Clinical Study Protocol
Version 2.0

Item	Details
Project Title	Clinical Efficacy and Peripheral Ultrasound Mechanism of Trigger Point Bloodletting Therapy for Primary Trigeminal Neuralgia
Project Source	Beijing Hospital Administration Center
Sponsor (if applicable)	Not applicable
Investigator (Signature)	Liu Huilin
Responsible Department	Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University
Leading Site	Beijing Hospital of Traditional Chinese Medicine, Capital Medical University
Participating Site	Beijing Hospital of Traditional Chinese Medicine, Capital Medical University
Contact Person and Telephone	Liu Huilin, 18827062868
Email Address	306916598@qq.com
Study Duration	August 31, 2026 – August 31, 2027

Study 1 Ultrasonic Characteristics of Trigger Points in Patients with Primary Trigeminal Neuralgia

1. Study Subjects

1.1 Source of Cases

All cases are recruited from the outpatient clinic of the Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University. All patients meet the diagnostic criteria of the International Headache Society for primary trigeminal neuralgia and satisfy the inclusion criteria.

1.2 Sample Size

This study adopts an exploratory self-controlled paired design with no prior reference literature available. Considering the sample continuity of subsequent clinical studies, the accessibility of primary trigeminal neuralgia outpatients in this center and a potential dropout rate of 15% to 20%, it is preliminarily estimated that 60 patients with primary trigeminal neuralgia need to be enrolled to ensure the statistical power of correlation analysis and the stability of results.

1.3 Case Selection Criteria

1.3.1 Diagnostic Criteria

Primary trigeminal neuralgia includes classic trigeminal neuralgia and idiopathic trigeminal neuralgia, diagnosed in accordance with the International Classification of Headache Disorders, 3rd edition issued by the International Headache Society in 2018.

Classic Trigeminal Neuralgia

1. Recurrent paroxysmal unilateral facial pain, which may be bilateral in some cases, occurring within the distribution of one or more branches of the trigeminal nerve, without radiating pain outside the trigeminal nerve distribution area, and meeting the following two criteria.
2. The pain meets three characteristics: lasting from an instant to 2 minutes or longer; pain intensity reaching severe level or above; manifesting as electric shock-like, tearing, knife-cutting or stabbing severe pain.
3. Induced by benign stimulation of the affected side of the face.
4. Cannot be better explained by other diagnoses in the International Classification of Headache Disorders, 3rd edition.
5. MRI or intraoperative confirmation of neurovascular compression rather than simple contact, accompanied by morphological changes of the trigeminal nerve root.

Idiopathic Trigeminal Neuralgia

1. Recurrent paroxysmal unilateral facial pain meets the diagnostic criteria for trigeminal neuralgia, presenting as purely paroxysmal, persistent or nearly persistent.
2. Confirmed by MRI or electrophysiological examination that it is neither classic trigeminal neuralgia nor secondary trigeminal neuralgia.

Common benign stimuli that trigger pain include chewing, tooth brushing, face washing and touching. The corners of the mouth, nasal alae, cheeks or tongue are sensitive areas where light touch can induce pain, which is defined as a trigger point.

1.3.2 Inclusion Criteria

- Patients meeting the diagnostic criteria for classic trigeminal neuralgia or idiopathic trigeminal neuralgia, regardless of gender.
- Patients with no obvious organic lesions shown by cranial MRI.
- Patients with daily pain attacks in the past month, with an average visual analogue scale score of no less than 4 points in the past 48 hours. For patients taking carbamazepine at enrollment, the average visual analogue scale score in the past 48 hours shall be no more than 8 points.
- Patients aged 18 to 75 years.
- Patients who have never received surgical treatment for primary trigeminal neuralgia, including percutaneous radiofrequency thermocoagulation of the trigeminal ganglion, balloon compression of Meckel's cave, glycerol injection of Meckel's cave, gamma knife therapy and microvascular decompression.
- Patients with definite trigger points in the orbicularis oris muscle of the upper lip, with unilateral onset of trigeminal neuralgia symptoms only.
- Patients who sign the written informed consent form and voluntarily participate in this study.

