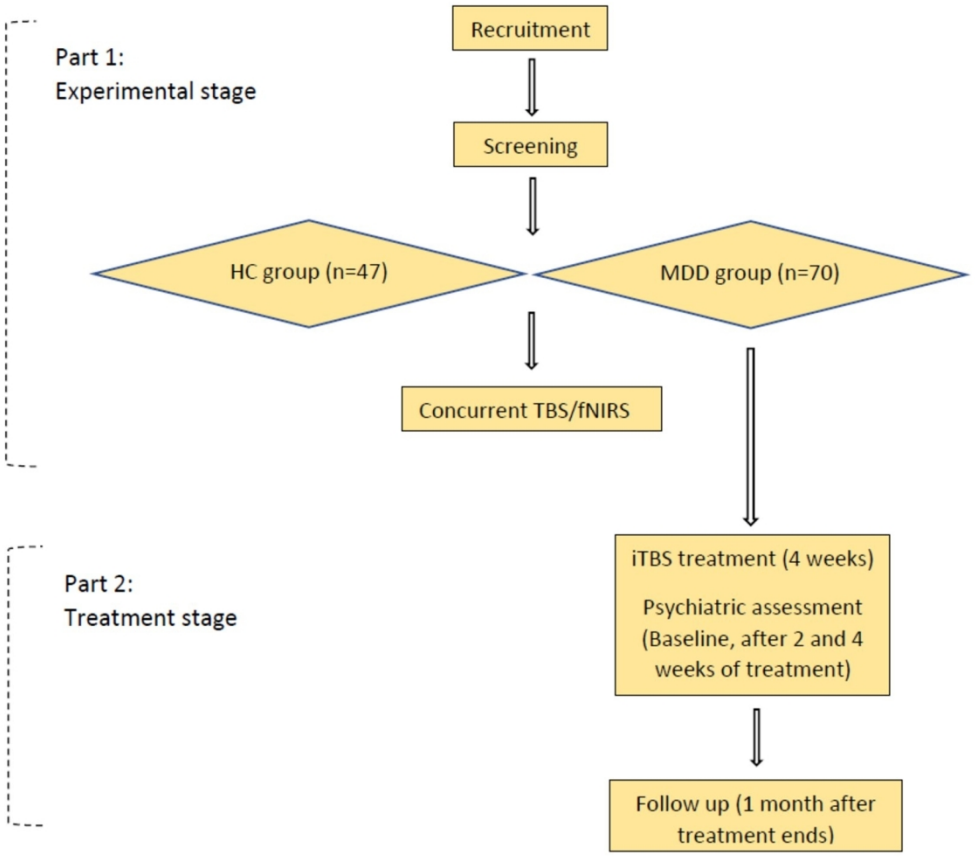

Figure 1. Study design

140x121mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	Yes (available in clinicaltrials.gov Identifier: NCT04526002)
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 9, 17
	5b	Name and contact information for the trial sponsor	N/A
	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
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	7
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, noninferiority, exploratory)	7, 8
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	9
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)	8, 9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
	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)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11-13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8

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	12, 13
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)	8, 13, 16
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target size	9, 10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	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	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16
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	16
Statistical methods	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	14-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15, 16
	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)	N/A
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	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
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	14
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
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
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)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10, 16
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	16

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	10, 16
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

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Protocol for a Prospective Open-label Clinical Trial to Investigate the Utility of Concurrent TBS/fNIRS for Antidepressant Treatment Optimization

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Abstract:

Introduction: Repetitive transcranial magnetic stimulation (rTMS) with theta bursts (i.e. TBS) of the dorsolateral prefrontal cortex (DLPFC) is an innovative treatment for major depressive disorder (MDD). However, fewer than 50% of patients show sufficient response to this treatment; markers for response prediction are urgently needed. Research shows considerable individual variability in the brain responses to rTMS. However, whether differences in individual DLPFC modulation by rTMS can be utilized as a predictive marker for treatment response remains to be investigated. Here, we present a research program that will exploit the combination of functional near-infrared spectroscopy (fNIRS) with brain stimulation. Concurrent TBS/fNIRS will allow us to systematically investigate TBS-induced modulation of blood oxygenation as a proxy for induced brain activity changes. The findings from this study will (1) elucidate the immediate effects of excitatory and inhibitory TBS on prefrontal activity in TBS treatment-naïve patients with MDD and (2) validate the potential utility of TBS-induced brain modulation at baseline for the prediction of antidepressant response to four weeks of daily TBS treatment.

