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Complete List of Authors:	Sun, Mingsheng; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Tao, Siyuan; Sichuan University, West China School of Public Health Geng, Guoyan; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Peng, Jieru; Sichuan University, West China School of Public Health Ma, Xingsha; Chengdu University of Traditional Chinese Medicine Yan, Mingxi; Chengdu University of Traditional Chinese Medicine Chen, Jiao; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Cai, Dingjun; Chengdu University of Traditional Chinese Medicine Zheng, Hui ; Chengdu University of Traditional Chinese Medicine, Yang, Chunxia; Sichuan University, West China School of Public Health Zhao, Ling; Chengdu University of Traditional Chinese Medicine, Acupuncture and Tuina School Liang, Fan-rong; Chengdu University of Traditional Chinese Medicine, Acu-moxibustion and Tuina school; Chengdu University of Traditional Chinese Medicine
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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

Ming-Sheng Sun [1†], Si-Yuan Tao[2†], Guo-Yan Geng[1†], Jie-Ru Peng[2], Xing-Sha Ma[1], Ming-Xi Yan[1], Jiao Chen[1], Ding-Jun Cai[1], Hui Zheng[1], Chun Xia Yang[2], Ling Zhao[1*], Fan-Rong Liang[1*]

† Ming-Sheng Sun, Si-Yuan Tao, and Guo-Yan Geng contributed equally to this work.

[1] College of Acupuncture and Tuina, Chengdu University of Traditional Chinese Medicine, Chengdu, 610075, China

[2] West China School of Public Health, Sichuan University, Chengdu, 610041, China

***Corresponding authors**

Professor Ling Zhao

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13568801429

Email: ling94@163.com

Professor Fan-Rong Liang

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13608058216

Email: acuresearch@126.com

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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.

► 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.

INTRODUCTION

Neck pain is a common clinical condition that is often accompanied by tenderness at sensitive points and limited function. The lifetime prevalence of neck pain is reportedly 48.5%.[1] Neck pain can be caused by cervical spondylosis, which is a common disease in China with a prevalence of 8.1–19.1%.[2] Neck pain imposes considerable personal and socioeconomic burdens. Furthermore, the incidence of cervical spondylosis has been increasing in recent years due to changes in work and lifestyle habits, leading to a prolonged work life and lifespan; this increased incidence has become a social concern. Neck pain is often treated with muscle relaxants and non-steroidal anti-inflammatory drugs; 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 neck pain. 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. 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 neck pain 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 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.[7] The present study will provide a basis for the selection of the optimal treatment points for neck pain in clinical practice.

METHODS AND ANALYSIS

Study design

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

Ethics

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

Patients and healthy subjects

Inclusion criteria

Patients are eligible for study inclusion if they: (1) have simple neck pain with symptoms that meet

the diagnostic criteria for cervical spondylosis; (2) are males or females aged 18–60 years; (3) provide written informed consent for all procedures in this study.

Healthy subjects are eligible if 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.

Exclusion criteria

Patients are not eligible if they: (1) have complicated neck or shoulder pain caused by cervical and intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder periarthritis, rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic system; (4) have difficulty in answering the questionnaires because of cognitive impairment; (5) have dermatopathic diseases; (6) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Healthy subjects are not eligible if they: (1) have serious disease related to the heart, liver, kidney, or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty in answering the questionnaires because of cognitive impairment; (4) have dermatopathic diseases; (5) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Recruitment strategies

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 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. 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 7th 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 7th cervical vertebra
Dazhu(BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1st thoracic vertebra
Jianwaishu(SI-14)	On the back,3 cun lateral to the lower border of the spinous process of the first thoracic vertebra
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
Jugu(LI-16)	In the upper portion of the shoulder, in the depression between the acromial end of clavicle and the scapular spine
Tianzong(SI-11)	In the region of the scapula, in the depression of the center of the subscapular fossa, on a level with the 4th thoracic vertebra
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 cudital crease
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
Zhongzhu(SJ-3)	On the dorsum of the hand, in the depression between the 4th and 5th metacarpal bones, proximal to the 4th metacarpalangeal joint
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

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 (AnalyzIR, 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 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

The pressure pain threshold (PPT) is widely used in clinical practice as a semi-objective method with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-fibers.[21] 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.[24 25]

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.

Sample size calculation

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

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

undergo training in accordance with the standard operating procedure of this study. 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.

DISCUSSION

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

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

As this will be the first study to evaluate the association between acupoint sensitization and neck pain, this observational study may have some limitations. 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; thus, quality control reviews will be conducted every 3 months.

In conclusion, this article describes the design and protocol of a study that aims to observe 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 identify the most prevalent sensitized forms of the sensitized points in patients with neck pain. The results will provide a basis for the selection of clinically optimal stimulation sites for the treatment of neck pain.

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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.

REFERENCES

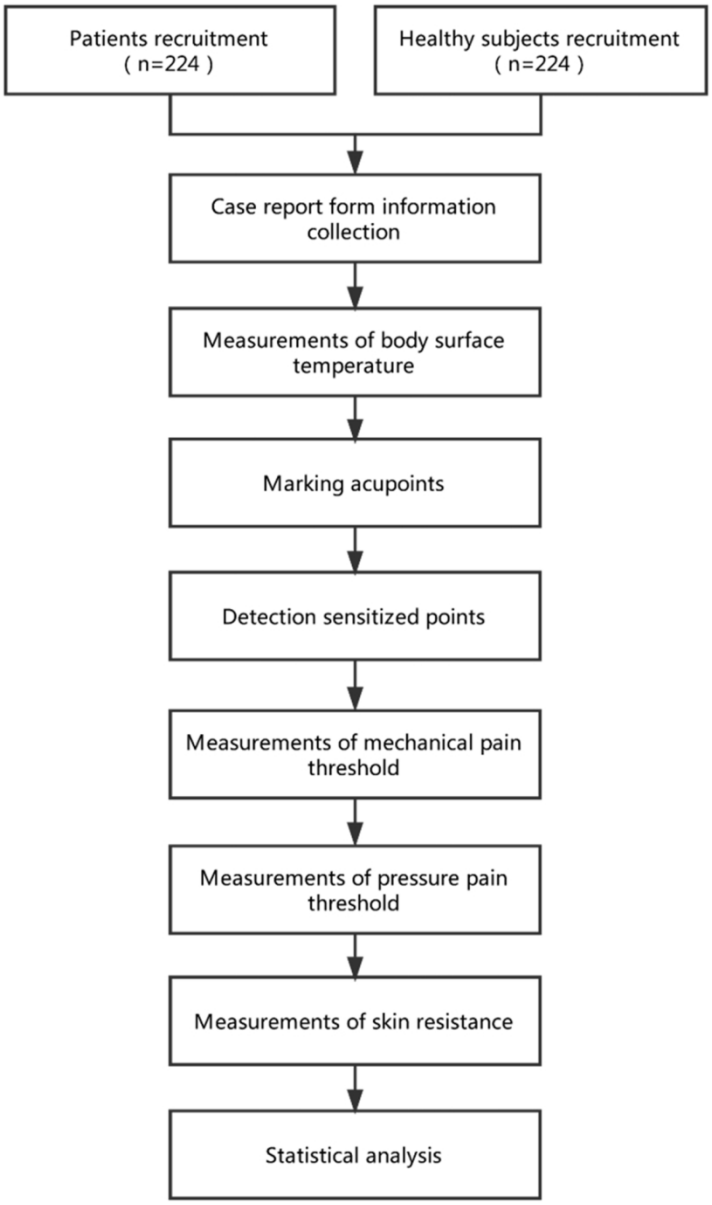
1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 2006;**15**(6):834-48 doi: 10.1007/s00586-004-0864-4[published Online First: Epub Date]|.
2. Guidelines for grading diagnosis and treatment of cervical spondylosis in Anhui Province, China（安徽省颈椎病分级诊疗指南）. Anhui Medical Journal 2017;**38**(9)
3. White AR, Ernst E. A systematic review of randomized controlled trials of acupuncture for neck pain. Rheumatology (Oxford, England) 1999;**38**(2):143-7
4. Mejuto-Vazquez MJ, Salom-Moreno J, Ortega-Santiago R, et al. Short-term changes in neck pain, widespread pressure pain sensitivity, and cervical range of motion after the application of trigger point dry needling in patients with acute mechanical neck pain: a randomized clinical trial. The Journal of orthopaedic and sports physical therapy 2014;**44**(4):252-60 doi: 10.2519/jospt.2014.5108[published Online First: Epub Date]|.
5. Irnich D, Behrens N, Gleditsch JM, et al. Immediate effects of dry needling and acupuncture at distant points in chronic neck pain: results of a randomized, double-blind, sham-controlled crossover trial. Pain 2002;**99**(1-2):83-9
6. Yan CQ, Zhang S, Li QQ, et al. Detection of peripheral and central sensitisation at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study. BMJ open 2017;**7**(6):e014438
7. Luo YN, Zhou YM, Zhong X, et al. Observation of Pain-Sensitive Points in Patients with Knee Osteoarthritis: A Pilot Study. European Journal of Integrative Medicine 2018
8. Chen RX, Kang MF. Acupoint heat-sensitization and its clinical significance. Journal of Traditional Chinese Medicine 2006;**2001**(4):488-9
9. Yao ZL, Liu F, Song NC. 40 Cases of Neck Type of Cervical Spondylosis Treated by Acupotomy Based on the Theory of Trigger point(基于激痛点理论应用针刀治疗颈型颈椎病 40 例). Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(11):1155-55
10. Zhang H, Xu N, Huang R, et al. Heat-Sensitive Moxibustion in Treatment of Cervical Spondylosis. Liaoning Journal of Traditional Chinese Medicine 2016(12):2631-34
11. Xiao L, Meng X, Du S, et al. Therapeutic Observation of Heavy-dose Moxibustion at Sha-petechiae for Neck-type Cervical Spondylosis Due to Cold Coagulation and Blood Stagnation. Shanghai Journal of Acupuncture and Moxibustion 2017(11):1336-39

