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Identification of the optimal points for the acupuncture treatment of neck pain in China: protocol for a cross-sectional, matched, case-control study

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4 **Identification of the optimal points for the acupuncture treatment of neck pain**
5 **in China: protocol for a cross-sectional, matched, case-control study**

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ABSTRACT

Introduction: Neck pain is a common condition that can be effectively treated by acupuncture. However, acupuncture for neck pain can be performed using various treatment point options (such as local acupoints, distal acupoints, and sensitized acupoints). It remains controversial which treatment point selection produces superior relief of neck pain. The present study aims to identify the types of sensitization (e.g. temperature, tenderness) and distribution of sensitized points in patients with neck pain, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients with neck pain, and to summarize the dominant forms of optimal sensitized points in patients with neck pain. This information will aid in the choice of optimal treatment points for neck pain.

Methods and analysis: This cross-sectional, matched, case-control study will include 224 patients with neck pain, and 224 healthy age- and sex-matched control subjects. Body surface temperature, mechanical pain threshold, pressure pain threshold, and skin resistance will be assessed at the 15 acupoints most frequently used to treat neck pain, and at the five body regions in which pain most frequently occurs. Hypothesis testing will be used to compare the differences in variables between cases and controls. In addition, receiver operating characteristic curve analysis will be used to explore the cutoff values of the sensitive states of heat, pain, and electrical resistance that indicate sensitization of the acupoint.

Ethics and dissemination: Ethical approval of this study has been granted by the Research Ethical Committee of the Teaching Hospital of Chengdu University of Traditional Chinese Medicine (ID: 2018KL-016). The outcomes of the study will be disseminated through peer-reviewed publications.

Trial registration: ChiCTR1800016220.

Strengths and limitations of this study

► This study will be the first observational study to evaluate the forms of sensitization and the distribution of sensitized points in patients with neck pain. To summarize the dominant forms of sensitized points related to neck pain, the study will evaluate the skin morphological changes, body surface sensations, and biophysical properties.

► This study will be the first to attempt to define the acupoint sensitization in patients with neck pain using a cutoff value for the identification of point sensitization.

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4 ▶ As this is the first study of its kind, only patients with simple neck pain were included; patients
5 with other types of neck pain, such as secondary neck pain, were excluded to reduce the bias caused
6 by other factors and ensure consistency. However, this will limit the representativeness and
7 generalizability of the study results.
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11 12 13 **INTRODUCTION**

14
15 Neck pain is a common clinical condition that is often accompanied by tenderness at sensitive points
16 and limited function. The lifetime prevalence of neck pain is reportedly 48.5%. [1] Neck pain can
17 be caused by cervical spondylosis, which is a common disease in China with a prevalence of 8.1–
18 19.1%. [2] Neck pain imposes considerable personal and socioeconomic burdens. Furthermore, the
19 incidence of cervical spondylosis has been increasing in recent years due to changes in work and
20 lifestyle habits, leading to a prolonged work life and lifespan; this increased incidence has become
21 a social concern. Neck pain is often treated with muscle relaxants and non-steroidal anti-
22 inflammatory drugs; however, such medications carry a risk of adverse effects, and neither drug is
23 better than non-pharmacological alternative treatments. [1]
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33 As non-pharmacological alternative treatments have reliable efficacy and availability, this type of
34 treatment is gaining an increasing amount of recognition worldwide. Acupuncture is one type of
35 non-pharmacological alternative treatment that can effectively treat neck pain. Although various
36 studies have confirmed that acupuncture is effective, the outcome of acupuncture treatment is
37 closely related to the point selections. [3] Previous studies have evaluated the use of local
38 acupoints [4] or distal acupoints, [5] with all types of treatment points reportedly having a certain
39 degree of clinical efficacy.
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47 Recent Chinese research has found that acupoints can be sensitized, with associated changes in
48 function and size, and thus can functionally switch from a relatively ‘silent’ state to a relatively
49 ‘active’ state. Treatment of an acupoint while it is in the ‘active’ state is thought to achieve a better
50 clinical outcome, and this has become a focus in acupoint research. [6-8] There are diverse types of
51 sensitivity manifested at sensitized points, including pain-sensitivity, heat-sensitivity, and form-
52 sensitivity. Recent clinical studies report that a superior outcome is obtained using acupuncture at
53 pain-sensitive points, [9] and moxibustion at heat-sensitive points [10] or visual-sensitive points. [11]
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60 However, most of the current observational studies have only focused on one form of sensitization

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4 in a small sample population, and thus are not comprehensive studies of various forms of
5 sensitization.[6 10] The choice of optimal treatment points for neck pain in clinical practice is still
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7 controversial.

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9 Herein, we describe the protocol for an observational study that aims to identify the different types
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11 of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients
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13 with neck pain, to analyze the cutoff values for acupoint sensitization in patients with neck pain,
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15 and to determine the most prevalent sensitization types seen at sensitized points in patients with
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17 neck pain. Previous small sample studies have confirmed the feasibility of sensitization testing.[7]
18
19 The present study will provide a basis for the selection of the optimal treatment points for neck pain
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21 in clinical practice.
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25 **METHODS AND ANALYSIS**

26 **Study design**

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28 This is a cross-sectional, age- and sex-matched, case-control study. The protocol was developed in
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30 accordance with the Strengthening the Reporting of Observational Studies in Epidemiology
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32 guidelines.[12] The study has been registered with ChiCTR at Current Controlled Trials
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34 (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.
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39 **Ethics**

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41 This study was designed in accordance with the principles of the Declaration of Helsinki. The study
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43 protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Chengdu
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45 University of Traditional Chinese Medicine (approval number: 2018KL-016), and is registered on
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47 the primary registry in the World Health Organization registry network (Chinese Clinical Trial
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49 Registry: no. ChiCTR1800016203). Signed consent will be obtained from each participant after
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51 they have been informed of the study procedures, possible risks, and their right to withdraw from
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53 the study.
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56 **Patients and healthy subjects**

57 **Inclusion criteria**

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59 Patients are eligible for study inclusion if they: (1) have simple neck pain with symptoms that meet
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4 the diagnostic criteria for cervical spondylosis; (2) are males or females aged 18–60 years; (3)
5 provide written informed consent for all procedures in this study.
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10 Healthy subjects are eligible if they: (1) have no history of neck pain and/or restricted neck
11 movement; (2) are males or females aged 18–60 years; (3) provide written informed consent for all
12 procedures in this study.
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16 17 **Exclusion criteria**

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19 Patients are not eligible if they: (1) have complicated neck or shoulder pain caused by cervical and
20 intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder peri-arthritis,
21 rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical
22 congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic
23 system; (4) have difficulty in answering the questionnaires because of cognitive impairment; (5)
24 have dermatopathic diseases; (6) are pregnant, breastfeeding, or planning a pregnancy during the
25 study period.
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35 Healthy subjects are not eligible if they: (1) have serious disease related to the heart, liver, kidney,
36 or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty in answering
37 the questionnaires because of cognitive impairment; (4) have dermatopathic diseases; (5) are
38 pregnant, breastfeeding, or planning a pregnancy during the study period.
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45 **Recruitment strategies**

46 We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and
47 Orthopedics in five clinical centers in China: Chengdu University of Traditional Chinese Medicine,
48 Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese
49 Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional
50 Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-
51 matched residents from the same communities as the patients. Recruitment strategies will include
52 posting advertisements on social media (such as WeChat, which is similar to Facebook) and at
53 community centers, or by word-of-mouth introductions through subjects already enrolled in the
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study. Patients or healthy subjects who consent to study participation will be examined and diagnosed by a hospital doctor.

Test regions, acupoints, and sensitized points

In accordance with the results of literature data-mining and expert consensus on the treatment of neck pain, we identified the 15 most frequently used acupoints (Table 1) and the five regions of the body with the most frequent occurrence of pain and the greatest degree of acupoint sensitization. The body was divided into five regions to standardize the treatment procedures and detection areas. Regions 1 and 2 are each bordered by the respective ipsilateral mastoid, sternal end of the clavicle, anterior axillary line, acromion, and C7 spinous process. Region 3 is the triangular region bordered by both sides of the mastoid and the C7 spinous process. Regions 4 and 5 are each bordered by the respective ipsilateral C7 spinous process, acromion, and axillary line; the two regions are divided by the posterior midline. These regions are the same as those used in previous studies on neck pain,[13] and include the upper trapezius, splenius capitis muscles, sternocleidomastoid, levator scapulae, infraspinatus, scalene, subscapularis muscles, and other neck muscles associated with neck pain.[14 15] These regions and acupoints are shown in Figure 2.

Investigators will mark the 29 acupoints on each patient. All investigators will attend training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls. The investigator will then palpate the detection area associated with each acupoint using the appropriate force (< 2,000 gf), and will identify the sensitized points that have pain/sourness/heaviness/fullness or nodules.

Table 1 Acupoints selected for use in the study

Acupoints	Location
Jianjing (GB-21)	On the shoulder, directly above the nipple, at the midpoint of the line connecting Dazhui(DU-14) with the acromial end of clavicle
Jianzhongshu(SI-15)	On the back, 2 cun lateral to the lower border of the spinous process of the 7 th cervical vertebra
Wangu(GB-12)	On the head, in the depression posterior and inferior to the mastoid process.
Fengchi(GB-20)	On the nape, below the occipital, on a level with Fengfu DU-16, in the depression between the upper portion of trapezius and the sternocleidomastoid
Tianzhu(BL-10)	On the nape, 1.3 cun lateral to the posterior hairline, in the depression of

		the posterior hairline lateral to the trapezius muscle
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4	Dazhui(DU-14)	On the posterior median line, in the depression below the spinous process of the 7 th cervical vertebra
5		
6	Dazhu(BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1 st thoracic vertebra
7		
8		
9	Jianwaishu(SI-14)	On the back, 3 cun lateral to the lower border of the spinous process of the first thoracic vertebra
10		
11	Tianliao(SJ-15)	On the region of scapula, at the midpoint of the line connecting Jianjing GB-21 with Quyuan SI-13, on the superior angle of the scapula
12		
13	Jugu(LI-16)	In the upper portion of the shoulder, in the depression between the acromial end of clavicle and the scapular spine
14		
15	Tianzong(SI-11)	In the region of the scapula, in the depression of the center of the subscapular fossa, on a level with the 4 th thoracic vertebra
16		
17	Shousanli(LI-10)	Flexing the elbow, on the dorsal radial side of the forearm, on the line connecting Yangxi LI-5 with Quchi LI-11, 2cun below the transverse cubital crease
18		
19	Lieque(LU-7)	On the radial margin of the forearm, 1.5 cun above the transverse crease of the wrist, between the branchioradial muscle and the long abductor muscle tendon of thumb
20		
21	Zhongzhu(SJ-3)	On the dorsum of the hand, in the depression between the 4 th and 5 th metacarpal bones, proximal to the 4 th metacarpalangeal joint
22		
23	Houxi(SI-3)	On the ulna side of the palm, proximate to the fifth metacarpophalangeal joint, at the end of transverse crease of metacarpophalangeal joint, at the dorsoventral boundary
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Outcome measurements

All data collection for each patient will be completed on the same day.

Body surface temperature

Thermal imaging technology has been widely applied to assess pain and muscle damage.[16 17] Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use Fotric thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to make measurements at each of the 29 acupoints in the five body regions. The temperature data of each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA).

Mechanical pain threshold

Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science,

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4 Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of
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6 the 29 acupoints in the five body regions in turn. Previous studies have found that the device can
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8 detect hyperalgesia and changes in sensory nerve function.[18-20] Mechanical pain caused by
9
10 punctate stimuli is related to A δ -fibers.[21] If there is a difference of more than 15 g between the
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12 two measurements of mechanical pain threshold made at one acupoint, the mechanical pain
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14 threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a
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16 rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each
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18 acupoint. To reduce the error associated with the effect of assessment at one point on the assessment
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20 of adjacent points, a method of alternate assessment of acupoints on the left and right sides of the
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22 body will be adopted.

23 24 25 **Pressure pain threshold**

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27 The pressure pain threshold (PPT) is widely used in clinical practice as a semi-objective method
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29 with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-
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31 fibers.[21] Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments,
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33 Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five
34
35 body regions in turn. If there is a difference of more than 500 gf between the two PPT measurements
36
37 performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive
38
39 pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated
40
41 for each acupoint. A method of alternate assessment of the acupoints on the left and right sides of
42
43 the body will again be adopted.

44 45 46 **Skin resistance**

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48 Investigators will use the Acupoint dynamics research instrument (LMR30-RIII, Peking
49
50 University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11,
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52 LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

53 54 55 56 **Pain**

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58 Neck pain will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain
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60 Questionnaire, and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to

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4 measure the severity of pain. The Northwick Park Neck Pain Questionnaire provides a reliable
5
6 outcome measure for patients with neck pain, while the McGill Pain Questionnaire is used to
7
8 measure the different qualities of the subjective pain experience; these questionnaires have been
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10 proven valid, and are convenient for patients to complete.[24 25]

11 12 13 **Neck function**

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15 The change in neck function will be evaluated by measuring the cervical range of motion before
16
17 and after treatment.

18 19 20 21 **General demographic information**

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23 This will include the collection of data regarding age, disease duration, medical history, and
24
25 medication type and dosage.

26 27 28 29 **Safety and adverse events**

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31 Non-invasive assessments of the body surface do not generally cause adverse events. However, we
32
33 will still record the number and type (e.g. serious pain, fainting) of adverse events in each group.
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35 Participants who experience adverse events will receive the appropriate intervention. Adverse
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37 events will be immediately reported to the primary investigator and the ethics committee, and the
38
39 affected participants will be withdrawn from the study.

40 41 42 43 **Sample size calculation**

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45 Sample size calculations for a matched case-control study design will be performed using PASS 11.
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47 Few previous studies have evaluated acupoint sensitization, especially in healthy subjects. Our
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49 previous small sample-sized study indicated that the rate of acupoint sensitization ranged from 20%
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51 to 70%, and so we set this rate at 50% to calculate the minimum sample size required for the
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53 proposed study. With a rate of healthy subjects of 20%, the odds ratio is 4. Thus, the smallest sample
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55 size is 108 with two-sided confidence, $\alpha=0.05$, $\beta=0.01$, and a ratio of control subjects to cases of 1.
56
57 Considering the potential non-response rate and sampling effectiveness, the final smallest sample
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59 size is 224 patients, plus 224 age- and sex-matched healthy subjects.
60

Statistical analysis

Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. SPSS 21.0 (SPSS Inc., Chicago, IL, USA) will then be used to complete the statistical analyses. The statistical data evaluation will be performed by the West China School of Public Health at Sichuan University, China. Missing data will be processed without imputation. First, the distribution of basic information in the case and control groups will be described, including age, sex, height, weight, job occupation, and education level. Data will be presented as means (SD) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent the skin morphological changes. The distributions of the intensity of neck pain and neck function will also be described. Hypothetical testing will then be performed to assess the differences between the case and control groups in body surface sensations and biophysical properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance. Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference between the case and control groups in body surface temperature, mechanical pain threshold, PPT, or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization rates of patients and healthy subjects will be calculated separately regarding the aspects of heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints.

Quality control

This is a multi-center observational study, and so quality control will play a vital role in the extrapolation of the conclusion. To ensure the integrity of the study and protect the rights and health of the participants, we will set up a Data and Safety Monitoring Board. The Data and Safety Monitoring Board will be developed in accordance with the Operational Guidelines for the Establishment and Functioning of the Data and Safety Monitoring Boards of the World Health Organization. Separate from the Data and Safety Monitoring Board, a quality control group will be established to guarantee the validity and reliability of results. Before study commencement, fieldwork operation manuals will be prepared, and pilot study participants from all centers will

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4 undergo training in accordance with the standard operating procedure of this study. Every 3 months,
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6 members of the quality control group will conduct a quality control review at each study site, and
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8 produce a report regarding the quality analysis of the whole data collection process.
9

11 **DISCUSSION**

13 Neck pain is the third-most common chronic pain condition, and the fourth leading cause of
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15 disability worldwide.[26] Acupuncture is a popular non-pharmacological modality used to relieve
16
17 pain. The analgesic properties of acupuncture may be mediated by the release of endogenous
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19 opioids.[27]

21 The latest research has found that the performance of acupuncture at sensitive points may provide
22
23 the most effective treatment.[8 28] When the body is in a diseased state, there will be morphological
24
25 form-sensitive point changes, such as nodules, pimples, uplifting, dimpling, and changes in skin
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27 color.[29] Thermal image detection has proved that the temperature of corresponding acupoints will
28
29 be obviously abnormal when a patient has visceral illness, such as diseases of the heart, lung, and
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31 stomach, which indicates that the temperature of acupoints can reflect the physiological and
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33 pathological phenomena of the affected organs.[30] Studies have confirmed that the PPT at
34
35 acupoints changes when patients are in a diseased state.[6 31 32] The degree of change in the PPT
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37 may reflect the intensity of acupoint sensitization, and may be related to the disease status.[33] This
38
39 proposed observational study will bridge the knowledge gap regarding the optimal sensitization of
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41 acupoints in various forms of point sensitivity in patients with neck pain.

