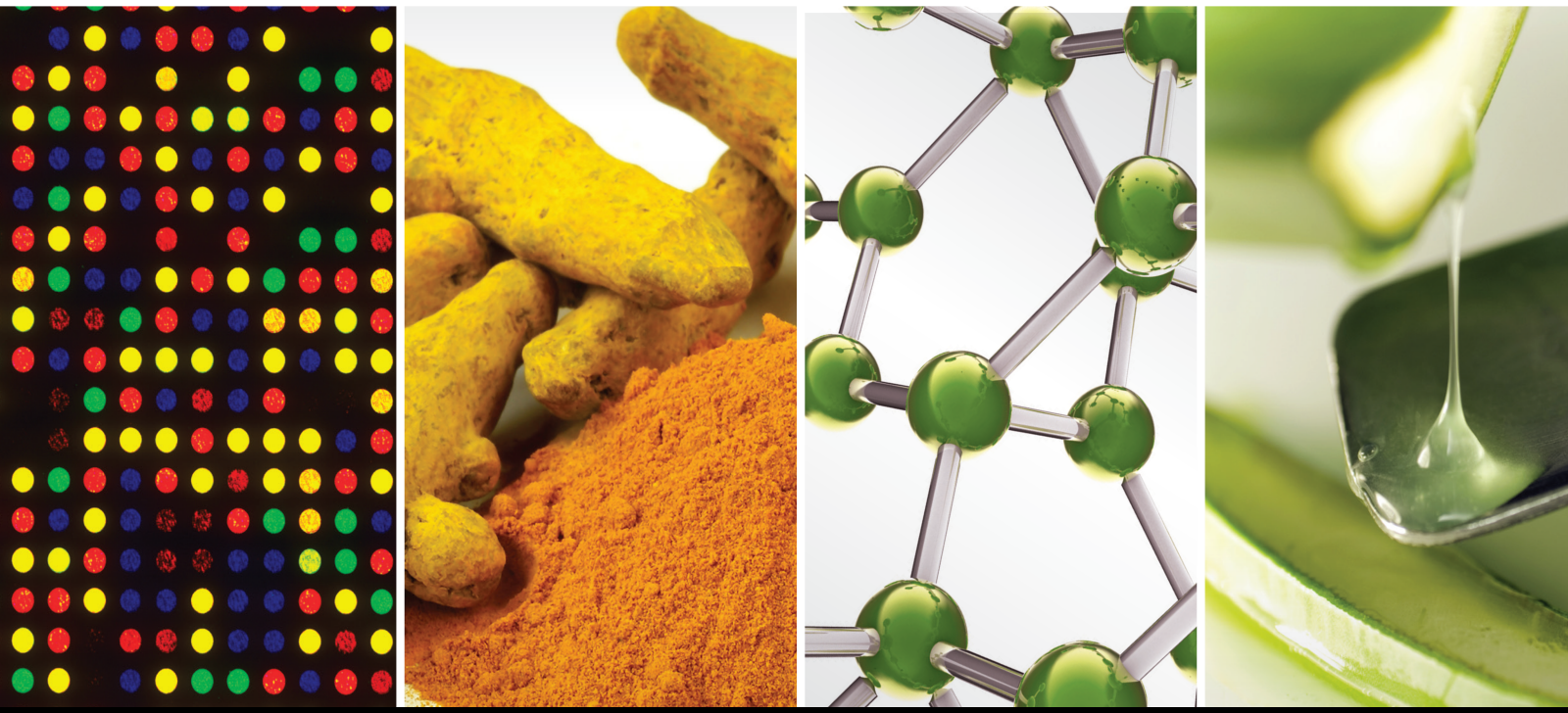
Evidence-Based Complementary and Alternative Medicine

Innovative Methods and Technologies for Efficacy Evaluation of Traditional Medicine

Lead Guest Editor: Hongcai Shang
Guest Editors: Zhaoxiang Bian, Dongran Han, Xinfeng Guo, Guihua Tian, and Nicola Robinson



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Research Article

Evaluating the Effects of Heat-Clearing Traditional Chinese Medicine in Stable Bronchiectasis by a Series of N-of-1 Trials

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Purpose. The purpose of this study is to study the effects of heat-clearing Traditional Chinese Medicine (TCM) in the stable stage of bronchiectasis via N-of-1 trials. *Methods*. The N-of-1 trials in this study were randomized and double-blinded with crossover comparisons consisting of three pairs. Each pair was of two 4-week periods. Each patient took the individualized decoction in the experimental period and the individualized decoction was removed of heat-clearing drugs, mainly including heat-clearing and detoxifying drugs, in the control period for three weeks. After three weeks, the patients stopped taking the decoction for one week. The primary outcome was from patients' self-reporting symptoms scores on a 1–7-point Likert scale. Mixed-effects models were used to conduct statistical analysis on these N-of-1 trials. *Results*. Of the 21 patients enrolled, 15 completed three pairs of N-of-1 trials (71.43%). (1) Seen from the individual level, no statistical difference between the experimental decoction and the control ($P > 0.05$) was observed. However, 5 patients found better decoctions according to the clinical criteria. (2) As revealed by the group data of all the N-of-1 trials, the control was better than the individualized decoction in terms of symptom scores on the Likert scale (1.94 ± 0.69 versus 2.08 ± 0.68, $P = 0.04$, mean difference, and 95% CI: 0.19 (0.01, 0.37)) and on CAT scores (13.66 ± 6.57 versus 13.95 ± 6.97, $P = 0.04$, mean difference, and 95% CI: 0.86 (0.042, 1.67)), but such differences were not clinically significant. The other outcomes, such as Likert scale score of respiratory symptoms and 24-hour sputum volume, showed no statistical difference. *Conclusion*. The experimental design of this study can make the TCM individualized treatment fully play its role and can detect the individualized tendencies according to the severity of phlegm and heat in some subjects. With the intermittent use or reduced use of heat-clearing drugs, most of the subjects, at the group level, enrolled in the series of N-of-1 trials may improve the symptoms and quality of life while saving the cost of TCM and reducing the potential side effects of heat-clearing TCM. This trial is registered with clinicaltrials.goc (NCT03147443).

1. Introduction

Bronchiectasis is a common chronic lung disease characterized by heterogeneous clinical features and outcomes [1]. In Europe and North America, the prevalence of bronchiectasis ranges from 67/100,000 to 566/100,000, while in China it is as high as 1200/100,000, which thus brings a serious and growing economic health burden [2–4]. There are, throughout the stable stage of bronchiectasis, still chronic coughing, purulent sputum, breathlessness, and other clinical symptoms, as well as extrapulmonary symptoms including fatigue, insomnia, constipation, or diarrhea. Traditional Chinese Medicine (TCM) has been proven to be capable of easing the clinical symptoms, reducing the frequency of acute exacerbation every year, and improving the quality of life of patients in the stable stage of bronchiectasis [5]. In TCM, the main syndromes in the stable bronchiectasis are deficiency of both Qi and Yin, deficiency of

lung-spleen Qi, and phlegm-heat syndrome [5–7]. Therefore, the basic therapeutic principles include strengthening the body's resistance by replenishing Qi, nourishing Yin, invigorating the spleen, removing phlegm, and clearing heat. Wu and Tang [8] considered that "phlegm-heat" syndrome is the most common clinical syndrome of bronchiectasis, thus indicating that "phlegm-heat" is the principal contradiction in the treatment of bronchiectasis. It is generally known that patients with bronchiectasis mainly cough up yellow sputum and are prone to hemoptysis. While heat-clearing and phlegm-transforming drugs, or blood-cooling and hemostatic drugs, are recommended, tonics or warming drugs should be used with caution to avoid furtherance of both heat and phlegm. Tian Zhengjian pointed out that [9] heat-clearing drugs should be administered no matter in the acute exacerbation or stable stage of bronchiectasis. However, according to the theory of TCM, such heat-clearing drugs should not be used excessively or in a protracted period, for fear that bitter dryness hurts Yin and coldness spoils the stomach which will then injure the spleen and inhibit the movement of Qi [8]. While strengthening the body's resistance and removing phlegm are generally regarded as the two therapeutic principles in the stable bronchiectasis, there has been controversy over the role and course of heat-clearing Traditional Chinese Medicine (TCM) in this stage. Microorganisms including *Pseudomonas aeruginosa* and other Gram-negative and Gram-positive microorganisms (to a lesser extent) identified in the culture are related to disease progression, adverse clinical outcome of bronchiectasis, and inflammation mediated by neutrophils in the driving airway [10]. Most heat-clearing and detoxifying Traditional Chinese Medicines have been proven to have certain antibacterial effects by clinical or in vitro experiments [11]. For example, Zaiyuan Pang and colleagues found that *Scutellaria baicalensis*, Forsythiae Fructus, *Coptis chinensis*, and honeysuckle have different degrees of bacteriostasis in vitro against widely resistant *Pseudomonas aeruginosa* [12]. Thus, it is important to investigate the function of heat-clearing TCM in the stable stage of bronchiectasis.

There was no randomized controlled comparison between the efficacy and side effects of decoctions containing heat-clearing drugs and decoctions removed of heat-clearing drugs in the stable stage of bronchiectasis. N-of-1 randomized controlled trials ("N-of-1 trials") were conducted with the subject himself/herself as the control. In a prospective, single-individual, planned, multiple-crossover trial, the patient undergoes paired treatment periods that are organized such that one period of each pair adopts the experimental therapy and the other period uses either an alternative treatment or placebo. The 2-cycle sequence in each pair is random. The individualized treatment concept represented by N-of-1 trials naturally conforms to the principle of syndrome differentiation in TCM (which must be applied individually) and provides a feasible way for the integration of TCM and Western medicine. Our previous studies have demonstrated a trend of beneficial effects of individualized herbal decoction compared to standard decoction through a series of N-of-1 trials [13]. However, the possibility of "carryover effects" reduces the reliability and sensitivity of the N-of-1 trials for TCM. In this study, we planned to use a mixed-effects model that accurately estimates the treatment effects and controls the "carryover effects" [14,15]. Given this, we intended to identify the role of heat-clearing drugs in the syndrome differentiation and treatment of bronchiectasis in its stable stage through a series of N-of-1 trials.

1.1. Hypotheses and Objective. It is predicted, on the group level, that the individualized decoction is more effective than the individualized decoction removed of heat-clearing drugs. However, at the individual level, the effect may vary with the severity of phlegm and the heat of the individual. The effects of individualized treatment based on TCM syndrome differentiation will be reflected. The results may have the guiding value for TCM clinical practice.

2. Methods

2.1. Study Design. The study design of these N-of-1 trials have been developed according to the literature of Guyatt et al. [16,17] and described in our previous study [13,18]. Briefly, the patients who met the inclusion criteria and received open preliminary trial treatment could be enrolled into the N-of-1 trials. During the run-in period, we got the onset time after drug administration and the efficacy maintenance time after drug withdrawal for the purpose of determining the length of the observation period and estimating the washout period. The observation period of the N-of-1 trials was set to be four weeks based on a comprehensive analysis of the results in the run-in periods and the results previously obtained [13,18].

We conducted three pairs of N-of-1 trials in one individual, and each pair consisted of two observation periods, that is, the experimental period and the control period, which were assigned randomly. Each observation period lasted for four weeks, and the medications were taken for three weeks. The medications were then stopped for one week at the last week of each observation period. The outcomes in this week were measured in each period. The washout period is the time before the measured week (fourth week) (Figure 1).

When the patients have acute exacerbations during the N-of-1 trials, they can receive antibiotics and other treatments routinely, and the data of this pair would not be statistically analyzed [19]. The study resumed when the disease returned to a stable stage. If a patient felt worse at any time during the trial, the current treatment period was terminated and, without breaking the blinding, the next treatment period began [17].

2.2. Patients and Diagnosis

2.2.1. Inclusion Criteria

(1) In line with the diagnostic criteria of bronchiectasis in accordance with the consensus of Chinese experts

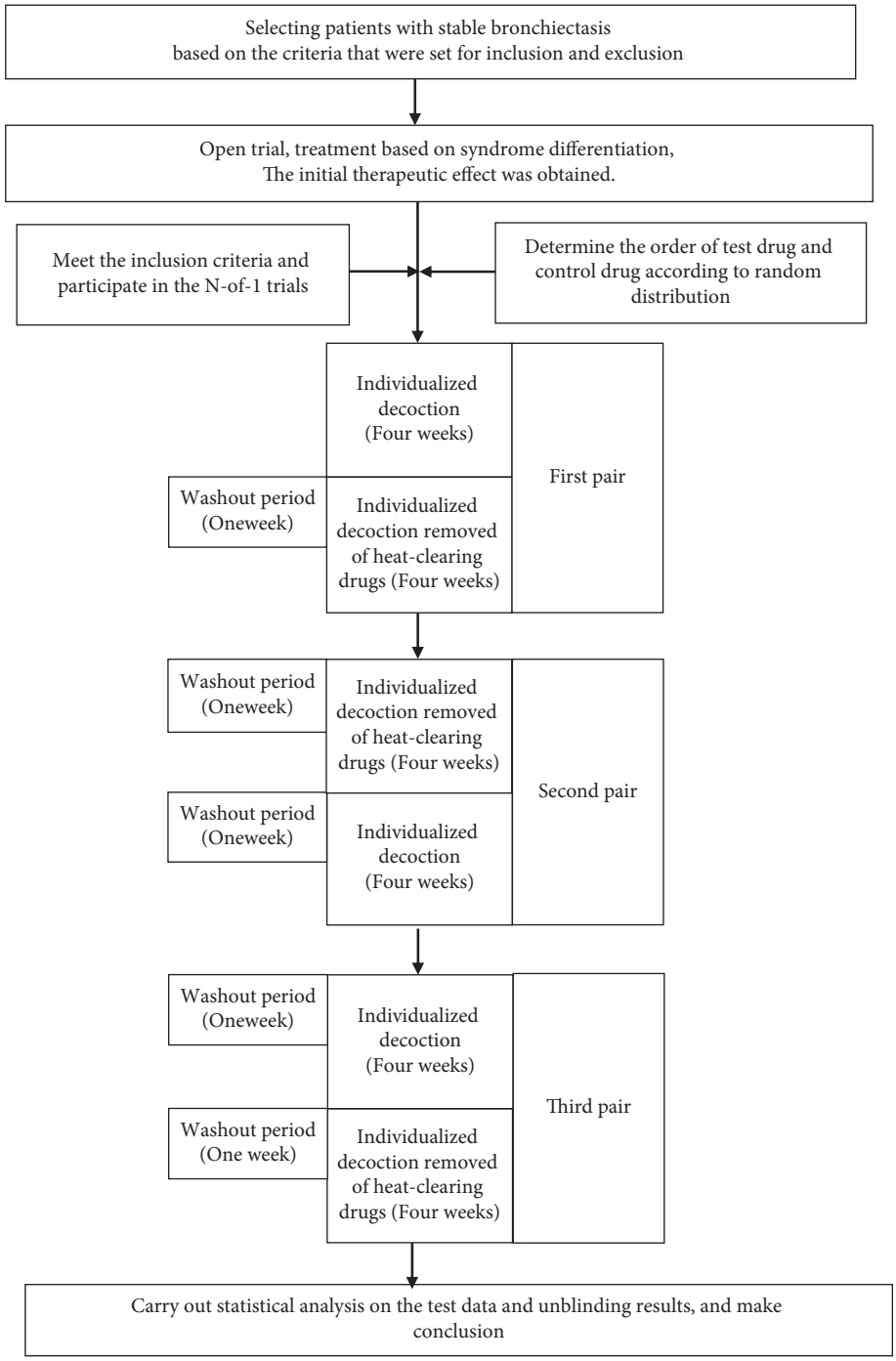

Figure 1: The flow chart of the N-of-1 trial of an individual patient in this study.

[20] and the guidelines for noncystic fibrosis bronchiectasis published by the British Thoracic Society in 2010 [21]

(2) Age of 18–70 years

(3) In the stable stage of bronchiectasis and no acute exacerbation within 3 weeks

(4) Frequency of acute exacerbation of bronchiectasis ≤3 times every year

(5) Signing informed consent

The diagnostic criteria for TCM syndrome differentiation were established in accordance with the "Criteria of Diagnosis and Therapeutic Effect of TCM Diseases" [22] and in combination with the research literature on the TCM syndrome differentiation rule of bronchiectasis [6]. It mainly includes lung and spleen deficiency syndrome, Qi and Yin deficiency syndrome, and phlegm-heat obstructing lung syndrome. Two main symptoms or more than two simultaneous symptoms showing corresponding tongue and pulse signs can constitute the TCM syndrome of each patient.

Clinically, the TCM syndrome differentiation of stable bronchiectasis is mostly the mixture of deficiency and excess of a certain syndrome type. There is a certain degree of phlegm and heat in each syndrome type of TCM. For example, for the syndrome of deficiency of both Qi and Yin combined with phlegm and heat syndrome or lung and spleen deficiency with phlegm and heat syndrome [5,8], the severity of intermingled phlegm and heat should be estimated based on clinical experience. In order to make sure that the treatment based on syndrome differentiation of TCM was accurate and effective, two chief physicians were invited to evaluate and conclude the TCM syndrome of each subject. In case of need, a third party would be invited (a distinguished veteran doctor of TCM).

The trial was carried out at the clinic of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine.

2.3. Randomization and Blinding. A randomized block design was adopted in this study, and the order of medication for three rounds of subjects in each single case trial was determined according to the random number generated by SPSS software, with a block number of 2 [13], such as AB-BA-AB or BA-BA-AB. First, the doctor simultaneously prescribed individualized decoction and control decoction. Then, the observer gave the random medication order together with the prescriptions to a specifically designated pharmacist in the TCM pharmacy. The pharmacist tossed a coin to determine which of A or B represented the individualized decoction or the control decoction and then recorded the blind code and kept it private. According to the random order of the subjects' medication, the herbal medicines were prepared according to the prescription and sent to the decoction room of the hospital for decoction. Finally, the drug dispensers distributed the drugs to the subjects and were responsible for medication records to ensure that drug trials were blind between doctors and patients.

2.4. Interventions. Chest physiotherapy was the primary treatment for stable bronchiectasis, which could promote the excretion of respiratory secretions. If the patient has complications with coronary heart disease, diabetes, or hypertension, the medicine for the concomitant diseases could be taken simultaneously. However, the medication should be relatively fixed, and the dosage and the administration of the medicine should be consistent with the experimental period and the control period of each cycle, as well as a detailed medication administration record.

2.4.1. Syndrome Differentiation Decoction (Individualized Decoction) Adopted in the Tested Drug Observation Period. In accordance with the therapeutic principles of strengthening body resistance, replenishing Qi, nourishing Yin, invigorating spleen, removing phlegm, and clearing heat, we prescribed the individualized decoction based on Bronchiectasis Stabilization Decoction [13] (Radix Lithospermi 15 g, Rhizoma Fagopyri Cymosi 30 g, Radix Ophiopogonis 15 g, Poria 15 g, Astragalus Astragali 20 g, Rhizoma Bletillae 10 g, *Platycodon grandiflorum* 10 g, and Semen Coicis 30 g). For subjects having the syndrome of lung and spleen Qi deficiency, Radix Codonopsis Pilosulae, Pericarpium Citri Reticulatae, and *Atractylodes macrocephala* Koidz. were added. For subjects having the syndrome of Qi and Yin deficiency, Radix Adenophorae, Radix Glehniae, and Radix Rehmanniae were added. For syndromes intermingled with phlegm and heat, we added heat-clearing and detoxifying drugs (e.g., *Houttuynia cordata*, *Viola mandshurica*, and Dandelion), heat-clearing and damp-drying drugs (e.g., *Scutellaria baicalensis* and *Coptis chinensis*), and heat-clearing and fire-purging drugs (e.g., Gardenia), depending on the clinician's estimation of the severity of intermingled phlegm and heat. Apart from that, decoctions can also be adjusted according to other symptoms of the subjects, such as constipation, diarrhea, fatigue, and insomnia. Besides, we also adjusted the individualized decoction in accordance with the change in the patient's condition throughout the study.

2.4.2. The Individualized Decoction Removed of Heat-Clearing Drugs (Control Decoction) Adopted in the Control Drug Observation Period. The heat-clearing TCM were removed from the individualized prescription as the control. The heat-clearing TCM include heat-clearing and detoxifying drugs (e.g., *Houttuynia cordata*, *Viola mandshurica*, and Dandelion), heat-clearing and damp-drying drugs (e.g., *Scutellaria baicalensis*), certain heat-clearing and blood-cooling drugs (e.g., Arnebia), and heat-clearing and fire-purging drugs (e.g., Gardenia). However, Radix Rehmanniae Recen and Radix Scrophulariae were not removed as heat-clearing and blood-cooling drugs due to their effect of nourishing Yin and generating Jin. Besides, reed root as medicine for clearing heat and purging fire was not removed, because it is good at nourishing Yin and quenching thirst. It is also an essential part of Qianjin reed stem soup, which is a prescription commonly used for the treatment of bronchiectasis.

In an observation period, both of the decoctions were taken for three weeks, and each was stopped for one week.

2.4.3. Herbal Preparation and Quality Assurance. The decoction of TCM was formulated according to the literature [23]. The traditional Chinese medicinal herbs which satisfied the national norms [18] were provided by the Shanghai Tongjitang Pharmaceutical Co., Ltd. The pieces of herbs were soaked in water for 30 min in nonwoven bags and decocted 1 time for 30 min at 110°C (pressure 0.1 MPa) in a TCM decocting machine produced by Beijing Donghuayuan Medical Equipment Co., Ltd. (model: YJ 20-G). The Chinese herbal decoctions were taken by one decoction a day and divided into 2 doses.

2.5. Outcome Measures. The doctors in charge of the treatment visited the patient and collected data prior to and

after each treatment period. Subjects identified the symptoms that bothered them and filled out patient diaries or questionnaires every day. The outcome measures are listed below.

2.5.1. Primary Outcome: Patient Self-Rated Symptom Score (7-Point Likert Scale). The patients were assessed for the severity of the symptoms (cough, expectoration, shortness of breath, chest pain, loss of appetite, fatigue, insomnia, and so on) using the 7-point Likert scale [13,16]. It is necessary to optimize the number of questions to make sure that the most important aspects of the patient's problem are examined (usually four to eight items). Each patient was graded daily for the severity of the problems on a 7-point Likert scale supplemented by Visual Analogue Scales (VAS). A higher score indicates more serious symptoms.

A 0.5 improvement in each question indicates a significant improvement in the patient's well-being. If there are seven questions, then in this case a total change of 3.5 or more points is considered to have clinical significance [16,17]. Therefore, the average difference of 0.5 points was defined as the "Minimal Clinically Important Difference (MICD)" for the 7-point scale.

2.5.2. Other Outcomes

(1) 24 h Sputum Volume. The average value of the 24-hour sputum volume for three consecutive days, that is, the three days before the start of the trial regimen or three days before the end of each observation period during the trial, was taken. In order to ensure the accuracy of the measurement of sputum volume, the subjects were instructed to spit the sputum into a special sputum cup with a scale of 100 mL from 8 am on the first day to 8 am the next day and finally recorded the sputum volume in the diary.

(2) COPD Assessment Test (Chronic Obstructive Pulmonary Disease Assessment Test, CAT). The CAT questionnaire consists of 8 questions. The score range for each item is from 0 to 5, so the range of the total score is from 0 to 40. The score of 0 denotes the best quality of life, and the score of 40 represents the worst quality of life. CAT was originally designed for evaluating the quality of life of chronic obstructive pulmonary disease patients. Recently, Lee [24] confirmed that CAT was valid and reliable in evaluating the quality of life of bronchiectasis patients. The MCID (the Minimal Clinically Important Difference) for the CAT has not been formally established, but it was estimated at approximately 2 points [13,25].

(3) TCM Syndrome Scores. In reference to "Guiding Principles for Clinical Research of New Drugs of Traditional Chinese Medicine" [26], the clinical TCM syndromes including cough, expectoration (color, quality, and quantity), hemoptysis, wheezing, fatigue, anorexia, dryness of mouth and throat, spontaneous sweating, night sweating, tongue coating (tongue body), and pulse condition were graded before trials and at each time point after treatment.

(4) Safety Outcome. Blood and urine routine, liver and kidney function, electrocardiogram, and so forth were measured prior to and after the trial, trial-related adverse events were observed, and the trial was unblinded or discontinued as necessary.

