The effects of individually tailored rTMS on hand function in chronic stroke: a protocol for an adaptive, phase II, randomized, sham-controlled clinical trial


*Corresponding author - Rodrigo Huerta. Spaulding Neuromodulation Center, Spaulding Rehabilitation Hospital. 96/79 13th Street Navy Yard, Charlestown MA, 02129, USA. Tel: 857-310-9754, Fax: 617-952-6060. E-mail: rhuerta@neuromodulationlab.org

Received Dec 15th, 2015; Revised Jan 3rd, 2016; Accepted Jan 6th, 2016

Abstract

Stroke is a leading cause of disability among adults. Existing rehabilitation programs haven’t been able to accomplish full motor recovery partially due to the pathologic plasticity exerted from the unaffected hemisphere to the affected one. This inhibition can be disrupted using non-invasive brain stimulation (NIBS). Transcranial magnetic stimulation (TMS) is a NIBS technique that has the capacity of depolarizing or hyperpolarizing neurons depending on the frequency of the pulses. Although several trials have been conducted to find the efficacy of low frequency rTMS for motor recovery after stroke, their results have been heterogeneous. One of the main variables that determine the response to rTMS is the dose, corresponding to the number of pulses delivered to the patients. However, due to the localization and the extension of the stroke, each patient responds differently to certain dose. Therefore, using the SPIRIT statement, we designed a protocol for an adaptive, phase II, randomized, sham-controlled clinical trial. The study proposed will include 75 patients between 45 and 80 years old, with hand function impairment after 1 to 3 years of stroke; it will exclude patients with severe cognitive or neuropsychiatric comorbidities, any previous stroke episode, Fugl Meyer (Upper limb) < 20, inability to understand the task or contraindications for rTMS. The study will have 3 arms: individually tailored (adaptive dosing) low frequency (1Hz) rTMS plus standard of care rehabilitation (physical therapy) compared to sham and fixed rTMS plus standard of care rehabilitation. The intervention will be applied during 6 weeks after which the main analysis will be performed. Subjects will be followed-up during 3 months and the results from this analysis will be exploratory. This protocol will use the results from the Pegboard test as a primary outcome and SF-36 questionnaire, hand strength, and responder’s rate as secondary outcomes.

Citation: Jannati A, Teixeira PEP, Lopes GHR, Mohamed BM, Huerta RA et al. The effects of individually tailored rTMS on hand function in chronic stroke. PPCR 2015, Nov-Dec;1(4):128-136.

Copyright: © 2015 Fregni F. The Principles and Practice of Clinical Research is an open-access article distributed under the terms of the Creative Commons Attribution License, which allows unrestricted use, distribution, and reproduction in any medium, providing the credits of the original author and source.

Introduction

Stroke is a leading cause of long-term disability among adults in developed countries. Over 60% of stroke survivors suffer from persistent neurological motor deficits that affect activities of daily living such as eating, dressing, and self-care (Carod-Artal, Egido, González, & Varela de Seijas, 2000). Decades of research have not resulted in full motor recovery after stroke. Therefore, novel treatments are needed to promote motor rehabilitation following a stroke.
After stroke, the affected areas of the brain have decreased cortical excitability. Simultaneously, the unaffected regions of the cortex inhibit the activity of the affected hemisphere in a process termed “interhemispheric inhibition” (Hoyer & Celnik, 2011). Therefore, two different approaches have been proposed to improve motor function in this scenario: inhibiting the unaffected hemisphere or activating the region abated by the lesion.

Due to its effect on cortical excitability, transcranial magnetic stimulation (TMS) has been studied as a treatment for post-stroke motor rehabilitation. Previously, high-frequency stimulation (3–10 Hz) to the ipsilesional primary motor cortex (M1) had been found to effectively increase cortical excitability, as well as improving motor function when combined with a movement-based physical therapy (Eman M. Khedr, Ahmed, Fathy, & Rothwell, 2003) (Kim et al., 2006) (Ennara et al., 2010) (P. Talelli, Greenwood, & Rothwell, 2006). On the other hand, low-frequency (1 Hz) repetitive TMS (rTMS) has shown to have an inhibitory effect on cortical activation and if applied to the unaffected motor cortex (M1) has demonstrated to improve motor function and control in the affected hand (Kakuda et al., 2011) (Liepert, Zittel, & Weiller, 2007) (Milly & Dinya, 2008) (Nowak, Bosl, Podubecká, & Carey, 2010) (Takeuchi, Tada, Toshima, Matsuo, & Ikoma, 2009). However, the response of the patients with stroke to TMS has proven to be variable. (Di Pino et al., 2014) One of the main factors that may contribute to this heterogeneity is the dosing of this intervention.

