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Effect of transcutaneous electrical acupoint stimulation on bone metabolism in patients with immobilisation after foot and ankle fracture surgery: a randomised controlled trial study protocol

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ABSTRACT

Introduction Fracture is a disease with a high incidence worldwide. Foot and ankle fractures are common among fractures of the lower extremities. Foot and ankle fractures usually require surgical fixation and a period of fixed treatment, which can lead to decreased bone density. Although transcutaneous electrical acupoint stimulation (TEAS) is widely used for movement system diseases, there is minimal evidence to show the effectiveness of TEAS on patients after surgical fixation of ankle and foot fractures. This trial aims to evaluate whether TEAS can reduce bone loss in patients with immobilisation after ankle and foot fractures.

Methods and analysis A randomised controlled trial will be conducted in which 60 patients will be randomly divided into two groups: (a) the control group will be treated according to the routine procedures of basic orthopaedics treatment; (b) in the treatment group, bilateral SP36, BL23 and ST36 will be performed on the basis of the control group, and the test will be performed for 30 min every other day for a total of 8 weeks. Bone turnover markers will be used as primary outcome. Secondary outcomes are composed of blood phosphorus, blood calcium and bone mineral density. Treatment safety will be monitored and recorded.

Ethics and dissemination This trial is approved by the Ethics Committee of Beijing University of Chinese Medicine (2020BZYLL0611) and the Ethics Committee of Beijing Luhe Hospital (2020-LHKY-055-02), and inpatients who meet the following diagnostic and inclusion criteria are eligible to participate in this study.

Trial registration number ChiCTR 2000039944.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is the first randomised controlled trial of transcutaneous electrical acupoint stimulation (TEAS) in the treatment of bone loss in patients after surgical fixation of ankle and foot fractures.
⇒ This study will provide evidence on the optimal option for external non-interventional therapy in the treatment of patients after surgical fixation of ankle and foot fractures.
⇒ The patients cannot be blinded due to the particularity of TEAS treatment.
⇒ We cannot judge the subjective feelings of patients due to the lack of subjective outcomes.
⇒ This is an exploratory study with a small sample size which has not been calculated.

INTRODUCTION

Fracture is one of the most common clinical diseases in the world, with an annual incidence rate of at least 5 per 10 000 people.[1 2] When the fracture is healing, patients with foot and ankle fractures will receive surgical fixation and a period of fixation treatment. This process will reduce the mechanical stress of patients and cause bone loss.[3–7] Studies have suggested that patients who are bedridden after fractures may experience bone mineral loss in their lower extremities, which is related to the reduction in weight bearing.[8–11] Other studies have shown that in individuals with lower extremities injuries, a significant correlation exists between the decrease in bone density and the acceleration of bone turnover.[12 13] Although there are several methods to prevent bone loss, such as hormone replacement therapy, bisphosphonates, denosumab and parathyroid hormone (PTH), the inherent side effects of their long-term use have drawn attention.[14] Therefore, it is necessary to find a safe and reliable method to treat bone loss. With an appropriate stimulation frequency, physiotherapy electrical

stimulation can cause passive contraction of muscles, promote osteoblast activity and inhibit bone absorption.[15]

Electrical stimulation can provide local irritation for bone-forming cells and reduce bone loss. As we all know, bone is a mechanical sensory organ that requires continuous strain to maintain its functional structure and prevent bone loss.[16–18] Transcutaneous electrical acupoint stimulation (TEAS) is derived from acupuncture, which bears the advantages of high safety and low risk, and it also can replace electric acupuncture. TEAS is becoming increasingly popular around the world.[19 20] It has been used in many diseases.[21–26] The therapeutic effect of TEAS is similar to that of acupuncture. Meanwhile, it has long been regarded as a potentially effective non-drug therapy for the treatment of fractures. TEAS can reactivate the biological process of bone regeneration, simplify fracture regeneration and shorten the duration of treatment.[27] It uses works by using pulsed electromagnetic fields (PEMF) together with traditional Chinese medicine acupoints.[28] To date, a large number of studies have suggested that PEMF can regulate bone metabolism in patients with fractures, enhance bone formation, increase bone density and reduce patients' pain.[29–32] Although it has been proved that the postoperative fixation of ankle and foot fractures can affect bone metabolism and lead to bone loss,[33 34] there is little evidence to show that TEAS can treat postoperative fixation of ankle and foot fractures.