1.3.3 Exclusion Criteria

- Patients with secondary trigeminal neuralgia caused by other diseases such as intracranial tumors and multiple sclerosis.
- Patients with painful trigeminal neuropathy secondary to herpes zoster or trauma.
- Patients with facial pain caused by other diseases such as glossopharyngeal neuralgia, temporomandibular joint disorder, sinusitis, apical periodontitis and cranial neuropathy.
- Pregnant or lactating women.
- Patients with severe diseases or poorly controlled underlying diseases.
- Patients with positive results of four infectious disease screenings, severe coagulation dysfunction or obvious platelet abnormalities.
- Patients who are participating in other medical trials.

2. Study Protocol

2.1 Study Design

An observational study design is adopted.

2.2 Instruments and Methods

Mindray Resona R9 color Doppler ultrasound diagnostic instrument with L11-3 high-frequency linear array probe is used, with a frequency range of 5 to 12 MHz and a resolution of ±0.05 mm. The instrument is equipped with shear wave elastography and superb microvascular imaging modules. Original images are stored via 4D-view software, and Image J 1.54f software is used for subsequent echo grayscale quantitative analysis.

All subjects take the supine position with lips naturally closed and no occlusal movement. Ultrasound coupling agent is applied to the upper lip and the probe surface.

The probe is placed transversely at the lower part of the philtrum for detection. Direct contact between the probe and the lips and air bubbles between the probe surface and the coupling agent shall be avoided.

2.3 Scanning and Image Acquisition

2.3.1 Two-dimensional Grayscale Ultrasound Structural Assessment

The standard section is defined as the image showing clear orbicularis oris muscle structure with visible philtrum dimple and bilateral philtrum ridges. Centered at 1 cm lateral to the philtrum ridge, the probe position is adjusted to obtain a sub-standard section that clearly displays the layers of the orbicularis oris muscle in this area. Two-dimensional dynamic images are collected and stored. Static images are collected three times at the same position with an interval of 5 minutes and output via high-density printing paper.

Image J 1.54f software is used to convert images into 8-bit grayscale images. A 5 mm × 5 mm sampling frame is selected for each image as the region of interest to reflect the echo characteristics of the soft tissue. The Measure function in the Analyze module is used to calculate the average grayscale value of the region of interest, ranging from 0 to 255.

2.3.2 Functional Imaging Acquisition

The probe is stabilized on the standard section of the upper lip. Shear wave elastography examination is performed first: the probe is rotated 90 degrees for longitudinal scanning along the muscle bundle direction of the orbicularis oris muscle without external pressure, with a preset shear wave velocity range of 0.5 to 10 m/s. Three quantitative analysis Q-boxes are set near the trigger point, and the measurement is repeated three times with an interval of 5 minutes.

After measurement of the main area, the probe is translated to the sub-standard section 1 cm lateral to the philtrum ridge. The longitudinal scanning direction is kept consistent with the muscle bundle course, and the above operations are repeated. The same measurement method is applied to the symmetrical area on the unaffected side without trigger points as a control.

Superb microvascular imaging examination is performed afterwards: the probe is reset to the transverse standard section, and the color superb microvascular imaging mode is adopted. The region of interest is set as 10×10 mm with the deep half matching the orbicularis oris muscle. Acquisition is started after the quality control area turns uniformly green.

During the examination, the same physician collects images three times for both the main and sub-areas of the bilateral orbicularis oris muscle, with an interval of 5 minutes each time, an acquisition duration of 20 seconds and a sampling rate of 30 frames per second, to reduce operational errors and soft tissue motion interference.

2.4 Observation Indicators

2.4.1 Ultrasound Parameters

1. **Thickness measurement**
2.

- Subcutaneous and superficial muscle thickness: thickness of subcutaneous connective tissue and superficial orbicularis oris muscle on the unaffected side and the corresponding thickness on the affected side.
- Deep muscle layer thickness: thickness of deep orbicularis oris muscle on the unaffected side and the corresponding thickness on the affected side.
- Deep connective tissue thickness: thickness of deep connective tissue on the unaffected side and the corresponding thickness on the affected side.
- Cross fiber bundle thickness: thickness of cross fiber bundle on the unaffected side and the corresponding thickness on the affected side.
- Angle measurement: angle between the cross fiber bundle of the orbicularis oris muscle and the horizontal reference line.

3. **Superb microvascular imaging indicators** Alder semi-quantitative grading is used to classify blood flow signals into four grades: grade 0 for no blood flow signal, grade 1 for punctate blood flow signal, grade 2 for short strip blood flow signal, grade 3 for long strip or tree-like blood flow signal.