The purpose of our study is to evaluate the individually tailored approach of rTMS in chronic stroke patients motor hand recovery after 6 weeks of treatment.

Mechanisms

Transcranial Magnetic Stimulation (TMS) is a non-invasive brain stimulation (NIBS) method that can deliver focused magnetic stimulation to a targeted brain area when applied to the skull. (Penelope Talelli & Rothwell, 2006) TMS is able to depolarize or hyperpolarize the neurons beneath the magnetic field that it generates. In healthy adults, TMS at frequencies less than 1 Hz suppresses the excitability of the motor cortex, causing an inhibitory effect. On the other hand, TMS at higher frequencies (>1 Hz) increases cortical excitability, causing a facilitatory effect. (Kohayashi & Pascual-Leone, 2003) The capacity of TMS to influence cortical excitability contributes to the rationale for its use to enhance post-stroke rehabilitation (Nowak, Grefkes, Ameli, & Fink, 2009) (Hummel & Cohen, 2005).

Existing knowledge

Several trials have investigated the effect of repetitive TMS (rTMS) on upper limb motor function in patients with stroke. High-frequency rTMS over the primary motor cortex (M1) in the affected hemisphere could improve motor learning performance in patients with chronic stroke (Kim et al., 2006) and have a positive, long-term effect on motor recovery in acute (E. M. Khedr, Etraby, Hemedu, Nasef, & Razek, 2010) and subacute (Chang et al., 2010) stroke. Previous investigations (Ameli et al., 2009) suggested that high-frequency rTMS has favorable effects in cases of subcortical rather than cortical stroke. Low-frequency rTMS to the unaffected hemisphere also has beneficial effects on hand dexterity (Liepert et al., 2007), pinch acceleration (Takeuchi, Chuma, Matsuo, Watanabe, & Ikoma, 2005), grip force (Takeuchi et al., 2008), reaction time (Dafoakis et al., 2008) and finger tapping (Fregni et al., 2006). However, other reports did not show measurable therapeutic effects of rTMS on motor function after stroke (Liepert et al., 2007) (Nowak et al., 2008) (Malcolm et al., 2007).

Need for innovation

Despite promising reports, there is a lack of consensus regarding the effect of rTMS on chronic post-stroke patients regarding hand motor recovery due to inconsistent findings and methodological discrepancies across previous trials. In addition, the literature also doesn’t address the therapeutic dose of rTMS needed to see results. It is highly probable that different patients need a different dose, which is why an individually tailored approach, meaning an adaptive dosing depending on the respond of the patient, is a therapeutic possibility that could improve the patient’s response. The purpose of our study is to evaluate the individually tailored approach of rTMS in chronic stroke patients motor hand recovery after 6 weeks of treatment.

Significance/impact of study

Stroke is a major cause of long-term disability and death worldwide. Two-thirds of stroke survivors have impaired motor function (Brooks, Lankhorst, Rumping, & Prevo, 1999). Functional motor impairment, particularly impaired manual dexterity, is one of the major sources of disability in stroke patients, despite the widespread use of physical therapy for rehabilitation (Kwakkel, Kollen, & Wagenaar, 2002).

rTMS can potentially be a valuable tool in the rehabilitation of stroke patients. By improving motor function and decreasing dependence in activities of daily living (ADL), it can improve patients’ quality of life and satisfaction. In turn, this can reduce long-term care costs while limiting family burden, societal burden and overall health care system costs (Gresham et al., 1997).

Methods

Choice of comparators

This study will have a three-arm design. The two intervention arms will receive rTMS plus physical therapy (PT). One group will receive an individually tailored
approach of dosing, and the other intervention group will receive a fixed rTMS dose. The comparison group will receive a sham coil simulating the rTMS plus physical therapy also. From an ethical point of view, the standard of care (PT) will be given to all subjects, so there isn’t any problem with the interventions.