Methods and analysis: Open-label, parallel-group experiment consisting of two parts. In part one, 70 patients and 37 healthy controls will be subjected to concurrent TBS/fNIRS. Intermittent TBS (iTBS) and continuous TBS (cTBS) will be applied on the left and right DLPFC, respectively. fNIRS data will be acquired before, during and several minutes after stimulation. In part two, patients who participated in part one will receive a 4-week iTBS treatment of the left DLPFC, performed daily for 5 days per week. Psychometric evaluation will be performed periodically and at 1 month treatment follow up. Statistical analysis will include a conventional, as well as a machine learning approach.

Ethics and dissemination: Ethics approval was obtained from the Institutional Review Board. Findings will be disseminated through scientific journals, conferences, and university courses.

Registration: clinicaltrials.gov Identifier: NCT04526002

Keywords: Theta-burst stimulation, major depression, treatment prediction, functional NIRS, concurrent TBS/fNIRS

Strengths and limitations of this study

- Concurrent application of TMS and fNIRS
- Investigation of the immediate effects of excitatory and inhibitory TBS on prefrontal activity in major depression
- Exploration of the utility of TBS-induced brain modulation at baseline for the prediction of the antidepressant response to four weeks of daily TBS treatment
- Concurrent TBS/fNIRS bears technical challenges that need to be remediated
- The NIRS probe used in our study covers only a small area underneath the coil, limiting the analysis of stimulation effects to a small region of interest

Introduction

Stratified medicine is still an unmet need for biological psychiatry. Despite major efforts by others and us in utilizing neuroimaging tools to uncover diagnostic and predictive markers (e.g.,[1]), psychiatrists are still lacking such indicators with clinical utility.[2] The urgency for developing biomarkers for psychiatric disorders such as major depressive disorder (MDD) is demonstrated by the fact that mental disorders are the leading global burden in terms of years lived with disability.[3] Moreover, mental disorders are associated with economic costs that are higher than cardiovascular disorders, cancer, and diabetes combined.[4] In light of the high percentage of treatment refractoriness, a particular need for psychiatry is to uncover markers that predict the outcome of treatments before or at an early stage after treatment start.

Theta burst stimulation (TBS), a special form of patterned repetitive transcranial magnetic stimulation (rTMS), has finally found its way into clinical practice for the treatment of MDD. TBS is safe, effective in depressed patients that are refractory to standard pharmacological treatments, and has the advantage of increased efficiency over standard rTMS. However, response rates for rTMS as well as TBS, while promising enough to offer this treatment (with only minor side effects) to patients with MDD, are still achieved in only about 50% of patients.[5] Several attempts to predict antidepressant response were made in recent years but they only succeeded at a group level, whereas markers that are sufficiently accurate to guide decisions on an individual level are still absent. For example, baseline functional connectivity between subgenual anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) has been proposed as a biomarker for the individualization of the stimulation target to optimize treatment response.[6 7] Yet, when functional connectivity-based target selection is implemented, response rates still do not exceed the 50% mark.[8] Other attempts to

predict response rates include measurements of cortical thickness.[9] or corticospinal excitability,[10] as well as many other patient-related, illness-related, and stimulation procedure-related factors, for a review, see [11 12].