42 As this will be the first study to evaluate the association between acupoint sensitization and neck
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44 pain, this observational study may have some limitations. The main limitation is that in order to
45
46 reduce the bias caused by other factors and to ensure consistency, this study will only include
47
48 patients with simple neck pain, while excluding patients with other types of neck pain (such as
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50 secondary neck pain). Furthermore, as the four participating centers are located in four different
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52 regions in China, it may be difficult to implement quality control; thus, quality control reviews will
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54 be conducted every 3 months.

56 In conclusion, this article describes the design and protocol of a study that aims to observe the
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58 different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized
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60 acupoints in patients with neck pain, to analyze the cutoff values for acupoint sensitization in

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4 patients with neck pain, and to identify the most prevalent sensitized forms of the sensitized points
5
6 in patients with neck pain. The results will provide a basis for the selection of clinically optimal
7
8 stimulation sites for the treatment of neck pain.
9

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12
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14
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16
17 investigators in each center. We thank Kelly Zammit, BVSc, from Liwen Bianji, Edanz Editing
18
19 China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.
20
21

23 **Authors' contributions**

24
25 MSS, SYT, JRP, DJC, CXY, HZ, LZ, and FRL participated in the design of the trial, in creating the
26
27 data analysis plan, and in drafting the manuscript. GYG, XSM, MXY, and JC collected the
28
29 information needed for the performance of this trial in each center. All of the authors discussed,
30
31 read, and revised the manuscript, and gave final approval for the publication of this study protocol.
32
33

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36
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38
39 81590950, 81590951, 81722050).
40
41

43 **Competing interests**

44
45 The authors declare that they have no competing interests.
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47

48 **Ethics approval**

49
50 The study protocol has been approved by the institutional review board and ethics committee of the
51
52 First Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (May 2018).
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56 **Provenance and peer review**

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58 Not commissioned; internally peer reviewed.
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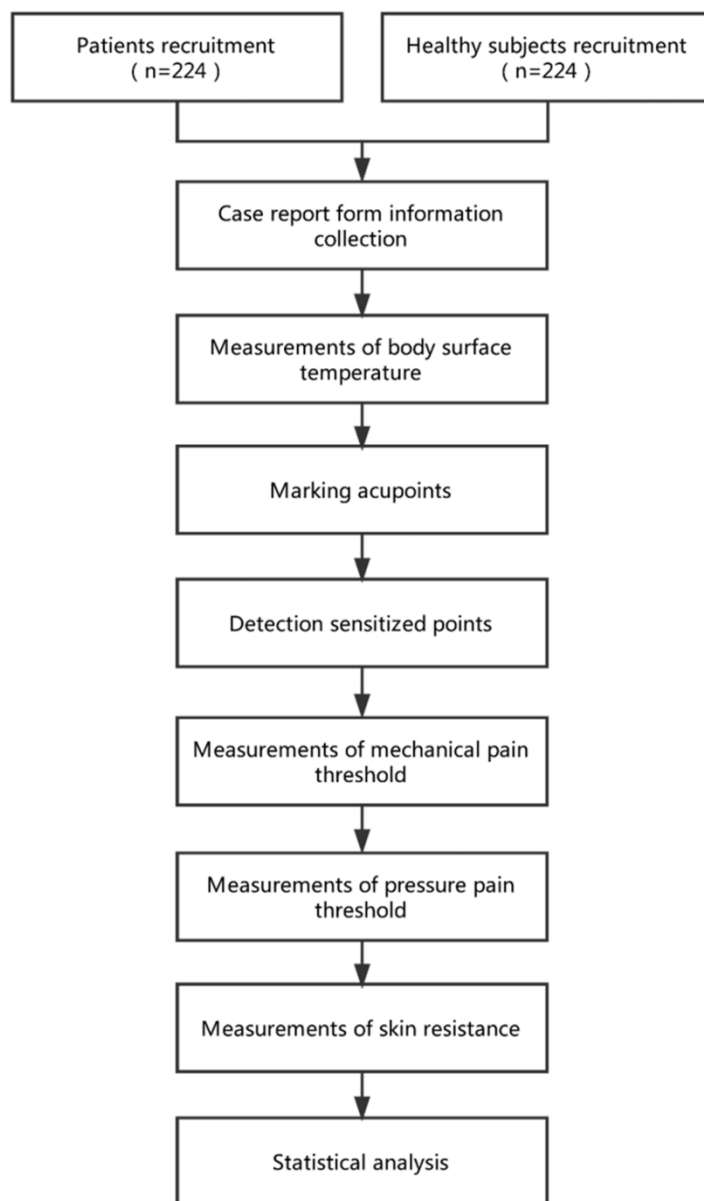
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Figure 1 Flowchart of the study design.

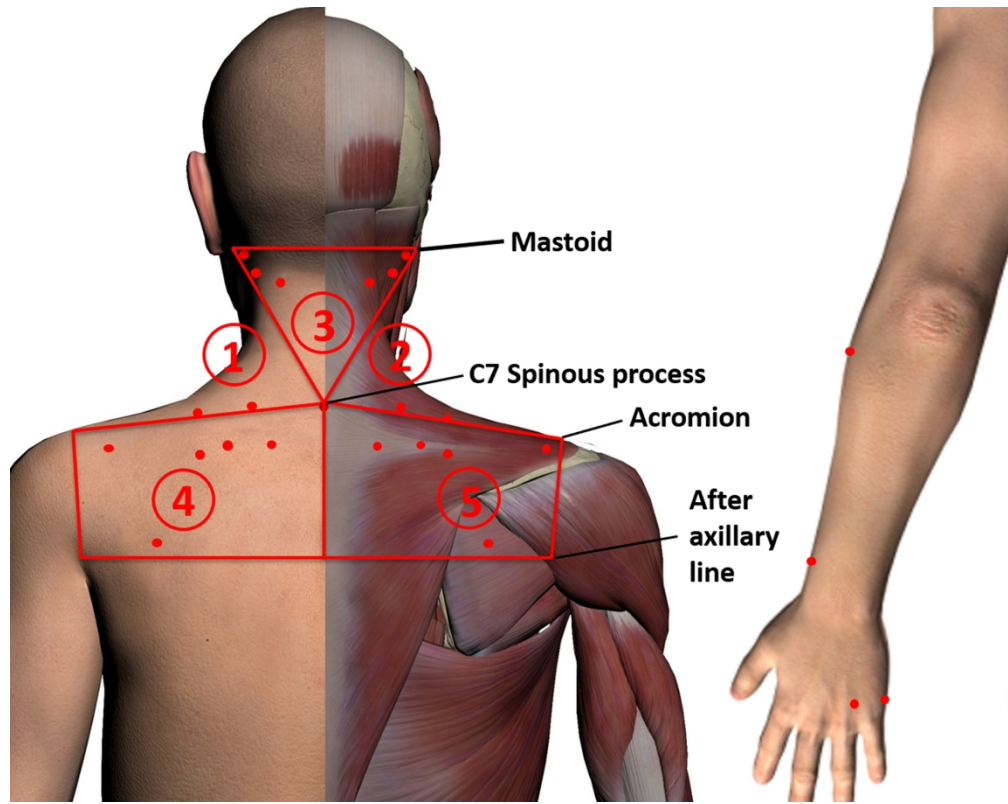
Figure 2 The test regions and acupoints that will be used in the study.



45 Flowchart of the study design.

46 53x89mm (600 x 600 DPI)

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The test regions and acupoints that will be used in the study.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a cross-sectional, matched, case-control study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	The present study aims to identify the types of sensitization (e.g. temperature, tenderness) and distribution of sensitized points in patients with neck pain, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients with neck pain, and to summarize the dominant forms of optimal sensitized points in patients with neck pain. This information will aid in the choice of optimal treatment points for neck pain.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4	Recent Chinese research has found that acupoints can be sensitized, with associated changes in function and size, and thus can functionally switch from a relatively 'silent' state to a relatively 'active' state. Treatment of an acupoint while it is in the 'active' state is thought to achieve a better clinical outcome, and this has become a focus in acupoint research. Recent clinical

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studies report that a superior outcome is obtained using acupuncture at pain-sensitive points, and moxibustion at heat-sensitive points or visual-sensitive points. However, most of the current observational studies have only focused on one form of sensitization in a small sample population, and thus are not comprehensive studies of various forms of sensitization. The choice of optimal treatment points for neck pain in clinical practice is still controversial.

Objectives

3

State specific objectives, including any prespecified hypotheses

4

We describe the protocol for an observational study that aims to identify the different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients with neck pain, to analyze the cutoff values for acupoint sensitization in patients with neck pain, and to determine the most prevalent sensitization types seen at sensitized points in patients with neck pain. Previous small sample studies have confirmed the feasibility of sensitization testing. The present study will provide a basis for the selection of the

optimal treatment points for neck pain in clinical practice.

Methods

Study design	4	Present key elements of study design early in the paper	4	This is a cross-sectional, age- and sex-matched, case-control study. The protocol was developed in accordance with the Consolidated Standards of Reporting Trials guidelines and the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6	We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and Orthopedics in five clinical centers in China: Chengdu University of Traditional Chinese Medicine, Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients. Recruitment strategies will include posting advertisements on social

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2				media (such as WeChat, which is
3				similar to Facebook) and at
4				community centers, or by word-of-
5				mouth introductions through
6				subjects already enrolled in the
7				study.
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10	Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of	4, 5, 9, 10
11			participants. Describe methods of follow-up	
12			<i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment	
13			and control selection. Give the rationale for the choice of cases and controls	
14			<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of	
15			participants	
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25			(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and	5, 9, 10
26			unexposed	
27			<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
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1 2 3 4 5 6 7 8	Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9	Body surface temperature, Mechanical pain threshold, Pressure pain threshold, Skin resistance, Pain, Neck function, General demographic information, Safety and adverse events.
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42	Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9	Body surface temperature: Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use Fotric thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to make measurements at each of the 29 acupoints in the five body regions. The temperature data of each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA). Mechanical pain threshold: Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science, Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of the 29 acupoints in the five body regions in turn. If there is a difference of more than 15 g between the two measurements of mechanical pain threshold made at one acupoint, the

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mechanical pain threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each acupoint. To reduce the error associated with the effect of assessment at one point on the assessment of adjacent points, a method of alternate assessment of acupoints on the left and right sides of the body will be adopted.

Pressure pain threshold:

Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments, Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five body regions in turn. If there is a difference of more than 500 gf between the two PPT measurements performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated for each acupoint. A method of alternate assessment of the acupoints on the left and right

sides of the body will again be adopted.

Skin resistance: Investigators will use the Acupoint dynamics research instrument (LMR30-RIII, Peking University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11, LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

Pain: Neck pain will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire, and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure the severity of pain. The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for patients with neck pain, while the McGill Pain Questionnaire is used to measure the different qualities of the subjective pain experience; these questionnaires have been proven valid, and are convenient for patients to complete.

Neck function: The change in neck function will be evaluated by measuring the cervical range of motion before and after treatment.

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General demographic information
This will include the collection of data regarding age, disease duration, medical history, and medication type and dosage.
Safety and adverse events: Non-invasive assessments of the body surface do not generally cause adverse events. However, we will still record the number and type (e.g. serious pain, fainting) of adverse events in each group. Participants who experience adverse events will receive the appropriate intervention. Adverse events will be immediately reported to the primary investigator and the ethics committee, and the affected participants will be withdrawn from the study.

Bias 9 Describe any efforts to address potential sources of bias

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All investigators will attend training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls.
Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. To ensure the integrity of the study and protect the rights and health of the participants, we will set up a

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2					Data and Safety Monitoring Board.
3					Every 3 months, members of the
4					quality control group will conduct a
5					quality control review at each study
6					site, and produce a report regarding
7					the quality analysis of the whole
8					data collection process.
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11	Study size	10	Explain how the study size was arrived at	9	Sample size calculations for a
12					matched case-control study design
13					will be performed using PASS 11.
14					Few previous studies have
15					evaluated acupoint sensitization,
16					especially in healthy subjects. Our
17					previous small sample-sized study
18					indicated that the rate of acupoint
19					sensitization ranged from 20% to
20					70%, and so we set this rate at 50%
21					to calculate the minimum sample
22					size required for the proposed
23					study. With a rate of healthy
24					subjects of 20%, the odds ratio is 4.
25					Thus, the smallest sample size is
26					108 with two-sided confidence,
27					$\alpha=0.05$, $\beta=0.01$, and a ratio of
28					control subjects to cases of 1.
29					Considering the potential non-
30					response rate and sampling
31					effectiveness, the final smallest
32					sample size is 224 patients, plus
33					224 age- and sex-matched healthy
34					subjects.
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41	Quantitative	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which	10	Parametric statistical testing (t-test)
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variables		groupings were chosen and why		will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon’s signed rank test) will be used.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10	First, the distribution of basic information in the case and control groups will be described, including age, sex, height, weight, job occupation, and education level. Data will be presented as means (SD) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent the skin morphological changes. The distributions of the intensity of neck pain and neck function will also be described. Hypothetical testing will then be performed to assess the differences between the case and control groups in body surface sensations and biophysical properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance. Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing

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(Wilcoxon's signed rank test) will be used. If there is a difference between the case and control groups in body surface temperature, mechanical pain threshold, PPT, or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization rates of patients and healthy subjects will be calculated separately regarding the aspects of heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints.

		(b) Describe any methods used to examine subgroups and interactions	N/A	
		(c) Explain how missing data were addressed	10	Missing data will be processed without imputation.
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	5,6	Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients.
		(e) Describe any sensitivity analyses	N/A	

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
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		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram	N/A	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A	
		(b) Indicate number of participants with missing data for each variable of interest	N/A	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	N/A	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A	
		(b) Report category boundaries when continuous variables were categorized	N/A	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A	
Discussion				
Key results	18	Summarise key results with reference to study objectives	N/A	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12	The main limitation is that in order to reduce the bias caused by other factors and to ensure consistency, this study will only include patients with simple neck pain, while excluding patients with other types of neck pain (such as secondary neck pain). Furthermore, as the four participating centers are located in four different regions in China, it may be difficult to implement quality control.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of	N/A	

		analyses, results from similar studies, and other relevant evidence		
Generalisability	21	Discuss the generalisability (external validity) of the study results	3	As this is the first study of its kind, only patients with simple neck pain were included; patients with other types of neck pain, such as secondary neck pain, were excluded to reduce the bias caused by other factors and ensure consistency. However, this will limit the representativeness and generalizability of the study results.
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12	This work was financially supported by the National Natural Science Foundation of China (no. 81590950, 81590951, 81722050).

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Identification of the optimal points for the acupuncture treatment of neck pain in China: protocol for a multicenter, matched, case-control study

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Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Complementary medicine
Keywords:	sensitized points, neck pain, study protocol, observational study

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4 **Identification of the optimal points for the acupuncture treatment of neck pain**
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6 **in China: protocol for a multicenter, matched, case-control study**

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8 Jiao Chen¹, Ding-Jun Cai¹, Hui Zheng¹, Chun Xia Yang², Ling Zhao^{1*}, Fan-Rong Liang^{1*}
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ABSTRACT

Introduction: Neck pain (NP) is a common condition that can be effectively treated by acupuncture. However, various treatment points prescription (such as local acupoints, distal acupoints, and sensitized acupoints) could be used. The present study aims to identify the types of sensitization and distribution of sensitized points in patients with NP, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients, and to summarize the dominant forms of optimal sensitized points in patients. This information will be helpful in the choice of optimal points for the treatment of NP.