2.6. Data Analysis

2.6.1. Sample Size Calculation. The main results of the study were patients' self-reported symptom scores on a 1–7-point Likert scale. The amount of samples required was estimated on the premise of having at least 80% power ($\beta = 0.20$) to detect a mean difference of 0.5 points (the "Minimal Clinically Important Difference (MICD)) in Patient Self-Rated Symptom Score, with a significance test at the $\alpha = 0.05$ level. The standard deviation (SD) of the data from this study was 0.53. Using a two-sided test, it was assumed that there was no period effect or treatment × time interaction under the given model parameters, so the ratio of the two groups was 1 : 1, with three cross-overs [27,28]. The sample size was calculated using the PASS 11.0 software (NCSS LLC, Kaysville, UT, USA). The result indicated that it took 12 patients to meet the same significance and power requirements. The final sample size was set as 16 due to the high drop-out rates of N-of-1 trial (30%).

2.6.2. Statistical Analysis. Aiming to reduce autocorrelation (that is, for data that are not independent) [29], the mean values of the data were collected from the last week of each observation period. SAS 9.4 (SAS Institute, Cary, NC) was adopted to conduct statistical analyses. For the data of normal distribution, paired t-test was used for single case and mixed-effects model for N-of-1 trials as a group. When there was a carryover effect, the mixed-effects model could be adopted to accurately estimate the treatment effects and control carryover effects [14,15]. In the construction of mixed-effects model, the intervention effect, stage effect, and residual effect were regarded as fixed effects, and the subject was regarded as random effects. If the stage effect and residual effect in the fixed effects were not significant, then one effect was deleted in turn until the result had statistical significance. If there was no statistical significance, only the intervention effect and the random effect of the subject were included for analysis. If the P value was smaller than 0.05, it was statistically significant for each test.

2.6.3. Clinical Efficacy Criteria. In order to make up for the limited power of statistical tests of individual N-of-1 trial, we also adopted the Clinical Efficacy Criteria proposed by Guyatt et al. for the definite answer of N-of-1 trial [17] (see Table 1).

2.7. Ethics. The trial protocol was approved by the Ethics Committee of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine (ethical review approval number: 2016-103). Volunteers from Shanghai city were

Table 1: Clinical criteria for definite N-of-1 trial.

1.	Clinicians have high confidence in the decisions made after the N-of-1 trials (1 or 2 on a 7-point scale)
2.	N-of-l trial interruption before completing three treatment pairs because of the clinician's belief that drug effectiveness had been established or refuted (perceived large treatment effect or severe side effects, both confirmed after breaking the code, or low frequency of treatment end-points)

recruited via advertisements and medical lectures. The informed consent of all subjects was obtained.

3. Results

3.1. General Information of the Study. From May 2016 to Oct. 2017, patients were recruited for this study in the Clinic of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine. A total of 25 patients with bronchiectasis satisfied the inclusion criteria, and 21 subjects were formally enrolled in this study and signed the informed consent forms. 15 of these patients (71.43%) completed the N-of-1 trials (71.43%), including three pairs of randomized, double-blind, and controlled trials. 4 subjects completed two pairs (19.05%), and 2 subjects completed only one pair (9.52%). The flow chart of the study and the reasons for withdrawal and exclusion are shown in Figure 2. The baseline data of the 21 subjects formally enrolled are listed in Table 2.

3.2. The Results of the Individual Data of the N-of-1 Trials

3.2.1. Results Based on Statistical Criteria. Nearly all of the outcomes, including the self-reported symptom scores on Likert scale, 24-hour sputum volume, CAT scores, and TCM syndrome scores, showed no statistical difference ($P > 0.05$) between individualized decoction based on syndrome differentiation and individualized decoction removed of heat-clearing drugs (Table 3).

3.2.2. Results Based on Clinical Efficacy Criteria. A total of 5 subjects identified the preferable decoctions according to Clinical Efficacy Criteria (Section 2.6.3), among which 4 subjects preferred individualized decoction and 1 subject preferred individualized decoction removed of heat-clearing drugs.

Case 4 (female). Syndrome differentiation indicated deficiency of both Qi and Yin, with obvious phlegm-heat. She felt the efficacy varied with each period of medication. The individualized decoction was superior to the individualized decoction removed of heat-clearing drugs after unblinding. The clinician judged that individualized decoction was better.

Case 7 (female). Syndrome differentiation indicated deficiency of both Qi and Yin, combined with mild phlegm-heat. She felt that the efficacy varied with each period of medication. The individualized decoction removed of heat-clearing drugs was superior to the individualized decoction after unblinding. The clinician judged that the individualized decoction removed of heat-clearing drugs was better.

Case 10 (female). Suffering from cough and a large amount of yellow sputum with a history of repeated hemoptysis, this case had the syndrome differentiation of Qi and Yin deficiency with heavy phlegm-heat. At the end of the first period of the second pair, massive hemoptysis suddenly occurred and the subject withdrew from the trial. The unblinding results showed that the patient had been given individualized decoction removed of heat-clearing drugs for nearly two consecutive periods in a random order. The etiology of hemoptysis may be due to the heavy phlegm-heat in the subjects and the long-term discontinuation of heat-clearing drugs. Since then, she has been given heat-clearing drugs to avoid using warm and dry TCM in her prescriptions.

Case 12 (female). This case had the syndrome differentiation of deficiency of Qi and Yin combined with obvious phlegm-heat, together with a history of hemoptysis. The subject perceived differences in the efficacy over the different periods of the N-of-1 trials. It was confirmed after unblinding the effect of the individualized decoction was superior to that of the individualized decoction removed of heat-clearing drugs. This case met the Clinical Efficacy Criteria.

Case 13 (female). The subject often had bloody sputum. She had the syndrome differentiation of deficiency of Qi and Yin combined with obvious phlegm-heat. After unblinding, the subject was found to be more inclined to individualized decoction in terms of hemostatic effect.

3.3. The Results of the Group Data

3.3.1. Comparison Results of Group Data between the Two Decoctions in Each Observation Outcome. We adopted the mixed-effects model to carry out the analysis. The intervention effect was statistically significant for both the self-reported symptom scores on the Likert scale (mean difference 0.19; 95% CI: 0.01, 0.37; $P = 0.04$) and the CAT scores (mean difference 0.86; 95% CI: 0.042, 1.67; $P = 0.04$), suggesting that the individualized decoction removed of heat-clearing drugs was superior to the individualized decoction on the two outcomes but not clinically significant (MCID < 0.5 on Likert scale score of symptoms and MCID< 2 on CAT scores, respectively). The other outcomes (Likert scale score of respiratory symptoms, 24-hour sputum volume, and TCM syndrome scores (including Qi and Yin deficiency syndrome and lung and spleen Qi deficiency syndrome) showed no statistical difference. Details are shown in Table 4.

3.4. Safety Outcome. Gastrointestinal reaction occurred in the third pair of Case 14, and the patient recovered after one

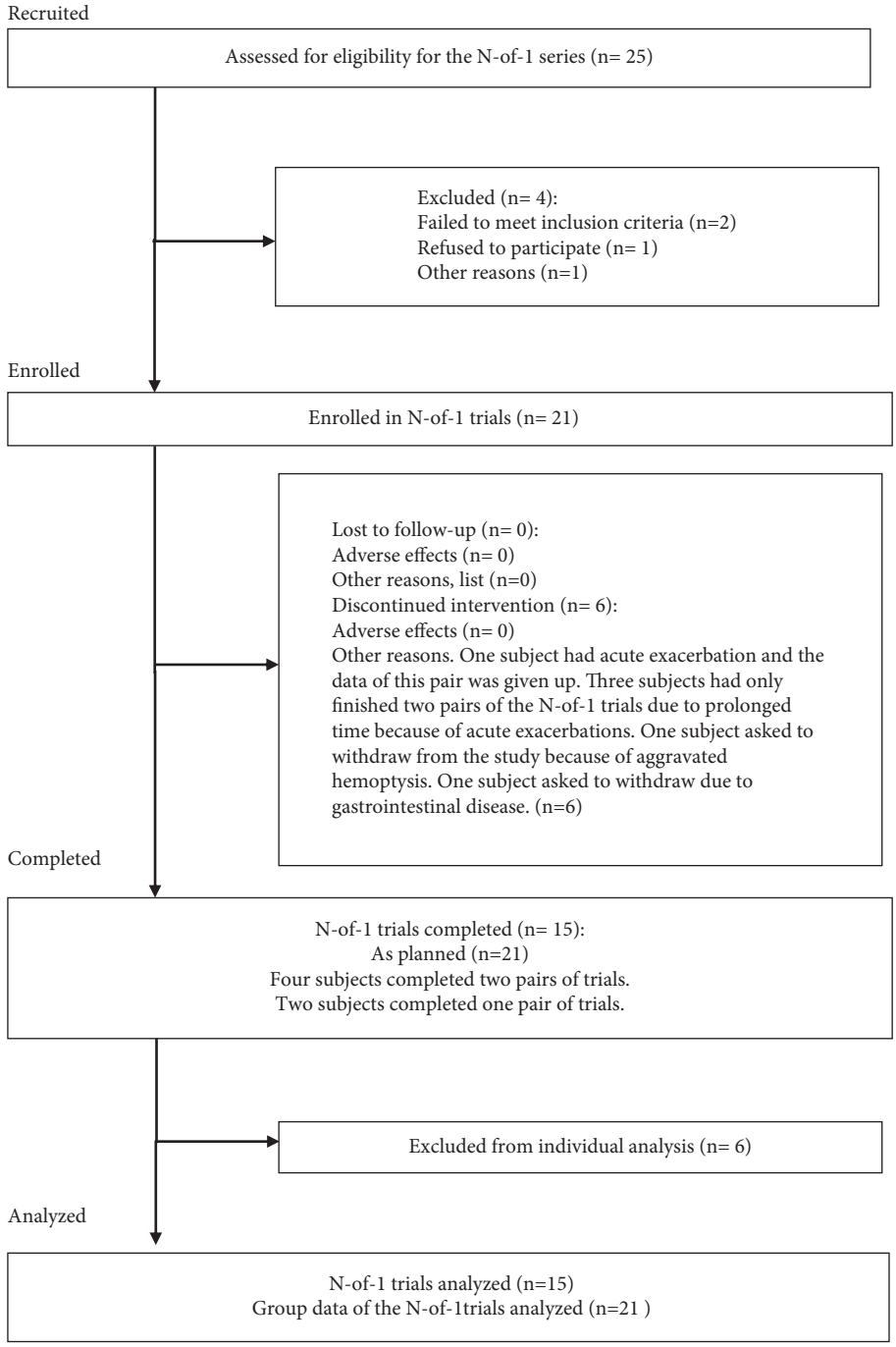

Figure 2: The flow chart of the whole process including the number of cases recruited, enrolled, and completed in this study.

Table 2: Clinical and demographic characteristics of the 21 completers/partial-completers.

Gender (male/female)	21 (13/8)
Age in years, mean (minimum/maximum)	49 (28/70)
Bronchiectasis in chest CT (unilateral/bilateral)	(15/6)
TCM syndrome differentiation (lung and spleen deficiency syndrome/Qi and Yin deficiency syndrome)	(5/16)
Concomitant medication (yes/no)	(4/17)
Baseline of the outcomes	Mean (SD)
Symptoms scores (scores)	2.78 (0.64)
CAT scores (scores)	17.33 (6.30)
24-hour sputum volume (ml)	50.05 (64.97)

Table 3: Comparison of the individual data (individualized decoction versus control decoction) of 15 cases who completed three pairs of the N-of-1 trials in each outcome.

Patient number	Likert scale score of symptoms mean difference and 90% CI	P	24-hour sputum volume mean difference and 90% CI	P	CAT scores mean difference and 90% CI	P	TCM syndrome scores mean difference and 90% CI	P
Case 1	−0.11 (−0.35, 0.13)	0.320	−10.00 (−26.86, 6.86)	0.225	0.17 (−4.48, 4.81)	0.926	−0.67 (−2.61, 1.28)	0.423
Case 2	0.14 (−0.23, 0.51)	0.389	−1.50 (−3.73, 0.73)	0.188	0.17 (−1.12, 1.45)	0.742	0.67 (−3.58, 4.91)	0.691
Case 3	0.06 (0.26, 0.38)	0.637	−23.33 (−65.76, 19.09)	0.250	1.33 (0.36, 2.31)	0.057	0.00 (−1.69, 1.69)	1.000
Case 4	−0.22 (−0.48, 0.05)	0.137	−4.00 (−16.16, 8.16)	0.438	−1.00 (−7.08, 5.08)	0.678	−2.33 (−7.48, 2.82)	0.317
Case 5	−0.30 (−0.88.0.29)	0.280	−1.67 (−14.54, 11.21)	0.742	1.33 (−2.07, 4.74)	0.371	−3.0 (−8.84, 2.84)	0.272
Case 6	0.35 (−0.89, 1.58)	0.497	1.667 (−8.46, 11.80)	0.678	1.33 (−4.65, 7.31)	0.582	4.00 (−5.39, 13.39)	0.339
Case 7	2.02 (−0.67, 4.71)	0.159	※	※	7.67 (0.065, 15.269)	0.099	7.67 (−0.30, 15.63)	0.107
Case 8	0.13 (−0.76, 1.01)	0.715	※	※	0.50 (−1.73, 2.73)	0.580	0.67 (−1.28, 2.61)	0.423
Case 9	0.19 (−0.40, 0.78)	0.444	7.22 (−19.78, 34.22)	0.517	1.33 (−2.66, 9.99)	0.233	5.00 (−0.84, 10.84)	0.130
Case 11	−0.08 (−1.25, 1.09)	0.859	−1.11 (−50.21, 47.99)	0.953	0.00 (−6.74, 6.74)	1.000	−0.33 (−6.25, 5.59)	0.885
Case 13	−0.11 (−0.27, 0.05)	0.184	0.56 (−7.56, 8.67)	0.860	−2.33 (−5.84, 1.18)	0.192	−1.00 (−5.46, 3.46)	0.580
Case 14	0.05 (−0.96, 1.06)	0.894	−3.33 (−29.09, 22.42)	0.742	−0.33 (−4.58, 3.91)	0.840	0.33 (−1.61, 2.28)	0.667
Case 15	0.03 (−0.34, 0.39)	0.839	−3.89 (−12.92, 5.14)	0.336	−0.67 (−1.64, 0.31)	0.184	0.33 (−2.24, 2.91)	0.742
Case 17	0.40 (−0.80, 1.61)	0.433	−6.11 (−26.44, 14.22)	0.473	0.00 (−7.35, 7.35)	1.000	3.00 (−3.08, 9.08)	0.286
Case 20	0.07 (−0.05, 0.19)	0.237	0.56 (−1.07, 2.18)	0.423	0.67 (−0.31, 1.64)	0.184	0.33 (−0.64, 1.31)	0.423

Note. ※ means not available.

Table 4: Comparison of group data between the two decoctions in each outcome.

Group level data ($n = 21$)	Individualized decoction $X \pm S$	Individualized decoction removed of heat-clearing drugs $X \pm S$	Mean difference and 95% CI	t	P values
Symptoms score on Likert scale	2.08 ± 0.68	1.94 ± 0.69	0.19 (0.01, 0.37)	2.20	0.040▲
Respiratory symptoms score on Likert scale	2.11 ± 0.75	1.98 ± 0.78	0.15 (−0.05, 0.35)	1.57	0.132
24-hour sputum volume (ml)	31.52 ± 40.54	33.74 ± 44.44	−1.46 (−3.59, 0.67)	−1.40	0.168
CAT scores	13.95 ± 6.97	13.66 ± 6.57	0.86 (0.042, 1.67)	2.19	0.040*
TCM syndrome scores (lung and spleen Qi deficiency syndrome)	7.50 ± 3.12	7.75 ± 4.69	−0.73 (−5.70, 4.24)	−0.40	0.704
TCM syndrome scores (Qi and Yin deficiency syndrome)	14.51 ± 6.46	13.70 ± 6.57	1.12 (−1.23, 3.47)	1.02	0.324

Note. ▲ indicates that symptom scores on the Likert scale of the individualized decoction were higher than those of individualized decoction removed of heat-clearing drugs. * indicates that the CAT score of individualized decoction was higher than that of the individualized decoction removed of heat-clearing drugs.

week of drug discontinuation, which was considered to be related to heat-clearing drugs after unblinding. Serious adverse events did not occur throughout the study. The results of blood and urine tests including liver and kidney functions were normal prior to and after the N-of-1 trials were normal.

4. Discussion

4.1. Summary of the Results in This Study

4.1.1. Summary of the Results of the Individual Data. Although there was no statistically significant difference on the individual level between the individualized decoction based on syndrome differentiation and the individualized decoction removed of heat-clearing drugs ($P > 0.05$), this did not mean that there was no difference between the two decoctions on individual level. Because a single N-of-1 trial had only a limited number of pairs (≤3), its statistical ability was low. Guyatt et al. thought that "using N-of-1 trials to enhance patient care is not dependent on the statistical analysis of the results." Therefore, they proposed the Clinical Efficacy Criteria for N-of-1 trials: "The N-of-l trial was interrupted before the completion of three treatment pairs because the clinician believed that the efficacy of the drug had been established or disproved (perceived greater therapeutic efficacy or severe side effects, both of which were confirmed after noncompliance)" [16,17]. From the perspective of individual analysis for each patient, there appeared some tendencies. A total of five subjects found out which of the two decoctions was better in accordance with clinical criteria. Among them, four were more inclined to the individualized decoction based on syndrome differentiation (A), and one was more inclined to the individualized decoction removed of heat-clearing drugs (B). While the four who were inclined to A were characterized by severe phlegm and heat as well as frequent hemoptysis, the one who was inclined to B was mild in terms of phlegm and heat.

4.1.2. Summary of the Results of N-of-1 Trials. Based on the analysis of group data, the results of this study were

inconsistent with the original assumption (the hypothesis of the study). It is believed that this situation may not be uncommon with the further development of N-of-l trials of TCM. The scores of self-reported symptoms and CAT of patients taking individualized decoction removed of heat-clearing drugs were better than those taking the individualized decoction ($P < 0.05$). However, the difference in Likert scale score for the overall symptoms between the two decoctions was only 0.19, which was not clinically significant. The other outcomes (Likert scale score of respiratory symptoms, 24-hour sputum volume, and TCM syndrome scores) showed no statistical difference.

We also observed whether the sequence of the first pair of medication would have an impact on the results. The results indicated that there was no statistically significant difference in the Likert scale score of self-evaluated overall symptoms between the two groups ($P > 0.05$). The disadvantage is that the number of cases is limited.

4.2. Comparing Findings with Other Studies. In recent years, there has been an increasing trend in the publication of the literature on N-of-1 trials of Traditional Chinese Medicine (TCM) at home and abroad, which indicates that it is feasible for the trials to be designed for clinical research of TCM and that it also demonstrates the features of treatment based on syndrome differentiation [30–35]. The number of cases in each study varies from 1 to 24 cases, with the observation period from 2 to 4 weeks and the administration time of Traditional Chinese Medicine from 3 days to 3 weeks. Many trials were designed to set a one-week washout period between the administration of the trial drugs and that of the control drugs. The outcome measures include TCM syndrome score, quality of life scale, or laboratory outcomes such as hemoglobin and platelet count. More studies have used blinding rather than blank control, indicating that the quality of N-of-1 trials of TCM is improving. Most of the studies showed that the treatment of TCM was effective ($P < 0.05$). However, some studies only carried out the group-based statistical analysis instead of individual analysis and statistics. Some of the studies used improper statistical methods, such as the use of the *t*-test in individual or group statistics.

In this study, the combination of individual and group analyses has been applied to enhance the sensitivity of N-of-1 trials of TCM. On the basis of the previous study [13], we reduced the medication duration of Traditional Chinese Medicine in each period from 4 weeks to 3 weeks, leaving the remaining one week as the washout time without medication, so that the whole washout period was actually prolonged to 4 weeks. In addition, we used the mixed-effects model for group-based statistical analysis, taking into account the possible stage effects and residual effects. The result showed that stage effect and residual effect had no statistical significance ($P > 0.05$).

4.3. Strengths and Limitations

4.3.1. Fully Utilizing the Individualized Treatment Based on Syndrome Differentiation. Since the design of the N-of-1 trial can be highly individualized, patients with different TCM syndromes or mixed syndromes could be included in the trial. While subjects received individualized TCM treatment based on syndrome differentiation, the prescription could also be adjusted according to the changes of symptoms or syndromes throughout the trial, which is identical to the real clinical practice. This is one of the most prominent superiorities of N-of-1 trials in the study of TCM.

4.3.2. Improving the Efficiency of Blind Method in the Clinical Trials of TCM. In this study, each subject was required to receive the intervention of two kinds of TCM decoctions at random, which made a high demand on blinding. However, due to the unique perception, taste, and order of TCM, finding a control drug that is completely consistent with the test drug can be extremely difficult [36]. In this study, the two TCM decoctions could be similar in appearance and size, but there may still be slight differences in taste and smell. To compensate for this difference, we informed subjects that both the test and control decoctions were likely to be effective regardless of taste and odor. This strategy proved effective in practice. Even if there were differences, most subjects did not know what type of decoction they were assigned to and most subjects did not show a preference for certain decoctions. The results of this study were not consistent with the initial expectations: no statistically significant difference between the experimental decoction and the control was observed at the individual level, and the control decoctions were even better in some outcomes (patient self-reported symptom scores on Likert scale and CAT scores) on the group level. Therefore, the data of this study has a relatively high degree of objectivity. We think that this strategy may be beneficial to improve the blinding of N-of-1 trials of TCM in the near future.

4.3.3. Fewer Interference Factors of Western Medicine. The treatment of Western medicine is essential in a considerable number of clinical studies of TCM. Still, it is difficult to avoid a certain degree of interference even if the control group is treated with the same Western medicine treatment. In this study, most of the subjects were patients with mild-to-moderate severity of bronchiectasis, so the basic treatment was expectoration or postural drainage. There is generally no use of Western medicine with the exception of rare cases. Therefore, fewer interference factors of Western medicine improve the reliability of this study.

4.3.4. Sensitivity Needs to be Further Improved. Because of the longer trial pair of the N-of-1 trials of TCM (about 8 weeks per pair), each case took about half a year, so usually only three pairs can be completed. For individual statistics, the statistical power was limited, and it was difficult for some patients to draw definite conclusions. The classic N-of-1 trials require the drugs studied to take effect quickly and have a short half-life [16,17], but the determination of the process of Chinese medicine metabolism can be very difficult. We had to run a preliminary trial, in conjunction with

the investigators' clinical experiences, to identify the observation period and the washout period [18]. The relatively longer observation period is also a common weakness in N-of-1 trials of TCM.

4.4. Implications for Clinical Practice and Future Research

4.4.1. Repositioning the Effect of Heat-Clearing Drugs in the Stable Stage of Bronchiectasis

(1) Intermittent Application of Heat-Clearing Drugs May Be Better. The most common TCM syndrome type of bronchiectasis is phlegm and heat obstructing the lung [3,4], indicating that "phlegm and heat" is an essential factor in the pathogenesis of bronchiectasis. Although the role of heat-clearing drugs is beyond all doubt, there is a lack of in-depth evidence-based medicine research on the application rules of heat-clearing drugs under the conditions of intermingled deficiency and excess as well as individual differences in the stable stage of bronchiectasis. According to our clinical experience in the stable stage of bronchiectasis, we tend to strengthen the body resistance while keeping in mind to clear lungs and resolve phlegm with both clearing and tonifying methods. However, Hong Guangxiang [37] believes that the asthenia in origin caused by bronchiectasis is dominated by the weakness of Qi and Yang and that phlegm is essentially the wet phlegm, which is the evil of Yin that cannot be removed without warmness. "The treatment of lungs does not reject warm drugs." The continuous application of heat-clearing drugs may cause gastrointestinal reactions and also have potential hepatorenal toxicity.

The results of this study, to a certain extent, have weakened the role and positioning of heat-clearing drugs in the stable stage of bronchiectasis. In accordance with the theory of TCM, the prolonged application of heat-clearing drugs may lead to the impairment of stomach due to bitter cold, the hindrance of the movement of Qi, the injury of Yin due to bitter dryness, and so on.

However, since phlegm and heat still exist in the stable stage of bronchiectasis, it is not practical to abandon heat-learning Traditional Chinese Medicine in the stable stage of bronchiectasis. In this study, the alternating application of individualized decoction on the basis of syndrome differentiation and the individualized decoction removed of heat-clearing drugs has achieved favorable clinical effects in most patients, which suggests that, in the stable stage of bronchiectasis, it is not necessary to use heat-clearing drugs in the whole process of the treatment and that intermittent use of the same may be taken into account. This has a certain clinical reference value for reducing the potential side effects of heat-clearing drugs as well as the cost of TCM.