Concurrent neuroimaging with TMS may be especially fruitful to probe diagnostic and predictive neuroimaging markers as it aims to uncover the immediate modulatory effects of stimulation. Indeed, the prevailing view on therapeutic brain stimulation is that modulation of prefrontal excitability mediates its antidepressant effect. Hence, direct modulatory effects of prefrontal excitability during and immediately after rTMS likely forecast long-lasting changes in cortical excitability by promoting synaptic plasticity, which, according to current theory, should accompany rTMS treatment response.[10] Technological advances within the last decade allowed for the application of concurrent brain measurements with TMS using functional magnetic resonance imaging (fMRI) or electroencephalogram (EEG).[13] Authors observed prefrontal activation upon 1Hz rTMS with BOLD responses correlating with increasing stimulation strength. Activations were observed during the 10-second stimulation blocks and lasting 4-6 seconds after the last stimulation.[14] However, although highly promising for future research, it is questionable whether such a sophisticated combination of TMS and fMRI will eventually translate into a routinely used clinical test. Moreover, simultaneous image acquisitions during the application of a stimulation-burst or train of high frequency (HF) rTMS is impossible in an fMRI setting.

Functional near-infrared spectroscopy (fNIRS) is cheap, easy and harmless to apply and a widely available method to measure superficial brain activity and connectivity by means of changes in

blood hemoglobin concentrations. Concurrent TMS/fNIRS may be clinically superior to TMS/fMRI in order to probe the direct modulatory effect of prefrontal excitability during and immediately after stimulation. Indeed, a recent study from Boston University attempted to predict the antidepressant response to rTMS by utilizing fNIRS (NCT01192685). Unfortunately, the study had to be terminated due to a technical failure of the neuronavigation system. However, more research is needed along this line because there is evidence indicating that cortical activity modulations before treatment commencement may be able to predict antidepressant treatment response. In a recent study published by Oliveira-Maia and colleagues, they measured stimulation-induced changes in motor evoked potentials (MEP) at baseline and found that modulations of corticospinal excitability predicted the antidepressant response of a two-week daily HF rTMS treatment.[10] The results of this study indicated that immediate modulatory effects of rTMS of the motor cortex forecast synaptic plasticity and associated antidepressant treatment effects. However, the motor cortex is not considered a key brain area in the neuropathology of MDD and predictions based on modulations of MEPs have been only moderately successful.[10] Therefore, research is needed that probes cortical modulations directly in the DLPFC; that is, the site where therapeutic brain stimulation is applied.

Noteworthy, studies measuring cortical and corticospinal activity reveal a substantial degree of individual variability in TMS-induced modulations.[10 15-18] For example, Maeda et al.[15] investigated the effects of 1 Hz, 10 Hz, 15 Hz and 20 Hz rTMS on MEP shortly after stimulation. Although responses were on average lowest at 1Hz and highest at 20 Hz, authors detected a high degree of variability, with some individuals even showing the opposite pattern, i.e., stronger excitatory responses at 1 Hz compared to 20 Hz. A high degree of variation in cortical excitability was also

reported in Oliveira-Maia et al.[10] and in a recent TMS/fMRI study.[18] The aim of the current proposal is to utilize such individual patterns of DLPFC modulations for personalized medicine in MDD.

There are two objectives in our proposal: The first objective is to provide mechanistic evidence for the direct effects of TBS of the healthy and presumed neuropathological prefrontal cortex. Specifically, we aim to assess excitability modulations of prefrontal oxyhemoglobin (HbO) by applying TBS on DLPFC in patients with MDD compared to healthy controls (HC). The second objective is to evaluate the relationship between immediate excitability modulations of the DLPFC and treatment response and thus provide a novel biomarker for individual patient selection. Given previous evidence and based on our line of reasoning given above, we will test the following operational hypotheses regarding our first objective: 1. We hypothesize an average (over all participants) increase in prefrontal HbO upon intermittent TBS (iTBS) and an average decrease in HbO upon continuous TBS (cTBS) in MDD and HC.[19 20] 2. We hypothesize average changes in prefrontal HbO will occur during stimulation, compared to baseline.[19 21] 3. We hypothesize significantly increased variance in HbO responses during and after stimulation compared to baseline.[10 15-18] 4. We hypothesize that patients with MDD have lower variability in TBS-induced HbO modulations compared to HC.[10 22 23] The following operational hypothesis will be tested regarding our second objective: 5. We hypothesize that individual TBS-induced HbO modulations predict the antidepressant response after treatment.[22 23]