Therefore, we have designed an open label, randomised, controlled trial to examine the effect of TEAS on patients with immobilisation after foot and ankle fractures. Our aim is to evaluate whether TEAS can reduce bone loss in patients with immobilisation after foot and ankle fractures.

METHODS

Study design and setting

The open-label and randomised controlled trial will be conducted from November 2020 to November 2021. The hospital will assign a multidisciplinary team (including acupuncturists, rehabilitative physicians, orthopaedic surgeons, nurses and physical therapists) to conduct the trial. Participants will receive transportation and blood sample subsidies during the trial, and all TEAS treatments, blood and urinary tests, dual energy X-ray absorptiometry (DXA) and quantitative computed tomography (QCT) examinations are free of charge. This trial has to follow Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines[35] and will conform with Consolidated Standards of Reporting Trials guidelines.[36] A SPIRIT checklist is provided as online supplemental file 1 . This trial was approved by the ethical committees of relevant hospitals and registered on www.chictr.org. The study was approved by the ethics committee of our institution and obtained the informed consent of all participants. The planned start date is April 2021 and end date is April 2022.

The flowchart of the study is presented in figure 1.

Sample size

Given that this is a pilot trial, a formal sample size calculation will be not necessary. Therefore, the sample size of 60 participants (30 persons per group) is determined to be sufficient for the purpose of the trial (preliminary verification of the effectiveness of TEAS treatment in terms of bone loss relief in patients with immobilisation after ankle and foot fractures after 8-week treatment). Due to the COVID-19 epidemic, we designed the 25% dropout rate to ensure the aimed sample size, so we plan to recruit 80 participants.

Participants

Diagnostic criteria

According to the *Guidelines for the Diagnosis and Treatment of Common Diseases in Orthopedics and Traumatology of Traditional Chinese Medicine*,[37] all cases will be diagnosed with foot and ankle fractures by imaging and related orthopaedics diagnosis in tertiary hospitals. Imaging examination: X-ray (CT or MRI can be used if necessary) diagnosed patients as foot and ankle fractures of the distal tibia, fibula, talus, metatarsal and ankle.

Inclusion criteria

1. Satisfaction with the above-mentioned key diagnosis points and immobilisation needs of foot and ankle injury.
2. The patient is aged 18–45 years old.
3. The patient has a clear consciousness.
4. The length of internal fracture fixation does not exceed 15 cm above ankle joint.
5. The patient is informed and agrees to sign an informed consent form.

Exclusion criteria

1. Patients with endocrine diseases (thyroid, parathyroid, gonad, adrenal diseases and etc) that affect bone metabolism, immune diseases (such as rheumatoid arthritis, digestive tract, liver and kidney diseases) that affect calcium and vitamin D absorption, and malignant diseases (such as multiple myeloma).
2. Long-term use of glucocorticoids, antiepileptic drugs, oestrogen, heparin, progesterone and other drugs affecting bone metabolism; various congenital and acquired bone metabolic disorders.
3. Patients who have taken calcium, calcitonin, vitamin D and other drugs affecting bone metabolism within 30 days.
4. Patients with severe infection of the limbs, or pathological, old fractures, or other fractures of the limbs, and severe heart, liver, kidney, lung, blood and endocrine system diseases.
5. Patients with histories of severe digestive system disease, kidney disease, connective tissue disease, or malignant tumour, or other disease affecting bone metabolic.
6. Pregnant or lactating women.

