Title: Pre-treatment biomarker for clinical response to neuronavigation repetitive transcranial magnetic stimulation (rTMS) in the acute phase treatment of refractory major depressive episode role of intrinsic functional connectivity

Research Plan and Methodology

i. Sampling

Participants will be referred by psychiatrists from specialist outpatient clinics based in the regional hospitals of New Territories East Cluster under the Hospital Authority of Hong Kong. Written informed consents will be obtained from all participants according to the Declaration of Helsinki.

ii. Pre-treatment assessment

a. Baseline clinical assessment------At baseline (before treatment), subjects' age, level of education, handedness will be noted by research assistant. A research psychiatrist will administer Montgomery-asberg Depression Rating Scale (MADRS), Hamilton Rating Scale for Depression (HDRS), Structured Clinical Interview for the Diagnostic and Statistical Manual for Mental Disorders to ascertain current/lifetime Axis I and II psychiatric diagnosis and score on Global Assessment of Functioning, age at onset of Major Depressive Disorder (MDD) and number of major depressive episodes, number of failed antidepressants, current life stressor(s) and the perceived severity on a visual analog scale of 0-10, and clinical global impression scale (CGI). Subjects are asked to complete self-administered questionnaires including 1) Beck Depression Inventory II that has been validated in local Chinese dialect; 2) a 60-item Neuroticism-Extraversion-Openness Five-Factor Inventory that measures five personality traits on neuroticism, extraversion, openness to experience, agreeableness and conscientiousness; 3) ruminative response subscale (Chinese version) (RRS) of the response style questionnaire, a 22-item questionnaire comprising of two distinct factors on brooding and reflection.

b. Pre-treatment functional and structural scans-------In each participant, a 6-minute resting-state fMRI brain scan is collected using a 3.0 tesla Philips Medical Scanner with an eight-channel SENSE head coil (Philips Healthcare, Best, The Netherlands) at the Prince of Wales hospital, Hong Kong. The scan consists of 170 volumes with the following parameters: time repetition (TR) = 2050ms, time echo (TE) = 25ms, flip angle =90°, 3.2mm3 voxels, slice thickness =3.2 mm, field of view (FOV) = 205 mm², and matrix size = 64 x 64. Participants are instructed to keep their eyes open to view a fixation cross and hold still. Structural data include a high-resolution T1-weighted anatomical image set with the following parameters: TR=7.54ms, TE=3.53ms, flip angle = 8°, 1.1x1.1 x0.6 mm voxels, number of slices = 285, slice orientation = sagittal, slice thickness =1.2mm, FOV = 250 mm3, and matrix size = 240x240. Slices are tilted about 30° clockwise along the anterior commissure-posterior commissure plane to obtain better signal in the orbitofrontal cortex. All the functional and anatomical MRI data are transferred to an offline workstation for image analysis. The non-commercial toolbox
Protocol CRE-2014.041 Version 3 November 13 2017

(http://www.fil.ion.ucl.ac.uk/spm/), which was developed using MATLAB by the Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London. Firstly, all fMRI images are realigned with the first scan, and then co-registered with the anatomical MRI of each patient. Subsequently, to enable group-based analysis, the spatially aligned MRI and fMRI data of the same subject will be re-oriented into the standardized Talairach space by registering with the Montreal Neurological Institute (MNI) brain template. The spatially normalized volumes are re-sliced and result in a voxel size of 2mm×2mm×2mm. The Gaussian smoothing with a kernel of 8 mm will be applied to increase the signal to noise ratio. Seed driven approach, guided by a priori regions of interest (ROIs), namely subgenual cingulate cortex and left DLPFC (Brodmann 9 and 46) will be adopted. Global signal regression will then be performed at individual subject level for which a statistical parametric map with time-series as a regressor of interest and the motion realignment parameters as regressors of non-interest will be computed, followed by a second-level t-test to examine the difference in functional connectivity of ROIs between subjects categorized by levels of clinical response to rTMS treatment.

iii. Neuro-navigated rTMS protocol

rTMS treatment suite and personnel: the treatment facilities are based in day-care centre where the physicians delivering the treatment are serving on site. Stimulator set-up: Neuro-navigated high-frequency rTMS over a specific area on left DLPFC will be provided using magnetic stimulator that generates short duration biphasic pulses. 70mm figure-of-eight coil will be held in place with a custom-made stand tangential to the scalp with the handle pointing back and away from the midline at 45 degrees. The resting motor threshold will be determined by the standard visual method.

