



Functional Near-Infrared Spectroscopy as a Target Navigator for rTMS Modulation in Patients with Hemiplegia: A Randomized Control Study

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ABSTRACT

Introduction: Although repetitive transcranial magnetic stimulation (rTMS) is efficacious for motor neuromodulation in stroke survivors, high interindividual variability for responsiveness remains a concern. Target probing on the skull using a proper brain-mapping technique may help overcome this challenge. This study

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assessed the feasibility of functional near-infrared spectroscopy (fNIRS) as a target navigator in rTMS treatment for motor facilitation in patients with stroke.

Methods: Fifty-one patients with stroke were enrolled in this randomized controlled study. The patients were assigned to three groups: fNIRS-guided rTMS treatment (fNIRS group, $n = 20$), motor evoked potential (MEP)-guided rTMS treatment (MEP group, $n = 16$), and sham ($n = 15$) group. Motor assessments, including Fugl–Meyer Assessment (FMA), Wolf Motor Function Test (WMFT), and muscle strength, were conducted at baseline and after the 10-session rTMS treatment.

Results: The fNIRS-guided hotspot (fNIRS-HS) was obtained for each patient, even those for whom the MEP-guided hotspot was undetectable. Both intervention groups exhibited significant improvements in muscle strength, FMA, and WMFT scores ($P < 0.001$) compared with the sham group. The fNIRS group achieved significantly greater improvement in elbow function ($P = 0.001$) than the MEP group.

Conclusion: fNIRS can be a reliable tool for hotspot navigation for motor neuromodulation in patients with stroke. With high sensitivity to cortical oxygenation changes, this navigation system achieved a superior outcome to the traditional MEP-based method in patients with stroke. fNIRS-based systems may also facilitate the integration of machine learning, thus

enabling precision medicine for neuromodulation.

Trial Registration: <https://clinicaltrials.gov>; Unique identifier: NCT02006615.

Keywords: Repetitive transcranial magnetic stimulation (rTMS); Near-infrared spectroscopy (fNIRS); Stroke; Cortical excitability; Motor function

Key Summary Points

Functional near-infrared spectroscopy (fNIRS) is a reliable tool for hotspot navigation for motor neuromodulation.

fNIRS-based hotspots can be detected in the post-stroke motor cortex with varied lesion types.

The fNIRS-based system achieved a superior outcome to the traditional motor evoked potential (MEP) method.

fNIRS may facilitate the integration of machine learning and precision medicine.

INTRODUCTION

Cerebrovascular diseases are among the ten leading causes of death in developed countries and usually result in devastating disability [1]. Although traditional neurorehabilitation is critical for post-stroke management, its general efficacy is unsatisfactory. Repetitive transcranial magnetic stimulation (rTMS), as a noninvasive brain stimulation, has become a promising tool in the treatment of post-stroke impairments under the putative mechanism of long-term potentiation (LTP) or long-term depression (LTD) [2]. rTMS delivers painless magnetic pulses to modulate cortex excitability, causing a change in the synaptic transmission followed by neuroplasticity [3]. Intermittent theta burst stimulation (iTBS), comprising compound stimulus frequencies, is stronger than the traditional paradigm in generating LTP-like or LTD-like phenomena under a shorter

conditioning time [4, 5]. iTBS over the primary motor cortex (M1) in the affected hemisphere may improve upper limb motor function in patients with chronic stroke [3, 6, 7].

Although rTMS intervention has grade A (definitely effective) to B (probably effective) evidence for motor improvement in patients with hemiplegia following stroke [8], considerable interindividual variation exists in the response rate, which is influenced by certain factors, such as the selected target, stimulation mode, and intensity. Among them, accuracy of target localization in the affected hemisphere is a major issue. Target localization can be navigated by a frameless stereotaxic system aimed at the neuroanatomical structure instead of the functional site. However, it is best accessed by TMS navigation on the skull to probe the functional hotspot where the highest amplitude of the motor evoked potential (MEP) is evoked by the lowest intensity of magnetic output. However, the challenge with this MEP method is that the hotspot is usually undetectable, particularly in the affected hemisphere of patients with stroke [9, 10].

Recently, functional near-infrared spectroscopy (fNIRS) has been used to measure cortical metabolism and brain-behavior interaction, which may be a novel approach for target selection in rTMS modulation [11]. fNIRS is a unique noninvasive neuroimaging tool for functional brain mapping by monitoring brain oxygenation and hemodynamic status; it has become a promising and high-sensitivity measurement in neuroscience research [12]. Multichannel fNIRS provides insights into neurophysiological activity by detecting the signal of wavelength absorption of oxygenated hemoglobin (HbO), deoxygenated hemoglobin (HbR), and total hemoglobin (total Hb) concentration. These signals reflect the anatomical vascular supply into the functional cortex, including capillaries in the activated cortex and the downstream veins of the cortical vascular structure [13, 14]. For instance, increased hemodynamic response in HbO and reduction in HbR during a motor task was observed in fNIRS channels covering the primary motor area M1 and the supplementary motor area in healthy people [15–17]. In another study, increased

HbO signal was observed shortly after the initiation of finger tapping, and this reaction was more prominent in the channel located in M1 [18]. These previous findings support the high specificity of fNIRS in capturing the center of activation during cortical mapping.

Although fNIRS mapping has a lower spatial resolution than functional magnetic resonance imaging (fMRI) in differentiating active brain regions, it is unknown whether fNIRS can be used as a navigator to locate the optimal region for rTMS treatment. While high-density fNIRS can achieve millimeter-scale resolution, the regular-density fNIRS (source detector distance: ~ 3 cm) can reach a spatial resolution of up to 1 cm [19, 20]. Cao et al. used the 24-channel fNIRS of diffuse optical tomography as the spatial prior to electroencephalography (EEG) and demonstrated high spatiotemporal resolution in detecting neuronal sources using a combined EEG-fNIRS system [20]. Recently, a set of fNIRS probes was designed to aid transcranial direct current stimulation in the feedback analysis of electric field distribution during cortical modulation [21]. Although fNIRS is sufficiently sensitive to detect cortical Hb change related to active central or electrophysiological drive, little is known about the feasibility of fNIRS as a surrogate for MEP to navigate targets for rTMS treatment in patients with hemiplegia.

Based on the rationale that fNIRS has high reliability in cortical activation measurement and high sensitivity to cortical excitability change, we hypothesized that for target determination, fNIRS incorporated into rTMS would be ideal for motor facilitation following stroke. In this randomized controlled trial (RCT), we divided hemiplegia patients into different groups to elucidate the possible differential effects of motor facilitation exerted by excitatory rTMS guided by fNIRS or the traditional MEP method.

METHODS

Participants

Individuals with stroke were recruited from a tertiary medical center from November 2018 to April 2020. Data on age, sex, stroke type, lesion

side, and duration of hemiparesis were obtained from interviews and medical charts. To be included in the study, participants with stroke had to satisfy the following criteria: (1) experienced a single stroke, with 2 months to 2 years since onset; (2) age between 30 and 80 years; and (3) hemiparesis with upper limb weakness and functional impairment. Participants were excluded if they had (1) a contraindication for rTMS treatment: presence of cardiac pacemakers or implantable cardioverter defibrillators, metal or magnetic objects in the brain, ear implants, aneurysm clips or coils, metal plates in the head/brain, and medication pumps for people; (2) a history of epilepsy; (3) visual defect or achromatopsia; or (4) the presence of other neurological diseases such as Parkinson's disease or dementia. All participants provided written informed consent. The study protocol was approved by the Institutional Review Board of Taipei Veterans General Hospital (201307011A). All procedures performed in this study involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration and its later amendments.