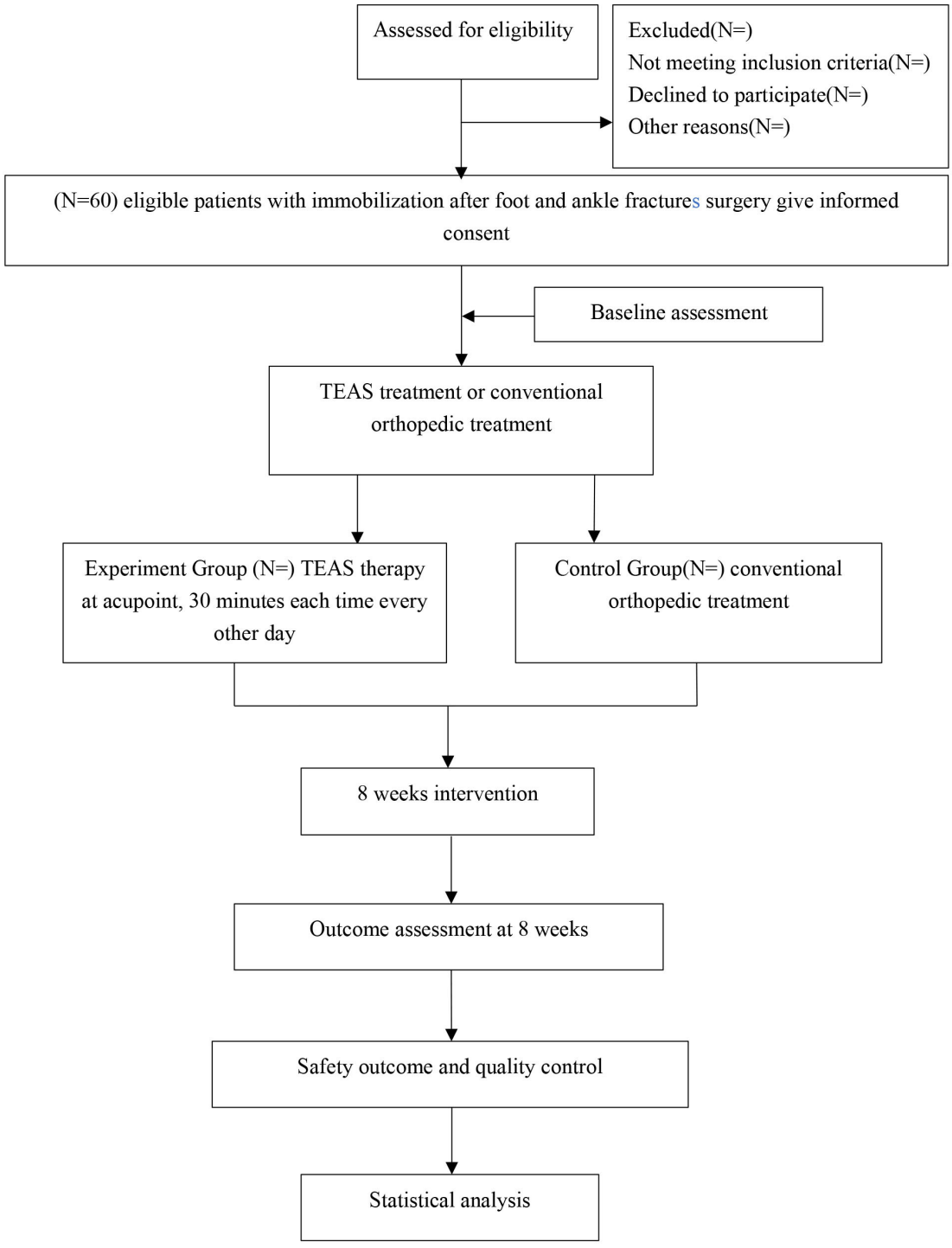

Figure 1 Flow diagram of study design.

7. Patients with a stent implanted in their body or a history of joint replacement.
8. Patients with known heart disease and patients with pacemakers.
9. Patients who have been treated by this study within the past 30 days.
10. Patients with allergies, poor compliance or mental disorders who cannot cooperate with the study.
11. Newly diagnosed patients who need calcium intervention.

Randomisation and allocation concealment

The trial will adopt computer software named SPSS V.26.0 to generate a random number table and put the random numbers and grouping information in an opaque, sealed and sequentially coded envelope, which will be kept by a dedicated person. Patients will receive corresponding envelopes in order of their enrolment. Random numbers ending in an odd number will be included in the treatment group, and those ending in an even number will be included in the control group. Because it is difficult

to implement a blinding principle in clinical research involving TEAS, special personnel will be responsible for grouping, treatment operation and data collection during the implementation process of this project; At the same time, as the outcome indicators of this trial are all objective indicators obtained from laboratory tests and machine measurement, the absence of blinding will not be considered as a factor affecting the results of the study.

Blinding

Due to the particularity of TEAS treatment, neither the patient nor the acupuncturist can be blinded. However, clinical assessors, rehabilitative physicians, orthopaedic surgeons, data collectors and analysts will be blinded to the treatment and grouping of patients.