Methods and analysis: This multicenter, matched, case-control study will enroll 224 patients with NP, and 224 age- and sex-matched healthy subjects for control. Body surface temperature, mechanical pain threshold, pressure pain threshold, and skin resistance will be assessed at the 15 acupoints most frequently used to treat NP, and at the five body regions in which pain most frequently occurs. We believe that in the state of disease, the sensitive points occurs with high frequency, and the sensitive points of different sensitizations will overlap, and the final optimal points from overlap may be closely related to the selection of clinical treatment points. Hypothesis testing will be used to compare the differences in variables between cases and controls. In addition, receiver operating characteristic curve analysis will be used to explore the cutoff values of the sensitive states of heat, pain, and electrical resistance that indicate sensitization of the acupoint. The optimal points will be comprehensively determined by the acupoint sensitization rate and OR value.

Ethics and dissemination: Ethical approval of this study has been granted by the Research Ethical Committee of the Teaching Hospital of Chengdu University of Traditional Chinese Medicine (ID: 2018KL-016). The outcomes of the study will be disseminated through peer-reviewed publications.

Trial registration: ChiCTR1800016220.

Strengths and limitations of this study

- ▶ This study will be the first observational study to evaluate the forms of sensitization and the distribution of sensitized points in patients with NP.
- ▶ This study will be the first to attempt to define the acupoint sensitization in patients with NP using a cutoff value for the identification of point sensitization.
- ▶ As this is the first study of its kind, only patients with nontraumatic NP with mobility deficits

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4 were included; patients with other NP with radiating pain or secondary NP, were excluded to reduce
5 the bias caused by other factors and ensure consistency. However, this will limit the
6 representativeness and generalizability of the study results.
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11 INTRODUCTION

13 Neck pain (NP) is a common clinical condition often accompanied by tenderness at sensitive points.
14 The global lifetime prevalence of NP was 48.5% in 2006.[1] The prevalence of white-collar workers
15 in China was 33.9-54.8% in 2016 and has an increase tendency in recent years,[2] imposing
16 considerable personal and socioeconomic burdens. Muscle relaxants and non-steroidal anti-
17 inflammatory drugs are used to treat this disease, however, such medications carry a risk of adverse
18 effects, and neither drug is better than non-pharmacological alternative treatments.[1]
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25 As non-pharmacological alternative treatments have reliable efficacy and availability, this type of
26 treatment is gaining an increasing amount of recognition worldwide. Acupuncture is one type of
27 non-pharmacological alternative treatment that can effectively treat NP. Although various studies
28 have confirmed that acupuncture is effective, the outcome of acupuncture treatment is closely
29 related to the point selections.[3] Previous studies have evaluated the use of local acupoints[4] or
30 distal acupoints,[5] with all types of treatment points reportedly having a certain degree of clinical
31 efficacy.
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38 Recent Chinese research has found that acupoints can be sensitized, with associated changes in
39 function and size, and thus can functionally switch from a relatively 'silent' state to a relatively
40 'active' state. Treatment of an acupoint while it is in the 'active' state is thought to achieve a better
41 clinical outcome, and this has become a focus in acupoint research.[6-8] There are diverse types of
42 sensitivity manifested at sensitized points, including pain-sensitivity, heat-sensitivity, and form-
43 sensitivity (appearance changes). Recent clinical studies report that a superior outcome is obtained
44 using acupuncture at pain-sensitive points,[9] and moxibustion at heat-sensitive points[10] or
45 visual-sensitive points.[11] However, most of the current observational studies have only focused
46 on one form of sensitization in a small sample population, and thus are not comprehensive studies
47 of various forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical
48 practice is still controversial.
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60 Herein, we describe the protocol for an observational study that aims to identify the different types

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4 of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients
5 with NP, to analyze the cutoff values for acupoint sensitization in patients with NP, and to determine
6 the most optimal sensitization types seen at sensitized points in patients with NP. We believe that
7 in the state of disease, the sensitive points occurs with high frequency (manifested as changes in
8 temperature, pain threshold etc), and the sensitive points of different sensitizations will overlap, and
9 the final optimal points from overlap may be closely related to the selection of clinical treatment
10 points. Previous small sample studies have confirmed the feasibility of sensitization testing.[7] The
11 present study will provide a basis for the selection of the optimal treatment points for NP in clinical
12 practice.
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23 **METHODS AND ANALYSIS**

24 **Study design**

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26 This is a multicenter, age- and sex-matched, case-control study. The protocol was developed in
27 accordance with the Strengthening the Reporting of Observational Studies in Epidemiology
28 guidelines.[12] The study has been registered with ChiCTR at Current Controlled Trials
29 (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.
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37 **Ethics**

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39 This study was designed in accordance with the principles of the Declaration of Helsinki. The study
40 protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Chengdu
41 University of Traditional Chinese Medicine (approval number: 2018KL-016), and is registered on
42 the primary registry in the World Health Organization registry network (Chinese Clinical Trial
43 Registry: no. ChiCTR1800016203). Signed consent will be obtained from each participant after
44 they have been informed of the study procedures, possible risks, and their right to withdraw from
45 the study.
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54 **Patients and healthy subjects**

55 **Inclusion criteria**

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57 Patients are eligible for study inclusion if they: (1) have nontraumatic NP with mobility deficits in
58 the acute and chronic stages (2) are males or females aged 18–60 years; (3) provide written informed
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4 consent for all procedures in this study.
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7 Healthy subjects are eligible if they: (1) have no history of NP and/or restricted neck movement; (2)
8 are males or females aged 18–60 years; (3) provide written informed consent for all procedures in
9 this study.
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14 15 **Exclusion criteria**

16 Patients are not eligible if they: (1) have complicated neck or shoulder pain caused by cervical and
17 intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder peri-arthritis,
18 rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical
19 congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic
20 system; (4) have difficulty in answering the questionnaires because of cognitive impairment; (5)
21 have dermatopathic diseases; (6) are pregnant, breastfeeding, or planning a pregnancy during the
22 study period.
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32 Healthy subjects are not eligible if they: (1) have serious disease related to the heart, liver, kidney,
33 or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty in answering
34 the questionnaires because of cognitive impairment; (4) have dermatopathic diseases; (5) are
35 pregnant, breastfeeding, or planning a pregnancy during the study period.
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43 **Recruitment strategies**

44 We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and
45 Orthopedics in five clinical centers in China: Chengdu University of Traditional Chinese Medicine,
46 Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese
47 Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional
48 Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-
49 matched residents from the same communities as the patients. Recruitment strategies will include
50 posting advertisements on social media (such as WeChat, which is similar to Facebook) and at
51 community centers, or by word-of-mouth introductions through subjects already enrolled in the
52 study. Patients or healthy subjects who consent to study participation will be examined and
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diagnosed by a hospital doctor.

Test regions, acupoints, and sensitized points

In accordance with the results of literature data-mining and expert consensus on the treatment of NP, we identified the 15 most frequently used acupoints (Table 1) and the five regions of the body with the most frequent occurrence of pain and the greatest degree of acupoint sensitization. The body was divided into five regions to standardize the treatment procedures and detection areas. Regions 1 and 2 are each bordered by the respective ipsilateral mastoid, sternal end of the clavicle, anterior axillary line, acromion, and C7 spinous process. Region 3 is the triangular region bordered by both sides of the mastoid and the C7 spinous process. Regions 4 and 5 are each bordered by the respective ipsilateral C7 spinous process, acromion, and axillary line; the two regions are divided by the posterior midline. These regions are the same as those used in previous studies on NP,[13] and include the upper trapezius, splenius capitis muscles, sternocleidomastoid, levator scapulae, infraspinatus, scalene, subscapularis muscles, and other neck muscles associated with NP.[14 15] These regions and acupoints are shown in Figure 2.

Investigators will mark the 29 acupoints on each patient. All investigators will attend training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls. The investigator will then palpate the detection area associated with each acupoint using the appropriate force (< 2,000 gf), and will identify the sensitized points that have pain/sourness/heaviness/fullness or nodules.

Table 1 Acupoints selected for use in the study

Acupoints	Location
Jianjing (GB-21)	On the shoulder, directly above the nipple, at the midpoint of the line connecting Dazhui (DU-14) with the acromial end of clavicle
Jianzhongshu (SI-15)	On the back, 2 cun lateral to the lower border of the spinous process of the 7 th cervical vertebra
Wangu (GB-12)	On the head, in the depression posterior and inferior to the mastoid process.
Fengchi (GB-20)	On the nape, below the occipital, on a level with Fengfu DU-16, in the depression between the upper portion of trapezius and the sternocleidomastoid
Tianzhu (BL-10)	On the nape, 1.3 cun lateral to the posterior hairline, in the depression of the posterior hairline lateral to the trapezius muscle

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3	Dazhui(DU-14)	On the posterior median line, in the depression below the spinous process of the 7 th cervical vertebra
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5	Dazhu(BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1 st thoracic vertebra
6		
7	Jianwaishu(SI-14)	On the back, 3 cun lateral to the lower border of the spinous process of the first thoracic vertebra
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9	Tianliao(SJ-15)	On the region of scapula, at the midpoint of the line connecting Jianjing GB-21 with Quyuan SI-13, on the superior angle of the scapula
10		
11	Jugu(LI-16)	In the upper portion of the shoulder, in the depression between the acromial end of clavicle and the scapular spine
12		
13	Tianzong(SI-11)	In the region of the scapula, in the depression of the center of the subscapular fossa, on a level with the 4 th thoracic vertebra
14		
15	Shousanli(LI-10)	Flexing the elbow, on the dorsal radial side of the forearm, on the line connecting Yangxi LI-5 with Quchi LI-11, 2cun below the transverse cubital crease
16		
17	Lieque(LU-7)	On the radial margin of the forearm, 1.5 cun above the transverse crease of the wrist, between the branchioradial muscle and the long abductor muscle tendon of thumb
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19	Zhongzhu(SJ-3)	On the dorsum of the hand, in the depression between the 4 th and 5 th metacarpal bones, proximal to the 4 th metacarpalangeal joint
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21	Houxi(SI-3)	On the ulna side of the palm, proximate to the fifth metacarpophalangeal joint, at the end of transverse crease of metacarpophalangeal joint, at the dorsoventral boundary
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Outcome measurements

All data collection for each patient will be completed on the same day.

Body surface temperature

Thermal imaging technology has been widely applied to assess pain and muscle damage.[16 17] Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use Fotric thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to make measurements at each of the 29 acupoints in the five body regions. The temperature data of each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA).

Mechanical pain threshold

Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science, Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of

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4 the 29 acupoints in the five body regions in turn. Previous studies have found that the device can
5 detect hyperalgesia and changes in sensory nerve function.[18-20] Mechanical pain caused by
6 punctate stimuli is related to A_δ-fibers.[21] If there is a difference of more than 15 g between the
7 two measurements of mechanical pain threshold made at one acupoint, the mechanical pain
8 threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a
9 rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each
10 acupoint. To reduce the consecutive assessment error for two adjacent points, a method of alternate
11 assessment of acupoints on the left and right sides of the body will be adopted.

21 **Pressure pain threshold**

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23 The pressure pain threshold (PPT) is widely used in clinical practice as a semi-objective method
24 with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-
25 fibers.[21] Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments,
26 Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five
27 body regions in turn. If there is a difference of more than 500 gf between the two PPT measurements
28 performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive
29 pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated
30 for each acupoint. A method of alternate assessment of the acupoints on the left and right sides of
31 the body will again be adopted.

42 **Skin resistance**

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44 Investigators will use the Acupoint dynamics research instrument (LMR30-RⅢ, Peking
45 University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11,
46 LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

52 **Pain**

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54 NP will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire,
55 and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure the severity
56 of pain. The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for
57 patients with NP, while the McGill Pain Questionnaire is used to measure the different qualities of
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4 the subjective pain experience; these questionnaires have been proven valid, and are convenient for
5 patients to complete.[24 25]
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9 **Neck function**

10 The change in neck function will be evaluated by measuring the cervical range of motion.
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14 **General demographic information**

15 This will include the collection of data regarding age, disease duration, medical history, and
16 medication type and dosage.
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23 **Safety and adverse events**

24 Non-invasive assessments of the body surface do not generally cause adverse events. However, we
25 will still record the number and type (e.g. serious pain, fainting) of adverse events in each group.
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27 Participants who experience adverse events will receive the appropriate intervention. Adverse
28 events will be immediately reported to the primary investigator and the ethics committee, and the
29 affected participants will be withdrawn from the study.
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37 **Patients and public involvement**

38 Patients and public were not involved.
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43 **Sample size calculation**

44 Previous study indicated that the rate of acupoint sensitization in patients ranged from 20% to
45 70%,[26] so we set this rate at 50% to calculate the minimum sample size required for the proposed
46 study. With acupoint sensitization rate of 20% in healthy subjects, the odds ratio is 4. According to
47 Chow's formula in comparing two sample proportion,[27] we assumed an α level of 0.05, a β of
48 0.01, thus, the smallest sample size is 408 with two-sided confidence and a ratio of control subjects
49 to cases of 1 (TrialSize package in R software). Considering the potential non-response rate and
50 sampling effectiveness as 10%, the final sample size is 448 (224 patients, plus 224 age- and sex-
51 matched healthy subjects).
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Statistical analysis

Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. SPSS 21.0 (SPSS Inc., Chicago, IL, USA) will then be used to complete the statistical analyses. The statistical data evaluation will be independently performed by the West China School of Public Health at Sichuan University, China. Missing data will be processed without imputation. First, the distribution of basic information in the case and control groups will be described, including age, sex, height, weight, job occupation, and education level. Data will be presented as means (SD) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent the skin morphological changes. The distributions of the intensity of NP and neck function will also be described. Hypothetical testing will then be performed to assess the differences between the case and control groups in body surface sensations and biophysical properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance. Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference between the case and control groups in body surface temperature, mechanical pain threshold, PPT, or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization rates of patients and healthy subjects will be calculated separately regarding the aspects of heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints. Meanwhile, OR represents the ratio of acupoint sensitization of patients and healthy subjects. Therefore, this study combines OR and sensitization rate of all acupoints to find out the optimal points in each sensitization.