12. von Elm E, Altman DG, Egger M, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ (Clinical research ed) 2007;**335**(7624):806-8 doi: 10.1136/bmj.39335.541782.AD[published Online First: Epub Date]|.
13. Croft PR, Lewis M, Papageorgiou AC, et al. Risk factors for neck pain: a longitudinal study in the general population. Pain 2001;**93**(3):317-25
14. Falla D, Lindstrøm R, Rechter L, et al. Effectiveness of an 8-week exercise programme on pain and specificity of neck muscle activity in patients with chronic neck pain: A randomized controlled study. European Journal of Pain 2013;**17**(10):1517-28
15. Cagnie B, Castelein B, Pollie F, et al. Evidence for the Use of Ischemic Compression and Dry Needling in the Management of Trigger Points of the Upper Trapezius in Patients with Neck Pain: A Systematic Review. American journal of physical medicine & rehabilitation 2015;**94**(7):573-83 doi: 10.1097/phm.0000000000000266[published Online First: Epub Date]|.
16. Ring EF, Ammer K. Infrared thermal imaging in medicine. Physiological measurement 2012;**33**(3):R33-46 doi: 10.1088/0967-3334/33/3/r33[published Online First: Epub Date]|.
17. Adamczyk JG, Krasowska I, Boguszewski D, et al. The use of thermal imaging to assess the effectiveness of ice massage and cold-water immersion as methods for supporting post-exercise recovery. Journal of thermal biology 2016;**60**:20-5 doi: 10.1016/j.jtherbio.2016.05.006[published Online First: Epub Date]|.
18. Neto MLP, Maciel LYS, Cruz KML, et al. Does electrode placement influence tens-induced antihyperalgesia in experimental inflammatory pain model? Brazilian journal of physical therapy 2017;**21**(2):92-99 doi: 10.1016/j.bjpt.2017.03.003[published Online First: Epub Date]|.
19. Yamamoto S, Ono H, Kume K, et al. Oxaliplatin treatment changes the function of sensory nerves in rats. Journal of pharmacological sciences 2016;**130**(4):189-93 doi: 10.1016/j.jphs.2015.12.004[published Online First: Epub Date]|.
20. Chen Y, Yao Y, Wu Y, et al. Transcutaneous electric acupoint stimulation alleviates remifentanil-induced hyperalgesia in patients undergoing thyroidectomy: a randomized controlled trial. International journal of clinical and experimental medicine 2015;**8**(4):5781-7
21. Beissner F, Brandau A, Henke C, et al. Quick discrimination of A(delta) and C fiber mediated pain based on three verbal descriptors. PloS one 2010;**5**(9):e12944 doi: 10.1371/journal.pone.0012944[published Online First: Epub Date]|.
22. Persson AL, Brogårdh C, Sjölund BH. Tender or not tender: test-retest repeatability of pressure pain thresholds in the trapezius and deltoid muscles of healthy women. Journal of rehabilitation medicine 2004;**36**(1):17
23. Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987;**30**(1):115-26
24. Chiu TT, Lam TH, Hedley AJ. Subjective health measure used on Chinese patients with neck pain in Hong Kong. Spine 2001;**26**(17):1884-9
25. Burckhardt CS, Jones KD. Adult measures of pain: The McGill Pain Questionnaire (MPQ), Rheumatoid Arthritis Pain Scale (RAPS), Short - Form McGill Pain Questionnaire (SF - MPQ), Verbal Descriptive Scale (VDS), Visual Analog Scale (VAS), and West Haven - Yale Multidisciplinary Pain. Arthritis Care & Research 2010;**49**(S5):0-0
26. Hoy D, March L, Woolf A, et al. The global burden of neck pain: estimates from the global burden of disease 2010 study. Annals of the rheumatic diseases 2014;**73**(6):968

27. Andersson S, Lundeberg T. Acupuncture--from empiricism to science: functional background to acupuncture effects in pain and disease. Medical hypotheses 1995;**45**(3):271-81
28. Zhang S, Wang W, Zou L, et al. [Acupoints heat-sensitive moxibustion in the application of traditional Chinese surgery]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(1):109-12
29. Zhou S, Yin H, Huai M. Progress and Prospect of the Study on the Correlation Mechanism of Surface and Visceral Phase. Acupuncture Research 1982(02)
30. Shui YL, Institute QH, Pasadena. Infrared imaging system as a versatile diagnostic and auxiliary treatment tool in Chinese Medicine.
31. Chen S, Miao Y, Nan Y, et al. The Study of Dynamic Characteristic of Acupoints Based on the Primary Dysmenorrhea Patients with the Tenderness Reflection on Diji (SP 8). Evidence-Based Complementray and Alternative Medicine,2015,(2015-6-16) 2015;**2015**:158012
32. Chae Y, Kim HY, Lee HJ, et al. The alteration of pain sensitivity at disease-specific acupuncture points in premenstrual syndrome. Journal of Physiological Sciences 2007;**57**(2):115-19
33. Tunks E, Crook J, Norman G, et al. Tender points in fibromyalgia. Pain 1988;**34**(1):11

Figure 1 Flowchart of the study design.

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



Flowchart of the study design.

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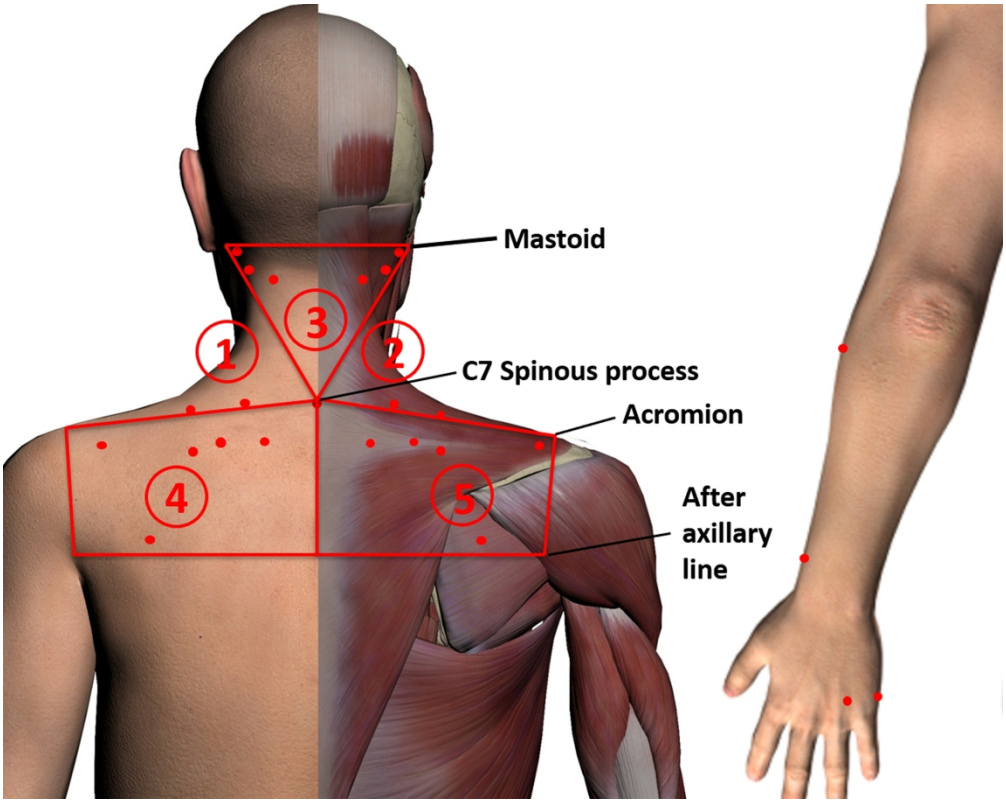

The test regions and acupoints that will be used in the study.

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 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

				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

				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	4, 5, 9, 10	Patients are eligible for study inclusion if they: (1) have simple neck pain with symptoms that meet the diagnostic criteria for cervical spondylosis; (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 unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	5, 9, 10	Healthy subjects are eligible if 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 (AnalyzIR, 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

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.

				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 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	Sample size calculations for a matched case-control study design will be performed using PASS 11. Few previous studies have evaluated acupoint sensitization, especially in healthy subjects. Our previous small sample-sized study indicated that the rate of acupoint sensitization ranged from 20% to 70%, and so we set this rate at 50% to calculate the minimum sample size required for the proposed study. With a rate of healthy subjects of 20%, the odds ratio is 4. Thus, the smallest sample size is 108 with two-sided confidence, α=0.05, β=0.01, and a ratio of control subjects to cases of 1. Considering the potential non-response rate and sampling effectiveness, the final smallest sample size is 224 patients, plus 224 age- and sex-matched healthy subjects.
Quantitative	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which	10	Parametric statistical testing (t-test)

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

				(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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	
		Cross-sectional study—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.

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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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Complete List of Authors:	Sun, Mingsheng; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Tao, Siyuan; Sichuan University, West China School of Public Health Geng, Guoyan; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Peng, Jieru; Sichuan University, West China School of Public Health Ma, Xingsha; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Yan, Mingxi; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Chen, Jiao; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Cai, Dingjun; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Zheng, Hui ; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Yang, Chunxia; Sichuan University, West China School of Public Health Zhao, Ling; Chengdu University of Traditional Chinese Medicine, Acupuncture and Tuina School Liang, Fan-rong; Chengdu University of Traditional Chinese Medicine
Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Complementary medicine
Keywords:	sensitized points, neck pain, study protocol, observational study

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

Ming-Sheng Sun [1†], Si-Yuan Tao[2†], Guo-Yan Geng[1†], Jie-Ru Peng[2], Xing-Sha Ma[1], Ming-Xi Yan[1], Jiao Chen[1], Ding-Jun Cai[1], Hui Zheng[1], Chun Xia Yang[2], Ling Zhao[1*], Fan-Rong Liang[1*]

† Ming-Sheng Sun, Si-Yuan Tao, and Guo-Yan Geng contributed equally to this work.