The G*Power program (v3.1.9.2; Franz Faul, University of Kiel, Kiel, Germany) was used to calculate the necessary sample size [22]. On the basis of a previous study using rTMS-iTBS on the ipsilesional M1 for upper limb motor improvement in patients with chronic stroke [23], an effect size of 0.53 was selected. In the one-way analysis of variance (ANOVA) test, an α -error of 0.05 and a β of 0.20 (power level of 0.80) were used, indicating a minimum of 39 patients. Assuming a dropout rate of 15% and the fact that we had three groups (one sham and two intervention), at least 15 patients per group were required.

Study Design

This was a prospective, double-blind, sham-controlled study. The randomization order was computer-generated and concealed in sequentially numbered opaque envelopes by an independent statistician. The patients were randomly assigned to one of the following three

groups in a 1:1:1 allocation ratio: those receiving rTMS-iTBS stimulation guided by fNIRS (fNIRS group), those receiving rTMS-iTBS stimulation guided by TMS-MEP (MEP group), or those receiving sham stimulation (sham group) guided by TMS-MEP. All groups underwent 10 daily sessions, 5 days per week, for 2 weeks. In the fNIRS and MEP groups, iTBS was applied on the ipsilesional fNIRS-guided hotspot (fNIRS-HS) and ipsilesional MEP-guided hotspot (MEP-HS), respectively. In cases where the hotspot was not obtainable in the affected hemisphere, the mirror site of contralesional M1 was used, with iTBS as the stimulation paradigm in the MEP group and the sham group. Both positions of fNIRS-HS and MEP-HS were recorded in the international 10–5 system, and their distances were calculated. Brunnstrom stage, Medical Research Council (MRC) scale, the Fugl–Meyer Assessment (FMA) score, the Wolf Motor Function Test (WMFT) score, and the grasping strength in the paretic upper limb were measured before and after the intervention in all patients (Fig. 1). Clinical assessment was conducted by an experienced physician blinded to patients' group allocation, and rTMS was performed by another investigator who was not involved in clinical assessment, patient follow-up, or data analysis.

fNIRS Imaging System

A multichannel wearable fNIRS imaging system (NIRSport, NIRx Medical Technologies, LLC, Glen Head, NY, USA) was used to simultaneously acquire dual-wavelength (760 and 850 nm) signals. The fNIRS optodes, including eight LED light sources and eight detectors in each hemisphere, were attached to participants' heads to monitor the hemodynamics of the bilateral motor cortex (Fig. 2).

The calculation of brain hemodynamic signals is provided as a supplementary document. In summary, the preprocessed signals were converted into concentration changes in oxygenated hemoglobin, Δ [HbO], and deoxygenated hemoglobin, Δ [HbR], using the modified Beer–Lambert law for each source-detector channel study [24].

The fNIRS head cap was specifically designed in compatibility with the international 10–5 system, which defines standard surface positions for a human head, with approximately 3.0 cm between any two adjacent positions. In the source and detector arrangements, 23 effective source-detector channels were generated for monitoring local blood oxygenation, with a sampling rate of 6.25 Hz. During signal acquisition, the time points for the beginning of each task condition were recorded.

To estimate the signal-to-noise quality of a data channel, the relative coefficient of variation (CV, in %) was calculated for the raw signals at 760 nm and 850 nm, which is a routine procedure for fNIRS measurement [23]. Data rejection based on two types of CV, CV_{chan} , and CV_{trial} , was used to reduce physical artifacts, such as motion-induced instability and blood pressure-induced hemodynamics [25].

$$CV = \sigma/\mu \times 100\%,$$

where μ is the mean value and σ is the standard deviation of the signal. CV_{chan} was calculated over the entire duration of the experiment (approximately 13 min) for each channel, and measurement channels with $CV_{\text{chan}} > 15\%$ were rejected. The CV_{trial} was then obtained for 60-s intervals of the individual trial block, and only trials for each remaining channel ($CV_{\text{chan}} < 15\%$) with $CV_{\text{trial}} < 10\%$ in both wavelengths were retained for subsequent analyses.

The remaining fNIRS signals were bandpass-filtered (low-cutoff frequency 0.005 Hz and high-cutoff frequency 0.03 Hz) to eliminate the effects of heartbeat, respiration, and low-frequency signal drifts for each wavelength [25]. Wavelet filtering was subsequently used to correct for the motion artifacts in each channel. The measured signal is assumed to be a linear combination of the physiological signal of interest (hemodynamics) and motion artifacts. Because the hemodynamic response is much slower than motion artifacts (such as a spike artifact), the wavelet coefficients for the evoked response are anticipated to be a Gaussian probability distribution [26, 27]. Motion artifacts usually cause a sudden change in signal

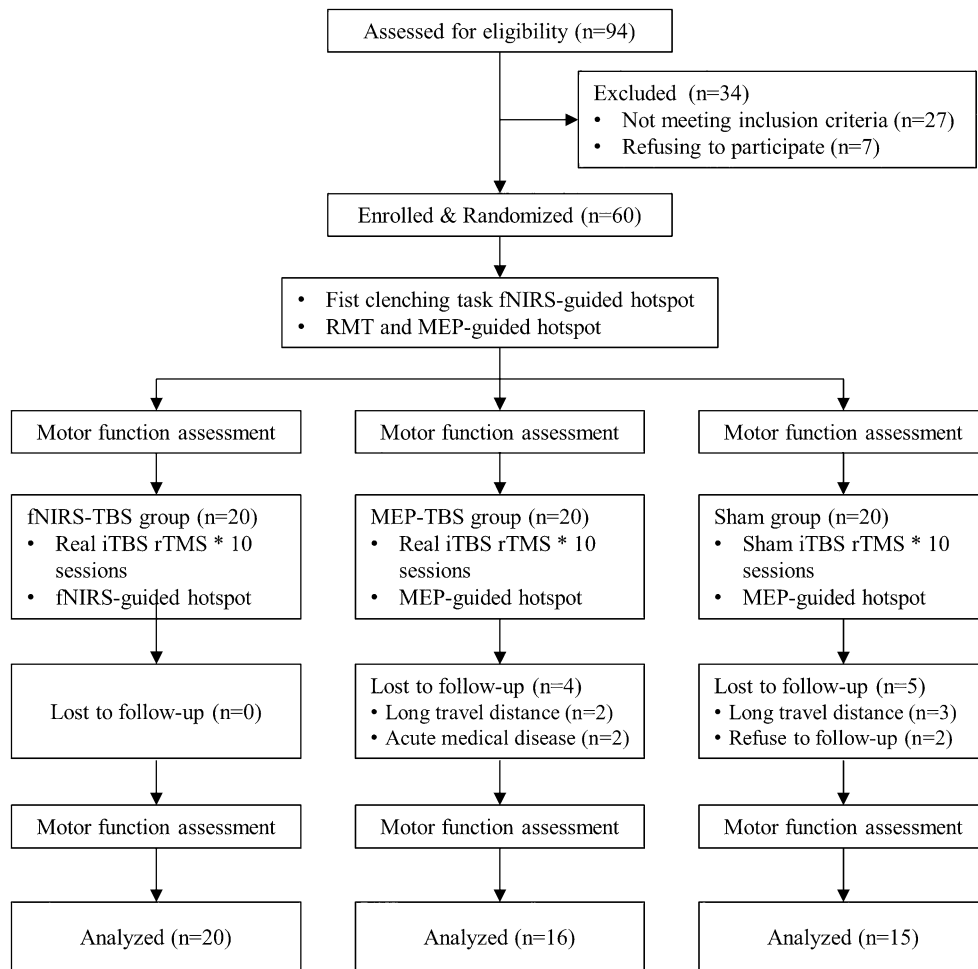


Fig. 1 CONSORT flowchart

amplitude with a large coefficient in the wavelet domain. Accordingly, the wavelet coefficients of hemodynamics are around zero, with smaller variance compared to the coefficients of motion artifacts which can be identified as outliers. The outlying coefficients (larger than a predefined threshold) were therefore eliminated before signal reconstruction using the inverse discrete wavelet to correct the corresponding motion artifacts. The selected threshold value for artifact removal is based on the level of artifact contamination. The removal threshold of the wavelet coefficient, α , was set to 0.1 in this study based on a previous study [28]. The preprocessed signals were converted to concentration changes in oxygenated hemoglobin, Δ [HbO], and deoxygenated hemoglobin, Δ [HbR], using

the modified Beer–Lambert law for each source-detector channel study [21]. Correlation-based signal improvement (CBSI) was then employed to improve the signal quality based on the findings that brain activation involves Δ [HbO] increases and Δ [HbR] decreases at the activated cortical regions [29]. The relative changes in [HbO] and [HbR] concentrations (i.e., Δ [HbO] and Δ [HbR]) were calculated based on a 5-s baseline and collected during the task of hand grasp. Participants were asked to keep clenching and relaxing their fists alternately for 20 s and then rest for 20 s, repeatedly for five cycles while fNIRS was recorded. The sequence of right or left hand examined was in random order for each patient. Patients with a completely paretic hand (i.e., unable to make a fist) were taught to