Methods and analysis

Study design

The study is designed as an open-label, parallel-group experiment and has two parts (see Figure 1). In part one, patients with MDD and HC will be subjected to concurrent TBS/fNIRS. iTBS will be applied on the left DLPFC, whereas cTBS will be applied on the right DLPFC. Each participant will receive iTBS first, followed by cTBS after a delay of 1 hour to exclude possible interaction effects.[24] fNIRS data acquisition will include a baseline measurement of a few minutes, will continue during the stimulation period and last for several minutes post-stimulation period. The length of the post-stimulation period will be optimized to cover the entire duration of anticipated facilitatory effects of iTBS.[24] The TMS operator, as well as the researchers performing data analyses will be blinded regarding group membership. In part two, patients who participated in part one will receive a 4-week brain stimulation treatment trial with iTBS of the left DLPFC, performed daily for 5 days per week. Patients and doctors prescribing and evaluating the treatment as well as TMS operators administering the treatment are kept blind to fNIRS results. Psychometric evaluation will be performed on the day of TBS/fNIRS measurements and at the day of treatment start, as well as after 2 and after 4 weeks of treatment. A follow-up assessment will be performed 1 month after treatment ends.

Participants

Seventy patients diagnosed with MDD in a current major depressive episode will be included. Key inclusion criteria are: MDD (DSM-5), 18≤age≤60, Hamilton depression rating scale (HAMD-17) ≥18, approval for TBS treatment by the physician in charge, stable antidepressive medication 4 weeks before treatment. Key exclusion criteria are: a history of brain surgery, head injury, stroke or neurodegenerative disorder, diagnosis of personality disorder, psychotic features, active suicidal

intent, severe somatic comorbidities, cardiac pacemakers, deep brain stimulation, intracranial metallic particles, history of seizures, antiepileptics and benzodiazepines corresponding to a dose of >1 mg lorazepam/d, substance dependence or abuse, if it is the primary clinical problem. For the HC group, key inclusion criteria are: age between 18 and 60, right-handedness. Key exclusion criteria are: a current or previous diagnosis of a psychiatric, neurological disorder or severe internal illness, common contraindications to rTMS,[25] and a psychiatric disorder in their first-degree relatives.

The sample size was determined based on previous studies demonstrating that motor cortex excitability modulation significantly predicts antidepressant response of a two-week rTMS treatment.[10] A minimum sample size of 37 was determined using an effect size of r=0.43,[10] a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (Point biseral correlation model, two-tailed). Given an expected dropout rate of 15%, conservative sample size was set to 43 participants. However, in order to ensure adequate power and the ability to have reliable estimates and replicable findings, we aim to include a sample size of 70 or above. In addition, we will recruit 47 HC to participate in the TBS/fNIRS measurement. The sample is based on the comparison of MEP facilitation obtained in MDD (8 ± 49%)[10] and HC (37.9 ± 53.6%),[15] determined using an effect size of d=0.58, a power of 0.8 and an alpha of 0.05 using G*Power 3.0.10 (unpaired two sample t-test, two-tailed). Four sites will be involved in patient recruitment: (1) the Department of Psychiatry, Chinese University of Hong Kong and its associated hospital, the Prince of Wales Hospital (Dr. Arthur Mak, Co-I); (2) the OT outpatient clinic at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University (Dr. Kenneth Fong, Co-I); (3) the Department of Psychiatry, Kowloon hospital (Dr. Wai Ching Yan, Dr. Athena K.Y. Chan) and (4) the Department of

Psychiatry, The University of Hong Kong and its associated hospital, the Queen Mary Hospital (Dr. Sherry K.W. Chan, Co-I). Healthy control participants will be recruited via posters and leaflets displayed at billboards on the University campus and community centers, community websites and social media. Participants will be screened by an experienced psychiatrist and the study including all study-related procedures will be explained to participants in oral and written form prior inclusion. The study will be performed in accordance with the Declaration of Helsinki,[26] including current revisions. All participants will be asked for written informed consent prior to inclusion in the study. Participants can decide to withdraw from the study at any time. The investigator may remove participants from the trial if exclusion criteria have been met or ending the participation is in the interest of the participant or study.