[1] College of Acupuncture and Tuina, Chengdu University of Traditional Chinese Medicine, Chengdu, 610075, China

[2] West China School of Public Health, Sichuan University, Chengdu, 610041, China

***Corresponding authors**

Professor Ling Zhao

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13568801429

Email: ling94@163.com

Professor Fan-Rong Liang

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13608058216

Email: acuresearch@126.com

Word count: 2779 words

Keywords: sensitized points, neck pain, study protocol, observational study

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

were included; patients with other NP with radiating pain or secondary NP, 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.

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 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. 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 AND ANALYSIS

Study design

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.[12] The study has been registered with ChiCTR at Current Controlled Trials (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.

Ethics

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

Patients and healthy subjects

Inclusion criteria

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

consent for all procedures in this study.

Healthy subjects are eligible 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.

Exclusion criteria

Patients are not eligible if they: (1) have complicated neck or shoulder pain caused by cervical and intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder periarthritis, rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic system; (4) have difficulty in answering the questionnaires because of cognitive impairment; (5) have dermatopathic diseases; (6) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Healthy subjects are not eligible if they: (1) have serious disease related to the heart, liver, kidney, or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty in answering the questionnaires because of cognitive impairment; (4) have dermatopathic diseases; (5) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Recruitment strategies

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 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. 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 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 7th 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 7th cervical vertebra
Dazhu(BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1st thoracic vertebra
Jianwaishu(SI-14)	On the back,3 cun lateral to the lower border of the spinous process of the first thoracic vertebra
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
Jugu(LI-16)	In the upper portion of the shoulder, in the depression between the acromial end of clavicle and the scapular spine
Tianzong(SI-11)	In the region of the scapula, in the depression of the center of the subscapular fossa, on a level with the 4th thoracic vertebra
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 cudital crease
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
Zhongzhu(SJ-3)	On the dorsum of the hand, in the depression between the 4th and 5th metacarpal bones, proximal to the 4th metacarpalangeal joint
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

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 (AnalyzIR, 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 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

The pressure pain threshold (PPT) is widely used in clinical practice as a semi-objective method with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-fibers.[21] 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

NP 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 NP, 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.[24 25]

Neck function

The change in neck function will be evaluated by measuring the 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 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.

Patients and public involvement

Patients and public were not involved.

Sample size calculation

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, thus, the smallest sample size is 408 with 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).

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

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 undergo training in accordance with the standard operating procedure of this study. 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.

DISCUSSION

NP is the third-most common chronic pain condition, and the fourth leading cause of disability worldwide.[28] Acupuncture is a popular non-pharmacological modality used to relieve pain. The latest research has found that the performance of acupuncture at sensitive points may provide the most effective treatment.[8 29] When the body is in a diseased state, there will be morphological form-sensitive point changes, such as nodules, pimples, uplifting, dimpling, and changes in skin color.[30] Thermal image detection has proved that the temperature of corresponding acupoints will be obviously abnormal when a patient has visceral illness, such as diseases of the heart, lung, and stomach, which indicates that the temperature of acupoints can reflect the physiological and pathological phenomena of the affected organs.[31] Studies have confirmed that the PPT at acupoints changes when patients are in a diseased state.[6 32 33] The degree of change in the PPT may reflect the intensity of acupoint sensitization, and may be related to the disease status.[34] This proposed observational study will bridge the knowledge gap regarding the optimal sensitization of acupoints in various forms of point sensitivity in patients with NP.

As this will be the first study to evaluate the association between acupoint sensitization and NP, this observational study may have some limitations. 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 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located in four different regions in China, it may be difficult to implement quality control; thus, quality control reviews will be conducted every 3 months.

In conclusion, this article describes the design and protocol of a study that aims to observe 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 identify the dominant sensitized forms of the sensitized points in patients with NP. The results will provide a basis for the selection of clinically optimal acupuncture points for NP.

Acknowledgments

We thank the staff of Chengdu CTC Tianfu Digital Technology Inc. for providing images. We acknowledge the help and contributions from the subjects, investigators, and experts in each center. We thank Kelly Zammit, BVSc, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

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.

Funding

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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.

REFERENCES

1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 2006;**15**(6):834-48 doi: 10.1007/s00586-004-0864-4[published Online First: Epub Date]|.
2. Guidelines for grading diagnosis and treatment of cervical spondylosis in Anhui Province, China（安徽省颈椎病分级诊疗指南）. Anhui Medical Journal 2017;**38**(9)
3. White AR, Ernst E. A systematic review of randomized controlled trials of acupuncture for neck pain. Rheumatology (Oxford, England) 1999;**38**(2):143-7
4. Mejuto-Vazquez MJ, Salom-Moreno J, Ortega-Santiago R, et al. Short-term changes in neck pain, widespread pressure pain sensitivity, and cervical range of motion after the application of trigger point dry needling in patients with acute mechanical neck pain: a randomized clinical trial. The Journal of orthopaedic and sports physical therapy 2014;**44**(4):252-60 doi: 10.2519/jospt.2014.5108[published Online First: Epub Date]|.
5. Irnich D, Behrens N, Gleditsch JM, et al. Immediate effects of dry needling and acupuncture at distant points in chronic neck pain: results of a randomized, double-blind, sham-controlled crossover trial. Pain 2002;**99**(1-2):83-9
6. Yan CQ, Zhang S, Li QQ, et al. Detection of peripheral and central sensitisation at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study. BMJ open 2017;**7**(6):e014438 doi: 10.1136/bmjopen-2016-014438[published Online First: Epub Date]|.
7. Luo YN, Zhou YM, Zhong X, et al. Observation of Pain-Sensitive Points in Patients with Knee Osteoarthritis: A Pilot Study. European Journal of Integrative Medicine 2018
8. Chen RX, Kang MF. Acupoint heat-sensitization and its clinical significance. Journal of Traditional Chinese Medicine 2006;**2001**(4):488-9
9. Yao ZL, Liu F, Song NC. 40 Cases of Neck Type of Cervical Spondylosis Treated by Acupotomy Based on the Theory of Trigger point(基于激痛点理论应用针刀治疗颈型颈椎病 40 例). Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(11):1155-55
10. Zhang H, Xu N, Huang R, et al. Heat-Sensitive Moxibustion in Treatment of Cervical Spondylosis. Liaoning Journal of Traditional Chinese Medicine 2016(12):2631-34
11. Xiao L, Meng X, Du S, et al. Therapeutic Observation of Heavy-dose Moxibustion at Sha-petechiae for Neck-type Cervical Spondylosis Due to Cold Coagulation and Blood Stagnation. Shanghai

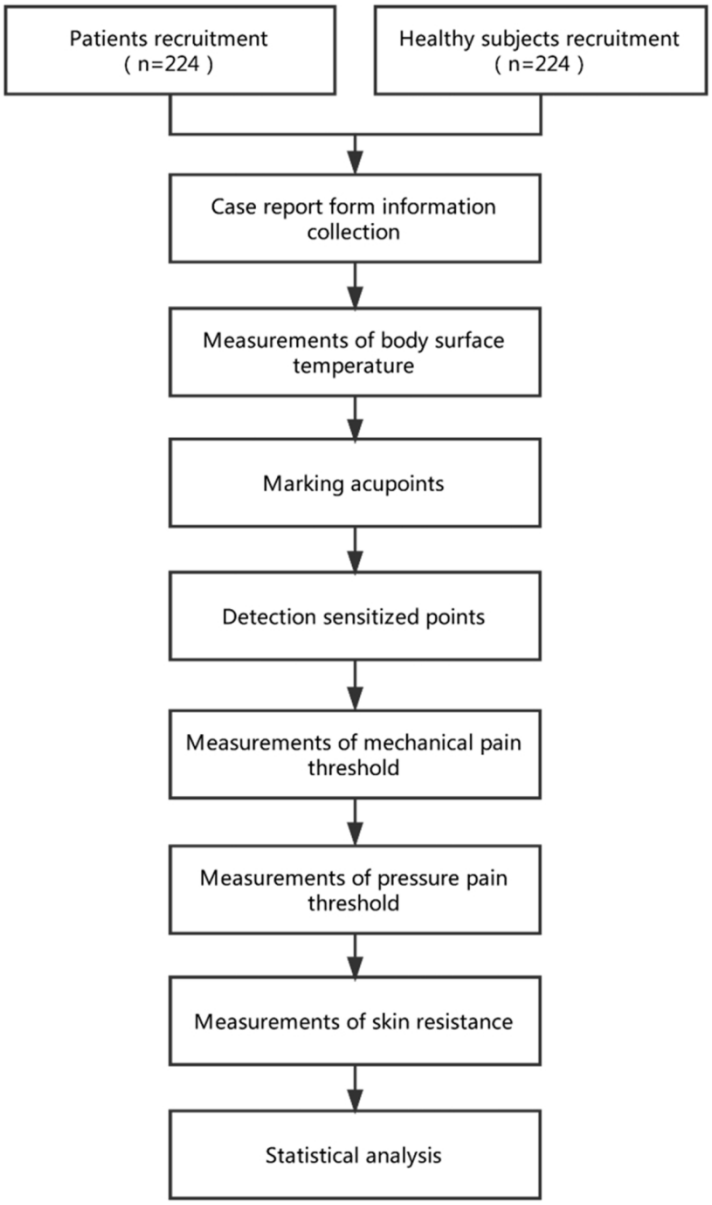
Journal of Acupuncture and Moxibustion 2017(11):1336-39