Stimulation parameters: 10 hertz, 120% motor threshold, thirty trains of 5 seconds with 25 seconds between trains, 3000 pulses per day delivered 5 days per week, totaling 60000 pulses over 4 weeks.

Stimulation localization: The coordinates of stimulator sites are determined individually for each participant. The localization procedure involves incorporation of anatomical MRI of brain into computer (Rogue Research Inc, 2007) to guide coil placement. For each subject, an MRI-to-head co-registration will be performed using software that identifies three anatomical landmarks (tip of the nose, bridge of the nose, superior-lateral edge of the tragus of left and right ears). The head surface landmarks are then identified using infrared tracking system (Polaris Northern Digital). Once successfully co-registered, infrared tracking will be used to monitor the coil position with respect to the desired brain target, which is chosen to be (y=45, x=-45 and z=35) on the MNI coordinates according to the conservative cortical landmarks approximating the cytoarchitectonic definitions of the junction of area 9 and area 46 in the original Brodmann Map.
iv. Management of decompensated mental states, serious adverse somatic reactions to rTMS and drop-outs

The research psychiatrists will be responsible for the detection of high-risk mental states (emergence of psychotic symptoms, catatonic state, suicide ideation and para-suicide state) at the regular follow-up assessments. Physical adverse effects will be checked at each follow-up and evaluated on the severity. In times of emergent risky mental state and adverse somatic reactions, the research psychiatrists will work collaboratively with the participant and if necessary the treating psychiatrists on the necessary psychiatric and medical management plans. All adverse effects will be reported to the "serious adverse effect monitoring sub-committee" under the local ethics committee. All drop-out participants will be assessed by phone followed by clinic-based interviews to work out the care plan as indicated clinically. At the end of the study, the subjects will receive the generic psychiatric service as they are originally entitled to. The research team will ensure a seamless aftercare will be arranged for all participants.

v. Clinical follow-up and outcome assessments

All participants are evaluated by a research psychiatrist who is blinded to the functional connectivity maps of participants. Follow-up assessments are scheduled at the end of week 2, 4, 6, 8 and 12.

Primary outcome measure is time-dependent change (trajectory) in depressive symptom scores measured with Montgomery-asberg Depression Rating Scale at baseline, week 2, 4, 6, 8, and 12.

Clinical response is defined as score of 2 on clinical global impression scale and 50% reduction of score on Montgomery-asberg Depression Rating Scale from baseline upon and after completion of intervention.

Clinical remission is defined as score of 7 or less on Montgomery-asberg Depression Rating Scale and 1 on clinical global impression scale upon and after completion of intervention.

vi. Sample size estimation

Sample size estimation is based on the parameter estimate that addresses the main objective. In our study it is the expected Pearson correlation coefficient between functional connectivity of the ROIs and quantitative measure of clinical efficacy (percentage drop in MADRS score). In Fox et al's study (2012), the correlation coefficient is -0.355 (p<0.05 one-tailed). According to Friedman's partial power table, the required sample size is at least 58 at alpha=0.8 and Pearson r = 0.35.
vii. Data analysis

Intention-to-treat analysis will be adopted with the last observation carried forward for participants with at least one post-baseline observation. Multiple regression to examine whether pre-treatment DLPFC-sgACC rs-fMRI connectivity can predict rTMS treatment response, multiple regression will be performed with percentage change in MADRS symptom score at week 12 as dependent variable using Statistical Package for the Social Sciences (SPSS) version 22. Several clinical and sociodemographic variables are included as covariates (see baseline measures). Backward elimination will be chosen as selection method, because our model is hypothesis-driven. To prevent multicollinearity, variance inflation factor and tolerance are calculated and multicollinearity would be considered a problem when tolerance values are 0.20 or less and/or variance inflation factor values are above 5.

To characterize the trajectory of symptom change and the underlying explanatory variables, the approach of Singer and Willet for the growth curve analysis will be applied using the mixed model procedure with maximum likelihood estimation in SPSS version 22. Individual changes over time are modeled by creating a two-level hierarchical model that nests time within individuals. In the level-1 model, the focus is on within-individual change in MADRS symptom score over time and several error covariance structures are examined. For the level-2 model, the focus is on inter-individual differences in symptom score change. More specifically, it is examined whether the rate of change in MADRS symptom score varies across individuals in a systematic way.