Quality control

This is a multi-center observational study, and so quality control will play a vital role in the extrapolation of the conclusion. To ensure the integrity of the study and protect the rights and health of the participants, we will set up a Data and Safety Monitoring Board. The Data and Safety Monitoring Board will be developed in accordance with the Operational Guidelines for the Establishment and Functioning of the Data and Safety Monitoring Boards of the World Health

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4 Organization. Separate from the Data and Safety Monitoring Board, a quality control group will be
5 established to guarantee the validity and reliability of results. Before study commencement,
6 fieldwork operation manuals will be prepared, and pilot study participants from all centers will
7 undergo training in accordance with the standard operating procedure of this study. Every 3 months,
8 members of the quality control group will conduct a quality control review at each study site, and
9 produce a report regarding the quality analysis of the whole data collection process.
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17 **DISCUSSION**

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19 NP is the third-most common chronic pain condition, and the fourth leading cause of disability
20 worldwide.[28] Acupuncture is a popular non-pharmacological modality used to relieve pain. The
21 latest research has found that the performance of acupuncture at sensitive points may provide the
22 most effective treatment.[8 29] When the body is in a diseased state, there will be morphological
23 form-sensitive point changes, such as nodules, pimples, uplifting, dimpling, and changes in skin
24 color.[30] Thermal image detection has proved that the temperature of corresponding acupoints will
25 be obviously abnormal when a patient has visceral illness, such as diseases of the heart, lung, and
26 stomach, which indicates that the temperature of acupoints can reflect the physiological and
27 pathological phenomena of the affected organs.[31] Studies have confirmed that the PPT at
28 acupoints changes when patients are in a diseased state.[6 32 33] The degree of change in the PPT
29 may reflect the intensity of acupoint sensitization, and may be related to the disease status.[34] This
30 proposed observational study will bridge the knowledge gap regarding the optimal sensitization of
31 acupoints in various forms of point sensitivity in patients with NP.
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44 As this will be the first study to evaluate the association between acupoint sensitization and NP, this
45 observational study may have some limitations. The main limitation is that in order to reduce the
46 bias caused by other factors and to ensure consistency, this study will only include patients with
47 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as
48 NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located
49 in four different regions in China, it may be difficult to implement quality control; thus, quality
50 control reviews will be conducted every 3 months.
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57 In conclusion, this article describes the design and protocol of a study that aims to observe the
58 different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized
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4 acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization in patients with
5 NP, and to identify the dominant sensitized forms of the sensitized points in patients with NP. The
6 results will provide a basis for the selection of clinically optimal acupuncture points for NP.
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10 11 **Acknowledgments**

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23 **Authors' contributions**

24 MSS, SYT, JRP, DJC, CXY, HZ, LZ, and FRL participated in the design of the trial, in creating the
25 data analysis plan, and in drafting the manuscript. GYG, XSM, MXY, and JC collected the
26 information needed for the performance of this trial in each center. All of the authors discussed,
27 read, and revised the manuscript, and gave final approval for the publication of this study protocol.
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36 81590950, 81590951, 81722050).
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43 **Competing interests**

44 The authors declare that they have no competing interests.
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48 **Ethics approval**

49 The study protocol has been approved by the institutional review board and ethics committee of the
50 First Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (May 2018).
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56 **Provenance and peer review**

57 Not commissioned; internally peer reviewed.
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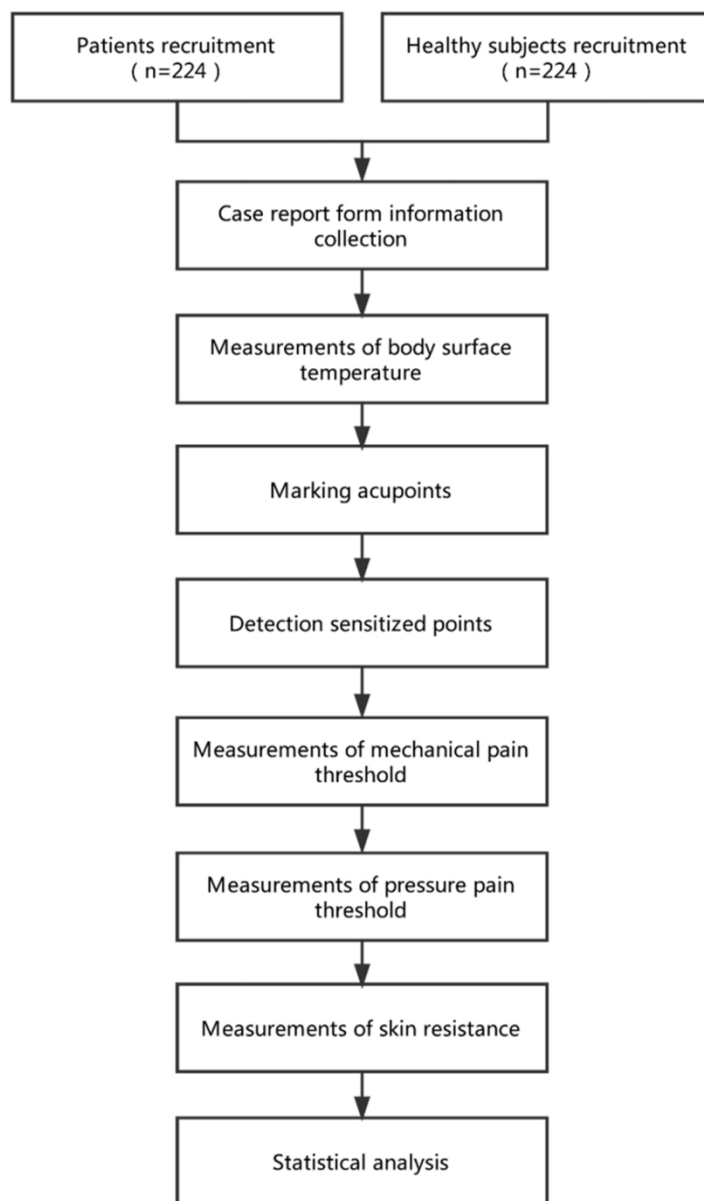
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31 **Figure 1** Flowchart of the study design.

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33 **Figure 2** The test regions and acupoints that will be used in the study.

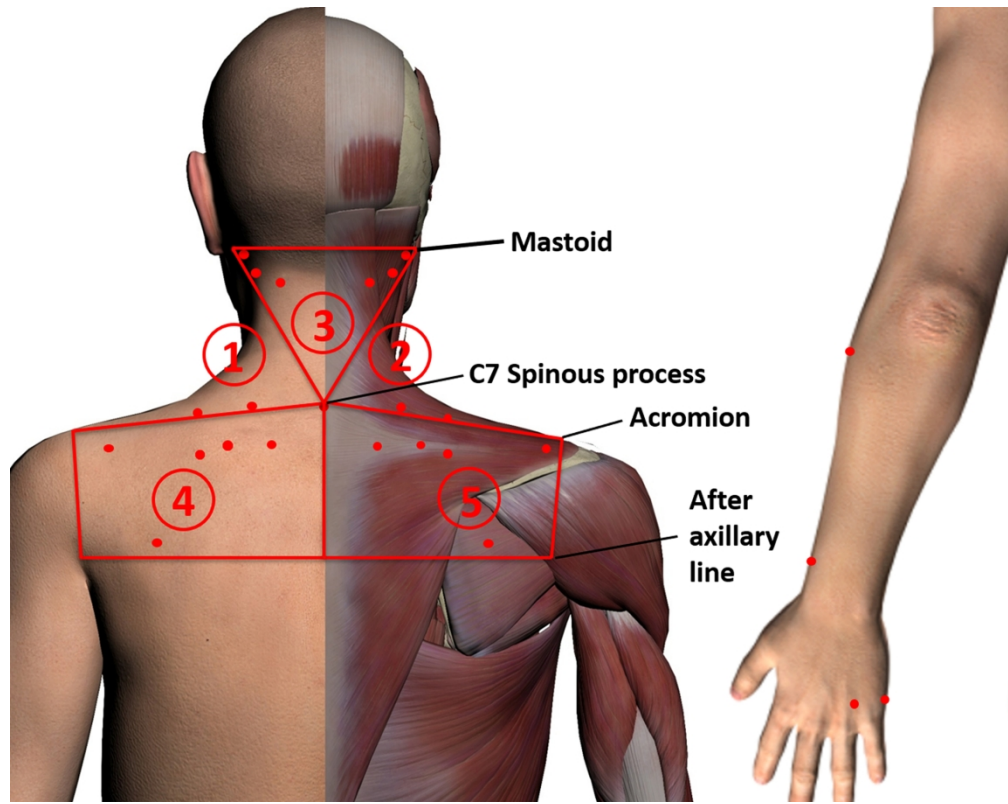
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45 Flowchart of the study design.

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The test regions and acupoints that will be used in the study.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a multicenter, matched, case-control study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	The present study aims to identify the types of sensitization (e.g. temperature, tenderness) and distribution of sensitized points in patients with NP, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients, and to summarize the dominant forms of optimal sensitized points in patients. This information will be helpful in the choice of optimal points for the treatment of NP.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	Recent Chinese research has found that acupoints can be sensitized, with associated changes in function and size, and thus can functionally switch from a relatively 'silent' state to a relatively 'active' state. Treatment of an acupoint while it is in the 'active' state is thought to achieve a better clinical outcome, and this has become a focus in acupoint research.[6-8] There are

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diverse types of sensitivity manifested at sensitized points, including pain-sensitivity, heat-sensitivity, and form-sensitivity (appearance changes). Recent clinical studies report that a superior outcome is obtained using acupuncture at pain-sensitive points,[9] and moxibustion at heat-sensitive points[10] or visual-sensitive points.[11] However, most of the current observational studies have only focused on one form of sensitization in a small sample population, and thus are not comprehensive studies of various forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical practice is still controversial.

Objectives 3 State specific objectives, including any prespecified hypotheses 4

We describe the protocol for an observational study that aims to identify the different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization in patients with NP, and to determine the most optimal sensitization types seen at sensitized points in patients with NP. We believe that in the

state of disease, the sensitive points occurs with high frequency (manifested as changes in temperature, pain threshold etc), and the sensitive points of different sensitizations will overlap, and the final optimal points from overlap may be closely related to the selection of clinical treatment points. And the different sensations of point may suggest selection of interventions. Previous small sample studies have confirmed the feasibility of sensitization testing.[7] The present study will provide a basis for the selection of the optimal treatment points for NP in clinical practice.

Methods

Study design	4	Present key elements of study design early in the paper	4	This is a multicenter, age- and sex-matched, case-control study. The protocol was developed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6	We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and Orthopedics in five clinical centers in China: Chengdu University of

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Traditional Chinese Medicine, Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients. Recruitment strategies will include posting advertisements on social media (such as WeChat, which is similar to Facebook) and at community centers, or by word-of-mouth introductions through subjects already enrolled in the study.

Participants 6 (a) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants

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Patients are eligible for study inclusion if they: (1) have no history of NP and/or restricted neck movement; (2) are males or females aged 18–60 years; (3) provide written informed consent for all procedures in this study. Patients or healthy subjects who consent to study participation will be examined and diagnosed by a hospital doctor.

(b) *Cohort study*—For matched studies, give matching criteria and number of exposed and

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Healthy subjects are eligible if

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unexposed

Case-control study—For matched studies, give matching criteria and the number of controls per case

they: (1) have no history of neck pain and/or restricted neck movement; (2) are males or females aged 18–60 years; (3) provide written informed consent for all procedures in this study. Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients. A ratio of control subjects to cases of 1.

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9	Body surface temperature, Mechanical pain threshold, Pressure pain threshold, Skin resistance, Pain, Neck function, General demographic information, Safety and adverse events.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9	Body surface temperature: Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use Fotric thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to make measurements at each of the 29 acupoints in the five body regions. The temperature data of each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA).

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Mechanical pain threshold:
Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science, Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of the 29 acupoints in the five body regions in turn. If there is a difference of more than 15 g between the two measurements of mechanical pain threshold made at one acupoint, the mechanical pain threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each acupoint. To reduce the consecutive assessment error for two adjacent points, a method of alternate assessment of acupoints on the left and right sides of the body will be adopted.

Pressure pain threshold:
Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments, Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five body regions in turn. If there is a

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difference of more than 500 gf between the two PPT measurements performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated for each acupoint. A method of alternate assessment of the acupoints on the left and right sides of the body will again be adopted.

Skin resistance: Investigators will use the Acupoint dynamics research instrument (LMR30-RIII, Peking University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11, LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

Pain: Neck pain will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire, and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure the severity of pain. The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for patients with

neck pain, while the McGill Pain Questionnaire is used to measure the different qualities of the subjective pain experience; these questionnaires have been proven valid, and are convenient for patients to complete.

Neck function: The change in neck function will be evaluated by measuring the cervical range of motion before and after treatment.

General demographic information This will include the collection of data regarding age, disease duration, medical history, and medication type and dosage.

Safety and adverse events: Non-invasive assessments of the body surface do not generally cause adverse events. However, we will still record the number and type (e.g. serious pain, fainting) of adverse events in each group.

Participants who experience adverse events will receive the appropriate intervention. Adverse events will be immediately reported to the primary investigator and the ethics committee, and the affected participants will be withdrawn from the study.

Bias	9	Describe any efforts to address potential sources of bias	6, 10, 11	All investigators will attend
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training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls.

Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. To ensure the integrity of the study and protect the rights and health of the participants, we will set up a Data and Safety Monitoring Board. Every 3 months, members of the quality control group will conduct a quality control review at each study site, and produce a report regarding the quality analysis of the whole data collection process.

Study size	10	Explain how the study size was arrived at	9	<p>Previous study indicated that the rate of acupoint sensitization in patients ranged from 20% to 70%[26], so we set this rate at 50% to calculate the minimum sample size required for the proposed study. With acupoint sensitization rate of 20% in healthy subjects, the odds ratio is 4. According to Chow's formula in comparing two sample proportion[27], we assumed an α level of 0.05, a β of 0.01, a delta of 0.3 (50%-20%=30%), thus, the smallest sample size is 408 with</p>
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				two-sided confidence and a ratio of control subjects to cases of 1 (TrialSize package in R software). Considering the potential non-response rate and sampling effectiveness as 10%, the final sample size is 448 (224 patients, plus 224 age- and sex-matched healthy subjects).
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10	Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon’s signed rank test) will be used.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10	First, the distribution of basic information in the case and control groups will be described, including age, sex, height, weight, job occupation, and education level. Data will be presented as means (SD) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent the skin morphological changes. The distributions of the intensity of NP and neck function will also be described. Hypothetical testing will then be performed to assess the differences between the

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case and control groups in body surface sensations and biophysical properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance. Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference between the case and control groups in body surface temperature, mechanical pain threshold, PPT, or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization rates of patients and healthy subjects will be calculated separately regarding the aspects of heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints. Meanwhile, OR represents the ratio of acupoint sensitization of patients and healthy subjects. Therefore, this study

				combines OR and sensitization rate of all acupoints to find out the optimal points in each sensitization.
		(b) Describe any methods used to examine subgroups and interactions	N/A	
		(c) Explain how missing data were addressed	10	Missing data will be processed without imputation.
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	5,6	Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients.
		(e) Describe any sensitivity analyses	N/A	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A	
		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram	N/A	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A	
		(b) Indicate number of participants with missing data for each variable of interest	N/A	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	N/A	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A	
		(b) Report category boundaries when continuous variables were categorized	N/A	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A	
Discussion				

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2	Key results	18	Summarise key results with reference to study objectives	N/A
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4	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
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22	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/A
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25	Generalisability	21	Discuss the generalisability (external validity) of the study results	3
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38	Other information			
39	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12
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Science Foundation of China (no. 81590950, 81590951, 81722050).

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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BMJ Open

Identification of the optimal points for the acupuncture treatment of neck pain in China: protocol for a multicenter, matched, case-control study

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Article Type:	Protocol
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Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Complementary medicine
Keywords:	sensitized points, neck pain, study protocol, observational study

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Manuscripts

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4 **Identification of the optimal points for the acupuncture treatment of neck pain**
5 **in China: protocol for a multicenter, matched, case-control study**

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7 Ming-Sheng Sun^{1†}, Si-Yuan Tao^{2†}, Guo-Yan Geng^{1†}, Jie-Ru Peng², Xing-Sha Ma¹, Ming-Xi Yan¹,
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9 Jiao Chen¹, Ding-Jun Cai¹, Hui Zheng¹, Chun Xia Yang², Ling Zhao^{1*}, Fan-Rong Liang^{1*}
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13 † Ming-Sheng Sun, Si-Yuan Tao, and Guo-Yan Geng contributed equally to this work.
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47 **Word count:** 2780 words
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51 **Keywords:** sensitized points, neck pain, study protocol, observational study
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ABSTRACT

Introduction: Neck pain (NP) is a common condition that can be effectively treated by acupuncture. However, various treatment points prescription (such as local acupoints, distal acupoints, and sensitized acupoints) could be used. The present study aims to identify the types of sensitization and distribution of sensitized points in patients with NP, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients, and to summarize the dominant forms of optimal sensitized points in patients. This information will be helpful in the choice of optimal points for the treatment of NP.

Methods and analysis: This multicenter, matched, case-control study will enroll 224 patients with NP, and 224 age- and sex-matched healthy subjects for control. Body surface temperature, mechanical pain threshold, pressure pain threshold, and skin resistance will be assessed at the 15 acupoints most frequently used to treat NP, and at the five body regions in which pain most frequently occurs. Hypothesis testing will be used to compare the differences in variables between cases and controls. In addition, receiver operating characteristic curve analysis will be used to explore the cutoff values of the sensitive states of heat, pain, and electrical resistance that indicate sensitization of the acupoint. The optimal points will be comprehensively determined by the acupoint sensitization rate and odds ratio (OR) value.