12. von Elm E, Altman DG, Egger M, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ (Clinical research ed) 2007;**335**(7624):806-8 doi: 10.1136/bmj.39335.541782.AD[published Online First: Epub Date]|.
13. Croft PR, Lewis M, Papageorgiou AC, et al. Risk factors for neck pain: a longitudinal study in the general population. Pain 2001;**93**(3):317-25
14. Falla D, Lindstrøm R, Rechter L, et al. Effectiveness of an 8-week exercise programme on pain and specificity of neck muscle activity in patients with chronic neck pain: A randomized controlled study. European Journal of Pain 2013;**17**(10):1517-28
15. Cagnie B, Castelein B, Pollie F, et al. Evidence for the Use of Ischemic Compression and Dry Needling in the Management of Trigger Points of the Upper Trapezius in Patients with Neck Pain: A Systematic Review. American journal of physical medicine & rehabilitation 2015;**94**(7):573-83 doi: 10.1097/phm.0000000000000266[published Online First: Epub Date]|.
16. Ring EF, Ammer K. Infrared thermal imaging in medicine. Physiological measurement 2012;**33**(3):R33-46 doi: 10.1088/0967-3334/33/3/r33[published Online First: Epub Date]|.
17. Adamczyk JG, Krasowska I, Boguszewski D, et al. The use of thermal imaging to assess the effectiveness of ice massage and cold-water immersion as methods for supporting post-exercise recovery. Journal of thermal biology 2016;**60**:20-5 doi: 10.1016/j.jtherbio.2016.05.006[published Online First: Epub Date]|.
18. Neto MLP, Maciel LYS, Cruz KML, et al. Does electrode placement influence tens-induced antihyperalgesia in experimental inflammatory pain model? Brazilian journal of physical therapy 2017;**21**(2):92-99 doi: 10.1016/j.bjpt.2017.03.003[published Online First: Epub Date]|.
19. Yamamoto S, Ono H, Kume K, et al. Oxaliplatin treatment changes the function of sensory nerves in rats. Journal of pharmacological sciences 2016;**130**(4):189-93 doi: 10.1016/j.jphs.2015.12.004[published Online First: Epub Date]|.
20. Chen Y, Yao Y, Wu Y, et al. Transcutaneous electric acupoint stimulation alleviates remifentanil-induced hyperalgesia in patients undergoing thyroidectomy: a randomized controlled trial. International journal of clinical and experimental medicine 2015;**8**(4):5781-7
21. Beissner F, Brandau A, Henke C, et al. Quick discrimination of A(delta) and C fiber mediated pain based on three verbal descriptors. PloS one 2010;**5**(9):e12944 doi: 10.1371/journal.pone.0012944[published Online First: Epub Date]|.
22. Persson AL, Brogårdh C, Sjölund BH. Tender or not tender: test-retest repeatability of pressure pain thresholds in the trapezius and deltoid muscles of healthy women. Journal of rehabilitation medicine 2004;**36**(1):17
23. Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987;**30**(1):115-26
24. Chiu TT, Lam TH, Hedley AJ. Subjective health measure used on Chinese patients with neck pain in Hong Kong. Spine 2001;**26**(17):1884-9
25. Burckhardt CS, Jones KD. Adult measures of pain: The McGill Pain Questionnaire (MPQ), Rheumatoid Arthritis Pain Scale (RAPS), Short - Form McGill Pain Questionnaire (SF - MPQ), Verbal Descriptive Scale (VDS), Visual Analog Scale (VAS), and West Haven - Yale Multidisciplinary Pain. Arthritis Care & Research 2010;**49**(S5):0-0
26. Chen R, Kang M. Acupoint Heat-Sensitization and Its Clinical Significance. Journal of Traditional

Chinese Medicine 2006;**2001**(4):488-9
27. Chow SC, Shao J, Wang H. *Sample size calculations in clinical research*. New York: Marcel Dekker, 2003.
28. Hoy D, March L, Woolf A, et al. The global burden of neck pain: estimates from the global burden of disease 2010 study. Annals of the rheumatic diseases 2014;**73**(6):968
29. Zhang S, Wang W, Zou L, et al. [Acupoints heat-sensitive moxibustion in the application of traditional Chinese surgery]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(1):109-12
30. Zhou S, Yin H, Huai M. Progress and Prospect of the Study on the Correlation Mechanism of Surface and Visceral Phase. Acupuncture Research 1982(02)
31. Shui YL, Institute QH, Pasadena. Infrared imaging system as a versatile diagnostic and auxiliary treatment tool in Chinese Medicine.
32. Chen S, Miao Y, Nan Y, et al. The Study of Dynamic Characteristic of Acupoints Based on the Primary Dysmenorrhea Patients with the Tenderness Reflection on Diji (SP 8). Evidence-Based Complementray and Alternative Medicine,2015,(2015-6-16) 2015;**2015**:158012
33. Chae Y, Kim HY, Lee HJ, et al. The alteration of pain sensitivity at disease-specific acupuncture points in premenstrual syndrome. Journal of Physiological Sciences 2007;**57**(2):115-19
34. Tunks E, Crook J, Norman G, et al. Tender points in fibromyalgia. Pain 1988;**34**(1):11

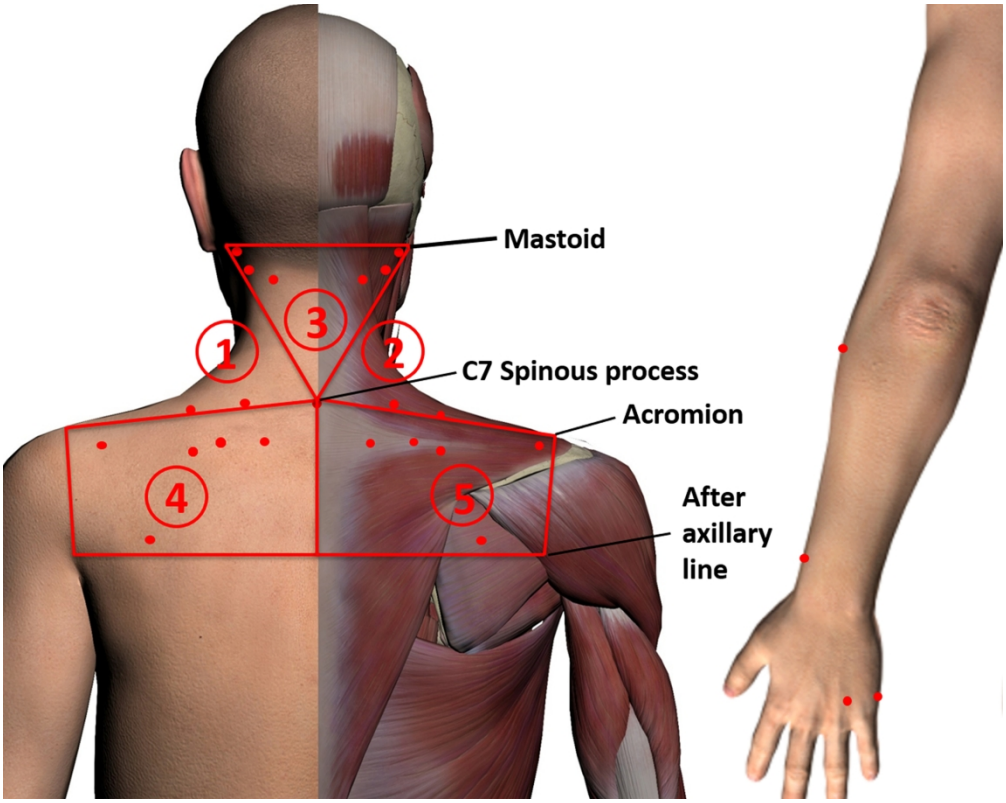
Figure 1 Flowchart of the study design.

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



Flowchart of the study design.

53x89mm (600 x 600 DPI)



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

				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

				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	4, 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.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and	5, 9, 10	Healthy subjects are eligible if

		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 (AnalyzIR, 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 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

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

				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	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

				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

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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	
		Cross-sectional study—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 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located in four different regions in China, it may be difficult to implement quality control; thus, quality control reviews will be conducted every 3 months.
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	
Generalisability	21	Discuss the generalisability (external validity) of the study results	3	As this is the first study of its kind, only patients with nontraumatic NP with mobility deficits were included; patients with other NP with radiating pain or secondary NP, 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.

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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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Complete List of Authors:	Sun, Mingsheng; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Tao, Siyuan; Sichuan University, West China School of Public Health Geng, Guoyan; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Peng, Jieru; Sichuan University, West China School of Public Health Ma, Xingsha; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Yan, Mingxi; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Chen, Jiao; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Cai, Dingjun; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Zheng, Hui ; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Yang, Chunxia; Sichuan University, West China School of Public Health Zhao, Ling; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Moxibustion and Tuina Liang, Fan-rong; Chengdu University of Traditional Chinese Medicine
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Identification of the optimal points for the acupuncture treatment of neck pain in China: protocol for a multicenter, matched, case-control study

Ming-Sheng Sun [1†], Si-Yuan Tao[2†], Guo-Yan Geng[1†], Jie-Ru Peng[2], Xing-Sha Ma[1], Ming-Xi Yan[1], Jiao Chen[1], Ding-Jun Cai[1], Hui Zheng[1], Chun Xia Yang[2], Ling Zhao[1*], Fan-Rong Liang[1*]

† Ming-Sheng Sun, Si-Yuan Tao, and Guo-Yan Geng contributed equally to this work.

[1] College of Acupuncture and Tuina, Chengdu University of Traditional Chinese Medicine, Chengdu, 610075, China

[2] West China School of Public Health, Sichuan University, Chengdu, 610041, China

***Corresponding authors**

Professor Ling Zhao

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13568801429

Email: zhaoling@cdutcm.edu.cn

Professor Fan-Rong Liang

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13608058216

Email: acuresearch@126.com

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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

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. Previous small sample studies have confirmed the feasibility of sensitization testing.[7] The present study will provide evidence for the selection of the optimal treatment points for NP in clinical practice.