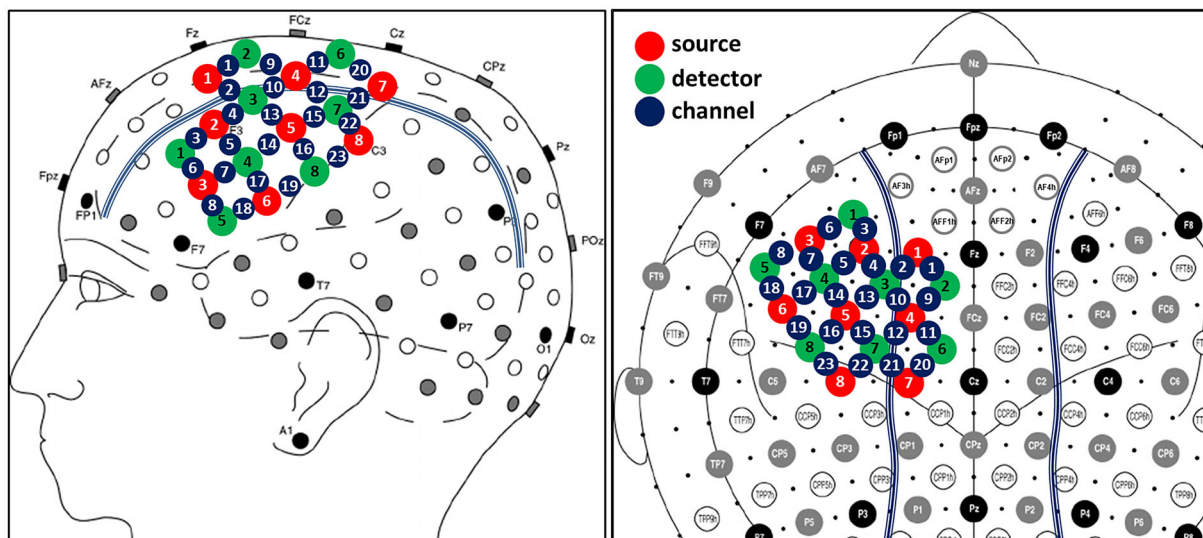


Fig. 2 Arrangement of fNIRS probes covering the bilateral premotor cortex, primary motor cortex (M1), and supplemental motor area (SMA). Δ [HbO] at S4D2

CH 9, S5D3 CH13, S5D8 CH16, and S8D7 CH 22 yielded significant correlation with RMT in the contralateral hemisphere

perform an imagery task (i.e., imagine they were holding a cup to drink or grooming their hair). The fNIRS signal preprocessing, including the motion artifact correction, bandpass filtering, and conversion of [HbO] and [HbR], were processed using the HOMER2 package [30]. The calculation of signal CVs and correlation analyses were performed using homemade scripts developed on MATLAB (MathWorks, Natick, MA, USA). The channel that yielded the most vigorous [HbO] response of the block average was chosen as the treatment target for the NIRS group. In the pilot study of our previous trial, the Δ [HbR] did not show a consistent response to the grasp task; therefore, we used Δ [HbO] as a biomarker of fNIRS for the following analyses in this study. The use of Δ [HbO] as a biomarker was based on studies whereby a significant change in [HbO] was noted in channels covering the primary motor cortex during the finger-tapping task in healthy subjects [31].

Measurements of Motor Function

We adopted the Brunnstrom stages, MRC scale, FMA, WMFT, and grip strength as the outcome evaluations for motor performance. WMFT served as the primary outcome measurement.

All the patients were assessed before the rTMS intervention and the next day after completing the treatment.

The Brunnstrom stages, known as the approach for stroke improvement, classify motor progress into a series of six distinct stages. We used the MRC scale to determine muscle strength ranging from grades 0 (no visible contraction) to 5 (normal) of six muscle groups of the shoulder flexor, elbow extensor, elbow flexor, wrist extensor, wrist flexor, and hand grasp muscles. The FMA is a well-known assessment tool for measuring post-stroke physical impairments related to functional recovery and assessing the ability to isolate movements at each joint and the influence of unwanted synergies on movement. The FMA has seven domains related to the upper extremity (upper extremity, wrist, hand, coordination/speed, sensation, passive range of motion, and joint pain) and includes 63 items (score range, 0–126) [32]. The WMFT evaluates upper extremity motor function through the use of timed and functional tasks. The widely used version of the WMFT consists of 16 items, namely forearm to table (side), forearm to box (side), extended elbow (side), extended elbow (to the side) with 1 lb weight, hand to

table (front), hand to box (front), weight to box, reach and retrieve, lift a can, lift a pencil, pick up paper clip, stack checkers, flip cards, turn a key in a lock, fold towel, and lift a basket. The first six items involve timed functional tasks, followed by one to measure strength, and the remaining nine items assess movement quality when completing various tasks [33]. The items are rated on a six-point scale [34]. The grip strength was tested using a digital hand dynamometer (Jamar Plus+, Patterson Medical, Cedarburg, WI, USA), which was set in line with the patient's forearm at the thigh level and squeezed as hard as possible.

A Dantec Keypoint electromyograph (Dantec, Skovlunde, Denmark) was connected to the stimulator to record MEP signals [35]. The amplified (100 μV –1 mV/div) and bandpass-filtered (20–2000 Hz) signals were digitized at a sampling rate of 20 kHz. Each patient sat in a comfortable reclining armchair, with both hands relaxed and keeping their eyes open. We recorded MEPs bilaterally from the first dorsal interosseous hand muscles using surface Ag/AgCl electrodes. A grid of 49 positions, spaced 1 cm on the interaural and sagittal lines, was tested on each motor cortex. A hotspot was defined as the position where the highest amplitude could be evoked with the lowest stimulation intensity. We defined the resting motor threshold (RMT) for MEP as the lowest intensity at which MEPs $\geq 50 \mu\text{V}$ amplitude could be elicited in half of 10 single-pulse consecutive TMS stimuli at the hotspot.

Repetitive Transcranial Magnetic Stimulation Protocol

We performed TMS with the Magstim Rapid² stimulator (Magstim Company Ltd, Whitland, Dyfed, UK) using a 70-mm figure-of-eight coil. iTBS consisted of bursts containing three 50-Hz pulses every 200 ms for 2 s and repeated every 10 s for 20 cycles and 600 pulses [4]. The intensity of iTBS was set at 80% of RMT over the ispielosomal target area in both the MEP and fNIRS groups. The stimulation protocol for sham control was identical to the experimental group, but a placebo coil (Magstim) was used for

the sham stimulation; this delivered < 5% of the magnetic output with audible click-on discharge. Because none of the patients had ever undergone rTMS, they could not identify whether the stimulation was real or sham. Each patient received iTBS intervention for 10 daily sessions (5 days per week for 2 weeks).