The standard of care will be based on a physical therapy program targeting motor function recovery after stroke. Although post-stroke rehabilitation often includes occupational and speech therapies, the standard of care intervention in this study will be performed by a physical therapist only. It will be based on movement training to avoid potential confounding of associated therapies. The techniques used will be determined according to the initial evaluation of each individual patient's impairments. They will have an emphasis on strength, flexibility and coordination. All subjects will be asked to attend a total of eighteen physical therapy sessions, each 30 minutes in duration, three times per week for six weeks.

Subjects in the comparison group will receive a sham rTMS simulated by a coil placed perpendicular to the scalp (Mennemeier et al., 2009). While the sham rTMS should not stimulate the cortex, the acoustic sensation and the sensation on skin should not differ from the real rTMS. This technique has been used in similar studies (Chang et al., 2010) (E. M. Khedr et al., 2010). Other reported sham procedures carried higher risks of unblinding since they did not reproduce the same sound and skin sensation as the rTMS. The coil perpendicular to scalp solves this issue. It is also simple and achieves the standards of an ideal sham rTMS. The details of this technique are described elsewhere (Fregni et al., 2006).

Research question
Does individually tailored rTMS combined with physical therapy (PT) improve hand function in comparison to fixed rTMS and sham rTMS combined with PT in chronic stroke patients after six weeks?

P: Chronic stroke patients (1-3 years after stroke).
E: Individually tailored rTMS + PT.
C: Fixed rTMS and sham rTMS + PT.
O: Hand motor function improvement by the PegBoard Test.
T: six weeks.

Primary aim
Evaluate the efficacy of individually tailored low frequency rTMS in hand motor function improvement detected by the PegBoard Test at six weeks in chronic stroke patients.

Secondary aim
Evaluate at six weeks:
- The responder's rate, being categorize as “responders” those subjects who have > 50% improvement in the PegBoard Test.
- The hand strength using a dynamometer.
- Quality of life by the SF-36 questionnaire.

Hypothesis
Individually tailored low frequency rTMS for six weeks is better than fixed rTMS or sham to improve hand motor function in chronic stroke patients.

Trial design and setting
This will be a 3-arm, phase II, double blind, randomized, sham-controlled superiority trial held on the Spaulding Rehabilitation Hospital in Boston, Massachusetts. The protocol will be presented to the Institutional Review Board and an Informed Consent will be obtained for each subject prior to the beginning of data collection. The sponsor will be Harvard Medical School, and we will apply to a NIH grand.

Eligibility criteria: Inclusion
- Diagnosis of stroke with hand function impairment.
- Chronic condition (1 to 3 years after stroke).
- Age: 45 to 80 years old.

Eligibility criteria: Exclusion
- Any previous stroke episode.
- Pregnancy.
- Severe cognitive disorders or neuropsychiatric comorbidities.
- Fugl Meyer (Upper limb) < 20.
- Inability to understand the task.
- Unstable medical conditions (e.g. uncontrolled diabetes, uncompensated cardiac disease, heart failure, pulmonary disease).
- Contraindications for rTMS (Neuronetics, Inc. Manual) such as: metal in the head; implantable medical devices.
- Epilepsy or disorders that raise the probability of having a seizure (brain tumor, metabolism disorders associated with the occurrence of seizures, moderate or severe traumatic brain injury, congenital birth defects leading to seizures).

Recruitment strategy
Patients with a chronic stroke state within 1 to 3 years after the event that fulfill the inclusion and exclusion criteria according to their physician will be screened by trained personnel after they agree to do so. Physicians that manage this type of patients will be given all the information they need to know if their patients could benefit from this trial.
Advertisements will be put on rehabilitation centers and neurology departments. Every patient will receive the intervention after the trial is over. Each patient who consent and meet all inclusion and exclusion criteria will be randomized into one of the groups.

Other recruitment strategies will include:
- Flyers posted in public areas across the Boston-land region, in the outpatient specialist clinics, or other private locations with given permission.
- Internet and newspaper advertisements.
- Advertisings in the public transportation.
- Via electronic patients records like Meditech.
- Presentations in stroke support groups.