However, individualized treatment should always be applied to the clinical practice. Heat-clearing drugs should still be used in large dose or prolonged period for those bronchiectasis patients who have heavy phlegm-heat syndrome or are prone to hemoptysis.

(2) Enlightenment on the Course of Treatment of TCM in the Stable Stage of Bronchiectasis. At present, there is a lack of in-depth and systematic study on the treatment courses of chronic diseases such as bronchiectasis in TCM as well as on the safety of long-term medication. In TCM medication practice, three months can be used as one course of treatment, or two courses can be used consecutively [2]. In addition to the current doubts about the safety of long-term application of TCM (including heat-clearing drugs such as *Houttuynia cordata* Thunb) at home and abroad, quite a few of TCM medications in clinical practice adopt a dose in excess of that recommended in Chinese Pharmacopoeia, along with large prescriptions and long courses of treatment from time to time, which not only increases medical costs but also leaves safety loopholes. Attention must be paid to this issue. We set each observation period of N-of-1 trials to 4 weeks, including a medication period of 3 weeks and a suspension of 1 week. After that, the medication is rotated. Our original intention was to extend the washout period. However, in this study, it has been widely recognized by the subjects. They believed that a week of suspension did not reduce efficacy but instead gave them a short rest during the half-year trial. Compared with the traditional continuous use of Traditional Chinese Medicine [5], this medication method may reduce the side effects and the costs of TCM, which unintentionally offers enlightenment on the clinical medication practice for bronchiectasis or other chronic diseases.

4.4.2. Exploring a More Efficient Statistical Method Suitable for N-of-1 Trials of TCM.

In this study, although 5 subjects had found out which of the two decoctions was better in accordance with clinical criteria, no statistical difference can be drawn from any individual case, indicating that the sensitivity of this statistical methodology needs to be further improved. Thanks to its remarkable characteristics, hierarchical Bayesian statistical method is an important statistical method in N-of-1 trials [38]. The Bayesian method has the following advantages over the frequentist statistical methods: (1) it can simultaneously carry out the integrated analysis of individual and group data; (2) it is easy to introduce confounding variables, like the physique or gene type of different subjects, or different TCM syndromes (which helps to distinguish between the different TCM syndromes and differences in the effects); (3) if many patients have completed similar N-of-1 trials and the variance within an individual patient is greater than that between patients, the results of other patients can be used to enhance the accuracy of an individual result, meaning to increase the sensitivity of N-of-1 trials without increasing the pairs of N-of-1 trials. Currently, although this statistic method is rarely used in N-of-1 trials of TCM, it can be used for reference in our future study [39,40].

In summary, the effect of TCM individualized treatment in the experimental design in this study can be fully mobilized. The experimental design was able to detect the individualized tendency depending on the severity of phlegm and heat in some subjects. At the group level, for most of the

subjects enrolled in the series of N-of-1 trials, intermittent use or reduced use of the heat-clearing drugs may improve the symptoms and quality of life, while saving the cost of TCM and reducing the potential side effects of heat-clearing TCM. More cases are needed to further substantiate the supposition.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest

All the authors declare that there are no conflicts of interest with respect to the publication of this paper.

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Research Article

Natural Language Processing Algorithms for Normalizing Expressions of Synonymous Symptoms in Traditional Chinese Medicine

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Background. The modernization of traditional Chinese medicine (TCM) demands systematic data mining using medical records. However, this process is hindered by the fact that many TCM symptoms have the same meaning but different literal expressions (i.e., TCM synonymous symptoms). This problem can be solved by using natural language processing algorithms to construct a high-quality TCM symptom normalization model for normalizing TCM synonymous symptoms to unified literal expressions. *Methods*. Four types of TCM symptom normalization models, based on natural language processing, were constructed to find a high-quality one: (1) a text sequence generation model based on a bidirectional long short-term memory (Bi-LSTM) neural network with an encoder-decoder structure; (2) a text classification model based on a Bi-LSTM neural network and sigmoid function; (3) a text sequence generation model based on bidirectional encoder representation from transformers (BERT) with sequence-to-sequence training method of unified language model (BERT-UniLM); (4) a text classification model based on BERT and sigmoid function (BERT-Classification). The performance of the models was compared using four metrics: accuracy, recall, precision, and F1-score. *Results*. The BERT-Classification model outperformed the models based on Bi-LSTM and BERT-UniLM with respect to the four metrics. *Conclusions*. The BERT-Classification model has superior performance in normalizing expressions of TCM synonymous symptoms.

1. Introduction

Traditional Chinese medicine (TCM) symptoms are recorded by TCM practitioners who sometimes use different words when recording the same symptoms, as a consequence of their diverse experience and educational background. These variations in words lead to the phenomenon known as "one symptom with different literal expressions," which is prevalent in TCM medical records. Wang et al. [1] reported that approximately 80% of TCM symptoms were recorded with multiple expressions. Although the literal expressions of these symptoms are different, they have the same meaning, and their use does not affect understanding. Thus, the use of these alternative symptoms does not affect the pathogenesis diagnosis. In summary, TCM symptoms that have the same meaning but different literal descriptions are known as TCM synonymous symptoms. For example, the symptom "lack of appetite" (纳减) can also be expressed as "loss of appetite" (纳差) or "decreased appetite" (食欲减低). They all mean a reduced desire to eat and are used in the description of spleen Qi deficiency (脾气虚).

It is essential to explore and analyze TCM medical records for the purpose of TCM modernization [2, 3]. However, the abundance of synonymous symptoms in TCM

medical records hinders systematic scientific knowledge discovery. Referring to the TCM terminology [4] published by relevant authorities, it is possible to establish a TCM thesaurus and then normalize each symptom in TCM medical records to a symptom that has the same meaning in the thesaurus, so that TCM synonymous symptoms would have uniform literal expressions. That is, TCM symptom normalization is a feasible method for handling TCM synonymous symptoms. However, manual TCM symptom normalization is time-consuming and labor-intensive because of the large and growing quantity of TCM electronic medical records.

Natural language processing (NLP), which has experienced extraordinary development in recent years, provides valuable support for the automatic processing of text data, such as language translation [5], question answering [6], and information processing of medical texts [7–10]. This success suggests that the NLP technology will be effective for normalizing the expression of TCM synonymous symptoms.

In previous work, researchers have proposed some NLP-based normalization models for biomedical fields, such as Word2Vec [11], Jaccard similarity [12], DNorm [13], and BERT-based ranking [14] from the perspective of similarity matching. In addition, from the perspective of named entity recognition (NER), there are transition-based [15] models and Bi-LSTM-CNNs-CRF [16]. Although the performance of these models is satisfactory according to the published reports, there are two problems that are worthy of further exploration, from the perspectives of their applicability to normalizing TCM symptoms and the modeling concepts of the NLP model:

(1) With regard to applicability, the above models are used for normalizing multiple synonymous terms to one term. However, they are not suitable for cases in which synonymous symptoms correspond to multiple normalized symptoms. For example, "less white sputum and difficult to expectorate" (痰少色白难咳) and "less white phlegm and not easy to expectorate" (少量白痰且不易咳出) are synonymous symptoms, should be normalized to "less phlegm" (痰少), "white phlegm" (痰白), and "expectoration difficulties" (痰难咳出).

(2) With regard to the modeling concept, approaches from the perspectives of similarity matching and NER have been reported. However, many models constructed from the perspectives of sequence generation and text classification have also shown excellent performance and applicability in NLP tasks [17, 18]. Therefore, it is necessary to explore the applicability of sequence generation and text classification to this normalization task and investigate whether better performance can be achieved.

According to the above statement, the objective of this study is to develop normalization models for normalizing the expressions of TCM synonymous symptoms from the perspective of sequence generation and text classification and to compare and analyze the applicability and performance of the models, so as to select the best model.

2. Methods

The workflow of this study is shown in Figure 1. It can be divided into three parts: (1) collecting TCM symptoms from medical records (referred to as sample collection), (2) preparing training, development, and test data sets (referred to as division of data sets), and (3) constructing models for normalizing expressions of TCM synonymous symptoms (referred to as model construction).

2.1. Data Sources and Labeling. In total, 3,252 medical records, recorded by 22 TCM doctors on the platform of the "Heritage Program of Chinese Well-Known Experts" [19], were collected. The symptoms in the medical records were regarded as the original symptoms, each of which was then labeled by the corresponding normalized symptom, according to the *TCM Thesaurus* (from the Beijing University of Chinese Medicine TCM Information Science Research Center). Two researchers, who had obtained the qualification of TCM practicing physician and been trained by the provider of the *TCM Thesaurus*, performed the labeling work. Two additional experts in the *TCM Thesaurus* checked the labeling results independently, and inconsistent labeling results were submitted to a third expert for review and discussion to ensure consistency.

There are two forms of original symptoms in medical records: single symptoms and complex symptoms. A single symptom is an original symptom that corresponds to only one clinical manifestation; such a symptom was labeled as one normalized symptom by referring to the *TCM Thesaurus*. For example, "thinning and shapeless stool" was labeled as "loose stool." A complex symptom is an original symptom that corresponds to multiple clinical manifestations; such a symptom was labeled as multiple normalized symptoms. For example, "dry and itchy throat" was labeled as "dry throat" and "itchy throat" by referring to the *TCM Thesaurus*.

In total, 16,808 nonrepetitive original symptoms were collected from the 3,252 medical records, corresponding to 1,501 normalized symptoms, of which 339 appeared only once. The collected original symptoms and labeled normalized symptoms served as the input and output data, respectively, of TCM symptom normalization models.

2.2. Partition of Data Sets. Two strategies were used to divide the collected data into training, development, and test data sets. The first strategy was to divide the medical records by source doctors randomly. The nonrepetitive original symptoms recorded by one randomly selected doctor, and the corresponding normalized symptoms were used as a development set to set the parameters of the model. The nonrepetitive original symptoms recorded by another

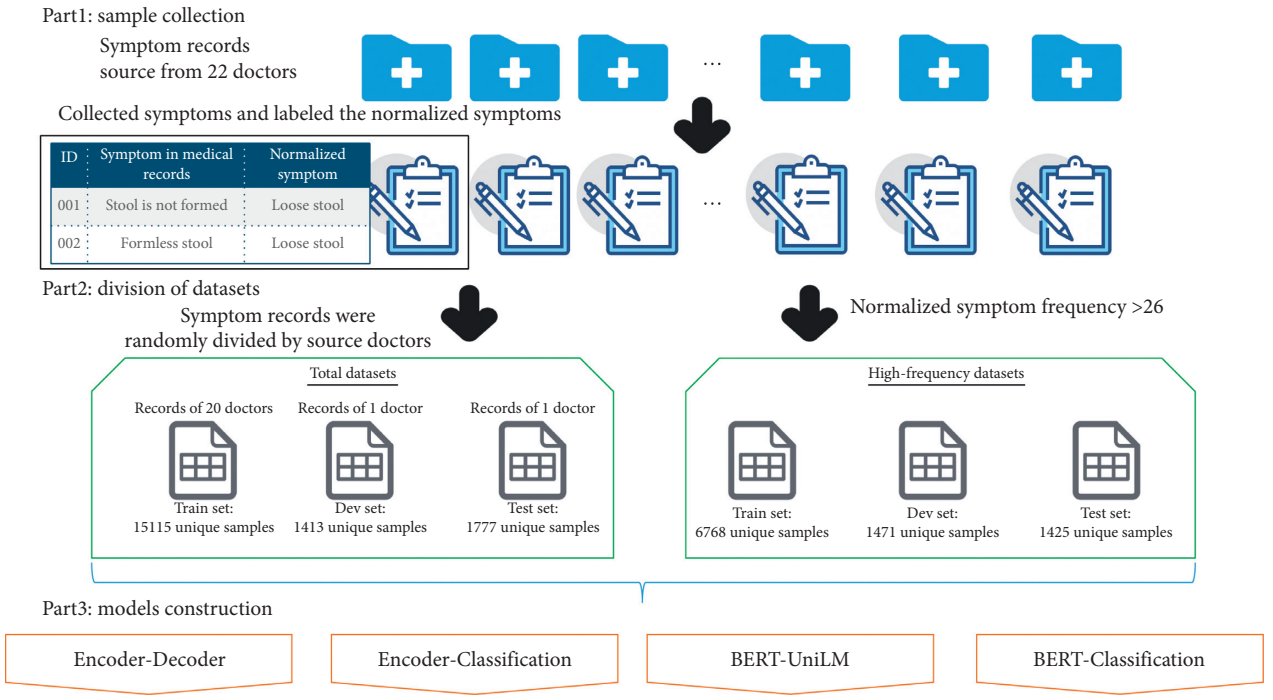


Figure 1: Workflow of the research process.

randomly selected doctor, and the corresponding normalized symptoms were used as a test set to observe the ability of the model to normalize the expression of TCM symptoms. The nonrepetitive original symptoms recorded by the 20 other doctors, and the corresponding normalized symptoms were used as the training set. These data sets were called total data sets (TDS). This data set division is suitable for evaluating the performance of the TCM symptom normalization models in practical applications.

The second strategy for dividing the collected data into training, development, and test data sets was based on high-frequency normalized symptoms. These data sets were called high-frequency data sets (HFDS). According to Zipf's law [20], $N = -1 + \sqrt{1 + 8 \times I_1}/2$ (N is the threshold between high-frequency and low-frequency, and I_1 is the number of normalized symptoms that only appeared once). Normalized symptoms with a frequency greater than 26 were defined as high-frequency normalized symptoms. The high-frequency normalized symptoms and the corresponding original symptoms were included in the HFDS. The ten most frequent normalized symptoms and their corresponding numbers of original symptoms are shown in Figure 2. In the HFDS, 70% of the data (6,768 original symptoms and the corresponding normalized symptoms) were randomly selected as a training set, 15% (1,471 original symptoms and the corresponding normalized symptoms) were as a development set, and 15% (1,425 original symptoms and the corresponding normalized symptoms) were as a test set. The numbers of samples in HFDS and TDS are shown in Table 1.

2.3. Model Construction. From the perspective of text sequence generation, the bidirectional long short-term memory (Bi-LSTM) recurrent neural network (RNN) with the encoder-decoder structure [21], combined with the Luong attention mechanism [22], was used to establish four models for TCM symptom normalization. (1) Encoder (Char)-Decoder (Char) model: the input of the original symptom and the output of the normalized symptom were in character form (multiple output normalized symptoms were separated by ","). (2) Encoder (Word)-Decoder (Word) model: the input of the original symptom and the output of the normalized symptom were in word form. (3) Encoder

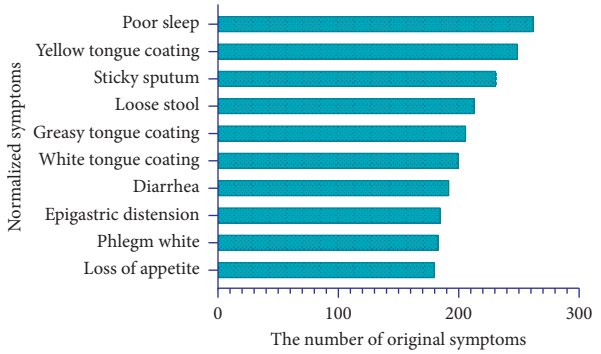


Figure 2: The ten most frequent normalized symptoms in the collected medical records.

Table 1: Numbers of samples in HFDS and TDS.

Data set	HFDS	TDS
Training	6,768	15,115
Development	1,471	1,413
Test	1,425	1,777
Total	9,664	18,305

(Char)-Decoder (Label) model: the input of the original symptom was in character form, and the output of the normalized symptom was in label form. (4) Encoder (Word)-Decoder (Label) model: the input of the original symptom was in word form, and the output of the normalized symptom was in label form. The structure of the four models was consistent; only the input and output forms were different, as shown in Figure 3(a).

This study also applied the Bi-LSTM and a full connection layer with sigmoid function to explore the feasibility of TCM symptom normalization from the perspective of text classification. In this case, the model output was in label form, and the input was in character or word form (see Figure 3(b)). In the Encoder (Char)-Classification model, the input was in character form; in the Encoder (Word)-Classification model, the input was in word form. The words that were input to the model were obtained from the original symptoms by a segmentation tool [23].

Chinese language pretraining weights, trained on a large number of Chinese corpora, can help achieve better results. Therefore, this study further used the unified language model (UniLM) based on the Chinese pretraining weights of bidirectional encoder representation from transformers (BERT) [18, 24] to construct the TCM symptom normalization model. The training process included first loading the Chinese pretraining weights of BERT (https://storage.googleapis.com/bert_models/2018_11_03/chinese_L-12_H-768_A-12.zip) and then training with the sequence-to-sequence method of UniLM [18]. This training method was based on text sequence generation. Two output forms were used in training: a character-based output form, namely the BERT-UniLM (Char) model, and a label-based output form, namely the BERT-UniLM (Label) model, as shown in Figure 4(a). BERT and a full connection layer with sigmoid function were also used to construct the TCM symptom normalization model, namely the BERT-Classification model, as shown in Figure 4(b). Because the input of the pretraining weights of BERT was in character form, the input of the BERT-based models was also in character form.

2.4. Model Parameters. The encoder-decoder models had initialization weights sampled from a random uniform distribution in the range of −0.05–0.05, the dimension of embedding was 300, and the training batch size was 256. Adam was the optimizer [25]. According to the F1-score of the encoder-decoder models on the development set, the best parameter combinations were selected for learning rate (selected from 0.0001, 0.0003, and 0.0005), dropout rate (selected from 0.3 and 0.5), and the number of memory cells (selected from 128, 256, and 512).

For the encoder-classification models, the training batch size was 256. According to the F1-score of the models on the development set, the best parameter combinations were selected for learning rate (selected from 0.005, 0.01, and 0.03), dropout rate (selected from 0.3 and 0.5), and the number of memory cells (selected from 128, 256, and 512).

For the BERT-UniLM and BERT-Classification models, the training batch size was 16, the optimizer was Adam [25], and the learning rate was 0.0003. The other parameters were the default settings of the BERT neural network [24].

The TensorFlow neural network framework (http://www.tensorflow.org/), developed by Google, was used to implement the above models and was combined with NVIDIA GeForce RTX 2080 (11 GB memory) to train the models. When the F1-score of the models in the development set had not improved for 20 epochs, the training was terminated. Even if a fixed random seed number was used, the results from different computers were still biased. Therefore, after setting the model parameters, the modeling process was repeated 10 times; the model performance was evaluated by four metrics and expressed as mean ± standard deviation (SD). The four metrics used were accuracy, precision, recall, and F1-score. Accuracy = P/T; Precision = TP/TP + FP; Recall = TP/TP + FN; and $F1 - \text{score} = 2 \times \text{Precision} \times \text{Recall}/\text{Precision} + \text{Recall}$.
Here, P (the correct normalized symptoms of model prediction) is the number of all correct results output by the model, and T (total correct normalized symptoms corresponding to the test set) is the number of all tests. TP (true positive) is the number of results produced by the model that were consistent with the actual results, FN (false negative) is the number of correct results that the model failed to output, and FP (false positive) is the number of results produced by the model that were incorrect. The key model parameters and development set results are shown in Tables 2 and 3.

2.5. Statistical Analysis. IBM SPSS 20.0 was used to analyze the results. When analyzing the indexes of each group, if the variance between groups was homogeneous and normal distribution was satisfied, one-way ANOVA was used. If the variance was not homogeneous or there was non-normal distribution among groups, the Kruskal–Wallis test was used.

3. Results

3.1. Performance of Models on Test Data Sets. Generally, the performance of models was better on the HFDS test data set than on TDS. With regard to the model structure, the BERT-UniLM models had more advantages than the Encoder-Decoder models, as shown in Tables 4 and 5. In addition, comparing the BERT-UniLM models with the BERT-Classification model, the BERT-Classification model had more advantages. That is, the BERT-Classification model was the best model for normalizing expressions of TCM synonymous symptoms in this study, on both the HFDS and TDS test data sets.

The performance of three classification models with different threshold values on HFDS and TDS was explored. With regard to HFDS, when the threshold value was 0.2, the performance of both BERT-Classification and Encoder-Classification was generally the best, as shown in Figure 5. With regard to TDS, the best threshold value was 0.1, as shown in Figure 6. When comparing the BERT-Classification model with the Encoder-Classification models, the BERT-Classification model achieved better results. The

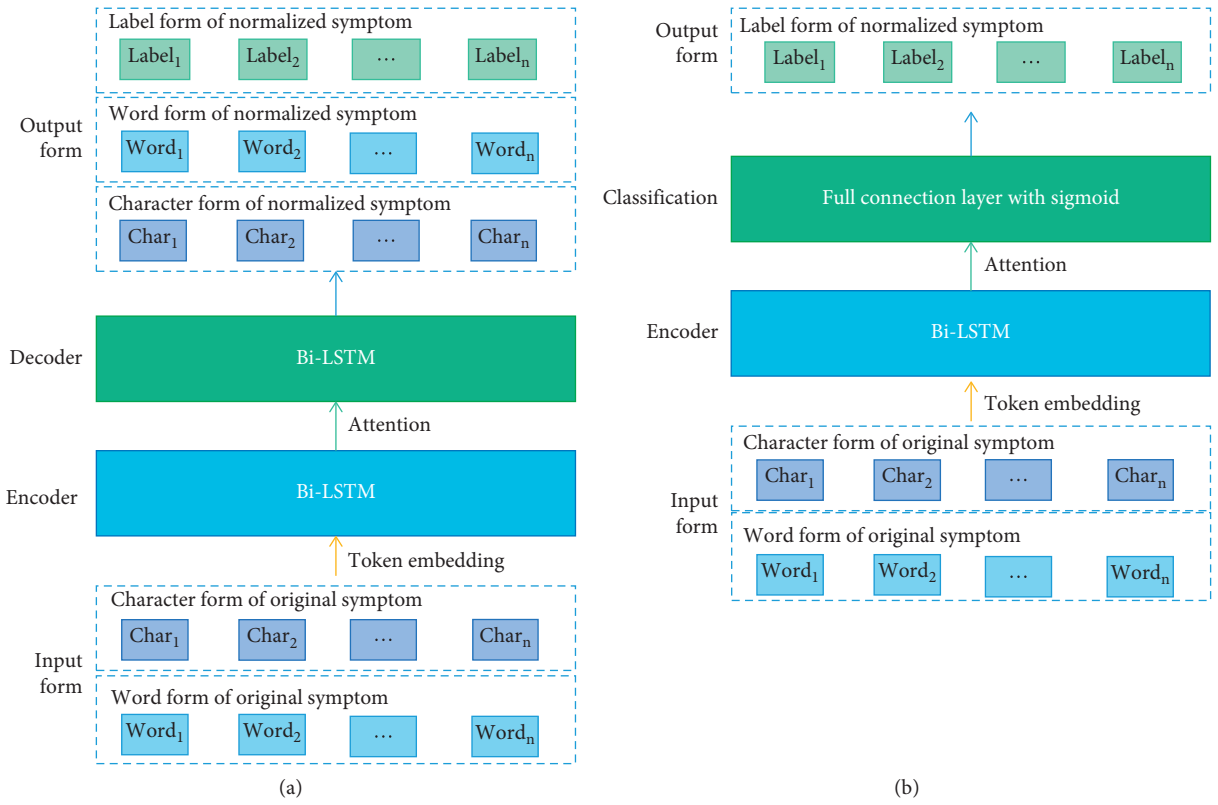


Figure 3: Examples of the (a) Encoder-Decoder and (b) Encoder-Classification models.

accuracy and F1-score were 0.9051 and 0.9073 on the HFDS and 0.8568 and 0.8574 on the TDS, respectively.

The classification-based models have the ability to adjust the output threshold to change the recall. We believe this capability can be used for the retrieval of normalized symptoms. Because retrieval focuses on higher recall, namely, focuses on the outputs contain the correct normalized symptoms. By lowering the output threshold, the models can output the top 5 and 10 normalized symptoms above the threshold. Therefore, the retrieval ability was evaluated by the top 5 and 10 recall, and the results are shown in Table 6.