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Demographic data including sex, involved laterality, type of stroke, and location of lesion were compared by group using a chi-square test. However, age, duration from stroke onset to the experimental day, and baseline upper limb motor assessment were compared using one-way ANOVA. For intragroup comparison, we used a paired *t* test to evaluate the group-wise motor improvement. To compare the change scores of motor performance among the three groups, we used one-way ANOVA and a post hoc analysis with Bonferroni's correction. To obtain the relationship between fNIRS expression and neurophysiological and clinical behavior, we used Spearman's correlation to analyze the values of $\Delta[\text{HbO}]$ and RMT. To clarify the relationship between the distance of the fNIRS-HS from the mean MEP-HS and the motor improvement, we correlated the distance and change scores of motor performance in the fNIRS group using Spearman's correlation analyses. The level of significance was set at $P < 0.05$. All data are expressed as mean \pm standard deviation.

RESULTS

Demographic and Clinical Characteristics

We recruited 94 patients with stroke and upper limb weakness. Of these, 27 did not meet the inclusion criteria, and seven declined participation. Finally, 60 patients were included in the study from November 2018 to April 2020 and were divided into the fNIRS (experimental group), the MEP (positive control), and the

Table 1 Demographic data and motor assessment scores at baseline for each group in mean (SD)

Characteristics	All (<i>n</i> = 51)	fNIRS (<i>n</i> = 20)	MEP (<i>n</i> = 16)	Sham (<i>n</i> = 15)	<i>P</i> value
Male sex, <i>n</i> (%)	34 (67)	12 (60)	10 (63)	12 (80)	0.422
Age (years)	58.4 (11.8)	58.9 (12.6)	54.9 (11.3)	61.4 (10.9)	0.303
Period post-stroke (months)	12.6 (8.5)	12.2 (7.9)	14.3 (9.0)	11.2 (9.0)	0.587
Lesion site (right/left)	31/20	12/8	12/4	7/8	0.270
Stroke type					0.342
Infarction, <i>n</i> (%)	32 (63)	14 (70)	9 (56)	9 (60)	
Hemorrhage, <i>n</i> (%)	14 (27)	3 (15)	5 (31)	6 (40)	
Mixed, <i>n</i> (%)	5 (10)	3 (15)	2 (13)	0 (0)	
Location					0.744
Cortical, <i>n</i> (%)	15 (29)	6 (30)	5 (31)	4 (27)	
Subcortical, <i>n</i> (%)	25 (49)	8 (40)	8 (50)	9 (60)	
Mixed, <i>n</i> (%)	11 (22)	6 (30)	3 (19)	2 (13)	
Brunnstrom, proximal UE (1–6)	3.3 (1.4)	3.4 (1.5)	3.4 (1.3)	3.1 (1.4)	0.835
Brunnstrom, distal UE (1–6)	3.1 (1.6)	3.2 (1.5)	3.3 (1.5)	2.9 (1.5)	0.844
MRC (0–5)					
Shoulder flexion	2.9 (1.3)	3.1 (1.2)	2.9 (1.5)	2.7 (1.4)	0.646
Elbow extension	2.5 (1.4)	2.4 (1.2)	2.8 (1.5)	2.4 (1.5)	0.706
Elbow flexion	3.1 (1.3)	3.3 (1.1)	3.3 (1.3)	2.7 (1.4)	0.277
Wrist extension	1.4 (1.2)	1.2 (1.0)	1.5 (1.3)	1.5 (1.3)	0.593
Wrist flexion	1.4 (1.3)	1.2 (1.1)	1.6 (1.4)	1.5 (1.3)	0.593
Hand grasping	2.0 (1.3)	1.8 (1.1)	2.4 (1.3)	1.9 (1.4)	0.299
FMA (0–126)	84.7 (19.4)	82.5 (18.8)	86.5 (18.7)	85.7 (21.8)	0.812
WMFT (0–75)	26.7 (21.9)	24.5 (21.0)	27.6 (20.6)	28.7 (25.3)	0.844
Grip strength (kg)	4.9 (4.4)	5.4 (4.2)	5.3 (5.2)	3.9 (3.8)	0.576

Analysis method with ANOVA and chi-square test

UE upper extremity, MRC Medical Research Council scale, FMA Fugl–Meyer Assessment, WMFT Wolf Motor Function Test

sham groups (negative control), with 20 patients each. No adverse events were reported during the treatment. No significant between-group differences were noted in demographics or baseline functional scores, including the period post-stroke, type of stroke, and location of lesion (Table 1).

fNIRS-Guided Hotspot (fNIRS-HS)

Figure 3 illustrates the motion correction performance of fNIRS signals in one of the patients as an example.

Figure 4 presents a comparison of paired MEP-HS and fNIRS-HS in the contralesional

hemisphere. The mean group locations of MEP-HS and fNIRS-HS were at (X: 35.4, Y: 22.7 mm) and (X: 32.0, Y: 19.8 mm) from Cz, respectively. The mean distance between MEP-HS and fNIRS-HS was 17.7 mm (SD: 7.5). The fNIRS-HS demonstrated a mean medial displacement of 2.5 mm (SD: 12.2) and a mean posterior displacement of 3.0 mm (SD: 15.4) compared with the MEP-HS. Notably, in cases where the hotspot could not be obtained through MEP probing ($n = 24$ of 51), we could still locate the fNIRS-HS, defined as the channel with the most vigorous change in [HbO] signal. The correlation analyses indicated that with a shorter distance between the fNIRS-HS and mean MEP-HS, the fNIRS-guided method achieved greater motor improvement in elbow extension strength as measured with WMFT ($r = -0.508$, $P = 0.026$).

Correlation Analyses for fNIRS Signals and RMT

To determine the reliability of fNIRS, we correlated the hemodynamic responses of Δ [HbO] in each channel and cortical excitability assessed using RMT in the contralesional hemisphere. Significant correlations were noted at the channels covering the M1 and S1 areas: S4D2 (CH9, $r = -0.663$, $P = 0.037$), S5D3 (CH13, $r = -0.672$, $P = 0.023$), S5D8 (CH16, $r = -0.720$, $P = 0.012$), and S8D7 (CH22, S1 area, $r = -0.782$, $P = 0.013$) (Fig. 2, Table 2). Because the MEP recorded in the paretic hand was only obtainable in four patients, the RMT was present in a very small number of cases, thus precluding the correlation for ipsilesional analysis.

Group-Wise Improvements

The fNIRS group exhibited significant improvement in proximalBrunnstrom stage following rTMS intervention ($P = 0.042$), whereas no significant change was observed in the MEP or sham groups (Table 3). For muscle strength, significant improvements were noted in proximal and distal muscles in both intervention groups: shoulder flexion ($P < 0.001$), elbow extension ($P = 0.002$), elbow flexion

($P < 0.001$), wrist extension ($P = 0.021$), wrist flexion ($P = 0.021$), and grasping ($P < 0.001$) in the fNIRS group, and shoulder flexion ($P = 0.001$), elbow flexion ($P = 0.003$), and grasping ($P < 0.001$) strength in the MEP group. No significant changes in muscle strength were observed in the sham group. For the FMA total score, both intervention groups exhibited significant improvement (fNIRS group: $P < 0.001$; MEP group: $P = 0.009$) compared with their baseline levels. For WMFT, we observed that total scores increased significantly in both intervention groups (both $P < 0.001$). For subtests of WMFT, the fNIRS group exhibited significant improvements in each sub-test for gross motor assessment ($P < 0.001$ – 0.01) compared with baseline levels. The MEP group exhibited significantly increased sub-scores in the forearm ($P = 0.002$) and reach and retrieve ($P = 0.001$). The sham group also improved in the forearm to table sub-test ($P = 0.041$). In addition, both the fNIRS ($P = 0.001$) and MEP ($P = 0.006$) groups had significant enhancement of grip strength (Table 3).