4. **2.4.2 Clinical Symptom Related Indicators**
   Pain diary scale including pain visual analogue scale score and pain attack frequency is used to record pain intensity and attack conditions.

2.5 Quality Control

- Operator qualification: Two physicians with more than 10 years of clinical experience and musculoskeletal ultrasound certification perform the operation independently. In case of inconsistent results, a third-party expert will conduct review.
- Equipment calibration: Equipment parameters such as probe frequency and shear wave elastography velocity range are checked daily to ensure consistent measurement conditions at different time points.
- Data management: Image data are stored anonymously, and dedicated personnel perform data analysis under double-blind conditions to reduce subjective bias.

2.6 Statistical Analysis

SPSS 29.0 statistical software is used for data analysis. To ensure the repeatability and stability of trigger point ultrasound parameter measurement, target ultrasound parameters are collected three times at 5-minute intervals under the same conditions for the same area, recorded as M0, M1 and M2 in sequence.

Repeated measurement error is calculated to evaluate data fluctuation: coefficient of variation equals the standard deviation of three measurements divided by the mean value of three measurements and multiplied by 100%. Intraclass correlation coefficient with one-way model is used to quantitatively evaluate test-retest reliability. An intraclass correlation coefficient approaching 1 indicates good consistency of multiple measurements.

Normally distributed measurement data are expressed as mean ± standard deviation. Data not conforming to normal distribution are expressed as median with interquartile range and analyzed by non-parametric tests. Paired sample t-test or paired Wilcoxon rank sum test is used for comparison between the affected side and the unaffected side.

Count data are expressed as frequency and percentage, and chi-square test is used for inter-group comparison. Pearson or Spearman correlation coefficient is used for correlation analysis between ultrasound parameters and clinical indicators. A P value less than 0.05 is considered statistically significant.

3. Study Flowchart

Study on ultrasonic characteristics of trigger points in patients with primary trigeminal neuralgia

- Enroll 60 patients with primary trigeminal neuralgia meeting inclusion and exclusion criteria
- Perform bilateral muscle ultrasound examination
- Collect clinical data including grayscale ultrasound, shear wave elastography and superb microvascular imaging results
- Collect pain diary data including pain visual analogue scale score and attack frequency
- Perform statistical analysis
- Compare ultrasonic characteristics of muscles between the affected side and the unaffected side, analyze their correlation with pain indicators, and explore imaging evidence for peripheral sensitization of trigger points

Study 2 Clinical and Ultrasonic Characteristics of Trigger Point Bloodletting Therapy for Primary Trigeminal Neuralgia

1. Study Subjects

1.1 Source of Cases and Diagnostic, Inclusion and Exclusion Criteria

Same as Study 1.

1.2 Withdrawal and Termination Criteria

Patients who violate the study treatment protocol, such as receiving additional acupuncture treatment outside the study or choosing surgical treatment, and thus affect efficacy judgment, will be excluded from the study.

Patients will be terminated from the trial under the following circumstances:

- Patients who cannot tolerate acupuncture pain or develop severe adverse reactions.
- Patients whose clinical symptoms deteriorate, defined as visual analogue scale score above 8 points under medication, and are evaluated by the investigator as unfit to continue the clinical trial.
- Patients who develop new diseases that require additional mandatory treatment.

2. Study Protocol

2.1 Study Design

This study is a prospective randomized controlled trial.

2.2 Randomization, Blinding Design and Implementation

Simple randomization is adopted. The estimated sample size is input into SPSS 29.0 statistical software to set a random seed, generate a random sequence and produce grouping results. Random cards are prepared and sealed in opaque envelopes. The envelope number strictly corresponds to the enrollment order of patients. During clinical implementation, investigators open envelopes sequentially and assign groups

according to the prompts on the random cards. Eligible subjects are randomly divided into the treatment group and the western medicine group at a 1:1 ratio.
Considering the intuitive difference between acupuncture and western medicine intervention and the particularity of acupuncture treatment, neither subjects nor acupuncturists can be blinded. Third-party evaluation and statistical analysis are implemented: research assistants unaware of grouping perform efficacy evaluation and follow-up observation, and blinded statistical analysis is adopted at the data summary stage.