3.2. Performance of Models in Normalizing Single and Complex Symptoms. In evaluating the various models for normalizing single symptoms (the original symptoms corresponding to one normalized symptom) and complex symptoms (the original symptoms corresponding to multiple normalized symptoms), we found that the performance of the BERT-Classification model was comprehensively superior, not only on HFDS but also on TDS, as shown in Figures 7 and 8.

3.3. Comparison with Other Normalization Models. We also compared the BERT-Classification model with several other models that perform well for normalization, including the state-of-the-art models reported by other researchers. These methods are the Jaccard similarity algorithm [12], Word2Vec with cosine [11], DNorm [13], the transition-based model [15], RNN-CNNs-CRF [16], and BERT-based ranking [14]. The above models were not designed for the normalization of complex symptoms. Therefore, we only compared the performance of models to handle single symptoms (4,555 single symptoms) taken from the HFDS. The 4,555 single symptoms, and their corresponding normalized symptoms, were divided into a training set (70%), a development set (15%), and a test set (15%). The development set was used to select the parameters of each model, except the Jaccard method, for which there is no need to select parameters. The test results showed that the BERT-Classification model performed better than the other methods, as shown in Table 7.

We note that Jaccard similarity, Word2Vec with cosine, DNorm, and BERT-based ranking can output the score of each normalized symptom. Therefore, the models can output the top 5 and 10 normalized symptoms by score ranking to achieve retrieval. We used recall to observe the ability of retrieval, as shown in Table 8. The results show that the BERT-Classification model has advantages in retrieval.

To further demonstrate the advantages of our model, we summarized the test results on HFDS. According to the results, we comprehensively compared the performance and

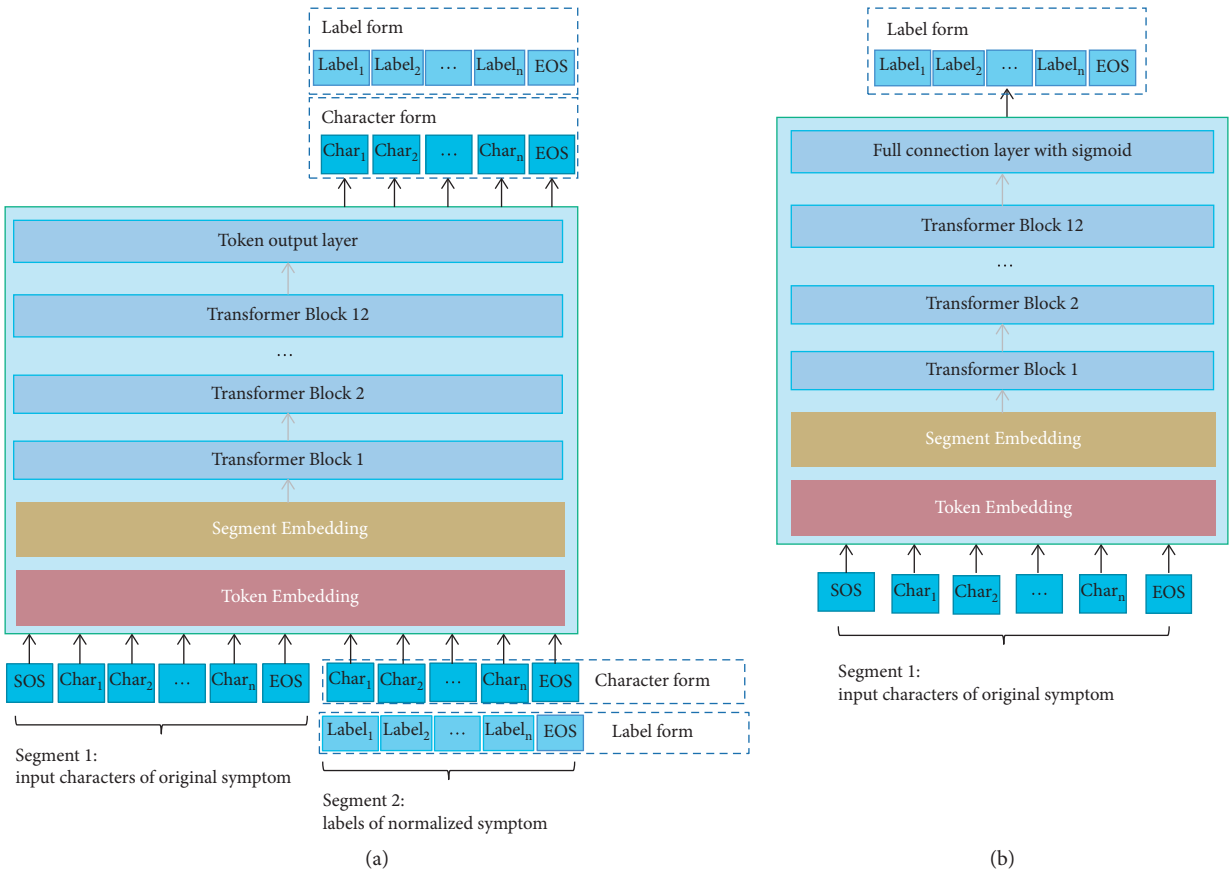


Figure 4: Examples of the BERT-UniLM and BERT-Classification models. (a) This model structure is consistent with BERT. There are 12 transformer blocks. According to the embedding composition of BERT, including segment embedding of segments 1 and 2 and character embedding of original symptom. The token output layer of the model outputs the normalized symptom in character form or label form through a fully connected layer with a softmax function. SOS is the symbol at the start of the sequence, and EOS is the symbol at the end of the sequence. This model was trained by the sequence-to-sequence method of UniLM. (b) This normalization model structure is also based on BERT. In contrast to (a), a full connection layer with the sigmoid function is used as the output layer.

Table 2: Model parameters and development set results on HFDS.

Model	LR	DR	MC	Accuracy	Precision	Recall	F1-score
Encoder (Char)-Decoder (Char)	0.0003	0.5	512	0.8631 ± 0.0042	0.8637 ± 0.0091	0.8587 ± 0.0038	0.8611 ± 0.0053
Encoder (Char)-Decoder (Label)	0.0005	0.5	256	0.8688 ± 0.0046	0.8812 ± 0.0070	0.8623 ± 0.0044	0.8716 ± 0.0048
Encoder (Word)-Decoder (Label)	0.0005	0.3	512	0.8631 ± 0.0042	0.8637 ± 0.0091	0.8587 ± 0.0038	0.8611 ± 0.0053
Encoder (Word)-Decoder (Word)	0.0005	0.3	512	0.8549 ± 0.0055	0.8596 ± 0.0047	0.8468 ± 0.0065	0.8531 ± 0.0052
Encoder (Char)-Classification	0.005	0.3	512	0.8377 ± 0.0060	0.9020 ± 0.0109	0.8414 ± 0.0062	0.8706 ± 0.0058
Encoder (Word)-Classification	0.005	0.5	512	0.8326 ± 0.0061	0.8978 ± 0.0068	0.8335 ± 0.0056	0.8645 ± 0.0043
BERT-UniLM (Char)	0.00003	0.1	N/A	0.8966 ± 0.0027	0.9013 ± 0.0064	0.8920 ± 0.0041	0.8966 ± 0.0025
BERT-UniLM (Label)	0.00003	0.1	N/A	0.8957 ± 0.0042	0.8996 ± 0.0063	0.8895 ± 0.0038	0.8945 ± 0.0039
BERT-Classification	0.00003	0.1	N/A	0.9087 ± 0.0029	0.9216 ± 0.0027	0.9084 ± 0.0034	0.9150 ± 0.0018

Note. LR: learning rate; DR: dropout rate; MC: number of memory cells of RNN; N/A: not applicable.

applicability of our model with that of existing models, as shown in Table 9.

4. Discussion

The normalization of expressions of TCM synonymous symptoms plays an important role in the collation of medical records, statistical mining, construction of TCM knowledge databases, and construction of TCM medical assistant decision-making systems [9]. The application of NLP technology improves the efficiency of normalization processing. NLP algorithms based on neural networks have been applied in normalizing biomedical texts [13, 14] but not in normalizing the expressions of TCM synonymous symptoms. In this study, multiple models were constructed with NLP algorithms based on Bi-LSTM and the BERT neural network to explore the normalization of expressions of TCM synonymous symptoms.

Table 3: Model parameters and development set results on TDS.

Model	LR	DR	MC	Accuracy	Precision	Recall	F1-score
Encoder (Char)-Decoder (Char)	0.0005	0.3	512	0.8212 ± 0.0038	0.8307 ± 0.0107	0.8011 ± 0.0044	0.8156 ± 0.0067
Encoder (Char)-Decoder (Label)	0.0003	0.5	512	0.8160 ± 0.0026	0.8379 ± 0.0060	0.7959 ± 0.0030	0.8164 ± 0.0033
Encoder (Word)-Decoder (Label)	0.0003	0.5	512	0.8091 ± 0.0045	0.8320 ± 0.0048	0.7875 ± 0.0052	0.8091 ± 0.0040
Encoder (Word)-Decoder (Word)	0.0001	0.3	256	0.8053 ± 0.0028	0.8167 ± 0.0076	0.7838 ± 0.0034	0.7999 ± 0.0049
Encoder (Char)-Classification	0.01	0.5	512	0.7681 ± 0.0058	0.8876 ± 0.0114	0.7503 ± 0.0051	0.8132 ± 0.0061
Encoder (Word)-Classification	0.01	0.3	512	0.7790 ± 0.0051	0.8913 ± 0.0074	0.7595 ± 0.0060	0.8201 ± 0.0034
BERT-UniLM (Char)	0.00003	0.1	N/A	0.8338 ± 0.0027	0.8399 ± 0.0047	0.8180 ± 0.0034	0.8288 ± 0.0033
BERT-UniLM (Label)	0.00003	0.1	N/A	0.8219 ± 0.0017	0.8388 ± 0.0056	0.8018 ± 0.0034	0.8199 ± 0.0028
BERT-Classification	0.00003	0.1	N/A	0.8547 ± 0.0027	0.9072 ± 0.0037	0.8405 ± 0.0026	0.8726 ± 0.0024

Note. LR: learning rate; DR: dropout rate; MC: number of memory cells of RNN; N/A: not applicable.

Table 4: Model performance on HFDS test data sets.

Model	Accuracy	Precision	Recall	F1-score
Encoder (Char)-Decoder (Char)	$0.8641 \pm 0.0065^{a,b}$	$0.8656 \pm 0.0084^{a,b}$	$0.8555 \pm 0.0062^{a,c}$	$0.8605 \pm 0.0056^{a,b}$
Encoder (Char)-Decoder (Label)	$0.8558 \pm 0.0070^{a,b}$	$0.8727 \pm 0.0038^{a,b}$	$0.8463 \pm 0.0062^{a,b}$	$0.8593 \pm 0.0043^{a,b}$
Encoder (Word)-Decoder (Label)	$0.8487 \pm 0.0046^{a,b}$	$0.8678 \pm 0.0076^{a,b}$	$0.8377 \pm 0.0054^{a,b}$	$0.8525 \pm 0.0059^{a,b}$
Encoder (Word)-Decoder (Word)	$0.8451 \pm 0.0035^{a,b}$	$0.8472 \pm 0.0056^{a,b}$	$0.8345 \pm 0.0036^{a,b}$	$0.8408 \pm 0.0023^{a,b}$
Encoder (Char)-Classification	0.8311 ± 0.0078^{c}	0.8937 ± 0.0072^{c}	0.8342 ± 0.0077^{c}	0.8629 ± 0.0045^{c}
Encoder (Word)-Classification	0.8294 ± 0.0079^{c}	0.8983 ± 0.0055^{c}	0.8302 ± 0.0070^{c}	0.8629 ± 0.0038^{c}
BERT-UniLM (Char)	0.8914 ± 0.0059^{c}	0.8983 ± 0.0042^{c}	0.8855 ± 0.0077^{c}	0.8918 ± 0.0043^{c}
BERT-UniLM (Label)	0.8829 ± 0.0046^{c}	0.8909 ± 0.0069^{c}	0.8773 ± 0.0044^{c}	0.8840 ± 0.0036^{c}
BERT-Classification	**0.9051 ± 0.0039**	**0.9118 ± 0.0033**	**0.9028 ± 0.0046**	**0.9073 ± 0.0033**

Note. The results are expressed as mean ± SD, and the threshold value of the sigmoid function was 0.2. [a]$P < 0.05$, compared with BERT-UniLM (Char); [b]: $P < 0.05$, compared with BERT-UniLM (Label). [c]$P < 0.05$, compared with BERT-Classification.

Table 5: Model performance on TDS test data sets.

Model	Accuracy	Precision	Recall	F1-score
Encoder (Char)-Decoder (Char)	$0.7980 \pm 0.0078^{a,b}$	$0.7876 \pm 0.0147^{a,b}$	$0.7678 \pm 0.0088^{a,b}$	$0.7775 \pm 0.0106^{a,b}$
Encoder (Char)-Decoder (Label)	$0.7974 \pm 0.0050^{a,b}$	$0.8060 \pm 0.0081^{a,b}$	$0.7690 \pm 0.0062^{a,b}$	$0.7870 \pm 0.0056^{a,b}$
Encoder (Word)-Decoder (Label)	$0.7892 \pm 0.0069^{a,b}$	$0.7979 \pm 0.0100^{a,b}$	$0.7595 \pm 0.0066^{a,b}$	$0.7782 \pm 0.0074^{a,b}$
Encoder (Word)-Decoder (Word)	$0.7904 \pm 0.0079^{a,b}$	$0.7805 \pm 0.0122^{a,b}$	$0.7594 \pm 0.0078^{a,b}$	$0.7698 \pm 0.0092^{a,b}$
Encoder (Char)-Classification	0.7559 ± 0.0056^{c}	0.8560 ± 0.0125^{c}	0.7278 ± 0.0057^{c}	0.7866 ± 0.0058^{c}
Encoder (Word)-Classification	0.7652 ± 0.0042^{c}	0.8557 ± 0.0065^{c}	0.7364 ± 0.0038^{c}	0.7915 ± 0.0028^{c}
BERT-UniLM (Char)	0.8274 ± 0.0087^{c}	0.8152 ± 0.0115^{c}	0.8043 ± 0.0082^{c}	0.8097 ± 0.0094^{c}
BERT-UniLM (Label)	0.8248 ± 0.0045^{c}	0.8230 ± 0.0066^{c}	0.7970 ± 0.0056^{c}	0.8098 ± 0.0037^{c}
BERT-Classification	**0.8568 ± 0.0029**	**0.8870 ± 0.0039**	**0.8298 ± 0.0037**	**0.8574 ± 0.0026**

Note. The results are expressed as mean ± SD, and the threshold value of the sigmoid function was 0.1. [a]$P < 0.05$, compared with BERT-UniLM (Char); [b]$P < 0.05$, compared with BERT-UniLM (Label). [c]$P < 0.05$, compared with BERT-Classification.

In TCM synonymous symptom normalization, the performance of normalization and the ability to handle one symptom corresponding to multiple normalized symptoms are crucial to the normalization model. The test results show that our BERT-Classification model outperforms previous models and has the ability as mentioned above, while previous models do not have. In addition, the model also supports retrieve normalized candidate symptoms. Our model can retrieve other candidate normalization symptoms according to original symptoms when the model does not provide suitably normalized symptoms.

These advantages of the model provide technical support for the efficient normalization of TCM synonymous symptoms and make the model highly adaptable in medical situations.

In this study, the accuracy, recall, precision, and F1-score metrics were used to evaluate the performance of each model. The results show that the BERT-Classification model outperformed other existing models with respect to various metrics; these models include the proposed Encoder-Decoder, Encoder-Classification, and BERT-UniLM designed in this study. This is because the performance of NLP models based on neural networks is strongly related to the extracted semantic features, and BERT excels in extracting semantic features [24]. Therefore, the BERT-Classification model, which extracts semantic features using BERT, is advantageous for normalization tasks. BERT-Classification, BERT-UniLM, and BERT-based ranking are all based on the BERT neural network; they differ only in their output layers due to their different modeling concepts. The results suggest that BERT-Classification performs best; therefore, the classification-based modeling concept may be the most conducive to normalizing TCM symptoms.

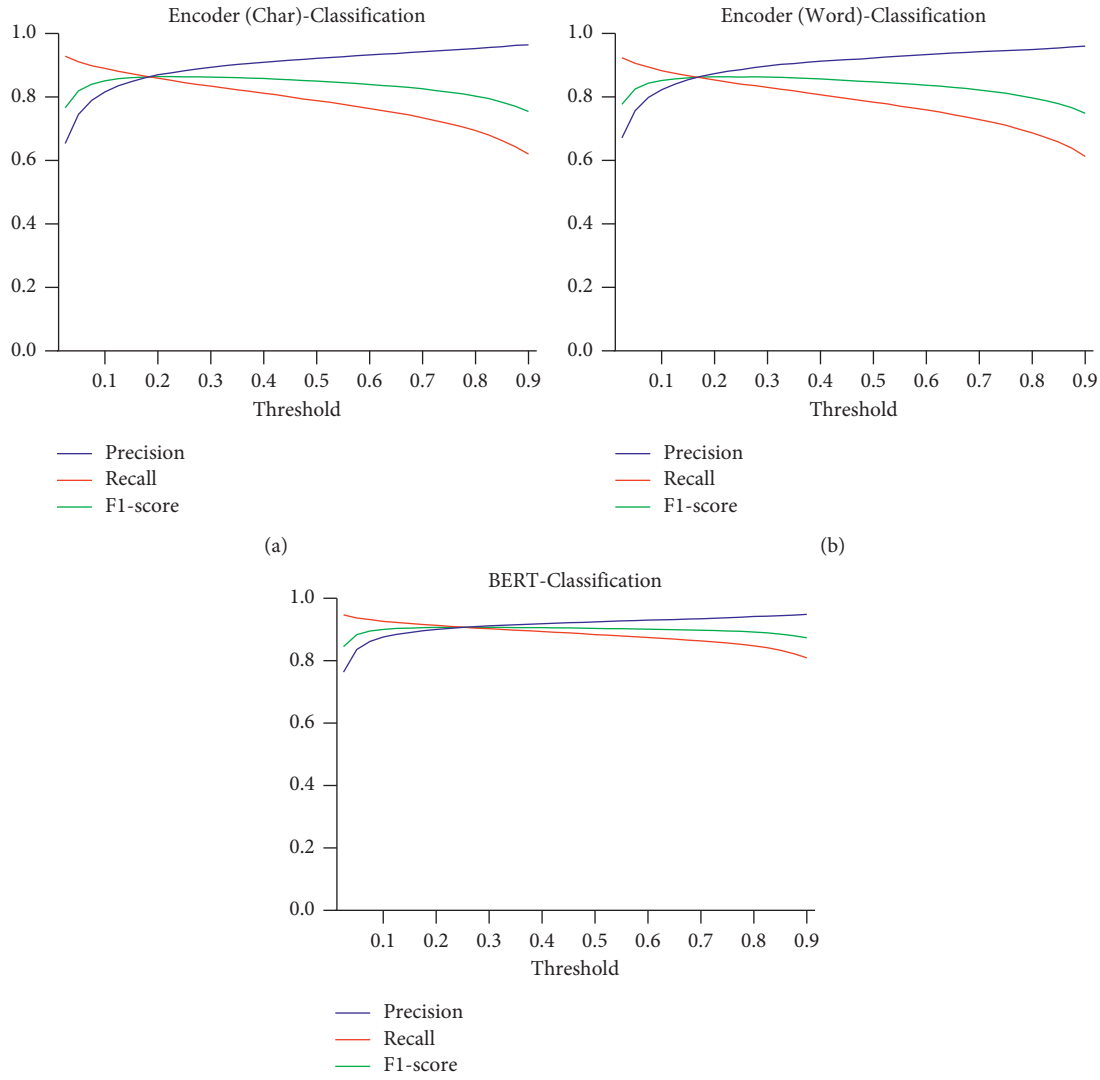
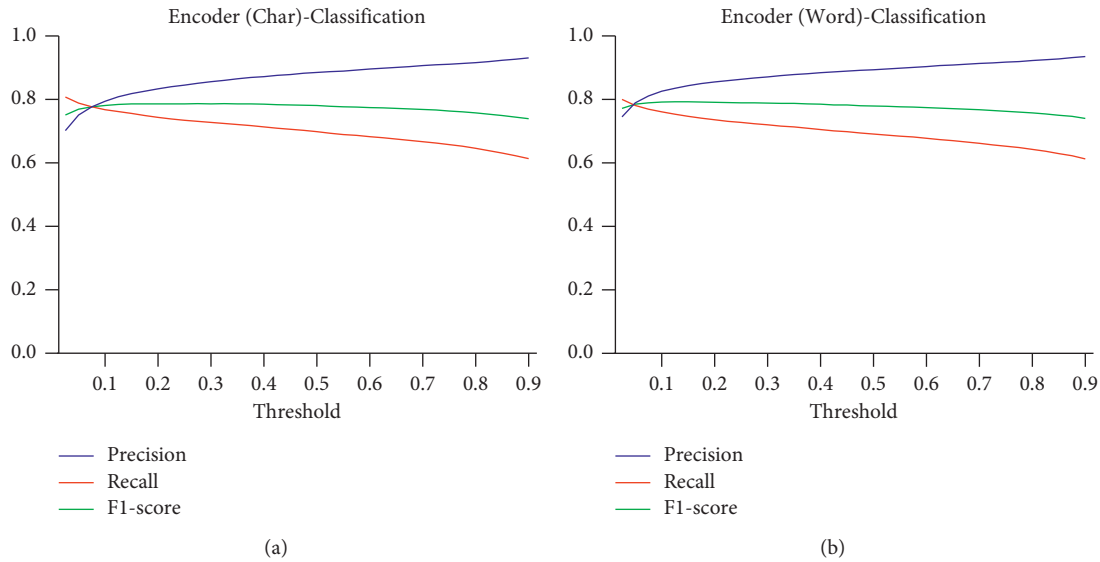


Figure 5: Effects of different thresholds on HFDS. The mean values of precision, recall, and F1-score of the (a) Encoder (Char)-Classification model, (b) Encoder (Word)-Classification model, and (c) BERT-Classification model at different sigmoid output thresholds.



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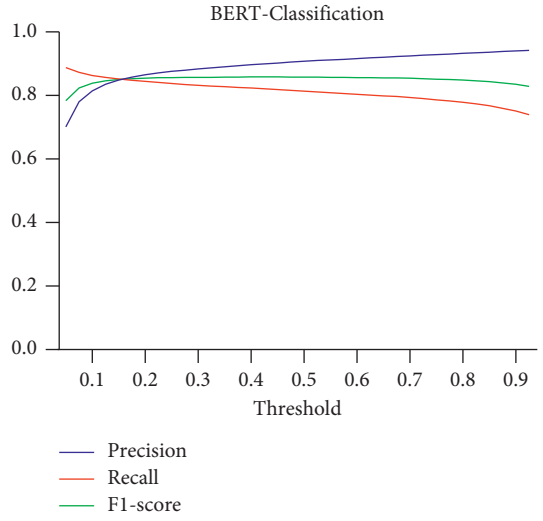

(c)

Figure 6: Effects of different thresholds on TDS. The mean values of precision, recall, and F1-score of the (a) Encoder (Char)-Classification model, (b) Encoder (Word)-Classification model, and (c) BERT-Classification model at different sigmoid output thresholds.

Table 6: The top 5 and 10 recall on the test set.

Model	HFDS		TDS	
	Top 5 recall	Top 10 recall	Top 5 recall	Top 10 recall
Encoder (Char)-Classification	0.9692 ± 0.0028*	0.9818 ± 0.0013*	0.8906 ± 0.0045*	0.9164 ± 0.0037*
Encoder (Word)-Classification	0.9635 ± 0.0025*	0.9785 ± 0.0015*	0.8928 ± 0.0046*	0.9195 ± 0.0038*
BERT-Classification	**0.9758 ± 0.0012**	**0.9858 ± 0.0011**	**0.9212 ± 0.0019**	**0.9426 ± 0.0015**

Note. The results are expressed as mean ± SD. *$P < 0.05$, compared with BERT-Classification.