Intergroup Comparisons

One-way ANOVA and post hoc analyses comparing the fNIRS and MEP groups revealed that the fNIRS group exhibited superior results in elbow extension assessed with weight resistance [$F(2,48) = 6.601$, $P = 0.003$, post hoc: $P = 0.010$] or without resistance [$F(2,48) = 12.471$, $P < 0.001$, post hoc: $P = 0.001$] in WMFT (Table 3, Fig. 5). Post hoc fNIRS and sham group comparison revealed significant differences in the following: FMA total score [$F(2,48) = 5.467$, $P = 0.033$], WMFT total score [$F(2,48) = 11.973$, $P < 0.001$], sub-tests of weight of grasping [$F(2,48) = 4.461$, $P = 0.016$], and strength of shoulder flexor [$F(2,48) = 6.729$, $P = 0.002$], elbow extensor [$F(2,48) = 4.316$, $P = 0.024$], elbow flexor [$F(2,48) = 3.431$, $P = 0.036$], and grasp muscles [$F(2,48) = 8.708$, $P < 0.001$]. For MEP and sham group comparison, significant differences were observed in the following: FMA total score [$F(2,48) = 5.467$, $P = 0.010$], WMFT total score [$F(2,48) = 11.973$, $P = 0.010$], upper extremity sub-score [$F(2,48) = 4.183$, $P = 0.029$],

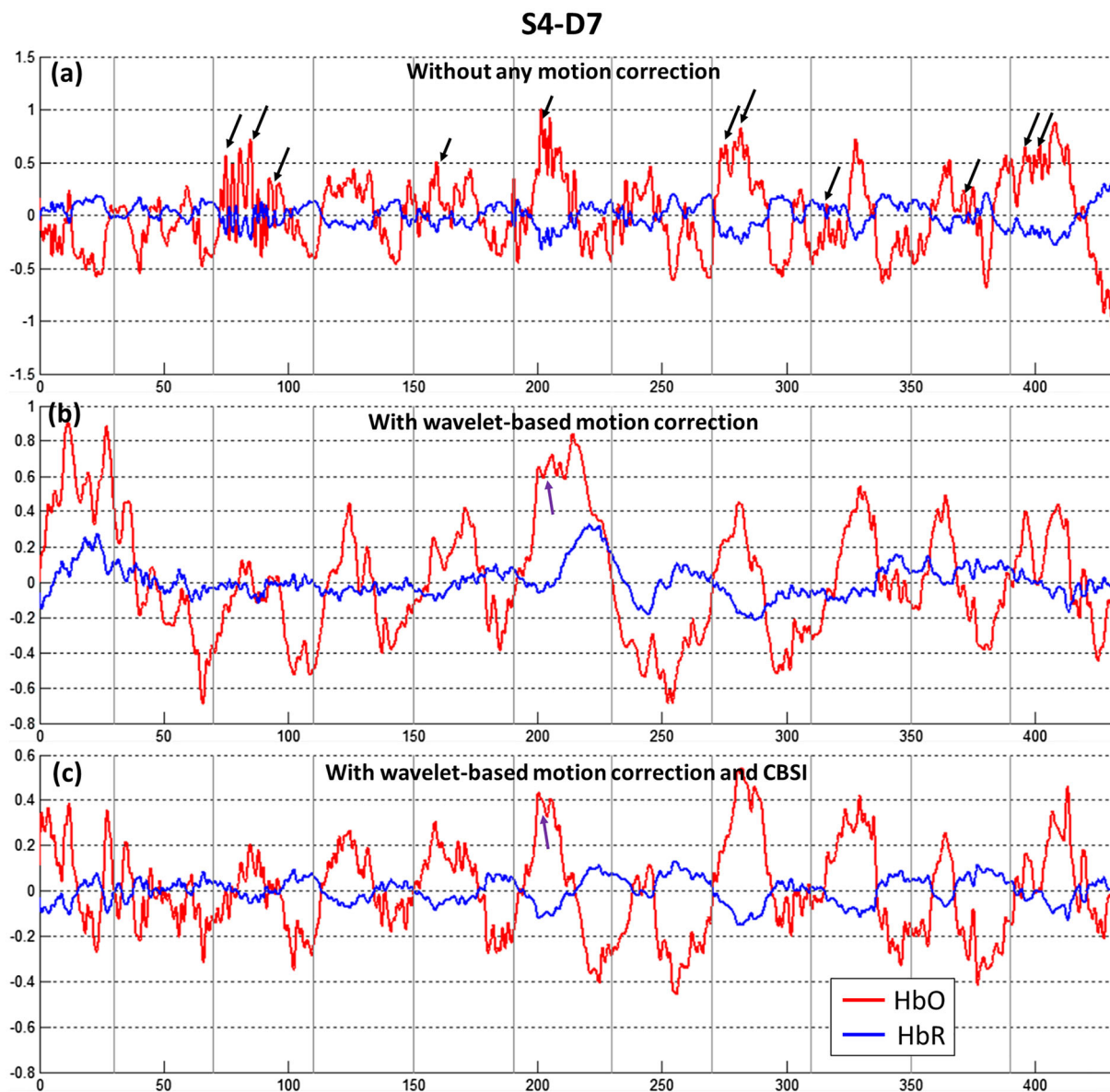


Fig. 3 **a** Shows that in the reconstructed [HbO] signals without any motion correction, contamination of spike noise is observed (black arrows). In **b**, the spikes are effectively eliminated using the wavelet-based method. However, an overestimation of [HbO] response (a relatively stronger amplitude compared to other blocks)

was identified (the purple arrow). In **c**, the additional CBSI processing, with the consideration of both [HbO] and [HbR] signal profiles, further reduces this overestimation of [HbO] response and restores the negative correlation between [HbO] and [HbR] changes

hand function sub-score [$F(2,48) = 3.809$, $P = 0.037$], and grasp strength [$F(2,48) = 8.708$, $P = 0.017$]. In summary, both intervention groups exhibited significant improvements in

proximal or distal upper limb function after iTBS modulation, with no intergroup differences except in elbow function, which had a superior outcome in the fNIRS group.

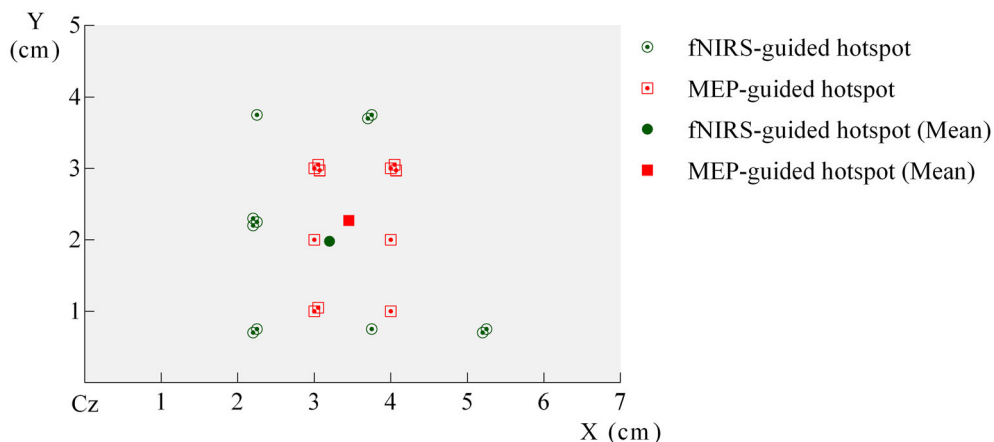


Fig. 4 Location of MEP-guided and fNIRS-guided hotspot related to Cz position on the skull surface. The same numbers indicate paired fNIRS-HS and MEP-HS. Green

color: fNIRS-HS, red color: MEP-HS, solid circle or solid square: mean group location