METHODS AND ANALYSIS

Study design

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.[12] The study has been registered with ChiCTR at Current Controlled Trials (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.

Ethics

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

Patients and healthy subjects

Inclusion criteria

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

Healthy subjects are eligible 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.

Exclusion criteria

Patients are not eligible if they: (1) have complicated neck or shoulder pain caused by cervical and intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder periarthritis, rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic system; (4) have difficulty in answering the questionnaires because of cognitive impairment; (5) have dermatopathic diseases; (6) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Healthy subjects are not eligible if they: (1) have serious disease related to the heart, liver, kidney, or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty in answering the questionnaires because of cognitive impairment; (4) have dermatopathic diseases; (5) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Recruitment strategies

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 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. 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

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 7th 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 7th cervical vertebra
Dazhu(BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1st thoracic vertebra
Jianwaishu(SI-14)	On the back,3 cun lateral to the lower border of the spinous process of the first thoracic vertebra

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
Jugu(LI-16)	In the upper portion of the shoulder, in the depression between the acromial end of clavicle and the scapular spine
Tianzong(SI-11)	In the region of the scapula, in the depression of the center of the subscapular fossa, on a level with the 4th thoracic vertebra
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 cudital crease
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
Zhongzhu(SJ-3)	On the dorsum of the hand, in the depression between the 4th and 5th metacarpal bones, proximal to the 4th metacarpalangeal joint
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

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 (AnalyzIR, 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

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

The pressure pain threshold (PPT) is widely used in clinical practice as a semi-objective method with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-fibers.[21] 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

NP 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 NP, 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.[24 25]

Neck function

The change in neck function will be evaluated by measuring the 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 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.

Patients and public involvement

Patients and public were not involved.

Sample size calculation

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, thus, the smallest sample size is 408 with 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).

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, odds ratio (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 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 undergo training in accordance with the standard operating procedure of this study. 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.

DISCUSSION

NP is the third-most common chronic pain condition, and the fourth leading cause of disability worldwide.[28] Acupuncture is a popular non-pharmacological modality used to relieve pain. The latest research has found that the performance of acupuncture at sensitive points may provide the most effective treatment.[8 29] When the body is in a diseased state, there will be morphological form-sensitive point changes, such as nodules, pimples, uplifting, dimpling, and changes in skin color.[30] Thermal image detection has proved that the temperature of corresponding acupoints will be obviously abnormal when a patient has visceral illness, such as diseases of the heart, lung, and stomach, which indicates that the temperature of acupoints can reflect the physiological and pathological phenomena of the affected organs.[31] Studies have confirmed that the PPT at acupoints changes when patients are in a diseased state.[6 32 33] The degree of change in the PPT may reflect the intensity of acupoint sensitization, and may be related to the disease status.[34] This proposed observational study will bridge the knowledge gap regarding the optimal sensitization of acupoints in various forms of point sensitivity in patients with NP.

As this will be the first study to evaluate the association between acupoint sensitization and NP, this observational study may have some limitations. 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 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located in four different regions in China, it may be difficult to implement quality control; thus, quality control reviews will be conducted every 3 months.

In conclusion, this article describes the design and protocol of a study that aims to observe 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 identify the dominant sensitized forms of the sensitized points in patients with NP. The 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.

REFERENCES

1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 2006;**15**(6):834-48 doi: 10.1007/s00586-004-0864-4[published Online First: Epub Date]|.
2. Guidelines for grading diagnosis and treatment of cervical spondylosis in Anhui Province, China（安徽省颈椎病分级诊疗指南）. Anhui Medical Journal 2017;**38**(9)
3. White AR, Ernst E. A systematic review of randomized controlled trials of acupuncture for neck pain. Rheumatology (Oxford, England) 1999;**38**(2):143-7
4. Mejuto-Vazquez MJ, Salom-Moreno J, Ortega-Santiago R, et al. Short-term changes in neck pain, widespread pressure pain sensitivity, and cervical range of motion after the application of trigger point dry needling in patients with acute mechanical neck pain: a randomized clinical trial. The Journal of orthopaedic and sports physical therapy 2014;**44**(4):252-60 doi: 10.2519/jospt.2014.5108[published Online First: Epub Date]|.
5. Irnich D, Behrens N, Gleditsch JM, et al. Immediate effects of dry needling and acupuncture at distant points in chronic neck pain: results of a randomized, double-blind, sham-controlled crossover trial. Pain 2002;**99**(1-2):83-9
6. Yan CQ, Zhang S, Li QQ, et al. Detection of peripheral and central sensitisation at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study. BMJ open 2017;**7**(6):e014438 doi: 10.1136/bmjopen-2016-014438[published Online First: Epub Date]|.
7. Luo YN, Zhou YM, Zhong X, et al. Observation of Pain-Sensitive Points in Patients with Knee Osteoarthritis: A Pilot Study. European Journal of Integrative Medicine 2018
8. Chen RX, Kang MF. Acupoint heat-sensitization and its clinical significance. Journal of Traditional Chinese Medicine 2006;**2001**(4):488-9
9. Yao ZL, Liu F, Song NC. 40 Cases of Neck Type of Cervical Spondylosis Treated by Acupotomy Based on the Theory of Trigger point(基于激痛点理论应用针刀治疗颈型颈椎病 40 例). Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(11):1155-55
10. Zhang H, Xu N, Huang R, et al. Heat-Sensitive Moxibustion in Treatment of Cervical Spondylosis. Liaoning Journal of Traditional Chinese Medicine 2016(12):2631-34
11. Xiao L, Meng X, Du S, et al. Therapeutic Observation of Heavy-dose Moxibustion at Sha-petechiae for Neck-type Cervical Spondylosis Due to Cold Coagulation and Blood Stagnation. Shanghai Journal of Acupuncture and Moxibustion 2017(11):1336-39
12. von Elm E, Altman DG, Egger M, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ (Clinical research ed) 2007;**335**(7624):806-8 doi: 10.1136/bmj.39335.541782.AD[published Online First: Epub Date]|.
13. Croft PR, Lewis M, Papageorgiou AC, et al. Risk factors for neck pain: a longitudinal study in the

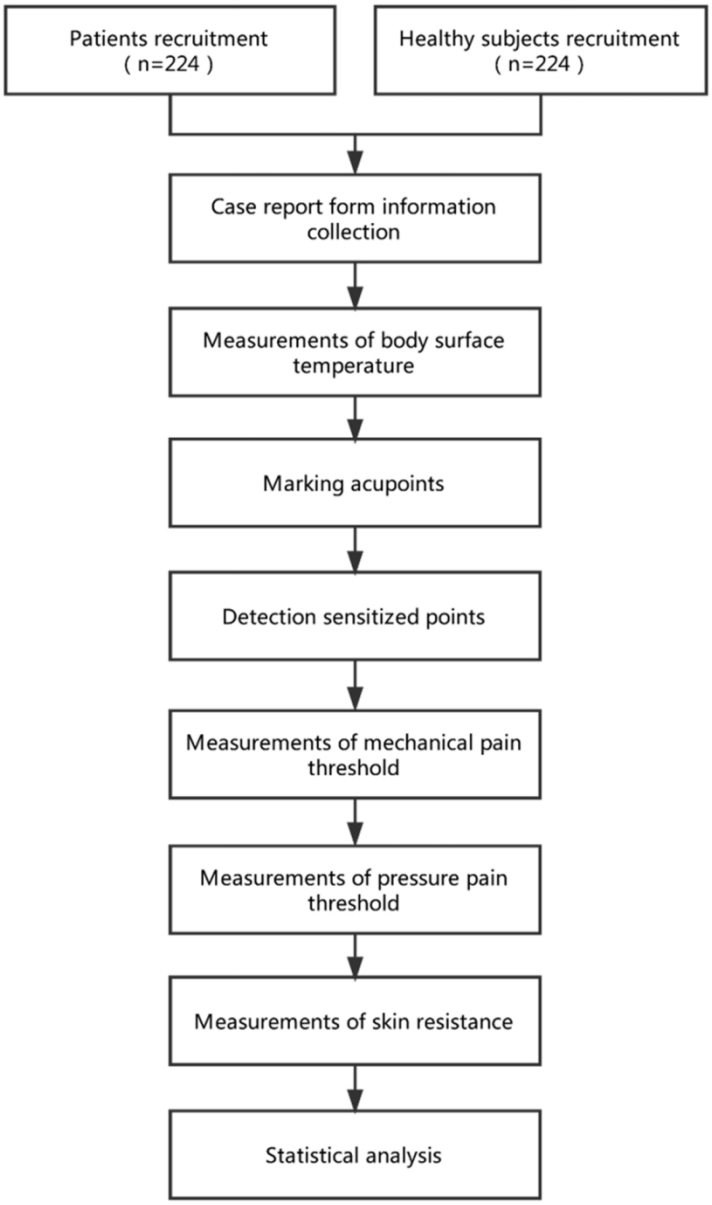
general population. Pain 2001;**93**(3):317-25