Patient and public involvement

There is no patient or public involvement in study design, recruitment for or conduct of the study.

Interventions

Control group

The conventional orthopaedic treatment: (a) no weight-bearing lower limb joint and muscle training for 4 weeks after operation, including active and passive exercises; (b) routine X-ray examinations 1 month and 2 months after operation would be performed to observe fracture healing and internal components (plates, screws).

Treatment group

Based on the conventional orthopaedic treatment in control group, TEAS treatment will be adopted bilaterally at SP36, BL23 and ST36. The acupoint locations refer to the PRC National Standard 'Acupoint Name and Location' (GB/T 12346-2006).[38] Operation: a patient takes the supine position, the acupoint locations are wiped and disinfected with a 75% alcohol cotton ball, and, after drying, the electrode pads are pasted on. During the treatment, one set of electrodes will be applied to the BL23 on both sides, and the other set of electrodes to the SP36 and ST36 on the same side of the lower limbs. In this way, a current loop will be formed. Electrical stimulation of the lower limbs focuses on stimulating the affected limb. If it is not possible to stimulate the affected side after fixation, a contralateral acupoint can be used. The power will be turned on and the parameters adjusted. The stimulation frequency will be set to 2 Hz/100 Hz and the default value to 10 mA; its actual value (5–10 mA) will be determined by keeping the patient comfortable for 30 min at a time, every other day, for 8 weeks. Before treatment, the knob will be adjusted to the lowest position and the power gradually increased. It should not be large and then small or suddenly large and small, which would make it difficult for the patient to accept. The methods and parameters of acupoint electrical stimulation are standard parameters, which ensure the maximum safety of TEAS. The operating instrument used is the INTI brand KWD-808 I pulse therapy instrument (Su Annotation 20152261330).

Study procedure

Treatment during hospitalisation

The patients with foot and ankle fractures in the trial group accept surgical treatment and can be discharged after 1 week of hospitalisation in accordance with routine procedure; so, the treatment in the first week is performed by a professionally qualified acupuncturist. At the same time, basic orthopaedic treatment is jointly completed by a professionally qualified orthopaedic doctor and a professionally qualified rehabilitation therapist. Before the project is launched, acupuncturists, rehabilitation physicians, orthopaedic surgeons, nurses and physiotherapists will be trained in the operation methods and procedures for transcutaneous electrical acupuncture and basic orthopaedic therapy, to unify and standardise their operation.

Treatment after discharge

Treatment after discharge: the patients and their families will get standardised operation process training after their enrolment. The training team consists of two acupuncturists, an orthopaedic doctor and a rehabilitation specialist who perform the first week of treatment. The training includes TEAS and basic orthopaedic treatment. Training content: (a) therapeutic point selection of SP36, BL23 and ST36 acupoint positioning (each patient will have an acupoint positioning guide); (b) operation procedures of TEAS (each patient will have an instrument operation manual); (c) basic orthopaedic treatment content (such as active and passive weight-bearing lower limb joint and muscle training).

OUTCOMES

Primary outcomes

After being randomly assigned, all patients will receive a baseline assessment and bone turnover marker (BTM) content to be evaluated over 2 months, including: (a) bone formation markers: alkaline phosphatase (ALP), bone glaprotein (BGP) and procollagen type I N-terminal propeptide (PINP); (b) bone resorption markers: β-CTX, urine calcium/creatinine (Ca/Cr); (c) PTH.

The primary outcome measures mean changes in BTM (BTM) and PTH from baseline to 8 weeks. Bone density provides only static parameters, which reflect changes in bone mass over a relatively long time. In order to understand the dynamic change process of bone metabolism, we need to evaluate bone metabolism markers.[39] Measuring BTMs is helpful for predicting rapid bone loss. In addition, subsequent changes in bone mineral density (BMD) can be predicted on the basis of the changes in bone turnover within 1 month, which can provide early and effective prognostic measures.[40] BTM can be divided into bone formation markers and bone resorption markers. Bone formation markers can reflect the activity of osteoblasts and the state of bone formation, and bone resorption markers reflect the activity of osteoclasts and the level of bone resorption.[41] Bone formation

markers include serum ALP, PINP, BGP. Bone resorption markers include: β-cross-linked C-telopeptide of type I collagen (β-CTX). The bone metabolism biochemical index can reflect the state of bone turnover over time, with high sensitivity and strong specificity.[42] The International Federation of Clinical Chemistry and Laboratory Medicine also recommends using serum PINP as a bone formation marker, and serum CTX as a bone resorption marker, making it a reference analyte for BTMs in clinical research.[43 44]