Table 2 Correlation analyses for RMT and Δ [HbO] signals in channels of fNIRS

Spearman's correlation between RMT and Δ [HbO] signal of NIRS												
Channel	S1D2	S1D3	S2D1	S2D3	S2D4	S3D1	S3D4	S3D5	S4D2*	S4D3	S4D6	S4D7
	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10	CH11	CH12
Spearman's <i>r</i>	-0.576	-0.527	-0.025	-0.310	-0.222	0.193	-0.201	-0.181	-0.663	-0.457	-0.393	-0.363
<i>P</i> value	0.064	0.095	0.943	0.354	0.511	0.570	0.553	0.595	0.037	0.184	0.232	0.273
Channel	S5D3*	S5D4	S5D7	S5D8*	S6D4	S6D5	S6D8	S7D6	S7D7	S8D7*	S8D8	
	CH13	CH14	CH15	CH16	CH17	CH18	CH19	CH20	CH21	CH22	CH23	
Spearman's <i>r</i>	-0.672	-0.377	-0.601	-0.720	-0.338	0.179	0.009	-0.335	-0.040	-0.782	-0.632	
<i>P</i> value	0.023	0.253	0.087	0.012	0.310	0.620	0.978	0.313	0.906	0.013	0.055	

DISCUSSION

This double-blind, randomized controlled study investigated the effectiveness of the fNIRS-aided navigation system in rTMS treatment for motor facilitation in stroke-related hemiplegia. We demonstrated that fNIRS can reliably assess cortical hemodynamic activity and can be a surrogate for conventional MEP-defined targets for rTMS treatment. In the bilateral motor cortex where the MEP-HS was unobtainable, fNIRS yielded excellent sensitivity and topographical resolution in hotspot localization, which compensates for the inadequacy of the conventional method. Furthermore, we demonstrated the superior therapeutic effect achieved by the fNIRS method reflected in elbow functional

performance after rTMS intervention compared with the MEP-guided method. With fNIRS assistance, patients demonstrated more favorable outcomes in elbow, wrist, and hand function compared with the sham group. We first explored the promising effect of the fNIRS navigation system for rTMS protocol refinement to optimize the neurostimulation effect in patients with hemiplegia.

Suppressive low-frequency (≤ 1 Hz) rTMS of contralesional M1 in the post-acute stage of stroke reached level A evidence (definitely effective) for hand motor recovery, whereas excitatory high-frequency rTMS (> 5 Hz) of ipsilesional M1 has reached level B evidence (probably effective) [8]. Because the phenomenon of interhemispheric activity

Table 3 Mean group data for motor assessments and group-wise and intergroup comparisons

	fNIRS (<i>n</i> = 20)		MEP (<i>n</i> = 16)		Sham (<i>n</i> = 15)	
	Pre-rTMS	Post-rTMS	Pre-rTMS	Post-rTMS	Pre-rTMS	Post-rTMS
Brunnstrom stage						
Proximal upper extremity (1–6)	3.40 ± 1.47	3.60 ± 1.31*	3.38 ± 1.26	3.38 ± 1.26	3.13 ± 1.41	3.13 ± 1.41
Distal upper extremity (1–6)	3.15 ± 1.60	3.25 ± 1.48	3.25 ± 1.48	3.25 ± 1.48	2.93 ± 1.53	2.93 ± 1.53
MRC						
Shoulder flexion (0–5)	3.13 ± 1.18	3.70 ± 1.21***†	2.88 ± 1.48	3.28 ± 1.34**	2.70 ± 1.41	2.83 ± 1.56
Elbow extension (0–5)	2.43 ± 1.22	2.85 ± 1.37**†	2.75 ± 1.52	2.91 ± 1.53	2.37 ± 1.49	2.43 ± 1.59
Elbow flexion (0–5)	3.33 ± 1.14	3.85 ± 0.99***†	3.34 ± 1.29	3.72 ± 1.28**	2.70 ± 1.41	2.83 ± 1.50
Wrist extension (0–5)	1.15 ± 1.01	1.28 ± 1.13*	1.53 ± 1.31	1.66 ± 1.45	1.47 ± 1.30	1.47 ± 1.30
Wrist flexion (0–5)	1.15 ± 1.11	1.28 ± 1.24*	1.56 ± 1.41	1.63 ± 1.46	1.47 ± 1.30	1.50 ± 1.30
Grasp (0–5)	1.78 ± 1.14	2.48 ± 1.14***†	2.41 ± 1.26	2.97 ± 1.26***#	1.87 ± 1.41	2.03 ± 1.48
FMA						
Total (0–126)	82.50 ± 18.82	85.95 ± 17.85***†	86.5 ± 18.68	90.69 ± 18.01***#	85.67 ± 21.85	86.00 ± 21.95*
Upper extremity (0–36)	19.80 ± 8.89	21.90 ± 8.06***	19.56 ± 11.08	22.06 ± 10.66*#	18.33 ± 11.94	18.47 ± 11.99
Wrist (0–10)	1.15 ± 2.43	1.25 ± 2.48	2.44 ± 3.20	2.56 ± 3.27	2.47 ± 2.97	2.47 ± 2.97
Hand (0–14)	3.10 ± 4.61	3.35 ± 4.48*	5.25 ± 4.93	5.88 ± 5.12***#	5.00 ± 5.18	5.13 ± 5.29
Coordination and speed (0–6)	2.20 ± 1.82	2.50 ± 2.09	2.25 ± 1.95	2.44 ± 1.86	2.40 ± 1.96	2.13 ± 2.00
Sensation (0–12)	10.90 ± 2.94	11.30 ± 1.72	11.69 ± 1.01	11.75 ± 1.00	11.87 ± 0.52	11.87 ± 0.52
Passive joint motion (0–24)	23.00 ± 1.49	23.10 ± 1.29	23.25 ± 1.29	23.63 ± 1.09	23.20 ± 2.60	23.20 ± 2.60
Joint pain (0–24)	22.35 ± 1.98	22.80 ± 1.54	22.06 ± 3.02	22.38 ± 2.99	22.40 ± 3.70	22.40 ± 3.70
WMFT						
Total (0–75)	24.50 ± 20.99	29.10 ± 21.96***†	27.63 ± 20.63	30.88 ± 21.97***#	28.67 ± 25.34	29.20 ± 25.43
Forearm to table (0–5)	2.65 ± 1.50	3.20 ± 1.40***	2.75 ± 1.44	3.25 ± 1.34**	2.67 ± 1.50	2.93 ± 1.67*
Forearm to box (0–5)	2.35 ± 1.81	2.85 ± 1.81***	2.31 ± 1.53	2.56 ± 1.67	2.40 ± 1.77	2.53 ± 1.89
Extend elbow (0–5)	1.90 ± 1.53	2.50 ± 1.57***†‡	2.25 ± 1.84	2.31 ± 1.85	2.27 ± 1.79	2.20 ± 1.74
Extend elbow (weight) (0–5)	1.65 ± 1.57	2.10 ± 1.86***†‡	2.19 ± 1.80	2.19 ± 1.80	2.07 ± 1.91	2.07 ± 1.87
Hand to table (0–5)	2.70 ± 1.46	3.10 ± 1.65**	2.63 ± 1.46	3.00 ± 1.76	2.47 ± 1.69	2.60 ± 1.77
Hand to box (0–5)	2.25 ± 1.80	2.60 ± 1.93**	2.19 ± 1.68	2.56 ± 1.79#	2.20 ± 1.934	2.20 ± 1.934
Weight to box	1.95 ± 1.67	2.25 ± 1.92***†	1.88 ± 1.75	2.00 ± 1.79	2.00 ± 1.85	2.00 ± 1.85
Reach and retrieve (0–5)	3.15 ± 1.14	3.90 ± 0.97***†	3.19 ± 1.28	3.75 ± 1.29***#	2.87 ± 1.77	3.00 ± 1.85
Lift can (0–5)	1.20 ± 1.58	1.35 ± 1.53	1.56 ± 1.71	1.69 ± 1.81	1.60 ± 1.96	1.80 ± 2.08
Lift pencil (0–5)	0.70 ± 1.30	0.80 ± 1.44	1.06 ± 1.29	1.19 ± 1.38	1.13 ± 1.51	1.13 ± 1.51
Lift paper clip (0–5)	0.70 ± 1.17	0.85 ± 1.35	0.88 ± 1.03	1.19 ± 1.47	1.07 ± 1.44	1.00 ± 1.36
Stack checkers (0–5)	0.70 ± 1.34	0.75 ± 1.37	0.75 ± 0.93	0.94 ± 1.24	1.00 ± 1.41	1.00 ± 1.41
Flip cards (0–5)	0.45 ± 0.95	0.55 ± 1.19	0.63 ± 0.89	0.75 ± 1.00	0.87 ± 1.30	0.80 ± 1.27
Turn key in lock (0–5)	0.65 ± 1.39	0.65 ± 1.39	1.00 ± 1.27	1.06 ± 1.34	1.13 ± 1.60	1.07 ± 1.62