14. Falla D, Lindstrøm R, Rechter L, et al. Effectiveness of an 8-week exercise programme on pain and specificity of neck muscle activity in patients with chronic neck pain: A randomized controlled study. European Journal of Pain 2013;**17**(10):1517-28
15. Cagnie B, Castelein B, Pollie F, et al. Evidence for the Use of Ischemic Compression and Dry Needling in the Management of Trigger Points of the Upper Trapezius in Patients with Neck Pain: A Systematic Review. American journal of physical medicine & rehabilitation 2015;**94**(7):573-83 doi: 10.1097/phm.0000000000000266[published Online First: Epub Date]|.
16. Ring EF, Ammer K. Infrared thermal imaging in medicine. Physiological measurement 2012;**33**(3):R33-46 doi: 10.1088/0967-3334/33/3/r33[published Online First: Epub Date]|.
17. Adamczyk JG, Krasowska I, Boguszewski D, et al. The use of thermal imaging to assess the effectiveness of ice massage and cold-water immersion as methods for supporting post-exercise recovery. Journal of thermal biology 2016;**60**:20-5 doi: 10.1016/j.jtherbio.2016.05.006[published Online First: Epub Date]|.
18. Neto MLP, Maciel LYS, Cruz KML, et al. Does electrode placement influence tens-induced antihyperalgesia in experimental inflammatory pain model? Brazilian journal of physical therapy 2017;**21**(2):92-99 doi: 10.1016/j.bjpt.2017.03.003[published Online First: Epub Date]|.
19. Yamamoto S, Ono H, Kume K, et al. Oxaliplatin treatment changes the function of sensory nerves in rats. Journal of pharmacological sciences 2016;**130**(4):189-93 doi: 10.1016/j.jphs.2015.12.004[published Online First: Epub Date]|.
20. Chen Y, Yao Y, Wu Y, et al. Transcutaneous electric acupoint stimulation alleviates remifentanil-induced hyperalgesia in patients undergoing thyroidectomy: a randomized controlled trial. International journal of clinical and experimental medicine 2015;**8**(4):5781-7
21. Beissner F, Brandau A, Henke C, et al. Quick discrimination of A(delta) and C fiber mediated pain based on three verbal descriptors. PloS one 2010;**5**(9):e12944 doi: 10.1371/journal.pone.0012944[published Online First: Epub Date]|.
22. Persson AL, Brogårdh C, Sjölund BH. Tender or not tender: test-retest repeatability of pressure pain thresholds in the trapezius and deltoid muscles of healthy women. Journal of rehabilitation medicine 2004;**36**(1):17
23. Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987;**30**(1):115-26
24. Chiu TT, Lam TH, Hedley AJ. Subjective health measure used on Chinese patients with neck pain in Hong Kong. Spine 2001;**26**(17):1884-9
25. Burckhardt CS, Jones KD. Adult measures of pain: The McGill Pain Questionnaire (MPQ), Rheumatoid Arthritis Pain Scale (RAPS), Short - Form McGill Pain Questionnaire (SF - MPQ), Verbal Descriptive Scale (VDS), Visual Analog Scale (VAS), and West Haven - Yale Multidisciplinary Pain. Arthritis Care & Research 2010;**49**(S5):0-0
26. Chen R, Kang M. Acupoint Heat-Sensitization and Its Clinical Significance. Journal of Traditional Chinese Medicine 2006;**2001**(4):488-9
27. Chow SC, Shao J, Wang H. *Sample size calculations in clinical research*. New York: Marcel Dekker, 2003.
28. Hoy D, March L, Woolf A, et al. The global burden of neck pain: estimates from the global burden of disease 2010 study. Annals of the rheumatic diseases 2014;**73**(6):968
29. Zhang S, Wang W, Zou L, et al. [Acupoints heat-sensitive moxibustion in the application of traditional

Chinese surgery]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(1):109-12
30. Zhou S, Yin H, Huai M. Progress and Prospect of the Study on the Correlation Mechanism of Surface and Visceral Phase. Acupuncture Research 1982(02)
31. Shui YL, Institute QH, Pasadena. Infrared imaging system as a versatile diagnostic and auxiliary treatment tool in Chinese Medicine.
32. Chen S, Miao Y, Nan Y, et al. The Study of Dynamic Characteristic of Acupoints Based on the Primary Dysmenorrhea Patients with the Tenderness Reflection on Diji (SP 8). Evidence-Based Complementray and Alternative Medicine,2015,(2015-6-16) 2015;**2015**:158012
33. Chae Y, Kim HY, Lee HJ, et al. The alteration of pain sensitivity at disease-specific acupuncture points in premenstrual syndrome. Journal of Physiological Sciences 2007;**57**(2):115-19
34. Tunks E, Crook J, Norman G, et al. Tender points in fibromyalgia. Pain 1988;**34**(1):11

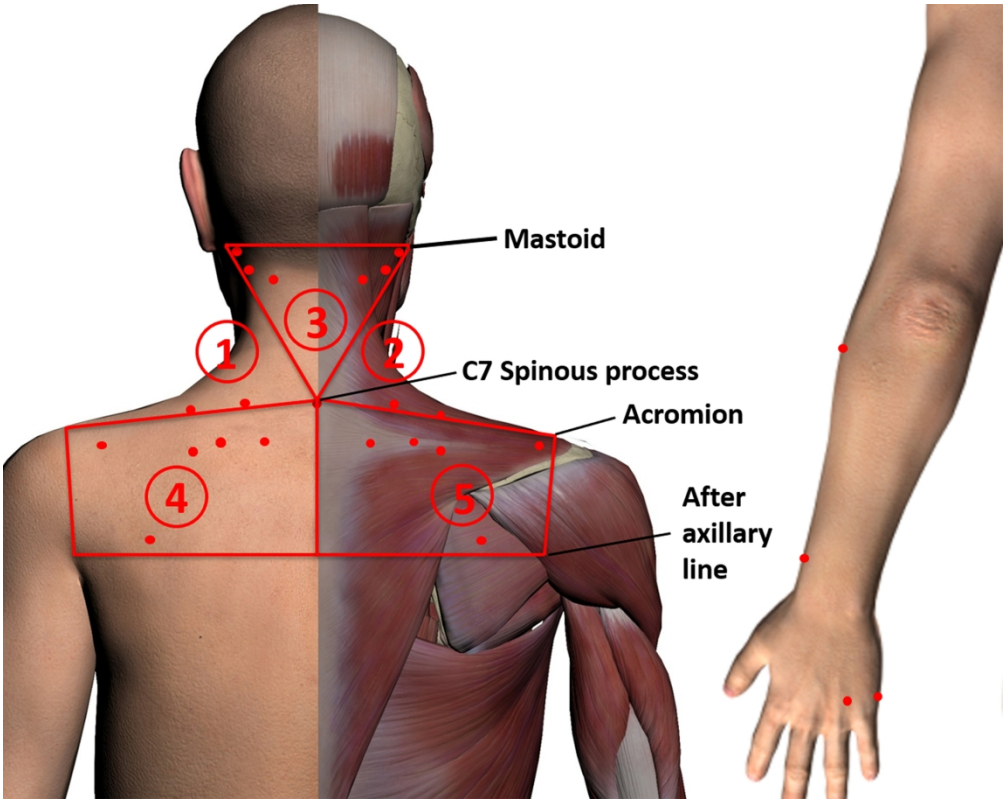
Figure 1 Flowchart of the study design.

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



Flowchart of the study design.

53x89mm (600 x 600 DPI)



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

				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

				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	4, 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.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and	5, 9, 10	Healthy subjects are eligible if

		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 (AnalyzIR, 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 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

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

				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	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

				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

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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	
		Cross-sectional study—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 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located in four different regions in China, it may be difficult to implement quality control; thus, quality control reviews will be conducted every 3 months.
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	
Generalisability	21	Discuss the generalisability (external validity) of the study results	3	As this is the first study of its kind, only patients with nontraumatic NP with mobility deficits were included; patients with other NP with radiating pain or secondary NP, 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.

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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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Complete List of Authors:	Sun, Mingsheng; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Tao, Siyuan; Sichuan University, West China School of Public Health Geng, Guoyan; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Peng, Jieru; Sichuan University, West China School of Public Health Ma, Xingsha; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Yan, Mingxi; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Chen, Jiao; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Cai, Dingjun; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Zheng, Hui ; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Tuina Yang, Chunxia; Sichuan University, West China School of Public Health Zhao, Ling; Chengdu University of Traditional Chinese Medicine, College of Acupuncture and Moxibustion and Tuina Liang, Fan-rong; Chengdu University of Traditional Chinese Medicine
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Secondary Subject Heading:	Complementary medicine
Keywords:	sensitized points, neck pain, study protocol, observational study

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

Ming-Sheng Sun [1†], Si-Yuan Tao[2†], Guo-Yan Geng[1†], Jie-Ru Peng[2], Xing-Sha Ma[1], Ming-Xi Yan[1], Jiao Chen[1], Ding-Jun Cai[1], Hui Zheng[1], Chun Xia Yang[2], Ling Zhao[1*], Fan-Rong Liang[1*]

† Ming-Sheng Sun, Si-Yuan Tao, and Guo-Yan Geng contributed equally to this work.

[1] College of Acupuncture and Tuina, Chengdu University of Traditional Chinese Medicine, Chengdu, 610075, China

[2] West China School of Public Health, Sichuan University, Chengdu, 610041, China

***Corresponding authors**

Professor Ling Zhao

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13568801429

Email: zhaoling@cdutcm.edu.cn

Professor Fan-Rong Liang

Postal address: 37 Shierqiao Road, Chengdu, Sichuan, China

Telephone: +86 13608058216

Email: acuresearch@126.com

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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 preliminarily 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

including only patients with nontraumatic NP with mobility deficits.

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 in white-collar workers in China was 33.9%-54.8% in 2016 and has increased in recent years,[2] imposing considerable personal and socioeconomic burdens. Muscle relaxants and non-steroidal anti-inflammatory drugs are used to treat this condition; however, these medications carry a risk of adverse effects, and neither drug is better than non-pharmacological alternative treatments.[1]

Because non-pharmacological alternative treatments have reliable efficacy and availability, this type of treatment is gaining increasing recognition worldwide. Acupuncture is one type of non-pharmacological alternative treatment that can effectively treat NP. Although several studies have confirmed that acupuncture is effective, outcomes are closely related to point selection.[3] Previous studies have evaluated the use of local acupoints[4] or distal acupoints,[5] with the treatment of both types 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 change 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] Several types of sensitivity manifest 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 visually-sensitive points.[11] However, most of the current observational studies have focused on only one form of sensitization in small-sized sample populations, and thus are not comprehensive studies of different forms of sensitization.[6 10] The choice of optimal treatment points for NP in clinical practice remain 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, and to determine the most optimal sensitization types seen at sensitized points. We believe that in the state of disease, sensitive points

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

METHODS AND ANALYSIS

Study design

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.[12] The study has been registered with ChiCTR at Current Controlled Trials (ChiCTR1800016203). A flowchart of the study design is shown in Figure 1.