Theta-burst stimulation (TBS)

TBS comprises 3-pulse 50-Hz bursts, applied every 200 ms (at 5 Hz) as described previously.[24] iTBS consists of 2-second trains with an inter-train-interval of 8 seconds. We will repeat trains (30 pulses; 10 bursts) for 20 times to reach a total number of 600 pulses (3x10x20). cTBS will comprise uninterrupted bursts to reach a total number of 600 pulses.[27] Concurrent TBS/fNIRS stimulation will be applied over the left (iTBS) and right (cTBS) DLPFC at an intensity of 90% resting motor threshold (RMT), this corresponds to ~110% of the active motor threshold, an intensity that also elicited robust DLPFC activation in a recent concurrent TMS/fMRI study.[14] Stimulation at 90% RMT will also ensure compliance, reduce sensory discomfort, and minimize dropout rates during the concurrent TBS/fNIRS experiment. Still, scalp discomfort will be recorded directly after the stimulation. We refrained from choosing an intensity of 120% RMT (which will be applied during

antidepressant treatment) for the concurrent TBS/fNIRS experiment because such intensity would unlikely be tolerated by all patients as they are stimulation-naïve at the time of the experiment. The stimulation site over the DLPFC will be determined using the international 10-20 system and corresponds to the F3 label, determined using the optimized method by Beam et al.[28] TBS of the prefrontal cortex (PFC) is generally well tolerated, even at higher stimulation intensities. Antidepressant treatment comprises daily sessions of iTBS of the left DLPFC, five times a week for four weeks. Stimulation intensity will be 120% RMT (titration to full therapeutic dose over the first three days), as approved by the FDA in the U.S.[5] The stimulation site will be the same as in the concurrent TBS/fNIRS stimulation. Treatment will be performed at the TMS treatment centers of the participating local clinics (Department of Psychiatry, The University of Hong Kong and The Chinese University of Hong Kong, and at the Department of Rehabilitation Sciences, The Hong Kong Polytechnic University).

Functional near-infrared spectroscopy (fNIRS)

We will make use of the fNIRS system ImagentTM from ISS Inc., Champaign, IL, US (http://www.iss.com/biomedical/instruments/imagent.html) to determine changes in hemoglobin concentrations before, during and after TBS. ImagentTM uses a sensor that is embedded in a rectangular rubber pad with prisms inside so that optical fibers are rested tangentially instead of perpendicularly on the head surface. This arrangement allows the placement of the sensor directly underneath the TMS coil in close proximity to the stimulation site. Concurrent TBS/fNIRS poses technical and conceptual challenges. Although NIRS has the advantage of being relatively insensitive to motion artifacts, pilot data from our lab show significant stimulation-related artifacts

if sensors and the TMS coil touch each other. Hence, a minimum distance of a few millimeters between probes and the TMS coil will be ensured before the start of measurements. The lowest fNIRS probes will match the Fp1-Fp2 line in order to cover most of the prefrontal cortex extending to the temporal lobes (DLPFC, ventrolateral, frontopolar and superior temporal regions). The device measures changes in HbO, deoxy-hemoglobin (HbR) and total hemoglobin (HbT) using two wavelengths of infrared light (695 and 830 nm). With a source-detector spacing of 3cm, changes in Hb can be measured at a depth of 2-3cm corresponding to the cerebral cortex. fNIRS is not a perfect measure of brain activity and there are several sources of artifacts that need to be carefully considered. We will primarily focus on HbO since it may closer reflect BOLD changes as measured by fMRI.[29] The primary imaging endpoint will be the mean HbO amplitude of left and right DLPFC during and after the TBS stimulation. Secondary endpoints include mean Hb amplitudes during and after iTBS and cTBS, the steepness in the decline of Hb and ascent of HbO values, as well as the area under curve of HbO and Hb values during stimulation.. During concurrent TBS/fNIRS, participants will comfortably sit in a chair. Participants will be carefully instructed about the nature of the experiment prior to the TBS/fNIRS run.