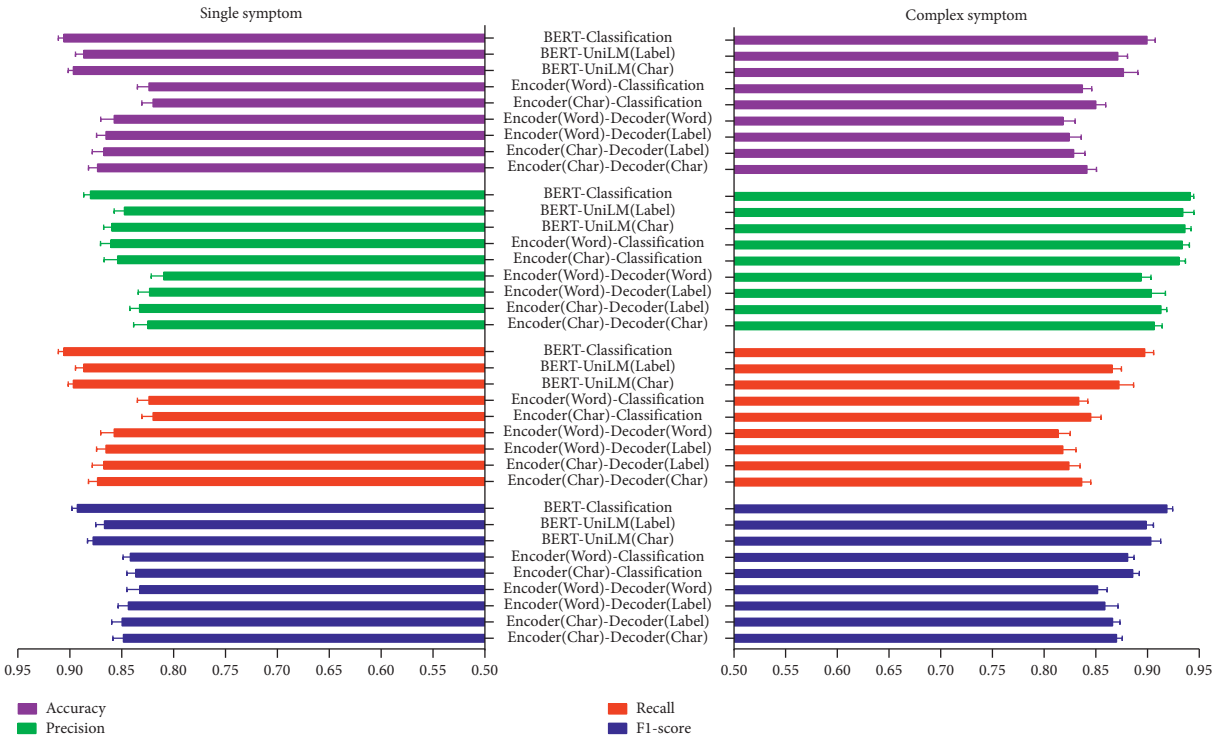

Figure 7: Normalization performance of single and complex symptoms on HFDS.

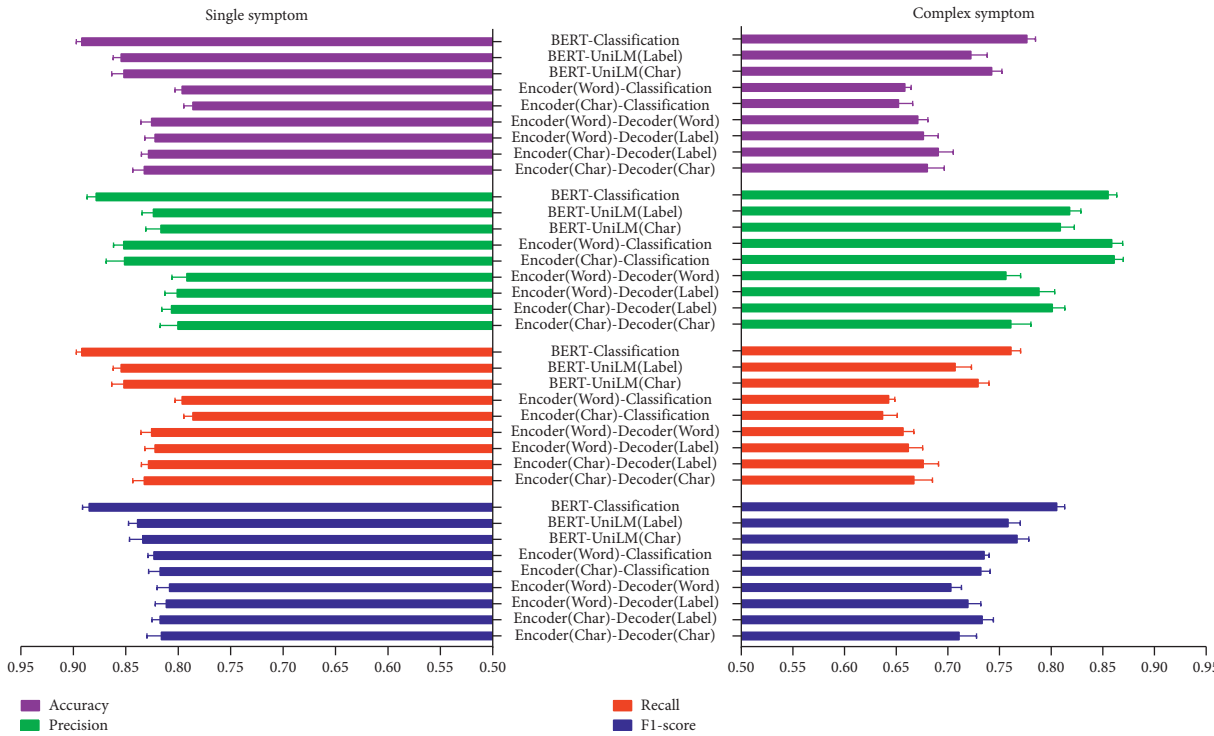

Figure 8: Normalization performance of single and complex symptoms on TDS.

Table 7: Comparison of the BERT-Classification model with other models.

Model	Accuracy	Precision	Recall	F1-score
Jaccard similarity	0.49188	0.65251	0.54722	0.54317
Word2Vec with cosine	0.6424 ± 0.0019*	0.7365 ± 0.0093*	0.6906 ± 0.0036*	0.6724 ± 0.0047*
DNorm	0.8572 ± 0.0050*	0.8694 ± 0.0087*	0.8602 ± 0.0072*	0.8555 ± 0.0061*
Transition-based model	0.7980 ± 0.0056*	0.8256 ± 0.0081*	0.7970 ± 0.0051*	0.7937 ± 0.0050*
RNN-CNNs-CRF	0.8852 ± 0.0036*	0.8755 ± 0.0035*	0.8724 ± 0.0032*	0.8645 ± 0.0034*
BERT-based ranking	0.9264 ± 0.0057	0.9413 ± 0.0056	0.9321 ± 0.0072*	0.9313 ± 0.0065*
BERT-Classification	**0.9300 ± 0.0019**	**0.9473 ± 0.0023**	**0.9380 ± 0.0021**	**0.9378 ± 0.0021**

Note. The test results are expressed as mean ± SD. Each model was repeated 10 times, except for Jaccard similarity. *$P < 0.05$, compared with BERT-Classification.

Table 8: The top 5 and 10 recall of models.

Model	Top 5 recall	Top 10 recall
Jaccard similarity	0.57312	60857
Word2Vec with cosine	0.9145 ± 0.0025*	0.9524 ± 0.0035*
DNorm	0.9702 ± 0.0006*	0.9858 ± 0.0008*
BERT-based ranking	0.9852 ± 0.0049	0.9910 ± 0.0050
BERT-Classification	**0.9864 ± 0.0044**	**0.9921 ± 0.0048**

Note. The test results are expressed as mean ± SD. Each model was repeated 10 times, except for Jaccard similarity. *$P < 0.05$, compared with BERT-Classification.

With regard to applicability, our proposed BERT-Classification model supports both the processing of the original symptoms that correspond to multiple normalized symptoms and the retrieval of normalized symptoms. We use sigmoid as an output function to handle the situation in which each original symptom corresponds to multiple normalized symptoms; this method is effective and outperforms sequence generation methods. Moreover, for the model to support the retrieval of normalized symptoms, it requires a higher recall. Our BERT-Classification model can increase the recall by reducing the output threshold of the sigmoid function and thereby support retrieval.

In contrast to BERT-Classification, the other reported models cannot support both of the above applications simultaneously. Jaccard similarity, DNorm, Word2vec with cosine, and BERT-based ranking pair an original symptom with each normalized symptom and rank the normalized symptoms by their pairing score. Although these models can

Table 9: Model comparison.

Model	Modeling concept	Complex symptoms [a]	Retrieval [b]	Overall performance [c]
Jaccard similarity	Similarity matching	×	√(0.61)	×
Word2vec with cosine	Similarity matching	×	√(0.95)	×
Encoder-Classification (our)	Text classification	√(0.89)	√(0.98)	√(0.86)
Encoder-Decoder (our)	Sequence generation	√(0.87)	×	√(0.86)
DNorm	Similarity matching	×	√(0.99)	×
Transition-based model	NER	×	×	×
Bi-LSTM-CNNs-CRF	NER	×	×	×
BERT-based ranking	Similarity matching	×	√(0.99)	×
BERT-UniLM (our)	Sequence generation	√(0.90)	×	√(0.89)
BERT-Classification (our)	**Text classification**	**√(0.92)**	**√(0.99)**	**√(0.91)**

Note. [a] means the ability to handle complex symptoms, if the model has this ability, it is evaluated for performance using F1-score; [b] means the ability to retrieve normalized symptoms, if the model has this ability, it is evaluated for performance using top 10 recall; [c] stands for the overall performance of normalizing single symptoms and complex symptoms, if the model has the ability of handling single symptoms and complex symptoms, it is evaluated by F1-score. √ indicates that the model has this ability or can be evaluated for overall performance. × indicates that the model does not have this ability or cannot be evaluated for overall performance.

output multiple normalized symptoms by ranking them for retrieval, when multiple normalized symptoms corresponding to the original symptoms need to be output precisely, it is difficult to decide whether the results (except for the normalized symptom with the highest score) should be output. The Bi-LSTM-CNNs-CRF model is only designed for outputting a single normalized symptom. In addition, because the model is based on the NER modeling concept, it cannot produce multiple candidate normalized symptoms, as the above models can, and therefore cannot be applied to the retrieval task. Although the Encoder-Decoder and BERT-UniLM models support the output of multiple normalized symptoms, they suffer from the same limitations as Bi-LSTM-CNNs-CRF and are not suitable for the retrieval of normalized symptoms.

The HFDS contained only high-frequency samples for modeling and testing, reflecting the performance of the BERT-Classification model under ideal conditions. Conversely, the TDS included both high-frequency and low-frequency samples, reflecting the performance of the model in practical applications. Comparing the results of the model on the two data sets, the performance on TDS was lower than that on HFDS. This suggests that the performance of the model can be improved by increasing the number of low-frequency samples.

5. Conclusions

This study constructed models to normalize TCM synonymous symptoms from the perspectives of text classification and sequence generation of NLP. The optimal model is the BERT-Classification model, which outperforms existing reported models in dealing with original symptoms that correspond to a single normalized symptom. Moreover, it also supports original symptoms that correspond to multiple normalized symptoms, and it has the ability to retrieve normalized symptoms. The limitation of this study is that the normalization models only explore symptoms. Whether the models can be used for normalizing other synonymous terms, such as TCM treatment terms and TCM disease terms, remains to be further studied. In addition, the pretrained BERT model based on large-scale corpora plays an important role in improving the model performance; the BERT model trained on corpora from professional medical fields is likely to achieve better results for normalization of medical terms. Therefore, the use of a large number of TCM literature corpora to construct the pretrained model, to improve the normalization performance, also needs further research.

Abbreviations

BERT: Bidirectional encoder representation from transformers
Bi-LSTM: Bidirectional long short-term memory
DR: Dropout rate
FN: False negative
FP: False positive
HFDS: High-frequency data sets
LR: Learning rate
MC: Memory cell
N/A: Not applicable
NLP: Natural language processing
RNN: Recurrent neural network
SD: Standard deviation
TCM: Traditional Chinese medicine
TDS: Total data sets
TP: True positive
UniLM: Unified language model.

Data Availability

All the data and materials used in the current study are available from the corresponding author on reasonable request.

Ethical Approval

Not applicable.

Consent

Not applicable.

Disclosure

The funder has no role in study design, data collection, analysis, decision to publish, or manuscript preparation.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

YH Li and FQ Xu guided the whole work; L Zhou and SQ Liu developed all models; CY Li, YD Li, FQ Xu, and YM Sun collected medical data; SQ Liu and YZ Zhang performed data labeling; Y Sun and YH Li checked all labels; Y Sun and HM Yuan calculated all metrics. All the authors read and approved the final manuscript. L Zhou and SQ Liu contributed equally to this work.

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Research Article

Identifying Chinese Medicine Patterns of Tension-Type Headache and Understanding Its Subgroups

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Tension-type headache (TTH) is common among adults. Individualized management strategies are limited due to lack of understanding of subtypes of TTH. Chinese medicine (CM) uses the pattern differentiation approach to subtype all health conditions. There is, however, a lack of evidence-based information on CM patterns of TTH. This study aimed to identity common CM patterns of TTH. TTH sufferers were invited for a survey, consisting of a validated Chinese Medicine Headache Questionnaire (CMHQ), Migraine Disability Assessment Test, and Perceived Stress Scale. The CMHQ consisted of information about headache, aggravating and relieving factors, and accompanying symptoms. Principal component analysis was used for factor extraction and TwoStep cluster analyses for identifying clusters. ANOVA was used to compare cluster groups with disability and stress. In total, 170 eligible participants took part in the survey. The commonest headache features were continuous pain (64%); fixed location (74%); aggravated by overwork (74%), stress (74%), or mental strain (70%); and relieved by sleeping (78%). The commonest nonpain symptoms were fatigue (71%) and neck stiffness (70%). Four clusters, differing in their key signs and symptoms, could be assigned to three different CM patterns including ascendant hyperactivity of liver yang (cluster 1), dual qi and blood deficiency (cluster 2), liver depression forming fire (cluster 3), and an unlabelled group (cluster 4). Additionally, over 75% participants in clusters 1 and 2 have episodic TTH, over one-third participants in cluster 3 have chronic TTH, and a majority of participants in cluster 4 have infrequent TTH. The three patterns identified also differed in levels of disability and some elements of coping as measured with PSS. The three CM patterns identified are common clinical presentations of TTH. The new information will contribute to further understanding of the subtypes of TTH and guide the development of targeted intervention combinations for clinical practice and research.

1. Introduction

Tension-type headache (TTH), described as a dull, pressing, or tight quality [1], is found to be the second most prevalent chronic disorders in the world [2]. TTH is regarded as a featureless headache, as most TTH sufferers have no associated symptoms when compared with other type of headaches. Due to its nonspecific and lacking specific and distinguishing feature [3], diagnosis of TTH is, therefore, largely based on negative features [3, 4] and by excluding symptoms, syndromes, and organ diseases mimicking other primary or secondary headache [5]. However, the treatment strategies for TTH remain unspecific as its underlying mechanisms are unknown [3, 6, 7]. In addition, it is well known that people with TTH have different headache features [8–11], yet there is no standard way to subtype TTH, except for categorizing into infrequent, episodic (ETTH), and chronic TTH (CTTH) based on the frequency of

headache within four weeks [1]. Such an approach does not lead to the development of effective, individualised treatment strategy [5].

Trigger factors could worsen TTH [12, 13]. Hence, identification of triggers and coping with these factors may be of values [14]. One hospital-based study confirmed that emotional stress was the major trigger factor of TTH, as outlined in other studies [8, 15]. Depression and anxiety levels in TTH sufferers have also been observed to be higher than headache-free controls [16, 17]. In addition, some triggers that are typically specific to migraine may also precipitate TTH [5], including a lack of sleep, fatigue, missing meals [18], menstruation, weather changes, relaxation after stress, exposure to bright lights, strong odors and loud noises, and ingestion of alcoholic beverages [18–20]. Presence of triggers for TTH is largely relied on patients' self-report, but has not been tested systematically [13].

Chinese medicine (CM), which has a long history of treating headaches, is viewed as "personalized medicine" as CM differential diagnostic approach guides the tailored treatment for each individual [21]. Given that TTH is not a diagnosis in CM, recognition and treatment of TTH must be based on the CM classification and common patterns of general headache. CM understands that the causes of most headaches are disorders of Qi and blood or lack of nourishment of the channels and collaterals [22]. The guiding principle of CM headache treatment is to enhance and strengthen any deficiency identified or eliminate and dispel the excessive pathogens, which vary from person to person. It is through addressing different forms of deficiency or excess that CM treatment is tailored to individuals.

Acupuncture, a key treatment modality of CM, has been reported to be effective for pain management [23, 24]. Acupuncture has been recommended as a prophylactic treatment for chronic TTH due to its effectiveness and safety profile [25, 26]. It is a valuable nonpharmacological option for patients suffering from frequent episodic or chronic TTH [27, 28]. In acupuncture treatment, each health condition is subdivided into a few common patterns based on signs and symptoms. Those patterns are important as they guide the selection of the supposed optimal individualised acupuncture protocol. Nevertheless, there is a lack of CM criteria for TTH patterns, which are important as they guide the determination of the optimal acupuncture protocol. Identifying CM patterns involves a complicated process of synthesising and analysing clinical symptoms and signs of the patient's condition to determine the location, cause, and nature of the condition [29]. Diagnosis of TTH largely relies on textbook information or expert opinion but not based on research evidence. Over the past several years, published CM studies specific to TTH in China proposed different TTH patterns based on clinical experiences and observations with some overlaps among them. Those include eleven (11) patterns of ascendant hyperactivity of liver yang, kidney deficiency, spleen deficiency, liver qi stagnation, stagnated gall bladder qi with disturbing phlegm, liver fire ascending, cold congealing in the jueyin meridian, static blood blocking collaterals, deficiency of heart and spleen, kidney yin deficiency, and deficiency of both liver yin and kidney yin [30]. Consequently, variations in the diagnosis of TTH among practitioners are common [31]. Studies have shown that it is possible to standardize and validate patterns using objective methods and evidence-based approaches [21, 32–37]. Cluster analysis has been recognized as a suitable technique to identify homogeneous subgroups for identifying CM patterns of diseases [38–40].

The aims of this study were (1) to explore CM patterns of TTH based on data collected using a validated Chinese Medicine Headache Questionnaire (CMHQ); and (2) to explore if identified CM patterns differed on information collected in modern TTH research and practice, including headache features, severity of headache-related disability assessed with Migraine Disability Assessment Test (MIDAS), and number of comorbidities, psychological profiles, such as anxiety, depression, and self-perceived level of stress. Findings of this research will lend a hand to understanding of subtypes of TTH.

2. Methods

2.1. Design. A bilingual cross-sectional survey was conducted from February 2011 to June 2012. A paper-based survey and an online survey were delivered in parallel. The online survey was performed via the SurveyMonkey® platform, whereas the paper-based survey was administrated at three sites: Melbourne, Beijing, and Chengdu. The Australian sample was from a clinical trial conducted from 2008–2012 entitled "Combined therapy of electroacupuncture and cognitive behavioural therapy for tension-type headache: a randomised controlled trial" (ANZCTR: ACTRN12608000239369) in Melbourne and from online survey. All Chinese samples were collected from two Chinese sites and through online. The two Chinese sites were of Beijing Hospital of Traditional Chinese Medicine (TCM) Affiliated to Capital Medical University and Affiliated Hospital of Chengdu University of TCM.

2.2. Ethics. The survey protocol was reviewed, assessed, and approved by the College Human Ethics Advisory Network of the College of Science Engineering and Health (CHEAN), RMIT University (BESHAPP10-11 HAO). The other two collaboration sites of Beijing and Chengdu were granted permission by the Department of Science Research of Beijing TCM Hospital and for the Chengdu site, by the Department of Science and Technology, Chengdu University of TCM, respectively. Those approvals were endorsed by CHEAN, RMIT.

2.3. Recruiting Criteria. Potential headache sufferers, aged from 18 to 65 years old, were eligible to participate if they were able to read English or Chinese; met the International Headache Society TTH diagnostic (ICHD-II) criteria of TTH or probable TTH [41]; and had one day or more of TTH attacks per month for at least one year. Exclusion criteria were TTH onset after 50 years old as those headaches are more likely to be secondary headache [42]; had more than 4 migraine attacks without aura per month, as

increased attacks of migraines should be classified under migraine, rather than TTH according to ICHD-II [41]; had any migraine attack with aura per month; had been hospitalized because of the head or neck injury; or had migraine attacks which were not able to be distinguished from TTH.

2.4. Measurements. Demographic characteristics of the participants collected from this survey included gender, age, ethnicity, marital status, and education. Each of the listed instruments included in the survey was available in both English and Chinese versions.

Chinese Medicine Headache Questionnaire (CMHQ): the CMHQ is a symptom-based data collection tool consisting of a total 193 items which are grouped into three broad categories of pain description, aggravating and relieving factors, and accompanying symptoms. It has been used to assist CM pattern identification for headache disorders and found to be reliable and valid in capturing essential clinical indicators for making a CM diagnosis [30]. Responses to each item presented were on a 5-point Likert scale rating from 0 to 4 indicating never, seldom, sometimes, often and almost always (Appendix A).

2.4.1. Migraine Disability Assessment (MIDAS) Questionnaire. The MIDAS was initially designed for the migraine population to evaluate the severity of migraine. Studies have shown it is also valid and reliable in evaluating disability associated with TTH [43–48].

2.4.2. Perceived Stress Scale (PSS). The PSS is a widely used instrument in measuring nonspecific psychological stress. Its 10-item version is among the most widely used tool to measure global perceived stress in relation to the health-related outcomes [49, 50].

2.4.3. Comorbidity Checklist. A comorbidity checklist was used to assess both somatic and mental comorbidity of TTH. Development of the checklist was based on the Cumulative Illness Rating Scale (CIRS) [51] and the World Mental Health Composite International Diagnostic Interview (WMH-CIDI) [52]. The items in this checklist were reformatted in a coherent manner to detect both somatic comorbidity and the mental comorbidity.

2.5. Data Analysis. SPSS 18.0 was used for data analysis. A P value <0.05 was considered to be statistically significant. Chi-squared tests were used to examine the difference in categorical outcomes. Factor analysis and cluster analysis were conjunctively applied to obtain effective clusters and identify meaningful CM patterns for TTH. Specifically, the principal component analysis (PCA) was used for factor extraction in condensing respondents' responses to diagnostic information obtained from CMHQ items, whereas the TwoStep cluster algorithm was then used for grouping these identified factors into clusters for further evaluation [53, 54]. For PCA factor extraction, a cutoff value of 0.5 on a coefficient ("factor loading") was adopted [55]. To determine if an identified factor was included in pattern identification based on the results from TwoStep cluster analyses, a cutoff value of 0.4 on clusters' mean scores was used. ANOVA was used to assess the cluster difference in MIDAS grades and in PSS levels. Chi-squared tests and ANOVA were employed to compare the characteristics of the resulting clusters, which enables further examination of the group differences among the CM pattern types, in MIDAS grades, and in PSS levels of the participants. Multiple comparisons were performed to compare group means via *post hoc* tests with Bonferroni correction when significant differences were observed in means across groups. For missing data handling, both case deletion and imputation methods were applied. Cases having more than 30% missing values within the total 193 items in CMHQ were deleted from the dataset, whereas cases having less than 30% missing values were remedied via the expectation-maximization algorithm [55].

Evaluation and interpretation of data for pattern identification had four sequential steps (Figure 1). The first step was to reduce the items of CMHQ into smaller datasets using factor analysis; the second step was to assess the factors extracted and to label those factors in a clinical meaningful manner; the third step was to group (clinical meaningful) factors into clusters using cluster analysis; the final step was the identification of TTH patterns, that is, to label the clusters into clinically meaningful CM patterns. Sixteen teaching and research staff across universities and hospitals with their professional backgrounds covering CM, acupuncture, modern medicine, statistics, etc., were invited to provide their experts' opinions in the 2nd and 3rd steps to ensure that the labels assigned to factors and clusters were of clinical relevance and significance. Only the labels that reached 70% agreement among 16 evaluators were retained.

3. Results

From February 2011 to June 2012, a total of 565 respondents took part in the survey and 497 completed it. 170 participants were eligible and included for data analysis. Figure 2 illustrates the participant selection process. Among them, 70.6% were female and 29.4% were male (F : M = 2.1 : 1). The average age was 38 years (SD = 12). Defined by headache days per month, a majority (63%) of the included participants suffered from ETTH, whereas 23% and 14% were of CTTH and infrequent subtypes, respectively. Sociodemographic characteristics including ethnicity, marital status, and education are shown in Table 1, which indicates a majority of participants were female with a higher level of education degree in the age range of 20 to 40.