**Intervention**

Patients will be randomized to any of the three arms using a block random size method. The three groups will receive rTMS 3 days a week for 6 weeks followed by 30 minutes of physical therapy with focus on strength, flexibility and coordination.

The intervention group will receive an individually tailored dose of rTMS. This means receiving 1 Hz (low frequency) of rTMS at 100% of the resting motor threshold (rMT) on the contralateral side of the lesion starting at 1,200 pulses. After each week, the subjects in this group will be assessed with the Pegboard Test. If they do not improve, the dose of rTMS will be increased 100 pulses. This will be done until the subject improves > 50% on their baseline score or the 6 week period of the trial ends.

The fixed arm will receive the same dose for the 6 weeks regardless of their response, which corresponds to 1,200 pulses of 1 Hz rTMS at 100% of the resting motor threshold (rMT) on the contralateral side of the lesion. Lastly, the sham arm will be a perpendicular coil that simulates the sensation of rTMS but doesn’t produce any effect, also for the same amount of time than the other two groups.

Reasons for discontinuing the intervention include:
- Occurrence of another stroke.
- Implantation of any metallic or incompatible device during the 6 weeks of rTMS intervention.
- Occurrence of seizures during the rTMS intervention.
- Patient's decision to stop participation in the trial.

**Adherence**

Every patient will receive the standard therapy (PT) and will be offered the intervention at the end of the trial if they participate during the whole study. Each complete week of 3 sessions will be paid with 50 American dollars. Parking will be provided as well as transportation to the center and back to the patient’s residence.

Other adherence strategies that will be used include:
- Screen all patients prior to initiation of the proposed treatment in order to identify risk factors for low adherence to allow for early risk modification.
- Help stabilize the patient’s living situation and social support system. We can collaborate with a case manager or social worker to maximize community resource. With the patient’s help, we can also identify a family member, friend, or partner who will assist with program adherence.
- Assess the patient’s beliefs and perceptions about the proposed treatment. We can consider the use of a support group, peer educator, or “treatment buddy” if the patient has negative perceptions about the treatment or does not believe that the method will work.
- Provide education and sufficient time to address any concerns. We can consider delaying initiation of the treatment until the patient is ready. Structured individualized or group educational sessions about the treatment and strategies for adherence have been found to be effective in a number of studies. A nurse, health educator, or other staff member can administer these. Oral and written material in each patient’s primary language and appropriate reading skills will also be provided.
- Simplify the regimen as much as possible. Shorter time of treatment enhance adherence. Thirty-minute PT sessions are chosen for efficacy and consideration of patient’s endurance.
- Individualize treatment regimens. Each patient will work with an individual physical therapist to choose a regimen that is tailored to his or her condition and schedule.
- Develop a warm, caring patient-provider relationship. Regular communication will be maintained through automated telephone or computer-assisted patient monitoring and counseling; manual telephone follow-up; and family visits.
- Make clinical site accessible. Patients should find it easy to call and obtain answers to their questions or make clinic appointments at a short notice if problems develop.
- Use of reminders. There will be an automated telephone reminder one day before each appointment and physical therapy session.
- Reinforcement or rewards for both improvement adherence and treatment response.
- Transportation vouchers will be provided to all patient participants to cover the transportation costs for their sessions.

Adherence will be measured by attendance to the sessions. Positive adherence will be indicated by attendance.
to 80% or more sessions. Lower than 80% will be considered negative adherence.

Outcomes: Primary
We will assess hand function improvement with the PegBoard Test, which measures time to finish a task (continuous variable). This decision is based on the extensive report of validity, reliability and responsiveness of this measurement in stroke outcome research. Additionally, we followed the recommendations of Baker et al (Baker, Cano, & Playford, 2011) of choosing a widely accepted scale for better interpretability. The latter is because one of the possible reasons for partial success reported in previous trials is attributed to the heterogeneity of outcome measurements.

Outcomes: Secondary
- Also using the Peg Board Test, we will categorize this variable and assess for responder’s rate, being a responder the patient who improve > 50%.
- Hand strength will be assessed using a grip dynamometer and quality of life with the SF-36 questionnaire.
- The Fugl Meyer Motor Score will be used to exclude patients with severe motor impairment.
- For safety purposes, cognition and mood will be evaluated through the Mini Mental Scale Examination and the Beck depression inventory.
- Questionnaires will be used to assess blinding and side effects.