Psychiatric assessment

HAMD17 is a standard instrument used in most clinical trials to screen for major depressive disorder. We will use a baseline score of HAMD17≥18 to apply a generally accepted definition of depression severity as inclusion criteria and to ensure comparability with other clinical trials. However, we will use the Montgomery-Asberg depression rating scale (MADRS) as the primary outcome measure because this symptom rating scale is more sensitive to changes over time.[30 31] In addition, we will

also use the Patient Health Questionnaire 9 (PHQ-9) as self-report questionnaire to assess subjective treatment effects over time. The PHQ-9 is widely used in psychiatric research. Therefore, we use different instruments for inclusion criteria and measurement of treatment response including both rater assessment and self-report inventories, a common practice in many clinical trials.

All psychometric scales used in this study are available in Chinese,[32-34] and show comparable psychometric qualities compared to the original scales. For example, the inter-rater reliability of the Chinese version of HAMD was r=0.94, as was the sensitivity (0.79) and specifictity (0.80).[32] The Chinese version of MADRS and QIDS-C showe a high correlation with the HAMD (0.853 and 0.75, respectively).[33] The Chinese version of all scales have been used in several previous clinical trials which involved Hong Kong populations.[35 36]

Psychiatric assessment includes a range of clinical scales, administered at baseline on the day of the TBS/fNIRS measurement, after 2 and 4 weeks of treatment, as well as at follow-up 1 month after treatment ends. The primary clinical outcome measure will be the response rate after treatment (defined by a C-MADRS reduction ≥50% of baseline). Secondary endpoints will be the remission rate after treatment (defined by a C-MADRS≤7), cutoff scores for the C-MADRS are based on Liu et al 2014.[33]. Further, secondary endpoints include the absolute reduction of mean C-HAMD17 and C-IDS-C after 2 and 4 weeks of treatment and at 1 month follow-up, as well as the response (C-IDS-C30 and C-HAMD17 ≥50% of baseline) and remission rates (C-IDS-C30≤12, C-HAMD17≤7) of patients after 4 weeks of treatment,[37 38] a reduction of 50% on a depression symptom rating scale is the most common response criterium in depression trials. Adverse events (AE) will be assessed according to good clinical practice (ICH/GCP) using an AE-questionnaire to detect unwanted side effects related to the treatment. Suicidality will be evaluated on each treatment day. Similarly,

depression severity will be evaluated at each treatment day using the Chinese version of the PHQ-9 (C-PHQ-9). Patients will be discontinued if they experience worsening in depression, defined as an increase in C-PHQ-9 from baseline of more than 25% during two consecutive assessments, or development of active suicidal intent or attempted suicide. Potentially occurring serious AEs (SAEs) will be recorded.

Data processing and statistical analysis

fNIRS data analysis will follow the standard processing steps. This includes spatial registration (recording of standard cranial landmarks nasion, inion, left and right ear, and the 3D locations of the fNIRS probes); transformation to MNI space; band-pass filtering for motion artifact removal; and estimation of the hemodynamic response function using GLM, as implemented in the NIRS Toolbox for MATLAB. Comparisons between HbO values at baseline and during/after stimulation will reveal TBS-related de/activations. A t-test between pre- and post-stimulation will be performed to test hypothesis 1. Hypothesis 1 will be supported if there is a significant increase and decrease in prefrontal HbO after iTBS and cTBS, respectively. A t-test between pre-stimulation and during stimulation will be performed to test hypothesis 2. Hypothesis 2 will be supported if there is a significant change in prefrontal HbO during stimulation. An F-test for the comparison of the variance in HbO values before versus after stimulation will be performed to test hypothesis 3. Hypothesis 3 will be supported if the F-test is significant. Similarly, an F-test for the comparison of the variance in HbO values in MDD versus HC will be performed to test hypothesis 4. Hypothesis 4 will be supported if the F-test is significant. Analyses will be performed using the IBM SPSS software (http://www-01.ibm.com/software/analytics/spss/). The alpha level will be set at 0.05,