According to the CMHQ, the key features of the headaches were pain with a fixed location (74%), of continuous (66.7%) and intermittent (52.7%) nature, with tight (35.3%), heavy (34.1%), and pulsating (34.1%) sensations, and affecting the neck (61.3%) and eyes (57.2%). Overwork (74.1%), stress (73.6%), mental strain (70%), being tired (68.1%), lack of sleep (68.1%), anger or irritability (65.8%), anxiety (excessive worry) (65.5%) nervousness (56.3%), and muscular strain (muscle tightness) (53.1%) were identified as

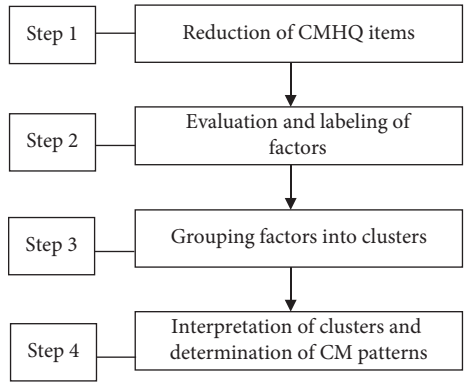


Figure 1: Process of CM pattern identification.

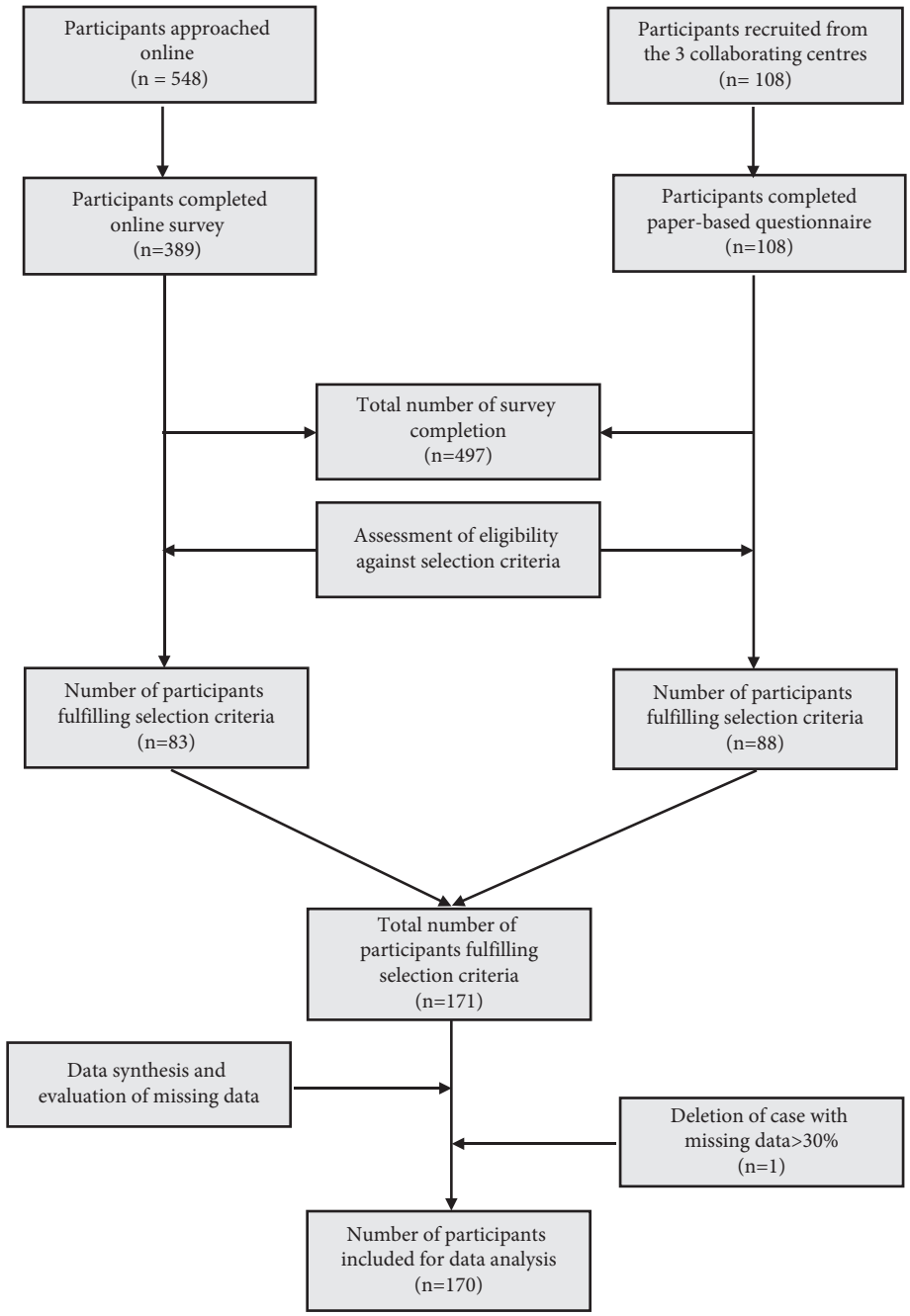


Figure 2: Flow chart of participant recruitment and screening process.

Table 1: Sociodemographic characteristics of the included participants.

	Frequency (n)	Percent
Gender ($n = 170$)		
Women	120	70.6
Men	50	29.4
Age range ($n = 166$)		
20–29	55	33.1
30–39	44	26.5
40–49	31	18.7
50–59	26	15.7
60+	10	6.0
Marital status ($n = 165$)		
Single	64	38.8
Married	77	46.7
Partnered	9	5.5
Divorced	13	7.9
Separated	2	1.2
Ethnicity distribution ($n = 164$)		
Asian	85	51.8
Oceanian	45	27.4
European	25	15.2
Arab	1	.6
Had >1 ethnicity	8	4.9
Level of education ($n = 164$)		
Postgraduate degree level	40	24.4
Graduate diploma and graduate certificate level	8	4.9
Bachelor degree level	72	43.9
Advanced diploma and diploma level	18	11.0
TAFE level	10	6.1
Secondary education	14	8.5
Primary education	2	1.2

Note: designing of the sociodemographic categories were referenced from the Australian Bureau of Statistics website (http://www.abs.gov.au).

the commonest aggravating factors of headache, whereas sleeping (77.7%), medication (62.7%), lying down (62.4%), pressing on the pain area (62.1%), and massage (50.9%) were the commonest relieving factors of the headaches. Apart from headaches, neck (60%), shoulder (45.3%), and lower back (35.3%) were the most common painful areas. Of the female-related items, bright red-coloured menstrual blood (50.5%), dark-coloured menstrual blood (62.4%), headache before period (51.6%), and abdominal pain during periods (52.7%) were common referred items. Overall, the most common accompanying symptoms were fatigue (71.3%), neck stiffness (70%), and neck pain (60%).

3.1. TTH Pattern Identification. The exploratory analytic methods of factor analysis and cluster analysis were conjointly used given the relatively large number of CMHQ items. Firstly, PCA was used to extract factors on each part of CMHQ separately. Based on CM theory, only 41 clinical meaningful factors, including 12 factors from CMHQ part 1, 13 from part 2, and 16 from part 3, were labelled and retained for TTH pattern identification (Appendix B and C). Secondly, using the TwoStep cluster analysis, four distinct cluster groups were identified. Lastly, experts analysed the clinical characteristics of each cluster and labelled them as ascendant hyperactivity of liver yang (cluster 1), dual qi and blood deficiency (cluster 2), liver depression forming fire (cluster 3), and an unlabelled group (cluster 4) (Table 2). The first three are common patterns of headache presented in CM clinical practice.

3.2. Cluster Comparisons. Table 3 summarizes the characteristics of participants according to the four clusters. The four clusters differed in the aspects of demographic characteristics, stress levels, pain intensity (indicated by MIDAS item B), disability grades (indicated by MIDAS), and TTH subtypes. There were no cluster differences in gender, marital status, or education level. There was statistical age difference among the clusters ($P < 0.001$). Participants in cluster 1 were older than those in clusters 3 and 4; however, those in cluster 2 were older than those in cluster 4. Statistically significant cluster differences were also found in ethnicity distribution. Over three quarters of participants in clusters 3 and 4 were of Asian origin, but over three quarters of those in clusters 1 and 2 were of non-Asian (Oceania and European) origin.

More than half of the participants were suffering from frequency TTH in all clusters. Cluster 1 had more infrequent ETTH headache than clusters 1–3, and cluster 3 had more CTTH than the other three clusters ($P < 0.001$). ANOVA results indicated no cluster differences in the overall MIDAS

Table 2: Summary of cluster characteristics according to the CMHQ data.

	Cluster 1 ($n = 46$)	Cluster 2 ($n = 34$)		Cluster 3 ($n = 46$)	Cluster 4 ($n = 44$)
Location and quality	(i) Forehead; Back of the head; Top of the head (ii) Pain quality: Throbbing; Pulsating; Pounding; Tight; A "band-like" sensation	(i) Forehead, Back of the head, Top of the head; Both side of the head (ii) Pain quality: Throbbing; Pulsating; Pounding (iii) Worse in the morning; Worse at night; All day		Whole head; No particular location	Explosive; Not dull; Sharp; Piercing
Aggravating and relieving factors	Aggravating by Dehydration; Hunger; Chocolate; Muscular strain (muscle tightness); Poor posture in sitting, standing or sleeping; Teeth grinding	Aggravating by Change of weather; Change in temperature; Hot weather; Cold weather; Dehydration; Hunger; Chocolate	Relieving by Exercise; Massage; Pressing the pain area; Warmth Coldness; Medication; Eating	Aggravating by Stress; Nervousness Irritability Excessive worry; Depression Tension or conflict-related	Aggravating by Windy days; Damp weather/ Humid weather; Rainy days
Accompanying symptoms	Sensitivity to light (or to bright lights); Sensitivity to sound	"Pins and needles" or numbness in the hands and feet; Faintness; Dizziness; Watery bowel motion; Loose bowel motion		Dry mouth; Thirst; Bitter taste in the mouth	Belching; Bloating/ Flatulence; Indigestion; Fear of being hot

Cluster 1: ascendant hyperactivity of liver yang. Cluster 2: dual qi and blood deficiency. Cluster 3: liver depression forming fire. Cluster 4: unlabelled group.

Table 3: Cluster comparisons of demographic data, TTH subtypes, MIDAS, PSS, and CIRS items.

		TTH clusters				Total (n)	Missing value (n)	P value† Chi-square	P value† ANOVA
		C1 (46)	C2 (34)	C3 (46)	C4 (44)	170			
Age (mean ± SD)		45 ± 12	39 ± 11	37 ± 12	30 ± 9	143	27	N/A	≤0.001*05
(n)		44	33	37	29				
Gender (n)	F	35	27	30	28	120	0	.307	N/A
	M	11	7	16	16	50			
Age range (n)	20–29	6	8	17	24	55	4	**0.001** *0125	N/A
	30–39	10	12	10	12	44			
	40–49	10	8	6	7	31			
	50–59	14	4	8	0	26			
	60+	5	2	2	1	10			
Marriage status (n)	Single	13	10	16	25	64	5	0.047	N/A
	Married	22	15	25	14	77			
	Partnered	3	4	0	2	9			
	Divorced	6	4	1	2	13			
	Separated	0	1	0	1	2			
Education level (n)	Postgraduate	13	8	8	11	40	5	0.968	N/A
	Graduate	3	2	1	2	8			
	Bachelor	17	14	21	20	72			
	Diploma	4	5	3	6	19			
	TAFE	4	1	3	2	10			
	Secondary edu	4	4	4	2	14			
	Primary edu	0	0	1	1	2			
Ethnicity a (n)	Oceania	19	18	4	4	45	6	≤0.001*0125	N/A
	European	14	7	2	2	25			
	Arab	0	0	1	0	1			
	Asian	7	5	35	38	85			
	had >1 ethnicity	5	3	0	0	8			
Ethnicity B (n)	Asian	7	5	35	38	85	5	≤0.001	N/A
	Non-Asian	39	28	7	6	80			

Table 3: Continued.

		TTH clusters				Total (n)	Missing value (n)	P value† Chi-square	P value† ANOVA
		C1 (46)	C2 (34)	C3 (46)	C4 (44)	170			
TTH subtypes (n)	Infrequent ETTH	1	1	7	15	24	0	≤0.001*0125	N/A
	Frequent ETTH	36	26	23	22	107			
	CTTH	9	7	16	7	39			
MIDAS item, n (%)	Q1	44 (1.07)	33 (2.30)	43 (2.74)	44 (3.86)	164 (7.771)		N/A	0.408
	Q2	44 (5.34)	33 (9.67)	43 (9.81)	44 (5.50)	164 (7.43)		N/A	0.157
	Q3	44 (3.95)	33 (6.82)	43 (4.00)	44 (3.23)	164 (4.35)		N/A	0.209
	Q4	44 (6.05)	33 (8.15)	43 (4.35)	44 (2.93)	164 (5.19)		N/A	**0.038*05**
	Q5	44 (2.18)	33 (4.24))	432.65	443.86	164 (3.17)		N/A	0.606
	MIDAS a	44 (20.45)	33 (26.73)	43 (20.53)	44 (15.82)	164 (20.49)		N/A	0.259
	MIDAS B	44 (5.45)	33 (6.30) v.4	43 (5.42)	44 (4.68) v.2	164 (5.41)		N/A	**0.015*05**
	MIDAS SUM (mean score)	44 (18.59)	33 (31.18)	43 (23.56)	44 (19.39)	164 (22.64)		N/A	.310
MIDAS grade, n (%)	Grade I	12 (27%)	3 (1%)	14 (33%)	22 (50%)	51 (31%)		**0.017*05**	N/A
	Grade II	5 (11%)	7 (21%)	3 (7%)	4 (9%)	19 (12%)			
	Grade III	14 (32%)	8 (24%)	8 (19%)	7 (16%)	37 (23%)			
	Grade IV	13 (30%)	15 (45%)	18 (42%)	11 (25%)	57 (35%)			
PSS score (score by item)	Sum	16.68	16.19	18.79	15.11	16.72		N/A	0.092
	Perceived distress	9.85	10.06	10.22	7.52	9.39		N/A	0.066
	Perceived coping	5.04v.3,4	5.18v.3,4	7.35*v.1,2	7.59*v.1,2	6.35		N/A	**≤0.001*017**
Comorbidity checklist (number of items)	Somatic comorbidity	46	34	46	44	42.9%		0.588	N/A
	Mental comorbidity	8	9	5	0	12.9%		0.060	N/A

Note 1: Australia is a county of immigration. In the section of ethnicity, the category of "had more than 1 ethnicity" indicated a group of participants in this country shares more than one ethnicity. For example, an Australian person may have his/her mother of Irish ethnicity and father of Greek. In such case, these participants may tick two options, and in data analysis, he/she was classified as participant had more than one ethnicity. Note 2: both Chi-square and ANOVA were applied to access cluster differences for comparison. Chi-square tests examine categorical outcomes, whereas ANOVA assesses the means of each cluster. P values correspond to comparisons between the clusters using Chi-square test or ANOVA, as appropriate. Note 3: in PSS-10, there are no cutoffs for "Perceived Distress" nor "Perceived Coping." A lower score in "Perceived Coping" factor reflects better coping ability since the four positively stated items (4, 5, 7, and 8) in this factor are reversed scored and then summing across all items when calculating the overall score. Note 4: *05—the mean difference is significant at the 0.05 level; *0125—the mean difference is significant at the 0.0125(0.05/4) level; *017—the mean difference is significant at the 0.017(0.05/3) level; "v" denotes the clusters differed with post hoc Bonferroni correction, whereas the "x (figure)" after "v" indicates specific cluster or clusters.

scores. The level of disability, which ranged from grade I to grade IV (from low to high), was classified based on the MIDAS scores. The mean MIDAS SUM score of the current sample was 22.64 lost days, at a severe disability level (grade IV). There was a statistically significant cluster difference in the disability level ($P = 0.17$). This was largely due to about 50% the participants in clusters 2 and 3 having a higher level of disability (grades III and IV), whereas 50% of cluster 4 had the lowest level of disability (grade I). There were statistically significant cluster group differences in MIDAS items 4, which indicate the reduced productivity in household because of headaches, and MIDAS B, detecting the average pain on a 0–10 scale. Post hoc t-tests with Bonferroni correction found clusters 2 and 4 were statistically different, with cluster 2 having more nonproductive days at home (8.2 days) due to headache and more severe headache (6.3) than cluster 4 (mean: 3 days, mean intensity: 4.7).

The average PSS score was 16.72. Compared with the normative data mean score of PSS-10 around 13 [49, 50], the existing sample had a relatively higher perceived stress than the general population. There was no cluster difference on PSS. PSS has two subscales: general distress (perceived distress; sum of items: 1, 2, 3, 6, 9, and 10) and coping ability (perceived coping; sum of items: 4, 5, 7, and 8) [56]. In this study, the average score for the "Perceived Distress" factor was 9.39, indicating a trend for a statistically significant cluster difference in this item ($P = 0.066$) with cluster 4 perceiving lower level of stress. A lower score of 6.35 was observed in "Perceived Coping" factor,

reflecting better coping ability. The cluster difference in this item was statistically significant ($P < 0.001$) with participants in clusters 1 and 2 coping with stress better than the other two clusters.

Comorbidities of TTH participants were calculated by counting the total number of somatic comorbidities and mental comorbidities separately. All participants had a low number of comorbidities (Table 4). There were no significant differences in somatic comorbidities among the identified four TTH clusters. Although there was no statistically significant cluster difference in mental comorbidity, cluster 4 participants reported no mental comorbidity at all (Table 3).

3.3. Profile of the Clusters. Table 5 illustrates the profile of the four clusters. Cluster 1 had a moderate level of pain, moderate level of disability, and moderate distribution in both physical and mental comorbidity. Participants in this cluster tended to perform the best in coping ability (PSS "Perceived Coping" factor) when compared with other three clusters. Cluster 2 had the highest pain intensity and severest disability among all four patterns. This cluster also had the largest number of participants having a physical comorbidity. Cluster 3 had a very similar pattern to cluster 2 with moderate headache intensity and severe disability. However, based on CM understanding, they differed significantly in their presentation of headache and nonpainful symptoms. In addition to the symptomatology, they were also being significantly different from their coping with stress (cluster 3 is significant among clusters, whereas cluster 2 is not). Cluster 4 was unlabelled as there were insufficient characteristics of the symptoms and signs for CM diagnosis. It had the lowest level (mild) of pain intensity and lowest disability level among the four clusters.

4. Discussion

4.1. Summary of Findings. The present study identified three distinct CM patterns of TTH through a cluster analysis of 170 TTH participants in a bilingual cross-sectional survey. The results of this study suggest that TTH can be subdivided based on symptoms and signs that are significant to the CM diagnostic process. Those clusters may or may differ in the subtypes of TTH (ETTH, frequent ETTH, and CTTH), stress level, pain intensity, and disability level. These findings expand the existing understanding of TTH symptomatology in Western medicine and TTH patterns in CM, which may help advance our understanding of the symptoms associated with TTH and subgroups of TTH as well as contribute to enhanced clinical practice in CM.

4.2. Pros and Cons of Explorative Analytic Methods for CM Pattern Identification. The essence of factor analysis and cluster analysis is to classify a set of observations into groups. Such an approach could be a suitable technique in supporting and verifying the CM patterns as it has been used to explore and study CM patterns in order to understand a series of diseases and conditions defined by modern medicine [57–60]. Generally, those studies identified explainable CM patterns and interpreted those modern illness/diseases in a reasonable fashion.

Although the explorative analysis could be a valuable method for the study of CM pattern identification, the results of such analysis cannot be used directly in research or clinical practice without integration with CM theory. Hence, it is necessary to incorporate experts' opinions and clinical experience in order to ensure the results being clinically meaningful. In this study, we combined the two approaches for pattern identification.

Initially, through the TwoStep cluster analysis, we identified a four-cluster solution and a five-cluster solution. Experts agreed that the patterns within the four-cluster solution tended to coincide more with the actual clinical observation and were meaningful for pattern identification. By contrast, the five-cluster solution did not lead to distinct sound/logical CM patterns. Finally, the four-cluster solution was used, resulting in three identifiable patterns and one unidentifiable cluster. However, it is likely the unidentifiable cluster including a few factors that are not powerful enough to form their own patterns. From the statistical perspective of the PCA results, we observed that the overall mean score ("power") of the factors in cluster 4 was relatively "weak" (lower than 0.4) with most of the factor mean sores between 0.04 and 0.09. Consequently, those factors were considered having little diagnostic value for CM pattern recognition. In addition, our relatively small sample size may have restricted the number of identifiable patterns.

In PCA, the coefficient, known as the "factor loading," refers to how strong each variable is associated with the proposed factor, is used to explain the correlation between the individual item and the overall factor [32]. As a rule of thumb, a factor loading below 0.4 indicates the loading condition is weak; 0.6, a moderate level; between 0.6 and 0.8, being large; and 0.8 or above, being very high [61]. We adopted 0.5 as the cutoff point. In contrast, in interpreting the results from TwoStep cluster analysis, the existing literature does not provide clear guiding rules for the cutoff mean score for including or excluding a factor within a cluster. In the present study, we used 0.4 of the cluster mean as the cutoff point. We then invited experts to interpret symptoms and signs and named each cluster to ensure clinical relevance. Three out of four clusters were labelled, reflecting this approach is workable (Appendix D).

4.3. Interpretation of Findings. The common TTH characteristics and associated symptoms identified in the present study are consistent with the findings of other studies [8–11]. The main similarities are the precipitating factors such as physical activity, stress/tension, when tired, lack of sleep, specific foods/drinks, alcohol, and skipping meals, and some accompanying symptoms such as fatigue, insomnia, and irritability. Emotion-related factors may have impacted on the presence of TTH. The present study found that stress and/or tension (73.6%) was the leading precipitating factors, and the finding is consistent with others (49.4% [8], 74.5% [62], and 63% in men and 77% [63] and 52.5% [11] in women). Only a small percentage of anxiety disorders and mood disorders

Table 4: Summary of comorbidity checklist results.

	Item	Frequency (n)	(%)
1	Cardiac	6	3.5
2	Vascular	11	6.5
3	Hematology	2	1.2
4	Respiratory	13	7.6
5	Ophthalmology and otorhinolaryngology	15	8.8
6	Upper gastrointestinal	16	9.4
7	Lower gastrointestinal	4	2.4
8	Hepatic and pancreatic	3	1.8
9	Renal	3	1.8
10	Genitourinary	3	1.8
11	Musculoskeletal-integumentary	13	7.6
12	Neurological	4	2.4
13	Endocrine-metabolic	6	3.5
14	Psychiatric	13	7.6
15	Female hormonal and reproductive	10	5.9
+1	Anxiety disorders	12	7.1
+2	Mood disorders	12	7.1
+3	Substance use disorders	2	1.2

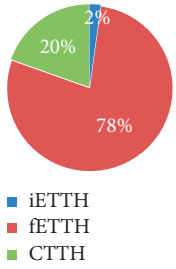
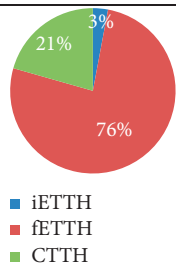
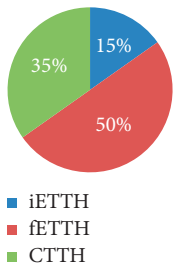
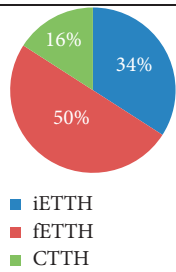
were detected (7.1%, respectively). This is probably due to more than three quarters of the respondents were ETTH sufferers, as it has been shown that psychiatric comorbidities are more common in CTTH patients [64, 65], whereas those having less frequent TTH tend to have less psychiatric comorbidity [66]. Such method has been used in other exploratory research [67, 68].

Very few studies have examined the differences between ETTH and CTTH beyond headache days. In the present study, the four CM patterns differed in the TTH subtypes. The three CM patterns not only differ in headache frequency but also in headache intensity and disability. Over three quarters of participants in clusters 1 and 2 had frequent ETTH, and about one-fifth had CTTH, whereas one-third in cluster 3 had CTTH, and half had frequent ETTH. All these three clusters had very few participants with infrequent ETTH, whereas one-third of cluster 4 was having infrequent ETTH (<1 day). Those results indicate that subtypes of TTH go beyond frequency of TTH. They could differ in clinical presentation of headache as well as accompanied signs and symptoms.