PTH has been specified in development and maturity in cartilage and bone as a crucial regulator of endochondral bone formation and bone remodelling.[45] Its target is found to be cells of the osteoblast lineage.[46] In addition, the only Food and Drug Administration-approved anabolic bone agent for the treatment of osteoporosis in the USA is PTH 1-34, or teriparatide.[47]

Secondary outcomes

Secondary results include: (a) BMD; (b) blood phosphorus and calcium in plasma. DXA and QCT will be used to measure the mean change in BMD at 8 weeks after baseline. We will use DXA to scan the participants' L1-L4 and femurs (femoral neck, greater tuberosity, and all femurs)[48] and QCT to scan the ankle of the affected limb to record and analyse changes in bone density. The measurement of BMD by DXA is the gold standard for the diagnosis of osteoporosis.[49] Although DXA is simple and fast to operate and does little harm to the body, its main measurement area is BMD; that is, the sum of cortical bone and cancellous bone BMD.[50] Therefore, the detection may be misdiagnosed due to tissue structure or abdominal aortic atherosclerotic and other diseases that affect the measurement accuracy.[51] The DXA vertebral body BMD measurement is therefore used only to predict overall fracture risk, although it is difficult to predict the absolute risk in some individuals. In recent years, it has been discovered clinically that QCT is of great value in diagnosing osteoporosis.[52] It uses phantom and analysis software to determine volume BMD on the basis of CT scans, and the measurements are not affected by cortical bone and hyperplasia.[53] Bone and other influences are of great significance for physicians in analysing bone mass changes of cancellous bone and providing cross-sectional data.[54]

The electrochemical method will be used to detect blood phosphorus and calcium in plasma. Blood phosphorus and calcium are general biochemical markers in bone metabolism. All the assessments are presented in table 1.

Safety evaluation

Adverse events occurring during the study will be recorded in detail, including the specific time, severity, duration and relationship with transcutaneous electrical acupuncture point stimulation. Five grading criteria will be used to evaluate the relationship between adverse events and treatment: (1) certain correlation; (2) possible correlation; (3) absolutely irrelevant; (4) may not be relevant; (5) uncertainty about the countermeasures taken and the final results.

Quality control of inpatients

All practitioners, evaluators and statisticians will receive uniform training before the test. The training courses include basic orthopaedic treatment operations, inclusion and exclusion criteria, standardised operating procedures, observation time, result measurement, completion, acupoint positioning, the operation of the transcutaneous acupoint electrical stimulator and training on technical data processing. The training of patients and their families involves the operation of basic orthopaedic treatment, acupoint positioning and the operation of the transcutaneous acupoint electrical stimulator. Clinical monitors will regularly monitor the study to ensure trial quality.

Telephone interview and WeChat video will be adopted to monitor and record the patients' feelings after treatment. The main content includes: (1) monitoring the operation process of the treatment group; (2) monitoring the accuracy of acupoint positioning; (3) inquiring about and recording of adverse reactions, and treatment instructions (severe pain: remove the electrode immediately; skin redness: reduce power or suspend use in time); (4) the use of the electrode sheet and whether it needs to be mailed; (5) re-checking time statistics.

Data collection and management

Two independent investigators will collect the baseline data, such as age, gender, height weight, cause of fracture, ankle fractured, the type of fixation (screw/plate or intramedullary nail), the severity of comminution of fractures, the use of bone graft, including autograft, allograft or artificial bone graft with/without osteoinductive activity, associated injury, for example, multiple traumas, brain injury, ambulation status (the activity limitation of included patients since weight-bearing status). All data will be carefully verified by these two investigators, then will be kept by a designated person.