Ethics and dissemination: Ethical approval of this study has been granted by the Research Ethical Committee of the Teaching Hospital of Chengdu University of Traditional Chinese Medicine (ID: 2018KL-016). The outcomes of the study will be disseminated through peer-reviewed publications.

Trial registration: ChiCTR1800016220.

Strengths and limitations of this study

- ▶ This study will be the first observational study to evaluate the forms of sensitization and the distribution of sensitized points in patients with NP.
- ▶ This study will be the first to attempt to define the acupoint sensitization in patients with NP using a cutoff value for the identification of point sensitization.
- ▶ As only patients with nontraumatic NP with mobility deficits were included, it would limit the representativeness and generalizability of the study results.

INTRODUCTION

Neck pain (NP) is a common clinical condition often accompanied by tenderness at sensitive points. The global lifetime prevalence of NP was 48.5% in 2006.[1] The prevalence of white-collar workers in China was 33.9-54.8% in 2016 and has an increase tendency in recent years,[2] imposing considerable personal and socioeconomic burdens. Muscle relaxants and non-steroidal anti-inflammatory drugs are used to treat this disease, however, such medications carry a risk of adverse effects, and neither drug is better than non-pharmacological alternative treatments.[1]

As non-pharmacological alternative treatments have reliable efficacy and availability, this type of treatment is gaining an increasing amount of recognition worldwide. Acupuncture is one type of non-pharmacological alternative treatment that can effectively treat NP. Although various studies have confirmed that acupuncture is effective, the outcome of acupuncture treatment is closely related to the point selections.[3] Previous studies have evaluated the use of local acupoints[4] or distal acupoints,[5] with all types of treatment points reportedly having a certain degree of clinical efficacy.

Recent Chinese research has found that acupoints can be sensitized, with associated changes in function and size, and thus can functionally switch from a relatively 'silent' state to a relatively 'active' state. Treatment of an acupoint while it is in the 'active' state is thought to achieve a better clinical outcome, and this has become a focus in acupoint research.[6-8] There are diverse types of sensitivity manifested at sensitized points, including pain-sensitivity, heat-sensitivity, and form-sensitivity (appearance changes). Recent clinical studies report that a superior outcome is obtained using acupuncture at pain-sensitive points,[9] and moxibustion at heat-sensitive points[10] or visual-sensitive points.[11] However, most of the current observational studies have only focused on one form of sensitization in a small sample population, and thus are not comprehensive studies of various forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical practice is still controversial.

Herein, we describe the protocol for an observational study that aims to identify the different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization in patients with NP, and to determine the most optimal sensitization types seen at sensitized points in patients with NP. We believe that in the state of disease, the sensitive points occurs with high frequency (manifested as changes in

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4 temperature, pain threshold etc), and the sensitive points of different sensitizations will overlap, and
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6 the final optimal points from overlap may be closely related to the selection of clinical treatment
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8 points. Previous small sample studies have confirmed the feasibility of sensitization testing.[7] The
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10 present study will provide evidence for the selection of the optimal treatment points for NP in
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12 clinical practice.

13 14 15 **METHODS AND ANALYSIS**

16 17 **Study design**

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19 This is a multicenter, age- and sex-matched, case-control study. The protocol was developed in
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21 accordance with the Strengthening the Reporting of Observational Studies in Epidemiology
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23 guidelines.[12] The study has been registered with ChiCTR at Current Controlled Trials
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25 (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.

26 27 28 29 **Ethics**

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31 This study was designed in accordance with the principles of the Declaration of Helsinki. The study
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33 protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Chengdu
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35 University of Traditional Chinese Medicine (approval number: 2018KL-016), and is registered on
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37 the primary registry in the World Health Organization registry network (Chinese Clinical Trial
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39 Registry: no. ChiCTR1800016203). Signed consent will be obtained from each participant after
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41 they have been informed of the study procedures, possible risks, and their right to withdraw from
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43 the study.

44 45 46 **Patients and healthy subjects**

47 48 **Inclusion criteria**

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50 Patients are eligible for study inclusion if they: (1) have nontraumatic NP with mobility deficits in
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52 the acute and chronic stages (2) are males or females aged 18–60 years; (3) provide written informed
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54 consent for all procedures in this study.

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58 Healthy subjects are eligible if they: (1) have no history of NP and/or restricted neck movement; (2)
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60 are males or females aged 18–60 years; (3) provide written informed consent for all procedures in

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4 this study.
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8 **Exclusion criteria**

9 Patients are not eligible if they: (1) have complicated neck or shoulder pain caused by cervical and
10 intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder periartthritis,
11 rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical
12 congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic
13 system; (4) have difficulty in answering the questionnaires because of cognitive impairment; (5)
14 have dermatopathic diseases; (6) are pregnant, breastfeeding, or planning a pregnancy during the
15 study period.
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25 Healthy subjects are not eligible if they: (1) have serious disease related to the heart, liver, kidney,
26 or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty in answering
27 the questionnaires because of cognitive impairment; (4) have dermatopathic diseases; (5) are
28 pregnant, breastfeeding, or planning a pregnancy during the study period.
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35 **Recruitment strategies**

36 We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and
37 Orthopedics in five clinical centers in China: Chengdu University of Traditional Chinese Medicine,
38 Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese
39 Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional
40 Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-
41 matched residents from the same communities as the patients. Recruitment strategies will include
42 posting advertisements on social media (such as WeChat, which is similar to Facebook) and at
43 community centers, or by word-of-mouth introductions through subjects already enrolled in the
44 study. Patients or healthy subjects who consent to study participation will be examined and
45 diagnosed by a hospital doctor.
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58 **Test regions, acupoints, and sensitized points**

59 In accordance with the results of literature data-mining and expert consensus on the treatment of
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NP, we identified the 15 most frequently used acupoints (Table 1) and the five regions of the body with the most frequent occurrence of pain and the greatest degree of acupoint sensitization. The body was divided into five regions to standardize the treatment procedures and detection areas. Regions 1 and 2 are each bordered by the respective ipsilateral mastoid, sternal end of the clavicle, anterior axillary line, acromion, and C7 spinous process. Region 3 is the triangular region bordered by both sides of the mastoid and the C7 spinous process. Regions 4 and 5 are each bordered by the respective ipsilateral C7 spinous process, acromion, and axillary line; the two regions are divided by the posterior midline. These regions are the same as those used in previous studies on NP,[13] and include the upper trapezius, splenius capitis muscles, sternocleidomastoid, levator scapulae, infraspinatus, scalene, subscapularis muscles, and other neck muscles associated with NP.[14 15] These regions and acupoints are shown in Figure 2.

Investigators will mark the 29 acupoints on each patient. All investigators will attend training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls. The investigator will then palpate the detection area associated with each acupoint using the appropriate force (< 2,000 gf), and will identify the sensitized points that have pain/sourness/heaviness/fullness or nodules.

Table 1 Acupoints selected for use in the study

Acupoints	Location
Jianjing (GB-21)	On the shoulder, directly above the nipple, at the midpoint of the line connecting Dazhui(DU-14) with the acromial end of clavicle
Jianzhongshu(SI-15)	On the back, 2 cun lateral to the lower border of the spinous process of the 7 th cervical vertebra
Wangu(GB-12)	On the head, in the depression posterior and inferior to the mastoid process.
Fengchi(GB-20)	On the nape, below the occipital, on a level with Fengfu DU-16, in the depression between the upper portion of trapezius and the sternocleidomastoid
Tianzhu(BL-10)	On the nape, 1.3 cun lateral to the posterior hairline, in the depression of the posterior hairline lateral to the trapezius muscle
Dazhui(DU-14)	On the posterior median line, in the depression below the spinous process of the 7 th cervical vertebra
Dazhu(BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1 st thoracic vertebra
Jianwaishu(SI-14)	On the back, 3 cun lateral to the lower border of the spinous process of the first thoracic vertebra

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3	Tianliao(SJ-15)	On the region of scapula, at the midpoint of the line connecting Jianjing
4		GB-21 with Quyuan SI-13, on the superior angle of the scapula
5		
6	Jugu(LI-16)	In the upper portion of the shoulder, in the depression between the
7		acromial end of clavicle and the scapular spine
8		
9	Tianzong(SI-11)	In the region of the scapula, in the depression of the center of the
10		subscapular fossa, on a level with the 4 th thoracic vertebra
11	Shousanli(LI-10)	Flexing the elbow, on the dorsal radial side of the forearm, on the line
12		connecting Yangxi LI-5 with Quchi LI-11, 2cun below the transverse
13		cuticular crease
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15	Lieque(LU-7)	On the radial margin of the forearm, 1.5 cun above the transverse crease
16		of the wrist, between the branchioradial muscle and the long abductor
17		muscle tendon of thumb
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19	Zhongzhu(SJ-3)	On the dorsum of the hand, in the depression between the 4 th and 5 th
20		metacarpal bones, proximal to the 4 th metacarpalangeal joint
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22	Houxi(SI-3)	On the ulna side of the palm, proximate to the fifth metacarpophalangeal
23		joint, at the end of transverse crease of metacarpophalangeal joint, at the
24		dorsoventral boundary
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Outcome measurements

All data collection for each patient will be completed on the same day.

Body surface temperature

Thermal imaging technology has been widely applied to assess pain and muscle damage.[16 17] Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use Fotric thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to make measurements at each of the 29 acupoints in the five body regions. The temperature data of each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA).

Mechanical pain threshold

Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science, Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of the 29 acupoints in the five body regions in turn. Previous studies have found that the device can detect hyperalgesia and changes in sensory nerve function.[18-20] Mechanical pain caused by punctate stimuli is related to A_δ-fibers.[21] If there is a difference of more than 15 g between the two measurements of mechanical pain threshold made at one acupoint, the mechanical pain

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4 threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a
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6 rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each
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8 acupoint. To reduce the consecutive assessment error for two adjacent points, a method of alternate
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10 assessment of acupoints on the left and right sides of the body will be adopted.

11 12 13 **Pressure pain threshold**

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15 The pressure pain threshold (PPT) is widely used in clinical practice as a semi-objective method
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17 with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-
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19 fibers.[21] Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments,
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21 Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five
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23 body regions in turn. If there is a difference of more than 500 gf between the two PPT measurements
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25 performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive
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27 pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated
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29 for each acupoint. A method of alternate assessment of the acupoints on the left and right sides of
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31 the body will again be adopted.

32 33 34 **Skin resistance**

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36 Investigators will use the Acupoint dynamics research instrument (LMR30-RⅢ, Peking
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38 University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11,
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40 LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

41 42 43 **Pain**

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45 NP will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire,
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47 and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure the severity
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49 of pain. The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for
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51 patients with NP, while the McGill Pain Questionnaire is used to measure the different qualities of
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53 the subjective pain experience; these questionnaires have been proven valid, and are convenient for
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55 patients to complete.[24 25]

56 57 58 59 60 **Neck function**

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4 The change in neck function will be evaluated by measuring the cervical range of motion.
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7 **General demographic information**

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9 This will include the collection of data regarding age, disease duration, medical history, and
10 medication type and dosage.
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14 **Safety and adverse events**

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16 Non-invasive assessments of the body surface do not generally cause adverse events. However, we
17 will still record the number and type (e.g. serious pain, fainting) of adverse events in each group.
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19 Participants who experience adverse events will receive the appropriate intervention. Adverse
20 events will be immediately reported to the primary investigator and the ethics committee, and the
21 affected participants will be withdrawn from the study.
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29 **Patients and public involvement**

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31 Patients and public were not involved.
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35 **Sample size calculation**

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37 Previous study indicated that the rate of acupoint sensitization in patients ranged from 20% to
38 70%,^[26] so we set this rate at 50% to calculate the minimum sample size required for the proposed
39 study. With acupoint sensitization rate of 20% in healthy subjects, the odds ratio is 4. According to
40 Chow's formula in comparing two sample proportion,^[27] we assumed an α level of 0.05, a β of
41 0.01, thus, the smallest sample size is 408 with two-sided confidence and a ratio of control subjects
42 to cases of 1 (TrialSize package in R software). Considering the potential non-response rate and
43 sampling effectiveness as 10%, the final sample size is 448 (224 patients, plus 224 age- and sex-
44 matched healthy subjects).
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54 **Statistical analysis**

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56 Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. SPSS
57 21.0 (SPSS Inc., Chicago, IL, USA) will then be used to complete the statistical analyses. The statistical
58 data evaluation will be independently performed by the West China School of Public Health at Sichuan
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4 University, China. Missing data will be processed without imputation. First, the distribution of basic
5 information in the case and control groups will be described, including age, sex, height, weight, job
6 occupation, and education level. Data will be presented as means (SD) for continuous variables, and
7 as frequency (percentage) for categorical variables. The distributions of sensitized points will be
8 shown in scatter plots to represent the skin morphological changes. The distributions of the intensity
9 of NP and neck function will also be described. Hypothetical testing will then be performed to assess
10 the differences between the case and control groups in body surface sensations and biophysical
11 properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance.
12 Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise,
13 non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference
14 between the case and control groups in body surface temperature, mechanical pain threshold, PPT,
15 or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-
16 sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be
17 detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization
18 rates of patients and healthy subjects will be calculated separately regarding the aspects of heat,
19 pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints.
20 Meanwhile, odds ratio (OR) represents the ratio of acupoint sensitization of patients and healthy
21 subjects. Therefore, this study combines OR and sensitization rate of all acupoints to find out the
22 optimal points in each sensitization.
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43 **Quality control**

44 This is a multi-center observational study, and so quality control will play a vital role in the
45 extrapolation of the conclusion. To ensure the integrity of the study and protect the rights and health
46 of the participants, we will set up a Data and Safety Monitoring Board. The Data and Safety
47 Monitoring Board will be developed in accordance with the Operational Guidelines for the
48 Establishment and Functioning of the Data and Safety Monitoring Boards of the World Health
49 Organization. Separate from the Data and Safety Monitoring Board, a quality control group will be
50 established to guarantee the validity and reliability of results. Before study commencement,
51 fieldwork operation manuals will be prepared, and pilot study participants from all centers will
52 undergo training in accordance with the standard operating procedure of this study. Every 3 months,
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4 members of the quality control group will conduct a quality control review at each study site, and
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6 produce a report regarding the quality analysis of the whole data collection process.
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9 **DISCUSSION**

10 NP is the third-most common chronic pain condition, and the fourth leading cause of disability
11 worldwide.[28] Acupuncture is a popular non-pharmacological modality used to relieve pain. The
12 latest research has found that the performance of acupuncture at sensitive points may provide the
13 most effective treatment.[8 29] When the body is in a diseased state, there will be morphological
14 form-sensitive point changes, such as nodules, pimples, uplifting, dimpling, and changes in skin
15 color.[30] Thermal image detection has proved that the temperature of corresponding acupoints will
16 be obviously abnormal when a patient has visceral illness, such as diseases of the heart, lung, and
17 stomach, which indicates that the temperature of acupoints can reflect the physiological and
18 pathological phenomena of the affected organs.[31] Studies have confirmed that the PPT at
19 acupoints changes when patients are in a diseased state.[6 32 33] The degree of change in the PPT
20 may reflect the intensity of acupoint sensitization, and may be related to the disease status.[34] This
21 proposed observational study will bridge the knowledge gap regarding the optimal sensitization of
22 acupoints in various forms of point sensitivity in patients with NP.
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36 As this will be the first study to evaluate the association between acupoint sensitization and NP, this
37 observational study may have some limitations. The main limitation is that in order to reduce the
38 bias caused by other factors and to ensure consistency, this study will only include patients with
39 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as
40 NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located
41 in four different regions in China, it may be difficult to implement quality control; thus, quality
42 control reviews will be conducted every 3 months.
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50 In conclusion, this article describes the design and protocol of a study that aims to observe the
51 different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized
52 acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization in patients with
53 NP, and to identify the dominant sensitized forms of the sensitized points in patients with NP. The
54 results will provide a basis for the selection of clinically optimal acupuncture points for NP.
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Authors' contributions

MSS, SYT, JRP, DJC, CXY, HZ, LZ, and FRL participated in the design of the trial, in creating the data analysis plan, and in drafting the manuscript. GYG, XSM, MXY, and JC collected the information needed for the performance of this trial in each center. All of the authors discussed, read, and revised the manuscript, and gave final approval for the publication of this study protocol.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval

The study protocol has been approved by the institutional review board and ethics committee of the First Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (May 2018).