We also found that, over three quarters of participants in clusters 3 and 4 were of Asian origin, but over three quarters of those in clusters 1 and 2 were of non-Asian (Oceania and European) origin. In our further analysis (Appendix E), over 40% of Asian participants were at MIDAS level 1, comparing with 20% in the non-Asian group, reflecting mild impact of TTH on function. Consistently, over one-fifth of Asian participants (22%) suffered from infrequent headache, compared with 5% of non-Asian participants did. The former also tended to have poor coping ability as assessed with PSS. Those findings are consistent with differences in four clusters identified in the current study. The ethnic difference in cluster is, therefore, likely due to the frequency and severity of headache and coping strategies, rather than differences in ethnicity. This question is, however, beyond the scope of this paper, which aims to assess if advanced statistical methods could help with TTH CM syndrome differentiation. Future studies with larger sample sizes could examine the impact of demographic features on TTH patterns.

4.4. Implications of the Pattern Exploration for Clinical Treatment. Currently, there is a significant gap in understanding subtypes of TTH. The IHS diagnostic criteria for TTH are designed to distinguish TTH from other types of headaches to some degree and to classify TTH into three subtypes based upon attack frequency only. Nonheadache symptoms associated with TTH are, however, not explained or accounted for. Furthermore, despite several epidemiological studies observing a series of aggravating and relieving factors and accompanying symptoms of TTH, clinical practice to date has not given adequate attention to TTH symptoms. The current study fills those gaps by using knowledge of pattern identification in CM and advanced statistical methods and identified three clinically meaningful subgroups of TTH. In addition, the identified four clusters not only differed in symptoms and signs but also in the level of disability and stress. Among them, cluster 2 had the most severe headache and highest disability level, whereas cluster 4 had mildest headache intensity, moderate disability, and was free from mental comorbidity. The presence of these subgroups of TTH indicates that there is a need to go beyond frequency of TTH, as it is possible to subcategorize TTH from a multidimensional perspective, but not just limited to the frequency of headache. Addressing headache as well as accompanying nonheadache symptoms may lead to more efficient, individualised treatment strategies. The PSS score in ascendant hyperactivity of liver yang and liver depression forming fire is high, indicating emotional stress. This needs to be acknowledged by CM practitioners. Whether CM treatment modalities are adequate for addressing those emotional difficulties is yet to be examined. In the West, psychological interventions are often used to specifically address those

Table 5: Relationship between patterns and other outcome measures.

Clusters	Corresponding TTH subtypes	MIDAS		Outcome measures		Comorbidity (%)	
				PSS (mean ± SD)			
		Intensity (0–10)	Disability level	By PSS items (scores of item 3: the higher, the worse) (reversed scoring of item 5 and 8: the lower, the better)	By "perceived coping" factor (combined items of 4, 5, 7, and 8; the lower, the better in coping)	Physical	Mental
Cluster 1: ascendant hyperactivity of liver yang ($n = 46$)		Moderate pain (5.5)	Moderate disability (grade III; 9 days)	Item 3: (2.46 ± 0.925) most often *'In the last month, how often have you felt nervous and "stressed"?'* **Item 5: (1.39 ± 0.755)** most often *'In the last month, how often have you felt that things were going your way?'*	(5.04 ± 3.025) Best performance in perceived coping among clusters. Cluster being different from clusters 3 and 4	43.40%	17.40%
Cluster 2: dual Qi and blood deficiency ($n = 34$)		Moderate pain (6.3)	Severe disability (grade IV; 31 days)	**Item 8: (1.44 ± 0.982)** most often *'In the last month, how often have you felt that you were on top of things?'*	(5.18 ± 3.070) Cluster being different from clusters 3 and 4	55.90%	26.50%
Cluster 3: liver depression forming fire ($n = 46$)		Moderate pain (5.4)	Severe disability (grade IV; 24 days)	**Item 5: (2.14 ± 1.104)** least often *'In the last month, how often have you felt that things were going your way?'* **Item 8: (2.26 ± 1.049)** LEASTLY often *'In the last month, how often have you felt that you were on top of things?'*	(7.35 ± 4.018)* Cluster being different from clusters 1 and 2	41.30%	10.90%
Cluster 4: unlabelled group ($n = 44$)		Mild pain (4.7)	Moderate disability (grade III; 19 days)	**Item 3: (1.39 ± 0.868)** LEASTLY often *In the last month, how often have you felt nervous and "stressed"?*	(7.59 ± 3.329)* Poorest performance in perceived coping. Cluster being different from clusters 1 and 2	34.10%	0.00%

Note 1: in this table, "iETTH" stands for "infrequent ETTH," whereas the "fETTH" is the abbreviation of "frequent ETTH." Note 2: "*": the mean difference of PSS is significant among clusters at the 0.17 (0.05/3) level.

problems. Patients presented with either of the two patterns may require additional psychological interventions to bring out the best therapeutic effects.

4.5. Strengths. To our best knowledge, the present investigation is the first study using exploratory statistical method to research TTH-related symptoms as well as identifying CM patterns of TTH. Our study is the first step towards a better understanding of TTH from both CM and modern medicine aspects. This study has a few important strengths. Firstly, our method provides an alternative to current modern medicine approaches in understanding features of TTH and its subgroups, contributing essential information for future research. These results expanded the common understanding of TTH symptomatology in terms of its pain description,

Table 6: In which areas does your headache mostly occur and how often? (Please tick (✓) one box for each item).

Location of headache	Never	Seldom	Sometimes	Often	Almost always
Forehead (front of the head)	☐	☐	☐	☐	☐
Side of the head (left side)	☐	☐	☐	☐	☐
Side of the head (right side)	☐	☐	☐	☐	☐
Back of the head	☐	☐	☐	☐	☐
Top of the head	☐	☐	☐	☐	☐
Whole head	☐	☐	☐	☐	☐
No particular location	☐	☐	☐	☐	☐
Others (please specify)					

Table 7: When you have a headache, do you ever have discomfort (pain, tension, or tenderness) in the following areas? (Please tick (✓) one box for each item).

Affected area	Never	Seldom	Sometimes	Often	Almost always
Neck	☐	☐	☐	☐	☐
Shoulders	☐	☐	☐	☐	☐
Ears	☐	☐	☐	☐	☐
Eyebrow	☐	☐	☐	☐	☐
Eyes	☐	☐	☐	☐	☐
Face	☐	☐	☐	☐	☐
Cheeks	☐	☐	☐	☐	☐
Jaw	☐	☐	☐	☐	☐
Nose/bridge of nose	☐	☐	☐	☐	☐
Others (please specify)					

Table 8: What is the sensation of your headache? (Please tick (✓) as many items as applicable).

☐ Pressing	☐ Explosive	☐ Burning	☐ Drilling	☐ Cutting
☐ Sharp	☐ Vague	☐ Heavy	☐ Dull	☐ Throbbing
☐ Pulling	☐ Empty	☐ Tight	☐ Distending	☐ Piercing
☐ Pulsating	☐ Radiating	☐ Pounding	☐ A "band-like" sensation around the head	
Others (please specify)				

Table 9: When you have a headache, what is the nature of the headache? (Please tick (✓) one box for each item).

	Never	Seldom	Sometimes	Often	Almost always
Fixed (headache with fixed location)	☐	☐	☐	☐	☐
Moves (headache moves around the head or shifting from side to side)	☐	☐	☐	☐	☐
Continuous (constant, persistent, nonstop headache)	☐	☐	☐	☐	☐
Intermittent (headache comes and goes, occasional, or periodically occurring)	☐	☐	☐	☐	☐

Table 10: Over the last 3 months, on average, how many days per month did you have a headache? (Please tick (✓) one of the three options).

☐ Less than one day	☐ Between 1 and 14 days	☐ 15 days or more

trigger factors, and accompanying symptoms. Secondly, our results fill the significant gap in the existing literature of CM on headaches, which there is lack of differentiation of TTH from other types of headaches, such as migraine or secondary headache. Through recruiting only TTH sufferers and using a validated questionnaire, we were able to collect comprehensive data of TTH that are of clinical significance to CM. Thirdly, the existing CM patterns of TTH in the literature relied on expert opinions alone. Our study used the evidence-based approach of combining exploratory data analysis with expert opinions to ensure the objectivity and clinical significance of our findings.

4.6. Limitations. There are several limitations of the current study. Firstly, the present results could be limited due to its sample size, as some other possible patterns may be observed with a larger sample size. Secondly, relying on exploratory analysis or expert opinion alone has its drawbacks. Statistically determined clusters can be affected by many factors. Expert opinions may be subjective. The present study combines both approaches to minimize this limit. Lastly, this study is a cross-sectional study, which only analyses the symptom distribution collected at a specific duration over the last 3 months. The presence and the severity of symptoms observed may change over time. Future studies may use

Table 11: During the course of the day, when does your headache get worse? (Please tick (✓) as many items as applicable).

☐ Worse in the morning	☐ Worse in the afternoon	☐ Worse at the end of the day
☐ Worse at night	☐ All day	☐ No particular time

Table 12: What aggravates your headache? (Please tick (✓) one box for each item).

Increased tension	Don't know	Never	Seldom	Sometimes	Often	Almost always
Overwork (e.g., prolonged working hours, long periods of studying/typing)	☐	☐	☐	☐	☐	☐
When tired	☐	☐	☐	☐	☐	☐
Mental strain (e.g., overthinking or other concentration)	☐	☐	☐	☐	☐	☐
Eyestrain (e.g., reading, computer, or TV)	☐	☐	☐	☐	☐	☐
Muscular strain (muscle tightness)	☐	☐	☐	☐	☐	☐
Physical labour	☐	☐	☐	☐	☐	☐
Lack of sleep	☐	☐	☐	☐	☐	☐
Poor posture in sitting, standing or sleeping	☐	☐	☐	☐	☐	☐
Diet						
Alcohol	☐	☐	☐	☐	☐	☐
Coffee	☐	☐	☐	☐	☐	☐
Dehydration	☐	☐	☐	☐	☐	☐
Hunger/being hungry	☐	☐	☐	☐	☐	☐
Chocolate	☐	☐	☐	☐	☐	☐
Cigarette smoking	☐	☐	☐	☐	☐	☐
Soft drink/sodas	☐	☐	☐	☐	☐	☐
Tea	☐	☐	☐	☐	☐	☐
Cheese	☐	☐	☐	☐	☐	☐
Dairy foods (e.g., milk, ice cream, etc.)	☐	☐	☐	☐	☐	☐
Monosodium glutamate (MSG)	☐	☐	☐	☐	☐	☐
Sugar/too much sugar	☐	☐	☐	☐	☐	☐
Spicy food	☐	☐	☐	☐	☐	☐
Overconsumption of oily food	☐	☐	☐	☐	☐	☐
Irregular diet (e.g., eating on the run, skip meals)	☐	☐	☐	☐	☐	☐
Weather						
Change of weather	☐	☐	☐	☐	☐	☐
Change in temperature	☐	☐	☐	☐	☐	☐
Exposure to bright lights or sunshine	☐	☐	☐	☐	☐	☐
Hot weather	☐	☐	☐	☐	☐	☐
Cold weather	☐	☐	☐	☐	☐	☐
Windy days	☐	☐	☐	☐	☐	☐
Damp weather/humid weather	☐	☐	☐	☐	☐	☐
Rainy days	☐	☐	☐	☐	☐	☐
Stress and emotional changes						
Stress	☐	☐	☐	☐	☐	☐
Nervousness	☐	☐	☐	☐	☐	☐
Anger or irritability	☐	☐	☐	☐	☐	☐
Anxiety (excessive worry)	☐	☐	☐	☐	☐	☐
Depression (feeling unhappy or depressed)	☐	☐	☐	☐	☐	☐
Tension or conflict-related (e.g., from financial constraints, family, relationship, and/or work)	☐	☐	☐	☐	☐	☐
Other factors						
Sneezing	☐	☐	☐	☐	☐	☐
Teeth grinding	☐	☐	☐	☐	☐	☐
Other (please specify)						

Table 13: What relieves your headache? (Please tick (✓) one box for each item).

	Don't know	Never	Seldom	Sometimes	Often	Almost always
Rest	☐	☐	☐	☐	☐	☐
Lying down	☐	☐	☐	☐	☐	☐
Sleeping	☐	☐	☐	☐	☐	☐
Medication	☐	☐	☐	☐	☐	☐
Exercise/light exercise	☐	☐	☐	☐	☐	☐
Massage	☐	☐	☐	☐	☐	☐
Pressing/applying pressure on the pain area	☐	☐	☐	☐	☐	☐
Eating	☐	☐	☐	☐	☐	☐
Warmth (e.g., warm environment, hot drink, hot pack, hot shower, etc.)	☐	☐	☐	☐	☐	☐
Coldness (e.g., cold environment, cold drink, cold pack, cold shower, etc.)	☐	☐	☐	☐	☐	☐
Others (please specify)						

Table 14: Do you have any of the following symptoms that may or may not be related to your headache? (Please tick (✓) one box for each item).

Eye-related	Never	Seldom	Sometimes	Often	Almost always
Sensitivity to light (or to bright lights)	☐	☐	☐	☐	☐
Dry eyes	☐	☐	☐	☐	☐
Teary eyes	☐	☐	☐	☐	☐
Blurred vision	☐	☐	☐	☐	☐
Sore eyes	☐	☐	☐	☐	☐
Red eyes	☐	☐	☐	☐	☐
Swollen eyelids	☐	☐	☐	☐	☐
Eye twitching	☐	☐	☐	☐	☐
Floaters in the eyes	☐	☐	☐	☐	☐
Burning sensation in the eyes	☐	☐	☐	☐	☐
Itchy sensation in the eyes	☐	☐	☐	☐	☐
Face related (mouth, ear, and nose)					
Sensitivity to sound (or to loud noises)	☐	☐	☐	☐	☐
Dry mouth	☐	☐	☐	☐	☐
Thirst	☐	☐	☐	☐	☐
Bitter taste in the mouth	☐	☐	☐	☐	☐
Runny nose	☐	☐	☐	☐	☐
Sore throat/feeling of foreign body in the throat	☐	☐	☐	☐	☐
Ear discharge	☐	☐	☐	☐	☐
Tinnitus (ringing in the ears)	☐	☐	☐	☐	☐
Flushed face/hot red face	☐	☐	☐	☐	☐
Digestion-related					
Nausea	☐	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐	☐
Reflux	☐	☐	☐	☐	☐
Belching	☐	☐	☐	☐	☐
Bloating/flatulence	☐	☐	☐	☐	☐
Indigestion	☐	☐	☐	☐	☐
Poor appetite/loss of appetite	☐	☐	☐	☐	☐
Urine and bowel related					
Yellowish urine	☐	☐	☐	☐	☐
Frequent urination (especially at night)	☐	☐	☐	☐	☐
Watery bowel motion	☐	☐	☐	☐	☐
Loose bowel motion	☐	☐	☐	☐	☐
Dry stools	☐	☐	☐	☐	☐
Constipation	☐	☐	☐	☐	☐
Muscle- and joint-related					
Joint stiffness	☐	☐	☐	☐	☐
Neck stiffness	☐	☐	☐	☐	☐
Muscle twitching	☐	☐	☐	☐	☐
Weak legs and knees	☐	☐	☐	☐	☐

Table 14: Continued.

Feeling weak in the lower back	☐	☐	☐	☐	☐
Feeling cold in the lower back or lower back pain worsened by coldness	☐	☐	☐	☐	☐
Cold hands and feet/cold limbs	☐	☐	☐	☐	☐
Hot sensation in the palms	☐	☐	☐	☐	☐
"Pins and needles" or numbness in the hands and/or feet	☐	☐	☐	☐	☐
Other symptoms					
Increased forgetfulness or poor memory	☐	☐	☐	☐	☐
Feeling depressed	☐	☐	☐	☐	☐
Irritability/irascibility (short-tempered, easily angered)	☐	☐	☐	☐	☐
Restlessness	☐	☐	☐	☐	☐
Fatigue/tiredness	☐	☐	☐	☐	☐
Faintness	☐	☐	☐	☐	☐
Heavy sensation in the body	☐	☐	☐	☐	☐
Insomnia (difficulty falling asleep or staying asleep)	☐	☐	☐	☐	☐
Sighing often	☐	☐	☐	☐	☐
Feverish sensation	☐	☐	☐	☐	☐
Shortness of breath	☐	☐	☐	☐	☐
Dizziness	☐	☐	☐	☐	☐
Excessive phlegm	☐	☐	☐	☐	☐
Palpitation (feeling the heart beats quickly or unusually)	☐	☐	☐	☐	☐
Inability to concentrate	☐	☐	☐	☐	☐
Night sweating	☐	☐	☐	☐	☐
Sweating upon mild activity	☐	☐	☐	☐	☐
Aversion to cold or fear of being cold	☐	☐	☐	☐	☐
Aversion to hot or fear of being hot	☐	☐	☐	☐	☐
Sensitive to temperature changes	☐	☐	☐	☐	☐
Others (please specify)					

Table 15: Apart from headache, do you experience pain in any other parts of your body? (Please tick (✓) as many items as applicable).

☐ Neck	☐ Shoulder	☐ Jaw	☐ Throat
☐ Ears	☐ Eyes	☐ Chest	☐ Breasts
☐ Upper back	☐ Middle back	☐ Lower back	☐ Abdomen
☐ Arms	☐ Legs	☐ Knees	☐ Heel
☐ Hips	☐ Buttocks	☐ Flank (side of the body)	☐ Hypochondria (lower abdomen)
Others (please specify)			

Table 16: Information related to women's health (Only female participants need to fill in this section. This section is about women's health which may relate to your headache.).

3.3.1. Do you still have periods?	
☐ No	If no, please ignore section (3.3.2) and tick the following reasons ☐ Menopause ☐ Hysterectomy ☐ Contraceptive pill ☐ Other medications ☐ Other underlying diseases ☐ Pregnancy ☐ Others (please specify)
☐ Yes	If Yes, please complete the next section (3.3.2)

Table 17: Information related to women's health (continued) (please tick (✓) one box for each item).

	Never	Seldom	Sometimes	Often	Almost always
Irregular period cycle	☐	☐	☐	☐	☐
Early periods (shortened menstrual cycle)	☐	☐	☐	☐	☐
Delayed periods (prolonged menstrual cycle)	☐	☐	☐	☐	☐
Light bleeding (bleeding less than normal)	☐	☐	☐	☐	☐
Excessive bleeding	☐	☐	☐	☐	☐

Table 17: Continued.

	Never	Seldom	Sometimes	Often	Almost always
Bleeding with clots	☐	☐	☐	☐	☐
Bright red-coloured menstrual blood	☐	☐	☐	☐	☐
Light red-coloured menstrual blood	☐	☐	☐	☐	☐
Dark-coloured menstrual blood	☐	☐	☐	☐	☐
Excessive watery discharge	☐	☐	☐	☐	☐
Yellow discharge	☐	☐	☐	☐	☐
Headache during period	☐	☐	☐	☐	☐
Headache after period	☐	☐	☐	☐	☐
Abdominal pain before periods	☐	☐	☐	☐	☐
Abdominal pain during periods	☐	☐	☐	☐	☐
Abdominal pain after periods	☐	☐	☐	☐	☐
Lower back pain before periods	☐	☐	☐	☐	☐
Lower back pain during periods	☐	☐	☐	☐	☐
Lower back pain after periods	☐	☐	☐	☐	☐
Others (please specify)					

Table 18: Do you have any other diseases or health conditions diagnosed by your medical doctor? ☐ No. ☐ Yes. If yes, please tick the following listed conditions that apply to you (Please tick (✓) as many items as applicable).

☐ Cardiac	Heart problem (such as cardiopathy, pericarditis, coronary heart disease, myocarditis, angina, myocardial infarction, arrhythmia, and valve problems)
☐ Vascular	Circulatory problem (such as peripheral atherosclerotic disease and aneurysm of the abdominal aorta), hypertension, or cholesterol problem
☐ Hematological	Blood problem (anemia, leukemia, hypercoagulability, or any other problem affecting the blood, the blood cells, the spleen, or the lymphatic system)
☐ Respiratory	Respiratory problem (such as asthma, emphysema, bronchitis, pulmonary embolism, or any problems related to the lungs, bronchi, and trachea)
☐ Ophthalmological and otorhinolaryngology	Problems of the eyes (such as glaucoma, cataract, and loss of vision); ears (such as important hearing impairment and otitis media); nose (such as sinusitis and rhinitis); throat (pharyngitis), and voice
☐ Upper gastrointestinal(does not include diabetes)	Problems of the stomach or digestion (such as problems of the esophagus, stomach, and duodenum (such as gastritis, peptic ulcer, and duodenal ulcer)
☐ Lower gastrointestinal	Intestinal problems (such as intestinal hernias, enteritis, intestinal tuberculosis, chronic diarrhea, colitis, constipation, anal problems and bowel incontinence)
☐ Hepatic and pancreatic	Problems of the liver (impairment in function, liver infection, etc.), pancreas (such as pancreatitis), gallbladder (such as cholecystitis)
☐ Renal	Problems of the kidneys (impairment in function, kidney infection, etc.)
☐ Genitourinary	Problems of the urination system, such as ureters, bladder, urethra, prostate, and genitals (such as kidney stone, urinary incontinence, bladder infection, prostate diseases, and sexual dysfunction)
☐ Musculoskeletal-integumentary	Problems of the muscles, joints, bones, connective tissue, and skin (such as fibromyalgia, rheumatoid arthritis, osteoarthritis, osteoporosis, and other forms of arthritis, carpal tunnel syndrome, Sjogren's syndrome, systemic lupus erythematosus, polymyositis etc.) and any skin (such as atopic dermatitis, eczema, and herpes) or other musculoskeletal problems
☐ Neurological	Neurological (brain, spinal cord, and nerves) problem (such as cerebrovascular accident, peripheral neuropathy, facial neuritis, polyneuropathy, Gearan–Kaizer syndrome, myelitis, myasthenia gravis, and multiple sclerosis)
☐ Endocrine-metabolic	Problems of the thyroid gland, obesity, diabetes, or any other hormonal problems
☐ Psychiatric	Problems of depression, anxiety, alcohol, drug abuse, or other problems
☐ Female hormonal and reproductive (for females only)	Problems of reproductive system (such as the uterus, ovary, and fallopian tubes) and other gynaecological problems (such as premenstrual syndrome, polycystic ovary syndrome, and pelvic inflammatory disease)
☐ Others (please specify)	

Table 19: Have you been diagnosed by a medical doctor with any of the following mental health conditions? ☐ No. ☐ Yes. If yes, please tick the following listed conditions that apply to you.

☐ Anxiety disorders	Includes anxiety, panic disorder, obsessive-compulsive disorder, posttraumatic stress disorder, agoraphobia, and social phobia
☐ Mood disorders	Includes depression, mania, and bipolar (affective) disorder
☐ Substance use disorders	Includes dependence or harmful use of alcohol, or drugs (opioids, sedatives, stimulants, cannabinoids, petrol, glue, etc.)
☐ Others (please specify)	

Table 20: Rotated component matrix for CMHQ factor extraction.

CMHQ item	Part I component																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.1.1	0.755																
1.1.2			0.830														
1.1.3			0.814														
1.1.4	0.731																
1.1.5	0.673																
1.1.6		0.718															
1.1.7		0.915															
1.2.1	0.937																
1.2.2	0.928																
1.2.3																	
1.2.4		0.723															
1.2.5		0.610															
1.2.6																	
1.2.7		0.759															
1.2.8																	
1.2.9																	
1.3.1					0.555												
1.3.2		0.734															
1.3.3					0.746												
1.3.4																	
1.3.5						0.804											
1.3.6			0.577														
1.3.7																	
1.3.8							-0.570										
1.3.9		−0.584															
1.3.10						0.627											
1.3.11							0.768										
1.3.12								0.882									
1.3.13	0.789																
1.3.14			0.800														
1.3.15				0.636													
1.3.16	0.674																
1.3.17					0.640												
1.3.18	0.744																
1.3.19				0.803													
1.6.1		0.811															
1.6.2	0.774																
1.6.3	0.706																
1.6.4		0.672															
1.6.5			0.984														
1.6.6	−0.765																

CMHQ item	Part II component																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2.1.1					0.816												
2.1.2					0.688												
2.1.3					0.689												
2.1.4									.								
2.1.5						0.719											

Table 20: Continued.