Statistical analysis

Statistical analysis will be performed using SAS V.9.4. For continuous outcomes, the Shapiro-Wilk test will be used to test the normal distribution of data. If quantitative data follow normal distribution, described as mean±SD, we will use the t-test to compare the differences. If quantitative data do not follow normal distribution, it will be described as median (IQR), and we will use the Wilcoxon rank-sum test to compare the differences. Categorical variables will be described as percentages (n%). We will use the χ^2 test to compare the differences on categorical variables (Fisher's exact test is used where appropriate). All statistical analyses will use two-sided tests, with $p<0.05$ considered as the difference required to be statistically significant.

Table 1 Trial flowchart

Items	Before enrolment	Baseline period	Treatment period	Treatment for 8 weeks
Recruitment	√			
Enrolment	√			
Inclusion criteria	√			
Exclusion criteria	√			
Informed consent	√	√		
Basic characteristic variables	√	√		√
Randomisation and allocation concealment	√	√		√
Primary outcomes				
BTM (bone formation)				
ALP		√		√
BGP		√		√
PINP		√		√
BTM (bone resorption)				
β-CTX		√		√
Urinary Ca/Cr		√		√
PTH (parathyroid hormone)		√		√
Secondary outcomes				
BMD (DXA)		√		√
BMD (QCT)		√		√
Blood phosphate		√		√
Blood calcium		√		√
Safety evaluation				
Pain		√	√	√
Redness and swelling of the skin		√	√	√

ALP, alkaline phosphatase; BGP, bone glaprotein; BMD, bone mineral density; BTM, bone turnover marker; DXA, dual-energy X-ray absorptiometry; PINP, procollagen type I N-terminal propeptide; QCT, quantitative computed tomography.

Data availability statement

Within 6 months of the trial's completion, the original data will be shared through the ResMan platform of the Chinese Clinical Trial Registry (http://www.medresman.org.cn).

DISCUSSION

The study aims to evaluate effectiveness and safety. We hypothesised that TEAS could reduce bone loss in patients with immobilisation after ankle and foot fractures. In clinical trials of TEAS, it has been proved that the combination of PEMF and acupuncture points can promote bone formation and slow bone resorption.[55 56] This study is the first randomised controlled trial of TEAS in the treatment of bone loss on patients after surgical fixation of ankle and foot fractures.

This study will provide evidence on the optimal option for external non-interventional therapy in the treatment of patients after surgical fixation of ankle and foot fractures. In addition, this is the first trial exploring whether the bone density will change after 2 months of TEAS.

Despite the potential strengths, there are some limitations in our trial. First, due to the particularity of patients hospitalised for only 1 week after orthopaedic treatment and surgery and then discharged, the post-discharge treatment can be performed only by patients and their families. In order to compensate for this limitation, professional acupuncturists and orthopaedic doctors will train patients in a rigorous and standardised method of TEAS, and basic orthopaedic treatment, during their week of hospitalisation. WeChat video and voice call will be used for quality monitoring, and questions about adverse reactions and statistical re-inspection time will be asked.

Second, the patients cannot be blinded due to the particularity of TEAS treatment. Patients will know whether they had received additional TEAS treatment,

which, in some cases, may have increase the risk of dropout in control patients.

Third, we cannot judge the subjective feelings of patients due to the lack of subjective outcomes.

Finally, this is an exploratory study with a small sample size which has not been calculated. No long-term follow-up will be performed to observe long-term treatment effects and adverse reactions.

Moreover, a review of the literature found that BMD may not change within 2 months, so we used bone metabolic markers as the primary outcome indicator. On the other hand, we hope to explore whether bone density will change in 2 months. The results of this trial will help provide evidence for the efficacy of TEAS in reducing bone loss for patients after fractures.

ETHICS AND DISSEMINATION

All participants who meet the diagnostic, inclusion criteria and exclusion criteria will be required to sign an informed consent form prior to participating in this study. This study was approved by Beijing University of Chinese Medicine (2020BZYLL0611) and the Ethics Committee of Beijing Luhe Hospital (2020-LHKY-055-02). Study findings will be published in peer-reviewed journals and presented at national congresses.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

		Reporting Item	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	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	15
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
Roles and	#5d	Composition, roles, and responsibilities of the	15

responsibilities: committees		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)	
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	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3-4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
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	4
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)	5-6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	8-9

		change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8-9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
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	9-11
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)	11
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	4-5
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	6

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	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12
Data collection plan: retention	#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	12
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	12
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	13

		outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
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	11-12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	14-15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria,	n/a

		outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	4
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	12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	13
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Uploaded file-consent

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a