Ethics

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

Patients and healthy participants (controls)

Inclusion criteria

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

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

informed consent for all procedures in this study.

Exclusion criteria

Patients are ineligible if they: (1) have complicated neck or shoulder pain caused by cervical and intervertebral disc degeneration, such as other types of cervical spondylosis, shoulder periarthritis, rheumatic myofibrillar inflammation; (2) have history of neck fracture or surgery, or cervical congenital abnormality; (3) have serious disease related to the heart, liver, kidney, or hematopoietic system; (4) have difficulty answering questionnaires because of cognitive impairment; (5) have dermatopathological diseases; or (6) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Healthy participants are ineligible if they: (1) have serious disease related to the heart, liver, kidney, or hematopoietic system; (2) have cervical congenital abnormality; (3) have difficulty answering the questionnaires because of cognitive impairment; (4) have dermatopathological diseases; or (5) are pregnant, breastfeeding, or planning a pregnancy during the study period.

Recruitment strategies

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 participants without neck or shoulder pain will comprise age- and sex-matched residents from the same communities as the patients, to act as controls. 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 participants already enrolled in the study. Patients or healthy participants who consent to study participation will be examined and diagnosed by a hospital doctor.

Test regions, acupoints, and sensitized points

Following 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 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 7th 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 7th cervical vertebra
Dazhu (BL-11)	On the back, 1.5 cun lateral to the lower border of the spinous process of the 1st thoracic vertebra
Jianwaishu (SI-14)	On the back,3 cun lateral to the lower border of the spinous process of the first thoracic vertebra

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
Jugu (LI-16)	In the upper portion of the shoulder, in the depression between the acromial end of clavicle and the scapular spine
Tianzong (SI-11)	In the region of the scapula, in the depression of the center of the subscapular fossa, on a level with the 4th thoracic vertebra
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 cudital crease
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
Zhongzhu (SJ-3)	On the dorsum of the hand, in the depression between the 4th and 5th metacarpal bones, proximal to the 4th metacarpalangeal joint
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

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 (AnalyzIR, 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 Aδ-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

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

Pressure pain threshold

The pressure pain threshold (PPT) is widely used clinically as a semi-objective method with which to quantify localized pain.[22 23] Pain caused by blunt pressure stimuli is related to C-fibers.[21] Investigators will use the FDIX Force Gauge (Force One™ FDIX, Wagner Instruments, Greenwich, CT, USA) to take two PPT measurements 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, and the average PPT will be calculated. An alternate assessment method for the acupoints on the left and right sides of the body will 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, bilateral (GB-21, GB-20, BL-11, LI-10, SI-3) and at the two most sensitive points (if there are two sensitive points).

Pain

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

Neck function

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

and control groups will be described for age, sex, height, weight, occupation, and education level. Data will be presented as means (standard deviation (SD)) for continuous variables, and as frequency (percentage) for categorical variables. The distributions of sensitized points will be shown in scatter plots to represent skin morphological changes. We will also describe the distributions of the intensity of NP and neck function. Hypothetical testing will then be performed to assess the differences between the case and control groups regarding 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 indices 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 the patients and healthy controls will be calculated separately for heat, pain, and electrical sensitivity to identify the most important form of sensitivity for all acupoints. The odds ratio (OR) will represent the ratio of acupoint sensitization in the patients and healthy controls; therefore, we will combine OR and the sensitization rate of all acupoints to determine the optimal points for each sensitization.

Quality control

This is a multi-center observational study; therefore, quality control will play a vital role in extrapolating the conclusions. To ensure the integrity of the study and to 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 undergo training in accordance with the standard operating procedure of this study. Every 3 months, members of the quality control group will perform a quality control review at each study site and

produce a report regarding the quality analysis of the entire data collection process.

DISCUSSION

NP is the third-most common chronic pain condition, and the fourth leading cause of disability worldwide.[28] Acupuncture is a popular non-pharmacological modality to relieve pain. The latest research has reported that acupuncture at sensitive points may provide the most effective treatment.[8 29] When the body is in a diseased state, there are morphological form-sensitive point changes, such as nodules, pimples, uplifting, dimpling, and changes in skin color.[30] Thermal imaging has proven that the temperature of corresponding acupoints will be obviously abnormal when a patient has visceral illness, such as diseases of the heart, lung, and stomach, indicating that the temperature of acupoints can reflect the physiological and pathological phenomena of the affected organs.[31] Studies have confirmed that the PPT at acupoints changes when patients are in a diseased state, [6 32 33] and that the degree of change in the PPT may reflect the intensity of acupoint sensitization, and may be related to the disease status.[34] This proposed observational study will increase knowledge of the different types of acupoint sensitization and the cutoff values for this sensitization in patients with NP.

As this will be the first study, to our knowledge, to evaluate the association between acupoint sensitization and NP, this observational study may have some limitations. The main limitation is that in order to reduce the bias caused by other factors and to ensure consistency, this study will include only patients with nontraumatic NP with mobility deficits, and will exclude patients with other types of NP (such as radiating neck pain or secondary NP). Furthermore, because the four participating centers are located in four different regions in China, it may be difficult to implement quality control; thus, quality control reviews will be performed every 3 months.

In conclusion, this article describes the design and protocol of a study that aims to observe 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, and to identify the dominant sensitized forms of the sensitized points. The results will provide a basis for selecting clinically-optimal acupuncture points for NP.

Acknowledgments

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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.

Funding

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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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REFERENCES

1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 2006;**15**(6):834-48 doi: 10.1007/s00586-004-0864-4[published Online First: Epub Date]|.
2. Guidelines for grading diagnosis and treatment of cervical spondylosis in Anhui Province, China（安徽省颈椎病分级诊疗指南）. Anhui Medical Journal 2017;**38**(9)
3. White AR, Ernst E. A systematic review of randomized controlled trials of acupuncture for neck pain. Rheumatology (Oxford, England) 1999;**38**(2):143-7
4. Mejuto-Vazquez MJ, Salom-Moreno J, Ortega-Santiago R, et al. Short-term changes in neck pain, widespread pressure pain sensitivity, and cervical range of motion after the application of trigger point dry needling in patients with acute mechanical neck pain: a randomized clinical trial. The Journal of orthopaedic and sports physical therapy 2014;**44**(4):252-60 doi: 10.2519/jospt.2014.5108[published Online First: Epub Date]|.
5. Irnich D, Behrens N, Gleditsch JM, et al. Immediate effects of dry needling and acupuncture at distant points in chronic neck pain: results of a randomized, double-blind, sham-controlled crossover trial. Pain 2002;**99**(1-2):83-9
6. Yan CQ, Zhang S, Li QQ, et al. Detection of peripheral and central sensitisation at acupoints in patients with unilateral shoulder pain in Beijing: a cross-sectional matched case-control study. BMJ open 2017;**7**(6):e014438 doi: 10.1136/bmjopen-2016-014438[published Online First: Epub Date]|.
7. Luo YN, Zhou YM, Zhong X, et al. Observation of Pain-Sensitive Points in Patients with Knee Osteoarthritis: A Pilot Study. European Journal of Integrative Medicine 2018
8. Chen RX, Kang MF. Acupoint heat-sensitization and its clinical significance. Journal of Traditional Chinese Medicine 2006;**2001**(4):488-9
9. Yao ZL, Liu F, Song NC. 40 Cases of Neck Type of Cervical Spondylosis Treated by Acupotomy Based on the Theory of Trigger point(基于激痛点理论应用针刀治疗颈型颈椎病 40 例). Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(11):1155-55
10. Zhang H, Xu N, Huang R, et al. Heat-Sensitive Moxibustion in Treatment of Cervical Spondylosis. Liaoning Journal of Traditional Chinese Medicine 2016(12):2631-34
11. Xiao L, Meng X, Du S, et al. Therapeutic Observation of Heavy-dose Moxibustion at Sha-petechiae for Neck-type Cervical Spondylosis Due to Cold Coagulation and Blood Stagnation. Shanghai Journal of Acupuncture and Moxibustion 2017(11):1336-39
12. von Elm E, Altman DG, Egger M, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ (Clinical research ed) 2007;**335**(7624):806-8 doi: 10.1136/bmj.39335.541782.AD[published Online First: Epub Date]|.
13. Croft PR, Lewis M, Papageorgiou AC, et al. Risk factors for neck pain: a longitudinal study in the general population. Pain 2001;**93**(3):317-25
14. Falla D, Lindstrøm R, Rechter L, et al. Effectiveness of an 8-week exercise programme on pain and

specificity of neck muscle activity in patients with chronic neck pain: A randomized controlled study. European Journal of Pain 2013;**17**(10):1517-28