2.3 Sample Size

This study is a parallel randomized controlled study with a superiority test design. The Brief Pain Inventory-Facial score of subjects is taken as the primary outcome indicator. According to previous study results, the mean Brief Pain Inventory-Facial score of the control group is 17.93±13.87, and that of the treatment group is 2.47±4.70. With two-sided alpha set at 0.05 and statistical power at 90%, calculation via PASS software shows that 19 subjects are required for each group.
Considering loss to follow-up and refusal rate at 20%, the actual sample size is adjusted to at least 24 cases per group. To ensure study stability and expand sample redundancy, 30 cases are allocated to each group, with a total of 60 patients with primary trigeminal neuralgia enrolled in this study.

2.4 Treatment Regimens

2.4.1 Treatment Group: Trigger Point Bloodletting

Trigger point exploration and recording method A trigger point refers to a site where light touch can induce pain. It is located in the cutaneous distribution area of trigeminal nerve branches, mostly distributed at the corners of the mouth, nasal alae, cheeks, forehead and inside the oral cavity. Common benign stimuli triggering pain include chewing, tooth brushing, face washing and touching.

The research team adopts repeated wiping with cotton swabs to explore trigger points in the trigeminal nerve distribution area covering the head, face and oral cavity. Trigger point exploration is performed before each treatment in both groups. The operator holds the cotton swab with the thumb and index finger, and wipes repeatedly across the full distribution area of trigeminal nerve branches in order from top to bottom, from outside to inside, and from facial skin to oral mucosa. The exploration covers frontotemporal scalp, forehead, eyelids, cheeks, auricles, nasal alae, lips and surrounding skin, and the lower margin of the mandible. The oral cavity examination includes buccal mucosa, gingiva, palate, anterior two-thirds of the tongue and oral floor mucosa.

The site that induces and continuously aggravates trigeminal neuralgia attack during repeated wiping is identified as a trigger point. The number of trigger points is usually one or more. All trigger points must be located and recorded. The location, number, exploration difficulty of trigger points, and the location, intensity, nature and duration

of pain induced by trigger points are recorded in detail at each treatment. Complete video recording is performed for each exploration procedure.

Instruments and specifications

- Needles: Disposable blood collection needles produced by Nipro Corporation, specification NM-21G.
- Cupping jars: Glass cupping jars produced by Beijing Guoyiyan Medical Technology Development Co., Ltd. No. 2 jar with 38 mm inner diameter of jar mouth, and No. 3 jar with 45 mm inner diameter of jar mouth.

Operation procedures The patient lies in supine position, and the operator stands beside the patient. After confirming all trigger point locations and disinfecting with 75% alcohol, the operator uses disposable blood collection needles to prick the skin vertically, with an interval of 3 to 5 mm between each prick and a depth of 1 to 3 mm. The depth is about 1 mm for thin soft tissue areas such as gingiva and eyelids, and about 3 mm for other areas.

For trigger points at anatomically suitable sites, cupping with flash fire method is performed immediately after pricking to increase bleeding volume. Either No. 2 or No. 3 glass jar is selected according to the bloodletting range and cupping feasibility. The bleeding volume per jar is controlled at 5 to 10 ml, and the jar is removed after 5 minutes of retention.

For trigger points unsuitable for cupping, such as frontotemporal scalp, eyebrows, nose and auricles, 75% alcohol cotton balls are used to wipe repeatedly immediately after pricking to dilate blood vessels and promote bleeding until hemostasis. For intraoral trigger points, the patient is instructed to open the mouth to fully expose the site. After pricking, the patient rinses the mouth with purified water until bleeding stops.

Post-treatment instructions: Patients are advised to keep the pinhole area clean and dry, avoid scratching, and do not apply oily or ointment medications. The bloodletting sites on the head and face shall avoid contact with water within 24 hours to prevent infection.

Treatment course: Twice weekly with a minimum interval of 2 days between sessions, for 8 consecutive weeks, totaling 16 treatment sessions.

2.4.2 Control Group: Oral Carbamazepine

Drug name and specification Carbamazepine tablets produced by Beijing Novartis Pharmaceutical Co., Ltd., with approval number Guo Yao Zhun Zi H11022279.

Medication regimen The regimen follows the Chinese Expert Consensus on the Diagnosis and Treatment of Trigeminal Neuralgia 2015 edition and the European Academy of Neurology guidelines on trigeminal neuralgia management 2020 edition.