adjusted for multiple comparisons using the Bonferroni-Holm procedure. For hypothesis 5, we will pursue two predictive modeling approaches, a conventional statistical analysis approach, as well as a machine learning approach.[39] First, a logistic regression analysis will be performed to define significant predictors of treatment response (defined as MADRS reduction ≥50% of baseline, see above). Logistic regression will be calculated as implemented in the generalized linear model function 'glm' of the statistical software 'R' (https://www.r-project.org/). Predictors will include imaging endpoints as given above, as well as sociodemographic and psychosocial variables (including the classification of patients as pharmacologically treatment resistant, TRD, defined by a failed treatment response after two or more consecutive antidepressants of adequate duration and dosage). Hierarchical multiple linear regression models will also be calculated to determine the relationship between MADRS reductions and secondary imaging endpoints as potential response predictors. Second, we will use machine learning algorithms for the classification of patients. We will test different algorithms since there is no established rule for the choice of an optimal machine learning approach. We will start with a dichotomous classification using the RandomForest (RF) package for R (ran.r-project.org/) and determine the most useful predictors for distinguishing responders from non-responders. RF is an ensemble tree classification tool that randomly selects subsamples of observations and builds a decision tree for the optimal splitting of these observations according to an outcome variable by a combination of predictors. For each split, the best performing predictor out of a random selection is applied. RF has the advantage of being straightforward and less susceptible to overfitting compared to other machine learning classifiers. To measure the predictive power of our classification model, we will use a five-fold cross-validation design. This allows for optimal validation in the absence of an independent test set.[40] There is no established

method of power calculation for RF. However, we will restrict HbO measurements to few prefrontal channels in order to keep the number of features for classification below the number of observations, thereby preventing the problem of hyperdimensionality. Receiver operating characteristics will be plotted using the ROCR package for the R-software.

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.

Ethics and dissemination

Ethics approval was obtained from the Institutional Review Board of the Hong Kong Polytechnic University (reference numbers HSEARS20200120005, CRESC202009), as well as from the Institutional Review Boards of participating hospitals. An information sheet will be provided to participants before the experiment and a consent form will be signed by both PI and participant to protect the right of both parties. Participants will receive reimbursement for their participation. The data will be stored in an encrypted way and the accessibility is restricted to the researcher team. The study will start in January 2022 and is expected to be completed in December 2023.

The findings of this study will be disseminated through scientific journals, academic conferences, and university courses.

Challenges and potential limitations of this study

Although fNIRS has the advantage of being relatively insensitive to motion artefacts, we expect

stimulation related artifacts caused by muscle contractions on the scalp given that sensors are in close proximity to the TMS coil. Furthermore, stimulation may have direct effects on superficial microvasculature. A challenge of this study will therefore be to minimize such artifacts. A variety of techniques have been proposed to resolve these and other issues related to TMS-fNIRS integration.[41] Moreover, utilizing NIRS in this study will limit the interpretation of our results due to the inherent limitations of the technique of fNIRS. This includes a restriction to mesurements of shallow cortical regions (compared to fMRI) and restrictions in temporal resolution (as compared to EEG). Finally, the NIRS probe proposed to be used in our study only covers a small cortical area underneath the coil, which limits the analysis of stimulation effects to a small region of interest.

Footnotes:

Contributions: GSK conceived the study and analysis. GSK and RLDK drafted the protocol and will conduct the data analysis. RLDK, BBBZ and KNKF will collect the data. ADPM and SKWC will recruit participants and perform the stimulation treatment. All the authors reviewed the protocol and agree to the final version being submitted.