Provenance and peer review

Not commissioned; internally peer reviewed.

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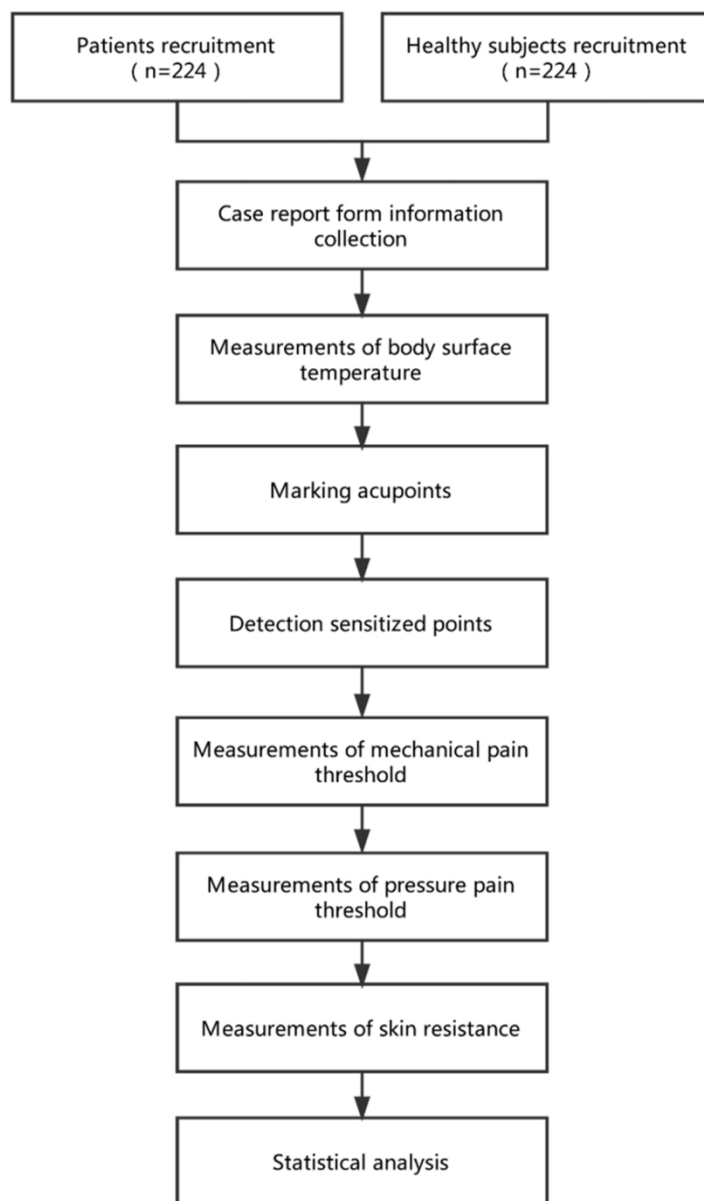
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22 **Figure 1** Flowchart of the study design.
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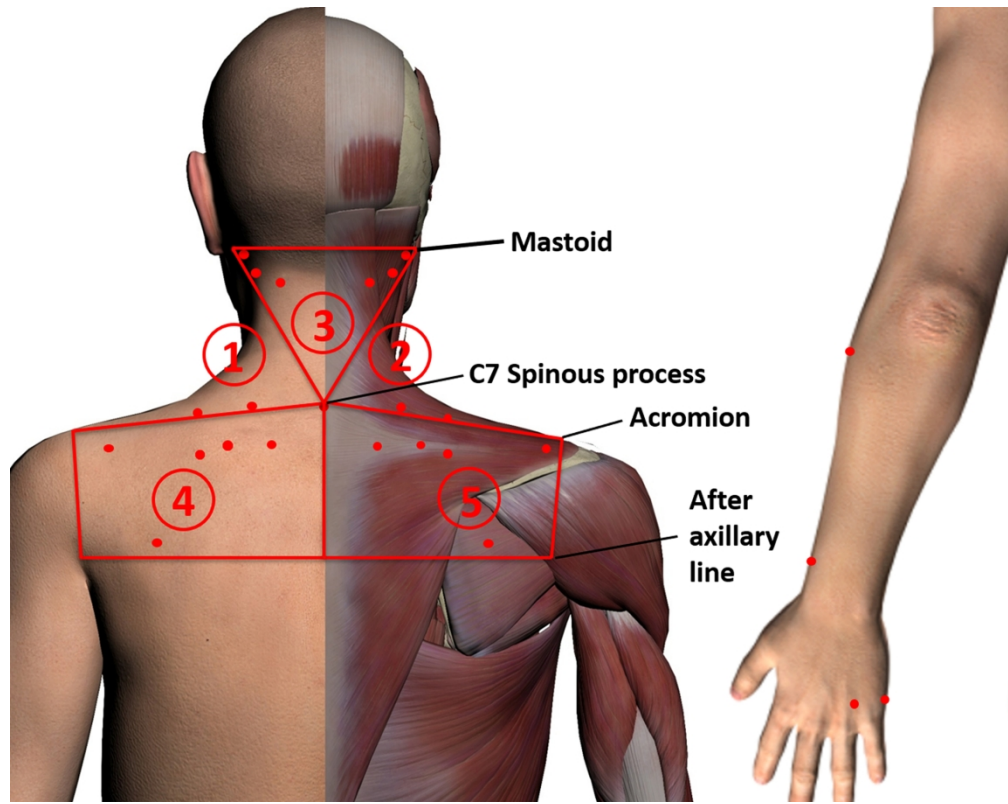
25 **Figure 2** The test regions and acupoints that will be used in the study.
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45 Flowchart of the study design.

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The test regions and acupoints that will be used in the study.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a multicenter, matched, case-control study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	The present study aims to identify the types of sensitization (e.g. temperature, tenderness) and distribution of sensitized points in patients with NP, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients, and to summarize the dominant forms of optimal sensitized points in patients. This information will be helpful in the choice of optimal points for the treatment of NP.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	Recent Chinese research has found that acupoints can be sensitized, with associated changes in function and size, and thus can functionally switch from a relatively 'silent' state to a relatively 'active' state. Treatment of an acupoint while it is in the 'active' state is thought to achieve a better clinical outcome, and this has become a focus in acupoint research.[6-8] There are

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diverse types of sensitivity manifested at sensitized points, including pain-sensitivity, heat-sensitivity, and form-sensitivity (appearance changes). Recent clinical studies report that a superior outcome is obtained using acupuncture at pain-sensitive points,[9] and moxibustion at heat-sensitive points[10] or visual-sensitive points.[11] However, most of the current observational studies have only focused on one form of sensitization in a small sample population, and thus are not comprehensive studies of various forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical practice is still controversial.

Objectives 3 State specific objectives, including any prespecified hypotheses 4

We describe the protocol for an observational study that aims to identify the different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization in patients with NP, and to determine the most optimal sensitization types seen at sensitized points in patients with NP. We believe that in the

state of disease, the sensitive points occurs with high frequency (manifested as changes in temperature, pain threshold etc), and the sensitive points of different sensitizations will overlap, and the final optimal points from overlap may be closely related to the selection of clinical treatment points. And the different sensations of point may suggest selection of interventions. Previous small sample studies have confirmed the feasibility of sensitization testing.[7] The present study will provide a basis for the selection of the optimal treatment points for NP in clinical practice.

Methods

Study design	4	Present key elements of study design early in the paper	4	This is a multicenter, age- and sex-matched, case-control study. The protocol was developed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6	We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and Orthopedics in five clinical centers in China: Chengdu University of

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Traditional Chinese Medicine, Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients. Recruitment strategies will include posting advertisements on social media (such as WeChat, which is similar to Facebook) and at community centers, or by word-of-mouth introductions through subjects already enrolled in the study.

Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4, 5, 9, 10
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and	5, 9, 10

Patients are eligible for study inclusion if they: (1) have no history of NP and/or restricted neck movement; (2) are males or females aged 18–60 years; (3) provide written informed consent for all procedures in this study. Patients or healthy subjects who consent to study participation will be examined and diagnosed by a hospital doctor. Healthy subjects are eligible if

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2		unexposed		
3		<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
4				they: (1) have no history of neck
5				pain and/or restricted neck
6				movement; (2) are males or
7				females aged 18–60 years; (3)
8				provide written informed consent
9				for all procedures in this study.
10				Healthy subjects without neck or
11				shoulder pain will comprise age-
12				and sex-matched residents from the
13				same communities as the patients.
14				A ratio of control subjects to cases
15				of 1.
16				
17	Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
18				Body surface temperature,
19				Mechanical pain threshold,
20				Pressure pain threshold, Skin
21				resistance, Pain, Neck function,
22				General demographic information,
23				Safety and adverse events.
24				
25	Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
26				Body surface temperature: Each
27				participant will be evaluated in a
28				room with a constant temperature
29				of 26°C. Investigators will use
30				Fotric thermal imaging cameras
31				(Fotric 226, IRS Systems Inc.,
32				Allen, TX, USA) to make
33				measurements at each of the 29
34				acupoints in the five body regions.
35				The temperature data of each point
36				on the images will be analyzed
37				using professional software
38				(AnalyzeIR, IRS Systems Inc.,
39				Allen, TX, USA).
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Mechanical pain threshold:
Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science, Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of the 29 acupoints in the five body regions in turn. If there is a difference of more than 15 g between the two measurements of mechanical pain threshold made at one acupoint, the mechanical pain threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each acupoint. To reduce the consecutive assessment error for two adjacent points, a method of alternate assessment of acupoints on the left and right sides of the body will be adopted.

Pressure pain threshold:
Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments, Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five body regions in turn. If there is a

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difference of more than 500 gf between the two PPT measurements performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated for each acupoint. A method of alternate assessment of the acupoints on the left and right sides of the body will again be adopted.

Skin resistance: Investigators will use the Acupoint dynamics research instrument (LMR30-RIII, Peking University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11, LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

Pain: Neck pain will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire, and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure the severity of pain. The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for patients with

neck pain, while the McGill Pain Questionnaire is used to measure the different qualities of the subjective pain experience; these questionnaires have been proven valid, and are convenient for patients to complete.

Neck function: The change in neck function will be evaluated by measuring the cervical range of motion before and after treatment.

General demographic information This will include the collection of data regarding age, disease duration, medical history, and medication type and dosage.

Safety and adverse events: Non-invasive assessments of the body surface do not generally cause adverse events. However, we will still record the number and type (e.g. serious pain, fainting) of adverse events in each group.

Participants who experience adverse events will receive the appropriate intervention. Adverse events will be immediately reported to the primary investigator and the ethics committee, and the affected participants will be withdrawn from the study.

Bias	9	Describe any efforts to address potential sources of bias	6, 10, 11	All investigators will attend
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training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls.

Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. To ensure the integrity of the study and protect the rights and health of the participants, we will set up a Data and Safety Monitoring Board. Every 3 months, members of the quality control group will conduct a quality control review at each study site, and produce a report regarding the quality analysis of the whole data collection process.

Study size	10	Explain how the study size was arrived at	9	<p>Previous study indicated that the rate of acupoint sensitization in patients ranged from 20% to 70%[26], so we set this rate at 50% to calculate the minimum sample size required for the proposed study. With acupoint sensitization rate of 20% in healthy subjects, the odds ratio is 4. According to Chow's formula in comparing two sample proportion[27], we assumed an α level of 0.05, a β of 0.01, a delta of 0.3 (50%-20%=30%), thus, the smallest sample size is 408 with</p>
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				two-sided confidence and a ratio of control subjects to cases of 1 (TrialSize package in R software). Considering the potential non-response rate and sampling effectiveness as 10%, the final sample size is 448 (224 patients, plus 224 age- and sex-matched healthy subjects).
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10	Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon’s signed rank test) will be used.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10	First, the distribution of basic information in the case and control groups will be described, including age, sex, height, weight, job occupation, and education level. Data will be presented as means (SD) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent the skin morphological changes. The distributions of the intensity of NP and neck function will also be described. Hypothetical testing will then be performed to assess the differences between the

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case and control groups in body surface sensations and biophysical properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance. Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference between the case and control groups in body surface temperature, mechanical pain threshold, PPT, or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization rates of patients and healthy subjects will be calculated separately regarding the aspects of heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints. Meanwhile, OR represents the ratio of acupoint sensitization of patients and healthy subjects. Therefore, this study

				combines OR and sensitization rate of all acupoints to find out the optimal points in each sensitization.
		(b) Describe any methods used to examine subgroups and interactions	N/A	
		(c) Explain how missing data were addressed	10	Missing data will be processed without imputation.
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	5,6	Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients.
		(e) Describe any sensitivity analyses	N/A	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A	
		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram	N/A	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A	
		(b) Indicate number of participants with missing data for each variable of interest	N/A	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	N/A	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A	
		(b) Report category boundaries when continuous variables were categorized	N/A	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A	
Discussion				

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2	Key results	18	Summarise key results with reference to study objectives	N/A
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4	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
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22	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/A
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25	Generalisability	21	Discuss the generalisability (external validity) of the study results	3
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38	Other information			
39	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12
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Science Foundation of China (no. 81590950, 81590951, 81722050).

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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BMJ Open

Identification of the optimal points for the acupuncture treatment of neck pain in China: protocol for a multicenter, matched, case-control study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-029194.R3
Article Type:	Protocol
Date Submitted by the Author:	19-Jul-2019
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Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Complementary medicine
Keywords:	sensitized points, neck pain, study protocol, observational study

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Manuscripts

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4 **Identification of the optimal points for the acupuncture treatment of neck pain**
5
6 **in China: protocol for a multicenter, matched, case-control study**

7 Ming-Sheng Sun^{1†}, Si-Yuan Tao^{2†}, Guo-Yan Geng^{1†}, Jie-Ru Peng², Xing-Sha Ma¹, Ming-Xi Yan¹,
8 Jiao Chen¹, Ding-Jun Cai¹, Hui Zheng¹, Chun Xia Yang², Ling Zhao^{1*}, Fan-Rong Liang^{1*}
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13 † Ming-Sheng Sun, Si-Yuan Tao, and Guo-Yan Geng contributed equally to this work.
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46 **Word count:** 2745 words
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50 **Keywords:** sensitized points, neck pain, study protocol, observational study
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ABSTRACT

Introduction: Neck pain (NP) is a common condition that can be effectively treated by acupuncture. However, several treatment point prescriptions (i.e., local acupoints, distal acupoints, and sensitized acupoints) may be used. The present study aims to identify the types of sensitization and the distribution of sensitized points in patients with NP, to analyze the cutoff values and sensitization rate for acupoint sensitization, and to summarize the dominant forms of optimally-sensitized points. This information will be helpful when choosing the optimal points to treat NP.

Methods and analysis: This multicenter, matched, case-control study will enroll 224 patients with NP, and 224 age- and sex-matched healthy participants as controls. Body surface temperature, mechanical pain threshold, pressure pain threshold, and skin resistance will be assessed at the 15 acupoints most frequently used to treat NP, and at the five body regions in which pain occurs most frequently. Hypothesis testing will be used to compare the differences in variables between cases and controls. In addition, receiver operating characteristic curve analysis will be used to explore the cutoff values of the sensitive states of heat, pain, and electrical resistance, which indicate sensitization of the acupoint. The optimal points will be comprehensively determined by the acupoint sensitization rate and odds ratio (OR).

Ethics and dissemination: Ethical approval of this study has been granted by the Research Ethical Committee of the Teaching Hospital of Chengdu University of Traditional Chinese Medicine (ID: 2018KL-016). The outcomes of the study will be disseminated through peer-reviewed publications.

Trial registration: ChiCTR1800016220.