Timeline
This project will recruit subjects during a 2 year period. The intervention will be performed during 6 weeks. After this period, the main analysis will be conducted. A follow-up of 3 months is planned for each patient. An exploratory analysis will be run after this period.

Randomization
A blinded statistician will apply a centralized web-based randomization system. Permuted block design with random 1:1:1 block sizes will assign 25 subjects for each arm, making a total of 75 patients. Regression adjustments can be used if needed to reduce baseline factor imbalances. Allocation will be concealed by the use of a centralized randomization system. Permuted block design with random 1:1:1 block sizes will assign 25 subjects for each arm, making a total of 75 patients. Regression adjustments can be used if needed to reduce baseline factor imbalances. Allocation will be concealed by the use of a centralized randomization system. A dedicated person assigned by the study coordinator will guide the subjects to their intervention. Physicians will be responsible for transmitting their patients the existence of this trial. If the patient accepts to know more, a researcher will approach the subjects with more information. Enrollment occurs after the completion of the informed consent. A statistician blinded to the purpose of the trial will handle the sequence generation.

Blinding
This study will be a double-blind trial. The technician in charge of applying the rTMS cannot be blinded due to the knowledge and skills needed when performing the intervention. The patients will be blinded using a coil sham in the placebo arm. The assessors, physical therapists and the statistician analyzing the results are also going to be blinded when performing their tasks. The principal investigator, who usually is unblinded, will be the one in charge of stopping the trial first if there is need to.

Emergency unblinding is reserved for special situations where a patient's health is at risk or situations where the exclusion criteria applies and could lead to termination of the patient's participation. These include:
- New stroke episode
- Seizures
- Hearing Loss
- Occurrence of a new unstable medical disorder
- New medical condition that require drugs which can be hazardous during application of rTMS
- Pregnancy

Data management
Data management procedures will follow the Data Management Plan, which outlines all procedures related to data collection, handling and auditing. This Data Management Plan complies with guidelines and standards of Good Clinical Data Management Practices. Electronic Case report forms (eCRF) will be used to collect patient data. Repetitive data such as protocol ID, site code, subject ID, and patient initials will be automatically generated by the system from the first page to all others, thus ensuring no duplication of CRF pages. The eCRF filling instructions will be provided to study investigators for error free data acquisition and will be documented at the Data Management Plan. Paper CRFs will be available at all centers in case of electronic system failure. This information will be translated to the electronic database as soon as possible.

A validated Clinical Data Management System (CDMS) will enable data management and control. According to the roles and responsibilities of each subject, multiple user IDs will be created with access limitation to data entry, medical coding, database designing, or quality check. This ensures that each user can access only the respective functionalities allotted to that user ID and cannot make any other change.
in the database. In conformity to global guidelines, the software will record the change made, the user ID that made the change and the time and date of change, for audit purposes (audit trail) (Krishnankutty, Bellary, Kumar, & Moodahadu, 2012).

For uniform medical terminologies associated with the clinical trial, Medical Dictionary for Regulatory Activities (MedDRA) will be used for the coding of adverse events and other illness. World Health Organization–Drug Dictionary Enhanced (WHODDE) will be used for coding of administered medications (Krishnankutty et al., 2012).

Data Management Plan will also contain procedures related to data validation, discrepancy management and database locking. All data management activities should be completed prior to database lock. Once the approval for locking is obtained from all stakeholders, the database is locked and clean data is extracted for statistical analysis. Generally, no modification is allowed after database lock. In case of a critical operational exception, at least two privileged users will be needed to override and modify the data after database lock. This exception will be recorded in the Data Management Plan.

As required per ICH Guideline for Good Clinical Practice, the sponsor will maintain all sponsor-specific essential documents for at least 2 years after formal discontinuation of the clinical development of the intervention or after the last approval of a marketing application. These documents should be retained for a longer period however if required by the applicable regulatory bodies or if needed by the sponsor. The sponsor should inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed (Branch, 2005).