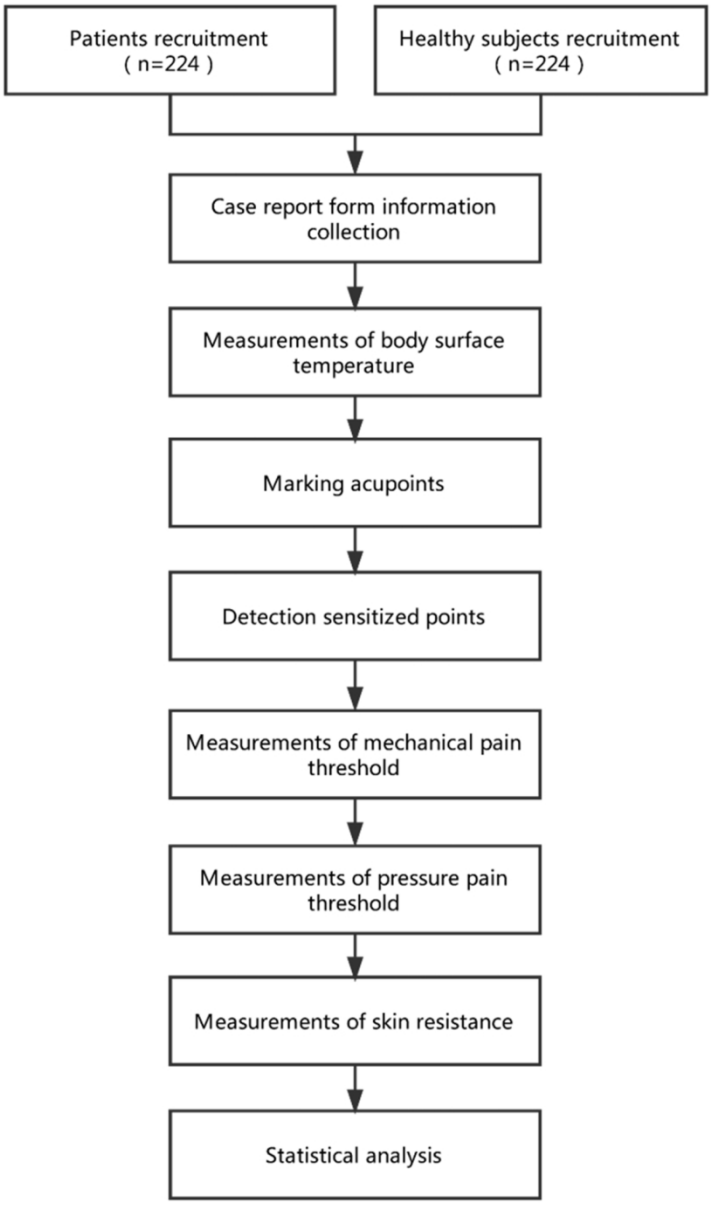
15. Cagnie B, Castelein B, Pollie F, et al. Evidence for the Use of Ischemic Compression and Dry Needling in the Management of Trigger Points of the Upper Trapezius in Patients with Neck Pain: A Systematic Review. American journal of physical medicine & rehabilitation 2015;**94**(7):573-83 doi: 10.1097/phm.0000000000000266[published Online First: Epub Date]|.
16. Ring EF, Ammer K. Infrared thermal imaging in medicine. Physiological measurement 2012;**33**(3):R33-46 doi: 10.1088/0967-3334/33/3/r33[published Online First: Epub Date]|.
17. Adamczyk JG, Krasowska I, Boguszewski D, et al. The use of thermal imaging to assess the effectiveness of ice massage and cold-water immersion as methods for supporting post-exercise recovery. Journal of thermal biology 2016;**60**:20-5 doi: 10.1016/j.jtherbio.2016.05.006[published Online First: Epub Date]|.
18. Neto MLP, Maciel LYS, Cruz KML, et al. Does electrode placement influence tens-induced antihyperalgesia in experimental inflammatory pain model? Brazilian journal of physical therapy 2017;**21**(2):92-99 doi: 10.1016/j.bjpt.2017.03.003[published Online First: Epub Date]|.
19. Yamamoto S, Ono H, Kume K, et al. Oxaliplatin treatment changes the function of sensory nerves in rats. Journal of pharmacological sciences 2016;**130**(4):189-93 doi: 10.1016/j.jphs.2015.12.004[published Online First: Epub Date]|.
20. Chen Y, Yao Y, Wu Y, et al. Transcutaneous electric acupoint stimulation alleviates remifentanil-induced hyperalgesia in patients undergoing thyroidectomy: a randomized controlled trial. International journal of clinical and experimental medicine 2015;**8**(4):5781-7
21. Beissner F, Brandau A, Henke C, et al. Quick discrimination of A(delta) and C fiber mediated pain based on three verbal descriptors. PloS one 2010;**5**(9):e12944 doi: 10.1371/journal.pone.0012944[published Online First: Epub Date]|.
22. Persson AL, Brogårdh C, Sjölund BH. Tender or not tender: test-retest repeatability of pressure pain thresholds in the trapezius and deltoid muscles of healthy women. Journal of rehabilitation medicine 2004;**36**(1):17
23. Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987;**30**(1):115-26
24. Chiu TT, Lam TH, Hedley AJ. Subjective health measure used on Chinese patients with neck pain in Hong Kong. Spine 2001;**26**(17):1884-9
25. Burckhardt CS, Jones KD. Adult measures of pain: The McGill Pain Questionnaire (MPQ), Rheumatoid Arthritis Pain Scale (RAPS), Short - Form McGill Pain Questionnaire (SF - MPQ), Verbal Descriptive Scale (VDS), Visual Analog Scale (VAS), and West Haven - Yale Multidisciplinary Pain. Arthritis Care & Research 2010;**49**(S5):0-0
26. Chen R, Kang M. Acupoint Heat-Sensitization and Its Clinical Significance. Journal of Traditional Chinese Medicine 2006;**2001**(4):488-9
27. Chow SC, Shao J, Wang H. *Sample size calculations in clinical research*. New York: Marcel Dekker, 2003.
28. Hoy D, March L, Woolf A, et al. The global burden of neck pain: estimates from the global burden of disease 2010 study. Annals of the rheumatic diseases 2014;**73**(6):968
29. Zhang S, Wang W, Zou L, et al. [Acupoints heat-sensitive moxibustion in the application of traditional Chinese surgery]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion 2016;**36**(1):109-12
30. Zhou S, Yin H, Huai M. Progress and Prospect of the Study on the Correlation Mechanism of Surface

and Visceral Phase. Acupuncture Research 1982(02)

31. Shui YL, Institute QH, Pasadena. Infrared imaging system as a versatile diagnostic and auxiliary treatment tool in Chinese Medicine.
32. Chen S, Miao Y, Nan Y, et al. The Study of Dynamic Characteristic of Acupoints Based on the Primary Dysmenorrhea Patients with the Tenderness Reflection on Diji (SP 8). Evidence-Based Complementray and Alternative Medicine,2015,(2015-6-16) 2015;**2015**:158012
33. Chae Y, Kim HY, Lee HJ, et al. The alteration of pain sensitivity at disease-specific acupuncture points in premenstrual syndrome. Journal of Physiological Sciences 2007;**57**(2):115-19
34. Tunks E, Crook J, Norman G, et al. Tender points in fibromyalgia. Pain 1988;**34**(1):11

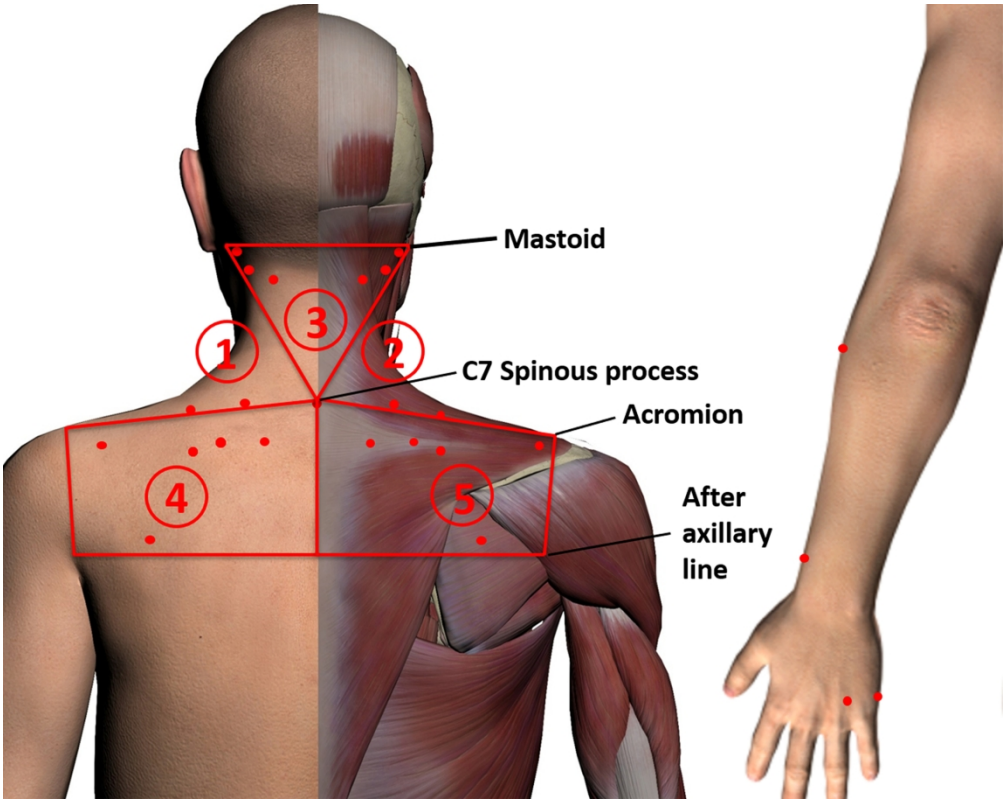
Figure 1 Flowchart of the study design.

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



Flowchart of the study design.

53x89mm (600 x 600 DPI)



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

				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

				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	4, 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.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and	5, 9, 10	Healthy subjects are eligible if

		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 (AnalyzIR, 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 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

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

				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	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

				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

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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	
		Cross-sectional study—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 nontraumatic NP with mobility deficits, while excluding patients with other types of NP (such as NP with radiating pain or secondary NP). Furthermore, as the four participating centers are located in four different regions in China, it may be difficult to implement quality control; thus, quality control reviews will be conducted every 3 months.
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	
Generalisability	21	Discuss the generalisability (external validity) of the study results	3	As this is the first study of its kind, only patients with nontraumatic NP with mobility deficits were included; patients with other NP with radiating pain or secondary NP, 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.