For patients not taking carbamazepine before enrollment, the initial dose is 0.2 g once daily. For patients already taking carbamazepine before enrollment, the original dose is maintained. For patients with insufficient pain control, the dose can be gradually increased by 0.2 g every 2 to 4 days, administered 1 to 3 times daily, with a maximum daily dose not exceeding 1.2 g. For patients requiring dose reduction, the dose is decreased by 0.1 g every 3 days as appropriate. Patients are instructed to record daily medication dose and frequency truthfully in the pain diary.

2.5 Observation Indicators and Evaluation Methods

2.5.1 General Information

Including age, gender, detailed medical history, family history and past medical history of subjects.

2.5.2 Primary Efficacy Indicator

The change of average total Brief Pain Inventory-Facial score in the past week after 8 weeks of treatment compared with baseline is defined as the primary efficacy indicator.

2.5.3 Secondary Efficacy Indicators

- Change of average total Brief Pain Inventory-Facial score compared with baseline at week 2, week 4 and week 6.
- Change of average pain visual analogue scale score in the past week compared with baseline at week 2, week 4, week 6 and week 8.
- Response rate: proportion of patients with 50% reduction in visual analogue scale score from baseline, evaluated at week 2, week 4, week 6 and week 8; proportion of patients with 70% reduction in visual analogue scale score from baseline, evaluated at the same time points.
- Change of Self-rating Depression Scale score compared with baseline at week 2, week 4, week 6 and week 8.
- Change of Self-rating Anxiety Scale score compared with baseline at week 2, week 4, week 6 and week 8.
- Change of 36-Item Short Form Health Survey score compared with baseline at week 2, week 4, week 6 and week 8.

2.5.4 Dynamic Observation of Trigger Points and Trigger-induced Pain

- Location, number and induction threshold of trigger points in both groups at baseline, week 2, week 4, week 6 and week 8.
- Location, nature, intensity and duration of pain induced by cotton swab stimulation of trigger points in both groups at baseline, week 2, week 4, week 6 and week 8.

2.5.5 Musculoskeletal Ultrasound Evaluation Indicators

Examination is performed at baseline and after 8 weeks of treatment. The operation method is the same as Study 1. Parameters include thickness, elastic modulus and microvascular blood flow of the affected orbicularis oris muscle and subcutaneous connective tissue, to evaluate soft tissue structural and microcirculatory changes after treatment.

2.5.6 Safety Evaluation

Acupuncture-related and drug-related adverse reactions are recorded throughout the study. Serious adverse events must be documented in the Adverse Event Report Form. Acupuncture-related adverse reactions include local subcutaneous ecchymosis, local hematoma, infection, scarring, sensory disturbance, facial muscle weakness, fear of bloodletting, acupuncture syncope, needle retention and needle breakage.

Drug-related adverse reactions include dizziness, drowsiness, fatigue, nausea, vomiting, ataxia and rash. The severity and duration of all symptoms are recorded in detail.

2.6 Statistical Methods

SPSS 29.0 statistical software is used for data analysis. Normally distributed measurement data are expressed as mean ± standard deviation and analyzed by t-test, one-way analysis of variance or analysis of covariance. Data not conforming to normal distribution are expressed as median with interquartile range and analyzed by non-parametric tests. Count data are expressed as frequency and percentage, and chi-square test is used for inter-group comparison.

For continuous outcome indicators with repeated measurements, such as score changes at different time points, a mixed-effects model is used for analysis, incorporating group effect, time effect, group-time interaction effect and individual random effect. Pearson or Spearman correlation coefficient is used for correlation analysis. A P value less than 0.05 is considered statistically significant.

3. Study Flowchart

- Enroll 60 patients with primary trigeminal neuralgia meeting inclusion and exclusion criteria
- Randomly assign subjects to two groups at a 1:1 ratio
- Treatment group: 30 subjects receiving trigger point bloodletting therapy
- Control group: 30 subjects receiving oral carbamazepine
- 8-week treatment course, twice weekly
- Perform musculoskeletal ultrasound examination to measure thickness, elasticity and blood flow signals
- Perform clinical efficacy evaluation, safety evaluation and dynamic trigger point assessment
- Evaluate structural and microcirculatory changes
- Perform statistical analysis
- Compare the clinical efficacy of trigger point bloodletting and western medicine for primary trigeminal neuralgia, evaluate changes in orbicularis oris muscle ultrasound characteristics before and after treatment, and verify the peripheral mechanism