Table 3 continued

	fNIRS (<i>n</i> = 20)		MEP (<i>n</i> = 16)		Sham (<i>n</i> = 15)	
	Pre-rTMS	Post-rTMS	Pre-rTMS	Post-rTMS	Pre-rTMS	Post-rTMS
Fold towel (0–5)	0.70 ± 1.42	0.80 ± 1.51	1.13 ± 1.46	1.31 ± 1.66	1.47 ± 1.69	1.40 ± 1.68
Lift basket (0–5)	0.75 ± 1.52	0.80 ± 1.58	1.25 ± 1.57	1.38 ± 1.75	1.47 ± 1.89	1.47 ± 1.89
Grip strength (kg)	5.35 ± 4.18	6.71 ± 5.36***†	5.28 ± 5.23	6.34 ± 5.98**	3.88 ± 3.81	3.96 ± 3.88

Intragroup comparison was made using a paired *t* test; intergroup comparison was made using one-way ANOVA and a post hoc analysis with Bonferroni's correction

UE upper extremity, MRC Medical Research Council scale, FMA Fugl–Meyer Assessment, WMFT Wolf Motor Function Test

Significance level: intragroup improvement ****P* < 0.001; ***P* < 0.01; **P* < 0.05

Intergroup comparison: fNIRS superior to sham (†*P* < 0.05), fNIRS superior to MEP (‡*P* < 0.05), MEP superior to sham (#*P* < 0.05)

**P* < 0.05, Spearman's correlation

imbalance exists in most patients with chronic stroke [36, 37], rTMS modulates cortical excitability and harnesses neuroplasticity under the operative mechanism of LTP or LTD. Regardless of the contralesional or ipsilesional approach, determination of RMT in the hotspot of the M1 area is key for accessing cortical excitability, and stimulation intensity is defined by RMT accordingly. However, the elicited MEP in hand muscles is usually absent in most cases, partly because of cortical necrosis and partly because of a high motor evoked threshold exceeding the maximal machine output. In such cases, the researchers set the mirror part of the contralesional hotspot as the treatment target, despite there being topographical asymmetry for bilateral M1. Furthermore, in some patients with vascular encephalopathy or elderly patients, the MEP could not be measurable in the unaffected cortex. Therefore, a surrogate for traditional MEP navigation is critical for optimizing rTMS treatment. As a reliable measure of regional cortical hemodynamic change, fNIRS has been widely used in the field of neuroscience or neurorehabilitation to investigate timely hemodynamic change following an active behavior task or passive transcranial magnetic or electric stimulation. Over the last three decades, numerous studies have been conducted on NIRS in a functional neuroimaging approach in several medical fields, including stroke [14], dementia [38], Alzheimer's disease [39], Parkinson's disease [40], psychiatric diseases, and cognition [41]. The hybrid EEG–fNIRS brain computer interface has

also enhanced the classification accuracy of hemodynamic change in neural source detection [20, 42]. The present study is the first to explore the efficacy of this tool as a topographical navigator for target localization prior to high-frequency rTMS treatment in the ipsilesional hemisphere of patients with stroke.

fNIRS-Guided Hotspot Exists in Each Patient

In this study, fNIRS-HS was observed in each patient, even though in some cases MEP was absent in the hand muscle. This implies that in patients with cortical or subcortical lesions, where MEP-HS is unobtainable, we can locate the most vigorous HbO change with the fNIRS method. fNIRS measures cortical oxygenation status to neural activity, whereas MEP-HS measures the loci with the strongest functional control through the corticospinal pathway to the terminal muscles [35]. The MEP from hand recording could be hampered by impaired cervical tract continuity or sedative medication consumption rather than a genu cortical lesion. From this viewpoint, fNIRS-HS may be more accessible than MEP-HS.

Patients with ischemic stroke have higher HbO and HbR levels in the recanalized or non-recanalized region compared with the neighboring nonischemic area [43]. In the case of recanalization, a preferential distribution of flow to the ischemic area where vascular resistance is lower would explain the higher concentration of HbO and HbR compared with the

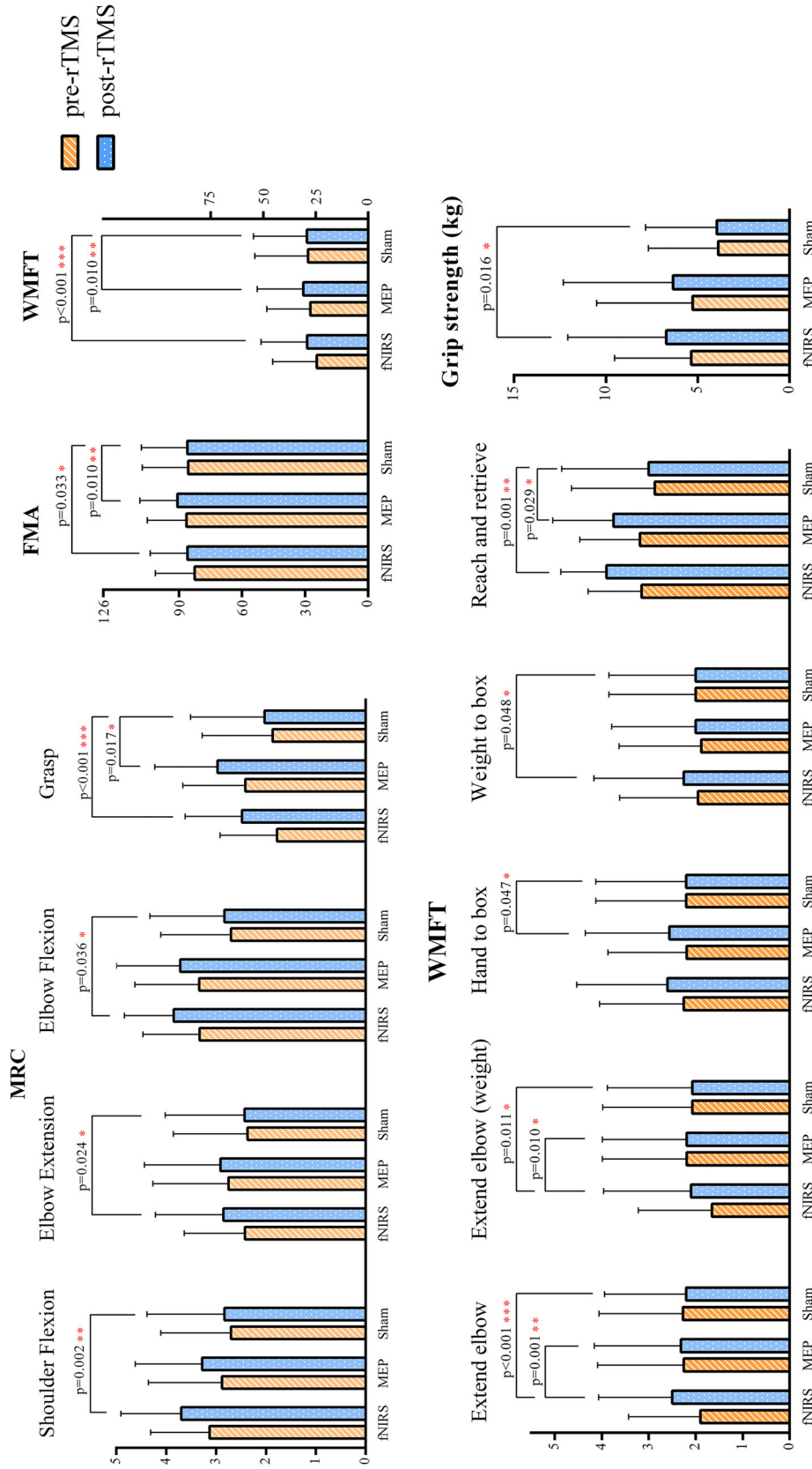


Fig. 5 Results of mean group data and intergroup comparisons for motor assessments in the three groups. The intergroup comparison was performed using one-way ANOVA and a post hoc analysis with Bonferroni's correction. Significance level: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