Sample size calculation
Sample size calculation is based on using an F-test one-way ANOVA for 3 groups. A significance level of 5% (alpha level of 0.05), and a power of 80% was assumed. Based on a recent meta-analysis (Thrane, Friborg, Anke, & Indredavik, 2014), an effect size of 0.4 was selected. An attrition rate of 15% was chosen. The resulting sample for each arm is 25 subjects, making a total of 75. Sample size

![Study outline diagram](image-url)
calculation was performed using smallSTATA 13.1 (StataCorp LP).

**Statistical analysis**

Statistical analysis will be performed using smallSTATA 13.1 software (StataCorp LP). All outcomes will be tested for normality with the Shapiro-Wilk test. The primary analysis for the main outcome will be assessed by hand function improvement with the PegBoard Test at baseline and at six weeks using one-way ANOVA with post hoc t-tests with Bonferroni correction.

The secondary outcome using the responder’s rate will be assessed also with the PegBoard Test but using the chi-square test for categorical variables. For the other two secondary outcomes, hand strength by dynamometer and quality of life with the SF-36 questionnaire, we will use one-way ANOVA. In the case of a not normal distribution of the data, baseline characteristics and outcome changes will be analyzed using Kruskal Wallis test.

**Missing data**

We will analyze the data with the Intention to treat (ITT) approach. Also, all the missing data will be manage using the last observation carried forward (LOCF) method. We believe it is conservative, approved method that will not produce bias results in our study.

**Data monitoring**

A Data Monitoring Committee (DMC) independent of the researchers and sponsors will assess this study. The committee will include a neurologist, a nurse, a biostatistician and a medical doctor with expertise in ethics. None of the members will have any conflict of interests. The DMC will determine the quality of the study analyzing protocol violations and deviations, patient adherence and withdrawal, and safety and efficacy of the intervention. Such information will be used to advise the sponsor and researchers to improve the quality of the study. They can recommend the continuation, termination or modification of the trial. All the managed information will be kept secure and private to prevent unblinding.

**Interim analysis**

Because of the short duration of the trial and the high safety profile of the intervention, we do not believe it is necessary to do a safety interim analysis. This doesn’t mean that the subjects aren’t going to be carefully assessed for any adverse event.

About an efficacy interim analysis, because the objective is to assess the improvement at 6 months and to know if increasing the dose corresponds to a better outcome, terminating the study before the trial ends would not be useful. Also, every patient will receive the standard of care and at the end of the trial the intervention with the better response, so we also believe is not necessary to implement this efficacy analysis.

**Ethics**

Institutional Reasearch Board and Ethical Research Committee approval will be required. An informed consent assignment will be based on a WHO model and obtained for all participants.

Reliability assurance will be made through the data protection of persons under the research. The author of the scientific survey may publish the result of the intervention but never data that reveals personal information about the person under clinical investigation.

The principal investigator is going to be the primary author of the research. This primary author have to assure that all authors meet basic standards for authorship and should prepare a simple written description of their contributions to the work, which will be approved by all authors. All authors needs to make substantial intellectual contributions to the work and participate of the writing, review and approval of the drafts and final version of the manuscript.

The order of authorship is going to be decided by the group before the study starts. It will take into account the contribution of each subject in the trial (West, 1997).

**Authors’ affiliations**

1. Berenson-Allen Center for Noninvasive Brain Stimulation, Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.
2. Research and Study Center Wilson Mello Institute
3. University of Brasilia, Brazil
4. Center of Transplantation Sciences, Massachusetts General Hospital / Harvard Medical School
5. Spaulding Neuromodulation Center, Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA, USA
6. ANVISA – Agência Nacional de Vigilância Sanitária, Brasil.
7. Partners in Health, Boston, USA.
8. Department of Clinical Pharmacy, College of Pharmacy, Umm Al Qura University, Holy Makkah, Kingdom of Saudi Arabia
9. Faculty of Medicine of the University of São Paulo.

**Conflict of interest and financial disclosure**

The authors followed the International Committee or Journal of Medical Journals Editors (ICMJE) form for disclosure of potential conflicts of interest. All listed authors concur with the submission of the manuscript, the final version has been approved by all authors. The authors have no financial or personal conflicts of interest.

**References**


134


Takeuchi, N., Tada, T., Toshima, M., Chuma, T., Matsuo, Y., & Konno, K. (2008). inhibition of the unaffected motor cortex by 1 Hz repetitive transcranial magnetic stimulation enhances motor performance and training effect of the paretic hand in patients with...