Strengths and limitations of this study

- ▶ This study will provide information about the forms of sensitization and the distribution of sensitized points in patients with NP using the largest known sample, in a strict observational study.
- ▶ Objective or semi-objective biological measure, body surface temperature, mechanical pain threshold, pressure pain threshold, and skin resistance, will be assessed as a preliminary exploration of the different forms of sensitization.
- ▶ This study will attempt to define acupoint sensitization in patients with NP using a cutoff value for identifying point sensitization.
- ▶ The representativeness and generalizability of the study results will be limited because we are

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4 including only patients with nontraumatic NP with mobility deficits.
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7 **INTRODUCTION**

9 Neck pain (NP) is a common clinical condition often accompanied by tenderness at sensitive points.
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11 The global lifetime prevalence of NP was 48.5% in 2006.[1] The prevalence in white-collar workers
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13 in China was 33.9%-54.8% in 2016 and has increased in recent years,[2] imposing considerable
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15 personal and socioeconomic burdens. Muscle relaxants and non-steroidal anti-inflammatory drugs
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17 are used to treat this condition; however, these medications carry a risk of adverse effects, and
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19 neither drug is better than non-pharmacological alternative treatments.[1]
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21 Because non-pharmacological alternative treatments have reliable efficacy and availability, this type
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23 of treatment is gaining increasing recognition worldwide. Acupuncture is one type of non-
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25 pharmacological alternative treatment that can effectively treat NP. Although several studies have
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27 confirmed that acupuncture is effective, outcomes are closely related to point selection.[3] Previous
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29 studies have evaluated the use of local acupoints[4] or distal acupoints,[5] with the treatment of both
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31 types reportedly having a certain degree of clinical efficacy.
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33 Recent Chinese research has found that acupoints can be sensitized, with associated changes in
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35 function and size, and thus can functionally change from a relatively "silent" state to a relatively
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37 "active" state. Treatment of an acupoint while it is in the "active" state is thought to achieve a better
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39 clinical outcome, and this has become a focus in acupoint research.[6-8] Several types of sensitivity
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41 manifest at sensitized points, including pain-sensitivity, heat-sensitivity, and form-sensitivity
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43 (appearance changes). Recent clinical studies report that a superior outcome is obtained using
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45 acupuncture at pain-sensitive points,[9] and moxibustion at heat-sensitive points[10] or visually-
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47 sensitive points.[11] However, most of the current observational studies have focused on only one
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49 form of sensitization in small-sized sample populations, and thus are not comprehensive studies of
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51 different forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical
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53 practice remain controversial.

54 Herein, we describe the protocol for an observational study that aims to identify the different types
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56 of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients
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58 with NP, to analyze the cutoff values for acupoint sensitization, and to determine the most optimal
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60 sensitization types seen at sensitized points. We believe that in the state of disease, sensitive points

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4 occur with high frequency (manifested as changes in temperature, pain threshold etc.), that sensitive
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6 points with different sensitization overlap, and that the optimal overlapping points may be closely
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8 related to selecting the clinical treatment points. Previous small sample-sized studies have
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10 confirmed the feasibility of sensitization testing.[7] The present study will provide evidence for
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12 selecting the optimal treatment points for NP in clinical practice.

13 14 15 **METHODS AND ANALYSIS**

16 17 **Study design**

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19 This is a multicenter, age- and sex-matched, case-control study. The protocol was developed in
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21 accordance with the Strengthening the Reporting of Observational Studies in Epidemiology
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23 guidelines.[12] The study has been registered with ChiCTR at Current Controlled Trials
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25 (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.

26 27 28 29 **Ethics**

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31 This study was designed in accordance with the principles of the Declaration of Helsinki. The study
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33 protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Chengdu
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35 University of Traditional Chinese Medicine (approval number: 2018KL-016), and is registered on
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37 the primary registry in the World Health Organization registry network (Chinese Clinical Trial
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39 Registry: no. ChiCTR1800016203). Signed consent will be obtained from each participant after
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41 they have been informed of the study procedures, possible risks, and their right to withdraw from
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43 the study.

44 45 46 **Patients and healthy participants (controls)**

47 48 **Inclusion criteria**

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50 Patients are eligible for study inclusion if they: (1) have nontraumatic NP with mobility deficits in
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52 the acute and chronic stages (2) are males or females aged 18–60 years; and (3) provide written
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54 informed consent for all procedures in this study.

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58 Healthy participants as controls subjects are eligible if they: (1) have no history of NP and/or
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60 restricted neck movement; (2) are males or females aged 18–60 years; and (3) provide written

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4 informed consent for all procedures in this study.
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7 **Exclusion criteria**

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9 Patients are ineligible if they: (1) have complicated neck or shoulder pain caused by cervical and
10 intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder periartthritis,
11 rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical
12 congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic
13 system; (4) have difficulty answering questionnaires because of cognitive impairment; (5) have
14 dermatopathological diseases; or (6) are pregnant, breastfeeding, or planning a pregnancy during
15 the study period.
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25 Healthy participants are ineligible if they: (1) have serious disease related to the heart, liver, kidney,
26 or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty answering
27 the questionnaires because of cognitive impairment; (4) have dermatopathological diseases; or (5)
28 are pregnant, breastfeeding, or planning a pregnancy during the study period.
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33 **Recruitment strategies**

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35 We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and
36 Orthopedics in five clinical centers in China: Chengdu University of Traditional Chinese Medicine,
37 Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese
38 Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional
39 Chinese Medicine. Healthy participants without neck or shoulder pain will comprise age- and sex-
40 matched residents from the same communities as the patients, to act as controls. Recruitment
41 strategies will include posting advertisements on social media (such as WeChat, which is similar to
42 Facebook) and at community centers, or by word-of-mouth introductions through participants
43 already enrolled in the study. Patients or healthy participants who consent to study participation will
44 be examined and diagnosed by a hospital doctor.
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58 **Test regions, acupoints, and sensitized points**

59 Following the results of literature data-mining and expert consensus on the treatment of NP, we
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identified the 15 most frequently used acupoints (Table 1) and the five regions of the body with the most frequent occurrence of pain and the greatest degree of acupoint sensitization. The body will be divided into five regions to standardize the treatment procedures and detection areas. Regions 1 and 2 are each bordered by the respective ipsilateral mastoid, sternal end of the clavicle, anterior axillary line, acromion, and C7 spinous process. Region 3 is the triangular region bordered by both sides of the mastoid and the C7 spinous process. Regions 4 and 5 are each bordered by the respective ipsilateral C7 spinous process, acromion, and axillary line; the two regions are divided by the posterior midline. These regions are the same as those used in previous studies of NP,[13] and include the upper trapezius, splenius capitis muscles, sternocleidomastoid, levator scapulae, infraspinatus, scalene, subscapularis muscles, and other neck muscles associated with NP.[14 15] These regions and acupoints are shown in Figure 2.

Investigators will mark the 29 acupoints on each patient. All investigators will attend training to ensure all practices are standardized. Investigators will be blinded regarding whether participants are patients or healthy controls. The investigator will palpate the detection area associated with each acupoint using the appropriate force (< 2,000 gf), and will identify sensitized points that have pain/sourness/heaviness/fullness or nodules.

Table 1 Acupoints selected for use in the study

Acupoints	Location
Jianjing (GB-21)	On the shoulder, directly above the nipple, at the midpoint of the line connecting Dazhui (DU-14) with the acromial end of clavicle
Jianzhongshu (SI-15)	On the back, 2 cun lateral to the lower border of the spinous process of the 7 th cervical vertebra
Wangu (GB-12)	On the head, in the depression posterior and inferior to the mastoid process.
Fengchi (GB-20)	On the nape, below the occipital, on a level with Fengfu (DU-16), in the depression between the upper portion of trapezius and the sternocleidomastoid
Tianzhu (BL-10)	On the nape, 1.3 cun lateral to the posterior hairline, in the depression of the posterior hairline lateral to the trapezius muscle
Dazhui (DU-14)	On the posterior median line, in the depression below the spinous process of the 7 th cervical vertebra
Dazhu (BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1 st thoracic vertebra
Jianwaishu (SI-14)	On the back, 3 cun lateral to the lower border of the spinous process of the first thoracic vertebra

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3	Tianliao (SJ-15)	On the region of scapula, at the midpoint of the line connecting Jianjing
4		(GB-21) with Quyuan (SI-13), on the superior angle of the scapula
5		
6	Jugu (LI-16)	In the upper portion of the shoulder, in the depression between the
7		acromial end of clavicle and the scapular spine
8		
9	Tianzong (SI-11)	In the region of the scapula, in the depression of the center of the
10		subscapular fossa, on a level with the 4 th thoracic vertebra
11	Shousanli (LI-10)	Flexing the elbow, on the dorsal radial side of the forearm, on the line
12		connecting Yangxi (LI-5) with Quchi (LI-11), 2cun below the transverse
13		cuticular crease
14		
15	Lieque (LU-7)	On the radial margin of the forearm, 1.5 cun above the transverse crease
16		of the wrist, between the branchioradial muscle and the long abductor
17		muscle tendon of thumb
18		
19	Zhongzhu (SJ-3)	On the dorsum of the hand, in the depression between the 4 th and 5 th
20		metacarpal bones, proximal to the 4 th metacarpalangeal joint
21		
22	Houxi (SI-3)	On the ulna side of the palm, proximate to the fifth metacarpophalangeal
23		joint, at the end of transverse crease of metacarpophalangeal joint, at the
24		dorsoventral boundary
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Outcome measurements

All data collection for each patient will be completed on the same day.

Body surface temperature

Thermal imaging technology has been widely used to assess pain and muscle damage.[16 17] Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to take measurements at each of the 29 acupoints in the five body regions. The temperature data for each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA).

Mechanical pain threshold

Investigators will use the electronic Von Frey esthesiometer (model 2390; IITC Life Science, Woodland Hills, CA, USA) to take two measurements of the mechanical pain threshold at each of the 29 acupoints in the five body regions in turn. Previous studies have found that this device can detect hyperalgesia and changes in sensory nerve function.[18-20] Mechanical pain caused by punctate stimuli is related to A δ -fibers.[21] If there is a difference of more than 15 g between the two measurements of mechanical pain threshold made at one acupoint, the mechanical pain threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a

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4 rate of 10 g/s at each acupoint, and we will calculate the average mechanical pain threshold for each
5 acupoint. To reduce the consecutive assessment error for two adjacent points, an alternate
6 assessment method for acupoints on the left and right sides of the body will be adopted.
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10 11 **Pressure pain threshold**

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13 The pressure pain threshold (PPT) is widely used clinically as a semi-objective method with which
14 to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-fibers.[21]
15 Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments, Greenwich,
16 CT, USA) to take two PPT measurements at each of the 29 acupoints in the five body regions in
17 turn. If there is a difference of more than 500 gf between the two PPT measurements performed at
18 one acupoint, the PPT at that acupoint will be measured a third time. Progressive pressure will be
19 applied at a rate of 100 gf/s at each acupoint, and the average PPT will be calculated. An alternate
20 assessment method for the acupoints on the left and right sides of the body will be adopted.
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31 **Skin resistance**

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33 Investigators will use the Acupoint Dynamics Research Instrument (LMR30-RIII, Peking
34 University, Beijing, China) to record the skin resistance at nine acupoints, bilateral (GB-21, GB-
35 20, BL-11, LI-10, SI-3) and at the two most sensitive points (if there are two sensitive points).
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41 **Pain**

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43 NP will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire,
44 and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure pain severity
45 . The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for patients
46 with NP, and the McGill Pain Questionnaire is used to measure the different qualities of subjective
47 pain; these questionnaires have been proven valid, and are convenient for patients to complete.[24
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56 **Neck function**

58 Changes in neck function will be evaluated by measuring cervical range of motion.

General demographic information

This will include the collection of data regarding age, disease duration, medical history, and medication type and dosage.

Safety and adverse events

Non-invasive assessments of the body surface do not generally cause adverse events. However, we will record the number and type (e.g. serious pain, fainting) of adverse events in each group. Participants who experience adverse events will receive the appropriate intervention. We will report adverse events immediately to the primary investigator and the ethics committee, and the affected participants will be withdrawn from the study.

Patients and public involvement

Patients and the public have not and will not be involved in the design and conception of this study.

Sample size calculation

A previous study indicated that the rate of acupoint sensitization in patients ranged from 20% to 70%;[26] therefore, we set this rate at 50% to calculate the minimum sample size required for the proposed study. With an acupoint sensitization rate of 20% in healthy participants, the odds ratio is 4. According to Chow's formula for comparing two sample proportions,[27] we assumed $\alpha = 0.05$, and $\beta = 0.01$, thus, the smallest sample size is 408 with two-sided confidence and a ratio of control participants to cases of 1 (TrialSize package in R; The R Project for Statistical Computing, Copenhagen, Denmark). Setting the potential non-response rate and sampling effectiveness at 10%, the final sample size is 448 (224 patients, plus 224 age- and sex-matched healthy controls).

Statistical analysis

Data will be blinded, double-entered in EpiData 3.1 software (EpiData Association, Odense, Denmark), and adequately checked for errors. SPSS 21.0 (IBM Inc., Armonk, NY, USA) will then be used to complete the statistical analyses. The statistical data evaluation will be performed independently by the West China School of Public Health at Sichuan University, China. Missing data will be processed without imputation. First, the distribution of basic information in the case

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4 and control groups will be described for age, sex, height, weight, occupation, and education level.
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6 Data will be presented as means (standard deviation (SD)) for continuous variables, and as
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8 frequency (percentage) for categorical variables. The distributions of sensitized points will be shown
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10 in scatter plots to represent skin morphological changes. We will also describe the distributions of
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12 the intensity of NP and neck function. Hypothetical testing will then be performed to assess the
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14 differences between the case and control groups regarding body surface sensations and biophysical
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16 properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance.
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18 Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise,
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20 non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference
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22 between the case and control groups in body surface temperature, mechanical pain threshold, PPT,
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24 or skin resistance, the cut-off values for these indices to distinguish between sensitive and non-
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26 sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be
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28 detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization
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30 rates of the patients and healthy controls will be calculated separately for heat, pain, and electrical
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32 sensitivity to identify the most important form of sensitivity for all acupoints. The odds ratio (OR)
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34 will represent the ratio of acupoint sensitization in the patients and healthy controls; therefore, we
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36 will combine OR and the sensitization rate of all acupoints to determine the optimal points for each
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38 sensitization.