neighboring nonischemic area. In cases of non-recanalization, HbO remains detectable because of residual blood flow from leptomeningeal arterial and venous collaterals, although reduced metabolism and oxygen consumption are noted. Noguchi et al. suggested a significant HbO increase after single-pulse TMS applied at M1 even though no MEP was detectable [44]. Consistent with these prior findings, we observed that HbO increased with the rising peak in each patient who had heterogeneous cortical or subcortical lesions. We demonstrated that fNIRS is a feasible tool for the detection of active cortical loci or a hub undergoing reorganization and demanding timely oxygen supply during motor activity.

Distance Between fNIRS-Guided and MEP-Guided Hotspots

In our study, we observed fNIRS-HS to be very close to MEP-HS. The mean distance between these targets is 2.5 mm on the X axis and 3 mm on the Y axis. fNIRS-HS sat more medially and posteriorly than MEP-HS. The reason for this trend could be related to the synergistic pattern from elbow co-activation when the patients were asked to clench their hands during fNIRS assessment. Most of our patients were in Brunnstrom stages II–IV in proximal (72.5%) and distal (62.7%) upper limbs, in whom a synergistic pattern was observed. In a cortical homunculus map representing body part control, M1 for elbow control lies medial and posterior, related to the M1 for hand control. A possible alternative explanation for the posterior shifting of the fNIRS-HS relative to the MEP-HS is the activated channels covering the post-central area (i.e., the somatosensory gyrus in addition to M1) during voluntary hand movement in healthy participants [31]. This is similar to the finding of Herwig et al., who reported that the voluntary finger movement during fMRI involved activation in the somatosensory cortex, which may account for the posterior location of the fNIRS-HS relative to MEP-HS [45]. Despite the spatial discrepancy between the fNIRS-HS and MEP-HS, we observed that a closer distance to the mean MEP-HS was

associated with greater improvement in elbow extensor strength. This result indicates that rTMS treatment assisted by fNIRS mapping is a reliable method. fNIRS-HS can serve as a surrogate for standard MEP-HS, particularly for patients in whom the MEP-HS is undetectable.

ΔHbO Correlates Inversely with RMT

We observed that some of the fNIRS channels exhibited significant correlations with RMT, which was distributed in the M1 and S1 regions. Our findings agree with previous results showing that Hb concentration changes were more prominent at M1 and areas posterior to it [18]. Another study investigating HbO change during fNIRS recording in healthy participants indicated that the channels with a significant increase in HbO during finger-tapping tasks mainly covered the primary motor, somatosensory cortex, and supplementary motor areas [29]. In our study, task-related coactivation as reflected in increased HbO was noted in surrounding M1 areas, including S1 and the prefrontal cortex. The coactivation in these regions may be caused by the interconnected pathway between the primary motor and sensory cortex and the prefrontal cortex [46]. The neurovascular coupling could be influenced by corticocortical and subcortico-cortical connections between the motor and somatosensory cortices [18]. Among the 23 channels in our fNIRS montage, four exhibited a significant correlation with the increase in HbO and the RMT values, with three lying in M1 and one in S1. The intimate relationship between HbO and RMT indicates the high specificity of fNIRS for use as an RMT surrogate. We assume that the channel with the most vigorous HbO increase in the M1 territory represents the optimal loci for efficient modulation–rTMS treatment.

fNIRS Navigation Achieved a Better Outcome in Elbow Strength than the MEP Method

After iTBS was applied to the ipsilesional hotspots, both intervention groups exhibited

significant a improvement in muscle strength assessed using MRC or grip dynamometer. The fNIRS group exhibited particularly improved elbow strength compared with the MEP group. Furthermore, both intervention groups achieved proximal and hand improvement in FMA and WMFT total scores and sub-tests, with the fNIRS method preferentially enhancing the elbow extension function compared with the MEP method. As mentioned, at the group level, fNIRS-HS tends to have a posteromedial deviation compared with the location of MEP-HS. The deviation pointing to the M1 controlling elbow movement in the cortical homunculus map may be the reason for this superiority. Empirically, the weakness of the elbow extensor complicated by biceps spasticity is the nidus that causes stagnation for post-stroke motor recovery. The functional restoration in the elbow extension is a key step for synergistic pattern breaking and subsequent progression to the next recovery stage.

Notably, although the fNIRS group achieved superior outcome in the elbow, this method improved hand function in magnitude comparable to that in the MEP group. With the experience from this study, in the future we may investigate the effects of fNIRS navigation to examine the influence if we instruct patients to co-contract the elbow muscles as little as possible.

In this study, we did not observe any alteration in scores assessing fine motor control in WMFT in any group, such as lifting a pencil, flipping cards, or turning a key in a lock. Because fine motor control is mediated by complex interregional connection and integration of secondary cortical motor or subcortical circuits, the dexterity function enhanced by the rTMS protocol may need more conditioning sessions to be addressed on these measuring scales. In our experience, the improvement in hand dexterity can be shown after the two-course rTMS protocol.

This study had some limitations. First, our sample size, albeit the largest for combined NIRS and rTMS studies in stroke so far, was limited. Second, the patients had different lesion types, locations, and post-stroke

durations, making it challenging to precisely determine the effect of fNIRS-guided rTMS treatment in specific subgroups. Further large-scale studies involving patients of specific stroke types are warranted. Third, compared with a previous study in which double-dense and quadruple-dense fNIRS were used to enhance spatial resolution at the centimeter level [47], we used a single standard density array, which may have increased spatial bias for fNIRS-HS detection. To improve the reliability of this novel guidance method, a double-dense array setting should be ideal. In addition, the effects of one-course rTMS conditioning may last for several months [7]. Fourth, we did not follow up with patients to investigate the enduring effect. Future studies should include a long-term follow-up to determine the overall efficacy of the fNIRS-aided system.

CONCLUSIONS

In this RCT, we successfully explored the feasibility of the fNIRS guidance system applied in patients with stroke for motor neuromodulation. This system has the advantages of being relatively cost-effective, portable, and reliable in navigation, with the possibility of digitalized software integration; therefore, it may serve as a surrogate biomarker for cortical excitability. This novel method achieved better functional restoration in the proximal upper limb than the MEP method. The incorporation of machine learning and automatic software analysis into the fNIRS system could facilitate precision medicine for individualized planning before rTMS treatment.

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Compliance with Ethics Guidelines. All procedures performed in this study involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration and its later amendments. This study has been approved by the Institutional Review Board of Taipei Veterans General Hospital (201307011A; Registration-URL: <https://clinicaltrials.gov>; Unique identifier: NCT02006615). Informed consent was obtained from all individual participants included in the study.

Data Availability. The datasets generated during and/or analyzed during the current

study are available from the corresponding author on reasonable request.

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