Abbreviations list: rTMS: repetitive transcranial magnetic stimulation, DLPFC: dorsolateral prefrontal cortex, MDD: major depressive disorder, fNIRS: functional near-infrared spectroscopy, TBS: theta burst stimulation, iTBS: intermittent TBS, cTBS: continuous TBS, fMRI: functional magnetic resonance imaging, EEG: electroencephalogram, HF: high frequency, MEP: motor evoked potentials, HC: healthy controls, HbO: oxy-hemoglobin, HbR: deoxy-hemoglobin, HbT: total hemoglobin, C-HAMD-17: Chinese version of Hamilton depression rating scale, RMT: resting motor threshold, PFC: prefrontal cortex, C-MADRS: Chinese version of Montgomery-Asberg depression rating scale, C-IDS-C30: Chinese version of Inventory of depression symptomatology-clinician, AE: adverse events, C-PHQ-9: Chinese version of Patient Health Questionnaire, RF: RandomForest.

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Competing interests: None declared.

Patient consent for publication: Not required.

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Figure legends

Figure 1. Study design

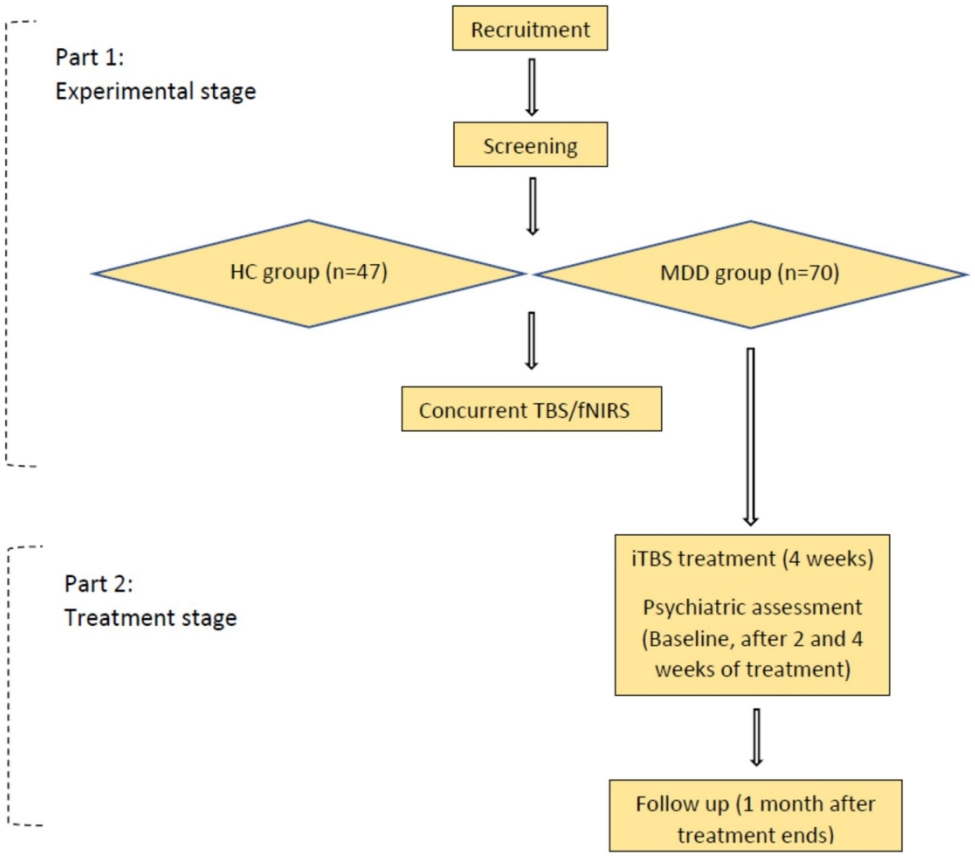

Figure 1. Study design

140x121mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	Yes (available in clinicaltrials.gov Identifier: NCT04526002)
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 9, 17
	5b	Name and contact information for the trial sponsor	N/A
	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
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	7
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, noninferiority, exploratory)	7, 8
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	9
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)	8, 9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
	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)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11-13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8

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	12, 13
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)	8, 13, 16
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target size	9, 10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	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	12-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16
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	16
Statistical methods	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	14-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15, 16
	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)	N/A
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	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
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	14
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
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
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)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10, 16
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	16

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	10, 16
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

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.