40 **Quality control**

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42 This is a multi-center observational study; therefore, quality control will play a vital role in
43
44 extrapolating the conclusions. To ensure the integrity of the study and to protect the rights and health
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46 of the participants, we will set up a Data and Safety Monitoring Board. The Data and Safety
47
48 Monitoring Board will be developed in accordance with the Operational Guidelines for the
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50 Establishment and Functioning of the Data and Safety Monitoring Boards of the World Health
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52 Organization. Separate from the Data and Safety Monitoring Board, a quality control group will be
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54 established to guarantee the validity and reliability of results. Before study commencement,
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56 fieldwork operation manuals will be prepared, and pilot study participants from all centers will
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58 undergo training in accordance with the standard operating procedure of this study. Every 3 months,
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60 members of the quality control group will perform a quality control review at each study site and

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4 produce a report regarding the quality analysis of the entire data collection process.
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7 **DISCUSSION**

9 NP is the third-most common chronic pain condition, and the fourth leading cause of disability
10 worldwide.[28] Acupuncture is a popular non-pharmacological modality to relieve pain. The latest
11 research has reported that acupuncture at sensitive points may provide the most effective
12 treatment.[8 29] When the body is in a diseased state, there are morphological form-sensitive point
13 changes, such as nodules, pimples, uplifting, dimpling, and changes in skin color.[30] Thermal
14 imaging has proven that the temperature of corresponding acupoints will be obviously abnormal
15 when a patient has visceral illness, such as diseases of the heart, lung, and stomach, indicating that
16 the temperature of acupoints can reflect the physiological and pathological phenomena of the
17 affected organs.[31] Studies have confirmed that the PPT at acupoints changes when patients are in
18 a diseased state, [6 32 33] and that the degree of change in the PPT may reflect the intensity of
19 acupoint sensitization, and may be related to the disease status.[34] This proposed observational
20 study will increase knowledge of the different types of acupoint sensitization and the cutoff values
21 for this sensitization in patients with NP.
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24 As this will be the first study, to our knowledge, to evaluate the association between acupoint
25 sensitization and NP, this observational study may have some limitations. The main limitation is
26 that in order to reduce the bias caused by other factors and to ensure consistency, this study will
27 include only patients with nontraumatic NP with mobility deficits, and will exclude patients with
28 other types of NP (such as radiating neck pain or secondary NP). Furthermore, because the four
29 participating centers are located in four different regions in China, it may be difficult to implement
30 quality control; thus, quality control reviews will be performed every 3 months.
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33 In conclusion, this article describes the design and protocol of a study that aims to observe the
34 different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized
35 acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization, and to identify
36 the dominant sensitized forms of the sensitized points. The results will provide a basis for selecting
37 clinically-optimal acupuncture points for NP.
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4 acknowledge the help and contributions from the subjects, investigators, and experts in each center.
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7
8 (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.
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13 **Authors' contributions**

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15 MSS, SYT, JRP, DJC, CXY, HZ, LZ, and FRL participated in the design of the trial, in creating the
16 data analysis plan, and in drafting the manuscript. GYG, XSM, MXY, and JC collected the
17 information needed for the performance of this trial in each center. All of the authors discussed,
18 read, and revised the manuscript, and gave final approval for the publication of this study protocol.
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24
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26
27 81590950, 81590951, 81722050).
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33 **Competing interests**

34 The authors declare that they have no competing interests.
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39 **Ethics approval**

40 The study protocol has been approved by the institutional review board and ethics committee of the
41 First Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (May 2018).
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46 **Provenance and peer review**

47 Not commissioned; internally peer reviewed.
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52 **Open Access**

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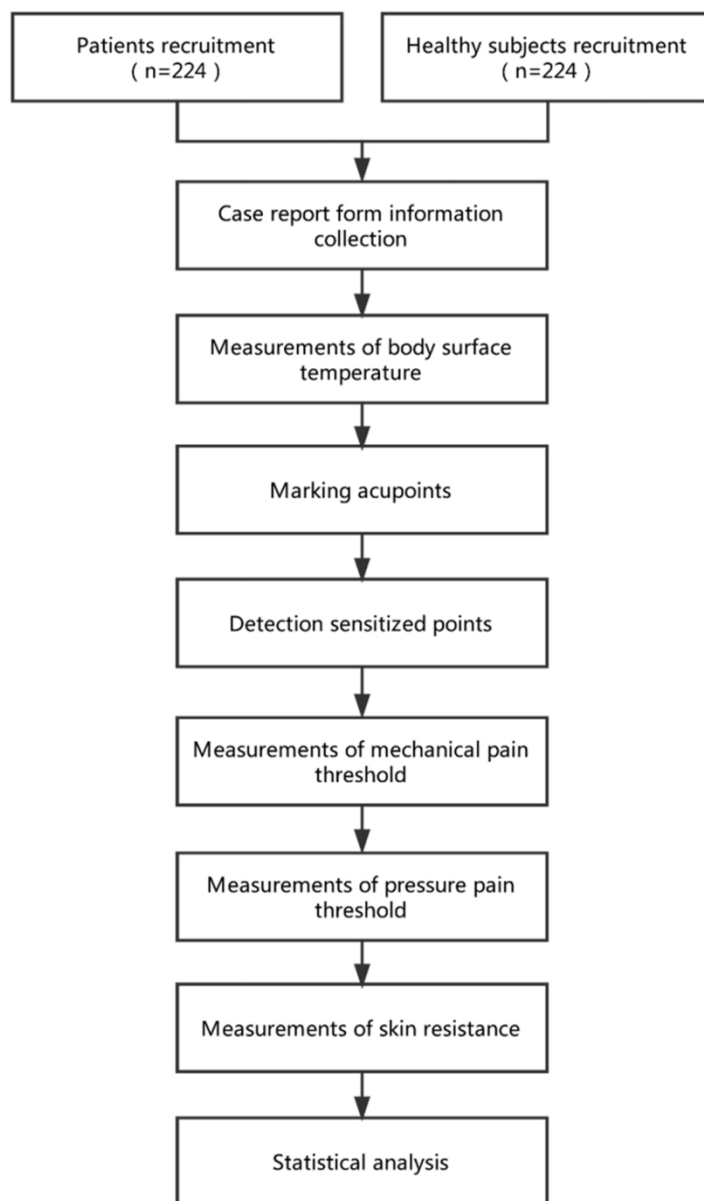
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Figure 1 Flowchart of the study design.

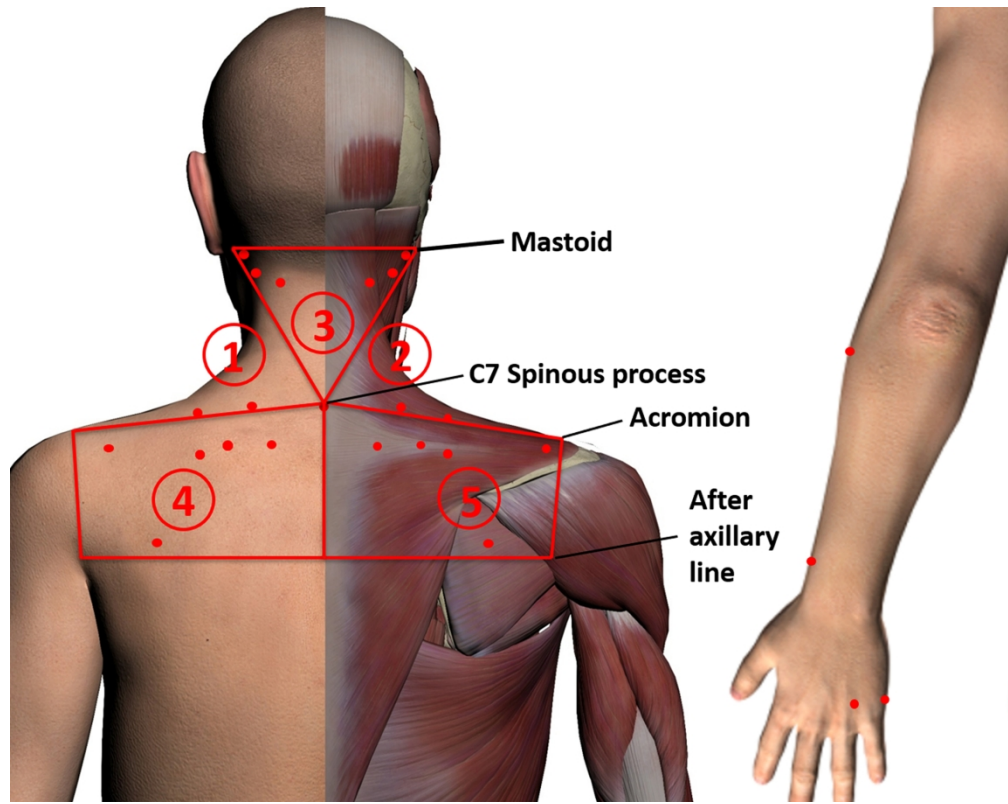
Figure 2 The test regions and acupoints that will be used in the study.



45 Flowchart of the study design.

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The test regions and acupoints that will be used in the study.

90x71mm (600 x 600 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a multicenter, matched, case-control study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	The present study aims to identify the types of sensitization (e.g. temperature, tenderness) and distribution of sensitized points in patients with NP, to analyze the cutoff values and sensitization rate for acupoint sensitization in patients, and to summarize the dominant forms of optimal sensitized points in patients. This information will be helpful in the choice of optimal points for the treatment of NP.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	Recent Chinese research has found that acupoints can be sensitized, with associated changes in function and size, and thus can functionally switch from a relatively 'silent' state to a relatively 'active' state. Treatment of an acupoint while it is in the 'active' state is thought to achieve a better clinical outcome, and this has become a focus in acupoint research.[6-8] There are

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diverse types of sensitivity manifested at sensitized points, including pain-sensitivity, heat-sensitivity, and form-sensitivity (appearance changes). Recent clinical studies report that a superior outcome is obtained using acupuncture at pain-sensitive points,[9] and moxibustion at heat-sensitive points[10] or visual-sensitive points.[11] However, most of the current observational studies have only focused on one form of sensitization in a small sample population, and thus are not comprehensive studies of various forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical practice is still controversial.

Objectives 3 State specific objectives, including any prespecified hypotheses 4

We describe the protocol for an observational study that aims to identify the different types of sensitization (e.g. temperature, tenderness) and the distribution of sensitized acupoints in patients with NP, to analyze the cutoff values for acupoint sensitization in patients with NP, and to determine the most optimal sensitization types seen at sensitized points in patients with NP. We believe that in the

state of disease, the sensitive points occurs with high frequency (manifested as changes in temperature, pain threshold etc), and the sensitive points of different sensitizations will overlap, and the final optimal points from overlap may be closely related to the selection of clinical treatment points. And the different sensations of point may suggest selection of interventions. Previous small sample studies have confirmed the feasibility of sensitization testing.[7] The present study will provide a basis for the selection of the optimal treatment points for NP in clinical practice.

Methods

Study design	4	Present key elements of study design early in the paper	4	This is a multicenter, age- and sex-matched, case-control study. The protocol was developed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6	We will recruit patients from the outpatient departments of Acupuncture and Moxibustion, and Orthopedics in five clinical centers in China: Chengdu University of

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Traditional Chinese Medicine, Hunan University of Traditional Chinese Medicine, Shaanxi University of Traditional Chinese Medicine, Shanxi University of Traditional Chinese Medicine, and Guiyang College of Traditional Chinese Medicine. Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients. Recruitment strategies will include posting advertisements on social media (such as WeChat, which is similar to Facebook) and at community centers, or by word-of-mouth introductions through subjects already enrolled in the study.

Participants 6 (a) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants

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Patients are eligible for study inclusion if they: (1) have no history of NP and/or restricted neck movement; (2) are males or females aged 18–60 years; (3) provide written informed consent for all procedures in this study. Patients or healthy subjects who consent to study participation will be examined and diagnosed by a hospital doctor.

(b) *Cohort study*—For matched studies, give matching criteria and number of exposed and

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Healthy subjects are eligible if

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unexposed

Case-control study—For matched studies, give matching criteria and the number of controls per case

they: (1) have no history of neck pain and/or restricted neck movement; (2) are males or females aged 18–60 years; (3) provide written informed consent for all procedures in this study. Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients. A ratio of control subjects to cases of 1.

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9	Body surface temperature, Mechanical pain threshold, Pressure pain threshold, Skin resistance, Pain, Neck function, General demographic information, Safety and adverse events.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9	Body surface temperature: Each participant will be evaluated in a room with a constant temperature of 26°C. Investigators will use Fotric thermal imaging cameras (Fotric 226, IRS Systems Inc., Allen, TX, USA) to make measurements at each of the 29 acupoints in the five body regions. The temperature data of each point on the images will be analyzed using professional software (AnalyzeIR, IRS Systems Inc., Allen, TX, USA).

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Mechanical pain threshold:
Investigators will use the electronic Von Frey esthesiometer (type 2390, IITC Life Science, Woodland Hills, CA, USA) to make two measurements of the mechanical pain threshold at each of the 29 acupoints in the five body regions in turn. If there is a difference of more than 15 g between the two measurements of mechanical pain threshold made at one acupoint, the mechanical pain threshold at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 10 g/s at each acupoint. The average mechanical pain threshold will be calculated for each acupoint. To reduce the consecutive assessment error for two adjacent points, a method of alternate assessment of acupoints on the left and right sides of the body will be adopted.

Pressure pain threshold:
Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments, Greenwich, CT, USA) to make two measurements of the PPT at each of the 29 acupoints in the five body regions in turn. If there is a

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difference of more than 500 gf between the two PPT measurements performed at one acupoint, the PPT at that acupoint will be measured a third time. Progressive pressure will be applied at a rate of 100 gf/s at each acupoint. The average PPT will be calculated for each acupoint. A method of alternate assessment of the acupoints on the left and right sides of the body will again be adopted.

Skin resistance: Investigators will use the Acupoint dynamics research instrument (LMR30-RIII, Peking University, Beijing, China) to record the skin resistance at nine acupoints (GB-21, GB-20, BL-11, LI-10, SI-3; bilateral) and at the two most sensitive points (if there are two sensitive points).

Pain: Neck pain will be measured using the Visual Analogue Scale, the Northwick Park Neck Pain Questionnaire, and the McGill Pain Questionnaire. The Visual Analogue Scale will be used to measure the severity of pain. The Northwick Park Neck Pain Questionnaire provides a reliable outcome measure for patients with

neck pain, while the McGill Pain Questionnaire is used to measure the different qualities of the subjective pain experience; these questionnaires have been proven valid, and are convenient for patients to complete.

Neck function: The change in neck function will be evaluated by measuring the cervical range of motion before and after treatment.

General demographic information This will include the collection of data regarding age, disease duration, medical history, and medication type and dosage.

Safety and adverse events: Non-invasive assessments of the body surface do not generally cause adverse events. However, we will still record the number and type (e.g. serious pain, fainting) of adverse events in each group.

Participants who experience adverse events will receive the appropriate intervention. Adverse events will be immediately reported to the primary investigator and the ethics committee, and the affected participants will be withdrawn from the study.

Bias	9	Describe any efforts to address potential sources of bias	6, 10, 11	All investigators will attend
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training to make sure all practices are standardized. And the investigator was not told whether the subject was a patient or a healthy controls.

Data will be blinded, double-entered in EpiData 3.1 software, and adequately checked for errors. To ensure the integrity of the study and protect the rights and health of the participants, we will set up a Data and Safety Monitoring Board. Every 3 months, members of the quality control group will conduct a quality control review at each study site, and produce a report regarding the quality analysis of the whole data collection process.

Study size 10 Explain how the study size was arrived at

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Previous study indicated that the rate of acupoint sensitization in patients ranged from 20% to 70%[26], so we set this rate at 50% to calculate the minimum sample size required for the proposed study. With acupoint sensitization rate of 20% in healthy subjects, the odds ratio is 4. According to Chow's formula in comparing two sample proportion[27], we assumed an α level of 0.05, a β of 0.01, a delta of 0.3 (50%-20%=30%), thus, the smallest sample size is 408 with

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				two-sided confidence and a ratio of control subjects to cases of 1 (TrialSize package in R software). Considering the potential non-response rate and sampling effectiveness as 10%, the final sample size is 448 (224 patients, plus 224 age- and sex-matched healthy subjects).
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10	Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon’s signed rank test) will be used.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10	First, the distribution of basic information in the case and control groups will be described, including age, sex, height, weight, job occupation, and education level. Data will be presented as means (SD) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent the skin morphological changes. The distributions of the intensity of NP and neck function will also be described. Hypothetical testing will then be performed to assess the differences between the

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case and control groups in body surface sensations and biophysical properties, including body surface temperature, mechanical pain threshold, PPT, and skin resistance. Parametric statistical testing (t-test) will be used for data that are normally distributed; otherwise, non-parametric statistical testing (Wilcoxon's signed rank test) will be used. If there is a difference between the case and control groups in body surface temperature, mechanical pain threshold, PPT, or skin resistance, the cut-off values for these indexes to distinguish between sensitive and non-sensitive states (including heat-sensitivity, pain-sensitivity, and electrical-sensitivity) will be detected using receiver operating characteristic curve analyses. Finally, the acupoint sensitization rates of patients and healthy subjects will be calculated separately regarding the aspects of heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints. Meanwhile, OR represents the ratio of acupoint sensitization of patients and healthy subjects. Therefore, this study

				combines OR and sensitization rate of all acupoints to find out the optimal points in each sensitization.
		(b) Describe any methods used to examine subgroups and interactions	N/A	
		(c) Explain how missing data were addressed	10	Missing data will be processed without imputation.
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	5,6	Healthy subjects without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients.
		(e) Describe any sensitivity analyses	N/A	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A	
		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram	N/A	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A	
		(b) Indicate number of participants with missing data for each variable of interest	N/A	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	N/A	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	N/A	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A	
		(b) Report category boundaries when continuous variables were categorized	N/A	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A	
Discussion				

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2	Key results	18	Summarise key results with reference to study objectives	N/A
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4	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
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22	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/A
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25	Generalisability	21	Discuss the generalisability (external validity) of the study results	3
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38	Other information			
39	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12
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Science Foundation of China (no. 81590950, 81590951, 81722050).

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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