CMHQ item	Part I component																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2.1.6									0.723								
2.1.7									0.607								
2.1.8						0.769											
2.1.9										0.606							
2.1.10		0.595															
2.1.11				0.771													
2.1.12				0.758													
2.1.13		0.564															
2.1.14										0.793							
2.1.15		0.585															
2.1.16		0.604															
2.1.17																	
2.1.18		0.792															
2.1.19																	
2.1.20		0.794															
2.1.21							0.698										
2.1.22							0.703										
2.1.23							0.695										
2.1.24			0.827														
2.1.25			0.838														
2.1.26																	
2.1.27			0.607														
2.1.28			0.620														
2.1.29								0.552									
2.1.30								0.592									
2.1.31								0.782									
2.1.32	0.742																
2.1.33	0.808																
2.1.34	0.828																
2.1.35	0.808																
2.1.36	0.682																
2.1.37	0.796																
2.1.38																	
2.1.39						0.507											
2.2.1	0.872																
2.2.2	0.900																
2.2.3	0.799																
2.2.4			0.772														
2.2.5		0.692															
2.2.6		0.736															
2.2.7		0.681															
2.2.8			0.655														
2.2.9		0.602															
2.2.10		0.567															

CMHQ item	Part III component																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3.1.1									0.679								
3.1.2		0.573															0.528
3.1.3		0.675															
3.1.4		0.654															
3.1.5		0.636															
3.1.6		0.679															
3.1.7		0.548															
3.1.8																	
3.1.9															0.776		
3.2.10																	
3.1.11																	
3.1.12									0.718								
3.1.13					0.855												

Table 20: Continued.

CMHQ item	Part I component																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3.1.14					0.744												
3.1.15					0.745												
3.1.16								0.605									
3.1.17								0.723									
3.1.18								0.546									
3.1.19																0.724	
3.1.20																	
3.1.21							0.704										
3.1.22							0.863										
3.1.23							0.517										
3.1.24				0.727													
3.1.55				0.748													
3.1.26				0.748													
3.1.27																	
3.1.28																	
3.1.29						0.697											
3.1.30														0.813			
3.1.31														0.743			
3.1.32											0.788						
3.1.33											0.793						
3.1.34			0.750														
3.1.35			0.694														
3.1.36			0.704														
3.1.37																	
3.1.38			0.506														
3.1.39			0.509														
3.1.40													0.724				
3.1.41																	
3.1.42												0.676					
3.1.43																	
3.1.44	0.673																
3.1.45	0.700																
3.1.46	0.798																
3.1.47	0.566																
3.1.48												0.614					
3.1.49	0.510																
3.1.50																	0.540
3.1.51	0.584																
3.1.52																	
3.1.53	0.509																
3.1.54												0.608					
3.1.55								0.537									
3.1.56																	
3.1.57	0.562																
3.1.58						0.545											
3.1.59						0.724											
3.1.60													0.731				
3.1.61										0.735							
3.1.62										0.661							
3.2.1	0.743																
3.2.2	0.792																
3.2.3			0.650														
3.2.4																	
3.2.5			0.589														
3.2.6						0.805											
3.2.7																	
3.2.8					0.766												
3.2.9																	
3.2.10																	

Table 20: Continued.

CMHQ item	Part I component																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3.2.11	0.584																
3.2.12					0.665												
3.2.13		0.700															
3.2.14		0.778															
3.2.15																	
3.2.16			0.639														
3.2.17																	
3.2.18																	
3.2.19				0.664													
3.2.20				0.744													
3.3.2.1					0.753												
3.3.2.2				0.548													
3.3.2.3					0.810												
3.3.2.4					0.591												
3.3.2.5		0.675															
3.3.2.6	0.638																
3.3.2.7																	
3.3.2.8		0.629															
3.3.2.9	0.521																
3.3.2.10		0.805															
3.3.2.11		0.763															
3.3.2.12				0.624													
3.3.2.13				0.791													
3.3.2.14				0.699													
3.3.2.15	0.782																
3.3.2.16	0.735																
3.3.2.17			0.801														
3.3.2.18			0.660														
3.3.2.19			0.622														
3.3.2.20			0.870														

Extraction method: principal component analysis. Rotation method: varimax with Kaiser normalization.

Table 21: Extraction of factors.

CMHQ part 1: pain description	CMHQ part 2: aggravating and relieving factors	CMHQ part 3: accompanying symptoms
FAC1.1F1CentralHead	FAC 2.1F1Mental	FAC 3.1F1Liver-Qi&Fire
FAC 1.1F2WholeHead	FAC 2.1F2Food	FAC 3.1F2Eye
FAC 1.1F3LateralHead	FAC 2.1F3WeatherChange	FAC 3.1F3BoneJointWind
FAC 1.3F1RhythmHeadache	FAC 2.1F4NoFood&Drink	FAC 3.1F4PoorDigestion
FAC 1.3F2ExplosiveNotDull	FAC 2.1F5MentalStrain	FAC 3.1F5LiverSpleenFire
FAC 1.3F3SharpHeadache	FAC 2.1F6MuscularStrain	FAC 3.1F6YinDeficiency
FAC 1.3F4TightHeadache	FAC 2.1F7Oil&Spicy	FAC 3.1F7LiverAttackStomach
FAC 1.3F7DistendingHeadache	FAC 2.1F8WindDamp	FAC 3.1F8ENT
FAC 1.3F8EmptyHeadache	FAC 2.1F9PhysicalStrain	FAC 3.1F9LightSound
FAC 1.5F1LateOfDay	FAC 2.1F10Alcohol-DragCigar	FAC 3.1F10TemperatureSensitivity
FAC 1.5F2BothEnd	FAC 2.2F1Rest	FAC 3.1F11Constipation
FAC 1.5F3AllDay	FAC 2.2F2PhysicalStimulation	FAC 3.1F12BloodDeficiency
	FAC 2.2F3EatingRelated	FAC 3.1F13YangDeficiency
		FAC 3.1F14SpleenDeficienyOfBowel
		FAC 3.1F16Tinnitus
		FAC 3.1F17Insomnia
Included: $n = 12$	Included: $n = 13$	Included: $n = 16$

*: In this table, "FAC" is the abbreviation of "factor," whereas the numbers 1.X after it indicate their section number. For instance, FAC1.6F1 denotes the extracted first factor of Table 11, which summarised items of forehead, back of the head, and top of the head.

Table 22: Results of the cluster analysis.

Clusters	Factors (PCA mean score)		
	CMHQ part 1 factors	CMHQ part 2 factors	CMHQ part 3 factors
Cluster 1: ascendant hyperactivity of liver yang	(i) 1.3F4TightHeadache (0.68) (ii) 1.3F1RhythmHeadache (0.53) (iii) 1.1F1CentralHead (0.51)	(i) 2.1F4NoFood&Drink (0.69) (ii) 2.1F6MuscularStrain (0.42)	(i) 3.1F9LightSound (0.61)
Cluster 2: dual Qi and blood deficiency	(i) 1.6F2BothEnd (0.72) (ii) 1.1F3LateralHead (0.66) (iii) 1.6F3AllDay (0.65) (iv) 1.3F1RhythmHeadache (0.56) (v) 1.1F1CentralHead (0.51)	(i) 2.2F2PhysicalStimulation (0.65) (ii) 2.1F3WeatherChange (0.55) (iii) 2.2F3EatingRelated (0.44) (iv) 2.1F4NoFood&Drink (0.42)	(i) 3.1F14SpleenDeficienyOfBowel (0.71) (ii) 3.1F12BloodDeficiency (0.49)
Cluster 3: liver depression forming fire	(i) 1.1F2WholeHead (0.66)	(i) 2.1F1Mental (0.42)	(i) 3.1F5LiverStomachFire (0.51)
Cluster 4: nonspecific cluster	(ii) 1.3F2ExplosiveNotDull (0.29) (iii) 1.3F3SharpHeadache (0.04)	(ii) 2.1F8WindDamp (0.08)	(ii) 3.1F4PoorDigestion (0.07) (iii) 3.1F10TemperatureSensitivity (0.09) (iv) 3.1F11Constipation (0.04)

*: this table reports the results of the TwoStep cluster analyses. Computed from the TwoStep cluster algorithm, mean scores of extracted factors that exceeded value 0.4 are listed for clusters 1, 2, and 3. The cutoff of 0.4 was determined as those clusters being considered to be clinically relevant.

Table 23: Group comparisons on MIDAS, PSS, and TTH subtypes between Asian and Non-Asian participants.

		Asian vs. Non-Asian		Chi	*t*-test
MIDAS		MIDAS LEVEL		0.007*	N/A
		MIDAS SUM		N/A	0.122
		MIDAS a (item)		N/A	0.073
		MIDAS B (item)		N/A	0.000*
PSS		**Asian vs. Non-Asian**		**Chi**	***t*-test**
		PSS SUM		N/A	0.615
		PSS perceived distress		N/A	0.445
		PSS perceived coping ability		N/A	0.000*
TTH subtypes	**Asian**	**Non-Asian**	Total	**Chi**	***t*-test**
(1) Infrequent ETTH	19	4	23	0.005*	N/A
(2) Frequent ETTH	47	56	103		
(3) Chronic TTH	18	20	38		
Total	84	80	164		

*: the mean difference is significant at the 0.05 level.

longitudinal cohort approaches to evaluate the stability of the identified CM patterns over time and to assess the effect of interventions.

5. Conclusions

This study provides new and critical information for determining the symptom patterns of TTH. The finding will contribute to the subgroup or pattern classification and guide targeted intervention design, including acupuncture, for future clinical practice and research. Future studies with a large sample size will identify other patterns in addition to those reported in the current study (Tables 6–23)

Abbreviations

ANOVA: One way analysis of variance
CIRS: Cumulative Illness Rating Scale
CM: Chinese medicine

CMHQ: Chinese Medicine Headache Questionnaire
CTTH: Chronic tension-type headache
ETTH: Episodic tension-type headache
ICHD-II: Headache Society TTH Diagnostic Criteria (2nd edition)
IHS: International Headache Society
MIDAS: Migraine disability assessment test
PCA: Principal component analysis
PSS: Perceived Stress Scale
SD: Standard deviation
TCM: Traditional Chinese medicine
TTH: Tension-type headache
WMH-CIDI: World Mental Health Composite International Diagnostic Interview.

Appendix

A. Chinese Medicine Headache Questionnaire (CMHQ)

Please tick the listed items that best describe your headache and other symptoms you experienced over the last 3 months.

Part I: Pain Description

Part II: Aggravating and Relieving Factors

Part III: Accompanying Symptoms

Part IV: Other Conditions

B. Rotated Component Matrix for CMHQ Factor Extraction

C. Extraction of Factors

D. Results of the Cluster Analysis

E. Group Comparisons on MIDAS, PSS, and TTH Subtypes between Asian and Non-Asian Participants

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The study was approved by the College Human Ethics Advisory Network (CHEAN) of Science Engineering and Health, RMIT University (protocol approval number: BSEHAPP 10–11 HAO) and subsequently ratified by collaboration sites. Participation to the study was on a voluntary basis. All data were anonymous.

Consent

All participants were provided information explaining the purpose of the study and informed consent was obtained from them before inclusion.

Disclosure

The authors have submitted a draft version of the manuscript to Research Square previously. The current manuscript is our updated version for submission, which is different from the preprint on Research Square https://www.researchsquare.com/article/rs-10234/v1.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Mechanism of Fei-Xian Formula in the Treatment of Pulmonary Fibrosis on the Basis of Network Pharmacology Analysis Combined with Molecular Docking Validation

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Objective. This study aimed to clarify the mechanism of Fei-Xian formula (FXF) in the treatment of pulmonary fibrosis based on network pharmacology analysis combined with molecular docking validation. *Methods*. Firstly, ingredients in FXF with pharmacological activities, together with specific targets, were identified based on the BATMA-TCM and TCMSP databases. Then, targets associated with pulmonary fibrosis, which included pathogenic targets as well as those known therapeutic targets, were screened against the CTD, TTD, GeneCards, and DisGeNet databases. Later, Cytoscape was employed to construct a candidate component-target network of FXF for treating pulmonary fibrosis. In addition, for nodes within the as-constructed network, topological parameters were calculated using CytoHubba plug-in, and the degree value (twice as high as the median degree value for all the nodes) was adopted to select core components as well as core targets of FXF for treating pulmonary fibrosis, which were subsequently utilized for constructing the core network. Furthermore, molecular docking study was carried out on those core active ingredients together with the core targets using AutoDock Vina for verifying results of network pharmacology analysis. At last, OmicShare was employed for enrichment analysis of the core targets. *Results*. Altogether 12 active ingredients along with 13 core targets were identified from our constructed core component-target network of FXF for the treatment of pulmonary fibrosis. As revealed by enrichment analysis, the 13 core targets mostly concentrated in regulating biological functions, like response to external stimulus (from oxidative stress, radiation, UV, chemical substances, and virus infection), apoptosis, cell cycle, aging, immune process, and protein metabolism. In addition, several pathways, like IL-17, AGE-RAGE, TNF, HIF-1, PI3K-AKT, NOD-like receptor, T/B cell receptor, and virus infection-related pathways, exerted vital parts in FXF in the treatment of pulmonary fibrosis. *Conclusions*. FXF can treat pulmonary fibrosis through a "multicomponent, multitarget, and multipathway" mean. Findings in this work lay foundation for further exploration of the FXF mechanism in the treatment of pulmonary fibrosis.

1. Introduction

Pulmonary fibrosis may take place in a variety of clinical settings and may threaten human life [1, 2]. It is featured by the disturbed cell homeostasis and changed cell composition in peripheral lungs, thus resulting in excess extracellular matrix (ECM) accumulation and lung dysfunction [3]. Over the last 10 years, investigators have identified that some molecular and cellular signal transduction pathways are related to the pulmonary fibrosis pathogenesis, which promotes to identify more novel therapeutic targets [4, 5]. Numerous treatments have been

utilized to treat pulmonary fibrosis, but few of them elevate patient survival or improve their quality of life [6]. Nintedanib and pirfenidone have been approved to be used to treat pulmonary fibrosis, yet they have serious diverse reactions (such as gastrointestinal symptoms, photosensitivity, and abnormal liver function) [7–10]. Such phenomenon may be ascribed to the complicated regulatory networks involved in pulmonary fibrosis, which suppress or promote associated target genes or pathway expression [11–13]. It is increasingly suggested that epigenetic, genetic, or proteomic factors play important roles in these regulatory networks in pulmonary fibrosis [14–16]. Besides, the networks modulate fibrosis-related gene expression through activating or inactivating the relevant pathways, but not through one single pathway [17].

Traditional Chinese medicine (TCM) exerts multitarget effects and has been used to treat pulmonary fibrosis by regulating oxidant stress, inflammation, and so on. The TCM pathogenesis of pulmonary fibrosis is Yin deficiency and blood stasis. Thus, the treatment principle is dominated by promoting blood circulation to remove blood stasis, enriching Yin and nourishing the lung [18]. Fei-Xian formula (FXF), the cipher prescription used to treat pulmonary fibrosis at our institution, is made up of 7 herbs, Salviae Miltiorrhizae Radix et Rhizoma (Danshen, DS), Persicae Semen (Taoren, TR), Hirudo (Shuizhi, SZ), Paeoniae Radix Rubra (Chishao, CS), Asparagi Radix (Tiandong, TD), Canarii Fructus (Qingguo, QG), and Oroxyli Semen (Muhudie, MHD). FXF is effective in relieving the pulmonary fibrosis symptoms, improving lung function (evaluated by vital capacity max (VC Max), total lung capacity (TLC), diffusion capacity for carbon monoxide-single breath method (DLCO-SB), and diffusion capacity for carbon monoxide/alveolar volume (DLCO/VA)), and enhancing the exercise tolerance as well as quality of life of patients [19, 20]. Nonetheless, the multigene and multi-pathway mechanism of FXF in the treatment of pulmonary fibrosis remains largely unclear.

In the treatment of disease, the traditional Chinese medicine (TCM) herbs are less toxic. Nonetheless, the mechanism of TCM remains unknown so far, which may thus spark criticism as it becomes increasingly popular nowadays. Therefore, it is of great importance to investigate those target genes and pathways for the sake of modernizing TCM. Network pharmacology, a critical technical approach for investigating TCM formulas in systems biology, can visualize the herb and formula pharmacological effects and the underlying molecular mechanisms through multidisciplinary integration, including computer technology (CT), high-throughput omics, network database retrieval, and pharmacology [21, 22]. In the present work, network pharmacology analysis was adopted to predict pharmacodynamic material basis together with the underlying molecular mechanism of FXF for the treatment of pulmonary fibrosis, and the results were then validated by molecular docking study. Findings in this work lay theoretical basis to conduct experimental study and to apply FXF in clinic.

2. Materials and Methods

2.1. Mining the Potential Pharmacodynamic Compounds and Related Targets of FXF. The names of 7 herbs were imported into the BATMAN-TCM, Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, and the analysis platform successively, to acquire the chemical compounds with corresponding information [23]. Thereafter, the drug likeness (DL) ≥ 0.18 and bioavailability (OB) $\geq 30\%$ were used as the thresholds to screen those possible pharmacodynamic compounds in FXF by the method in literature [24, 25]. Additionally, certain compounds that did not conform to the selection thresholds but had great levels in each herb or had wide pharmacological actions, or were utilized to identify single medicine in the pharmacopeia, were incorporated as the possible pharmacodynamic compounds as well, including hirudin, salvianolic acid A, amygdalin, and oroxin [26–29]. Moreover, for those active components, the possible targets were discovered against the TCSMP database based on drug structural similarity assessment as well as reverse molecular docking, which were later used for constructing a potential FXF pharmacodynamic target set.

2.2. Pulmonary Fibrosis-Associated Target Screening. Targets associated with the pathogenesis or treatment of pulmonary fibrosis were acquired based on DisGeNet (https://www.disgenet.org/) [30], TTD (http://db.idrblab.net/ttd/) [31], CTD (http://ctdbase.org/) [32], and GeneCards (https://www.genecards.org/) [33] databases with the following keyword: "pulmonary fibrosis." Targets were sorted out according to disease specificity index (DSI) from high to low against DisGeNet database, while targets larger than median were chosen. The abnormal drugs with related targets in TTD database were ruled out. For CTD database, those 200 most significant genes with the highest inference score were chosen. With regard to GeneCards, targets of Score ≥ 10 were chosen. Afterwards, targets retrieved based on these 4 databases were combined for the construction of a target set related to pulmonary fibrosis.

2.3. Establishment of the Core Component-Target Network of FXF for the Treatment of Pulmonary Fibrosis. Firstly, we chose the Uniprot database [34] in this work and chose species as "*Homo sapiens*"; then, target names acquired from these two steps were normalized for the acquisition of distinct Uniprot IDs and gene names. Later, both target sets were incorporated into Venny tool (https://bioinfogp.cnb.csic.es/tools/venny/), respectively, for obtaining overlapping targets, and they were regarded as the candidate FXF pharmacodynamic targets for the treatment of pulmonary fibrosis. Moreover, we applied Cytoscape in constructing the FXF candidate component-target network for the treatment of pulmonary fibrosis, where "component" was set as square, whereas "target" was set as circle. Thereafter, the node degree in candidate component-target network was calculated and ranked by CytoHubba plug-in [35], and the value (twice as

high as the median degree value for the nodes) was adopted to select core components as well as core targets of FXF for treating pulmonary fibrosis, which were subsequently utilized for constructing the core network.

2.4. Compound-Target Interaction Validation. AutoDock Vina [36] (version: 1.1.2) was used to validate the relationships of compounds with targets. The compound mol2 structures were obtained based on TCMSP database, and target protein 3D structures were acquired from RCSB PDB database (http://www.rcsb.org/) [37]. Prior to molecular docking, proteins and ligands were prepared using AutoDockTools [38] (version: 1.5.6) and the MOE method. With regard to target proteins, their crystal structures were pre-treated, including removal of water molecules, 3D hydrogenation, protonation, correction of protein structure, energy optimization, and retention of target active region. Additionally, the ligand structures must conform to the low-energy conformation. Moreover, the box size and coordinates in molecular docking were finally determined based on ligand position. To achieve higher calculation accuracy, the exhaustiveness parameter was set at 20. The remaining parameters were the defaults except as otherwise noted. Thereafter, the components were integrated to the target proteins in a semiflexible manner, which produced altogether 9 conformations. The most affinal conformation was chosen to be the eventual docking conformation.

2.5. GO and KEGG Enrichment Analysis for Core Targets. For better exploring those biological processes (BPs), molecular functions (MFs), cellular components (CCs), and regulatory signal transduction pathways related to the FXF core targets for the treatment of pulmonary fibrosis, we input the information of selected core targets. Thereafter, GO-BP along with KEGG enrichment analysis was carried out on these targets using OmicShare online software [39] (https://www.omicshare.com/) at a $p < 0.05$ threshold.

3. Results

3.1. Selection of Potential Pharmacodynamic Components and Targets of FXF. By searching against the TCMSP database, altogether 201 chemical components were obtained from 7 herbs in FXF and 126 were selected as the core components upon the DL ≥ 0.18 and OB ≥ 30% thresholds. Additionally, compounds with lower OB or DL value that had great contents in each herb were also collected as the potential pharmacodynamic components of FXF. At last, DS, TR, SZ, CS, TD, QG, and MHD had 61, 23, 1, 27, 9, 6, and 19 potential pharmacodynamic components, separately, of which, baicalin, beta-sitosterol, ellagic acid, stigmasterol, quercetin, and 2,5-dihydroxy-6,7-dimethoxyflavone were widely distributed in several herbs. Table S1 presents the information for the possible pharmacodynamic components of FXF.

Thereafter, the TCMSP database was searched to identify targets for the 130 potential pharmacodynamic components, and finally 286 were found (Table S2). DS, TR, SZ, CS, TD, QG, and MHD possessed 140, 71, 2, 114, 203, 207, and 131 potential targets, respectively. The 7 herbs obviously overlapped, regardless of the different target numbers in each herb, suggesting that those diverse herbs of FXF possibly exerted different actions by modulating similar targets.

To systemically and holistically understand the component-target network of FXF, Cytoscape was utilized to establish a network map, including 2969 edges and 416 nodes (Figure 1(a)). The node degree in the map represented the target or edge number related to nodes based on topological analysis (Figure 1(b)). There were 65 components discovered in the as-constructed network upon the threshold of median ≥14 degrees. Quercetin, beta-sitosterol, stigmasterol, baicalein, and luteolin of these components functioned in 326, 285, 132, 100, and 67 targets, separately, and they might serve as the obvious core components of FXF.

3.2. Selection of Core Targets in FXF for the Treatment of Pulmonary Fibrosis. Pulmonary fibrosis represents one of the polygenic genetic diseases, and its pathogenesis may be studied by examining the interactions between genes or between genes and the environment. In this study, the databases including CTD, TTD, DisGeNet, and GeneCards were retrieved, which discovered 318 targets related to pulmonary fibrosis (Table S3). Besides, 87 of these discovered targets were also recognized as the potential targets for the pharmacodynamic components in FXF, which suggested that FXF had some therapeutic effect (Figure 2(a) and Table S4). These 87 targets may be the candidate targets of FXF in treating pulmonary fibrosis, while the 88 pharmacodynamic active components that exert regulatory effect on these targets are the candidate components.

Additionally, the candidate component-target network of FXF for the treatment of pulmonary fibrosis was constructed using Cytoscape (Figure 2(b)). For better selecting the FXF core components and core targets for the treatment of pulmonary fibrosis, we adopted the CytoHubba plug-in for calculating and sorting those node topological parameters (degrees) in the as-constructed network (Table S5). Thereafter, nodes with degree value greater than or equal to twice as high as the median degree value (=5) for all the nodes were selected as the core components and core targets in FXF for the treatment of pulmonary fibrosis. Later, the core component-core target network (including 12 core components and 13 core targets) was established (Figure 2(c)). Of these core components, quercetin, beta-sitosterol, baicalein, luteolin, ellagic acid, wogonin, naringenin, tanshinone IIa, stigmasterol, and cryptotanshinone from DS, TR, CS, TD, QG, and MHD in FXF were the most significant in line with the degree values. Some of them were suggested previously to postpone pulmonary fibrosis course and improve the symptoms [40–43]. Besides, PTGS2, NOS2, CDK2, GSK3B, CCNA2, PPARG, MAPK14, CASP3, BCL2, and RELA ranked the top 10 places based on the degree value.

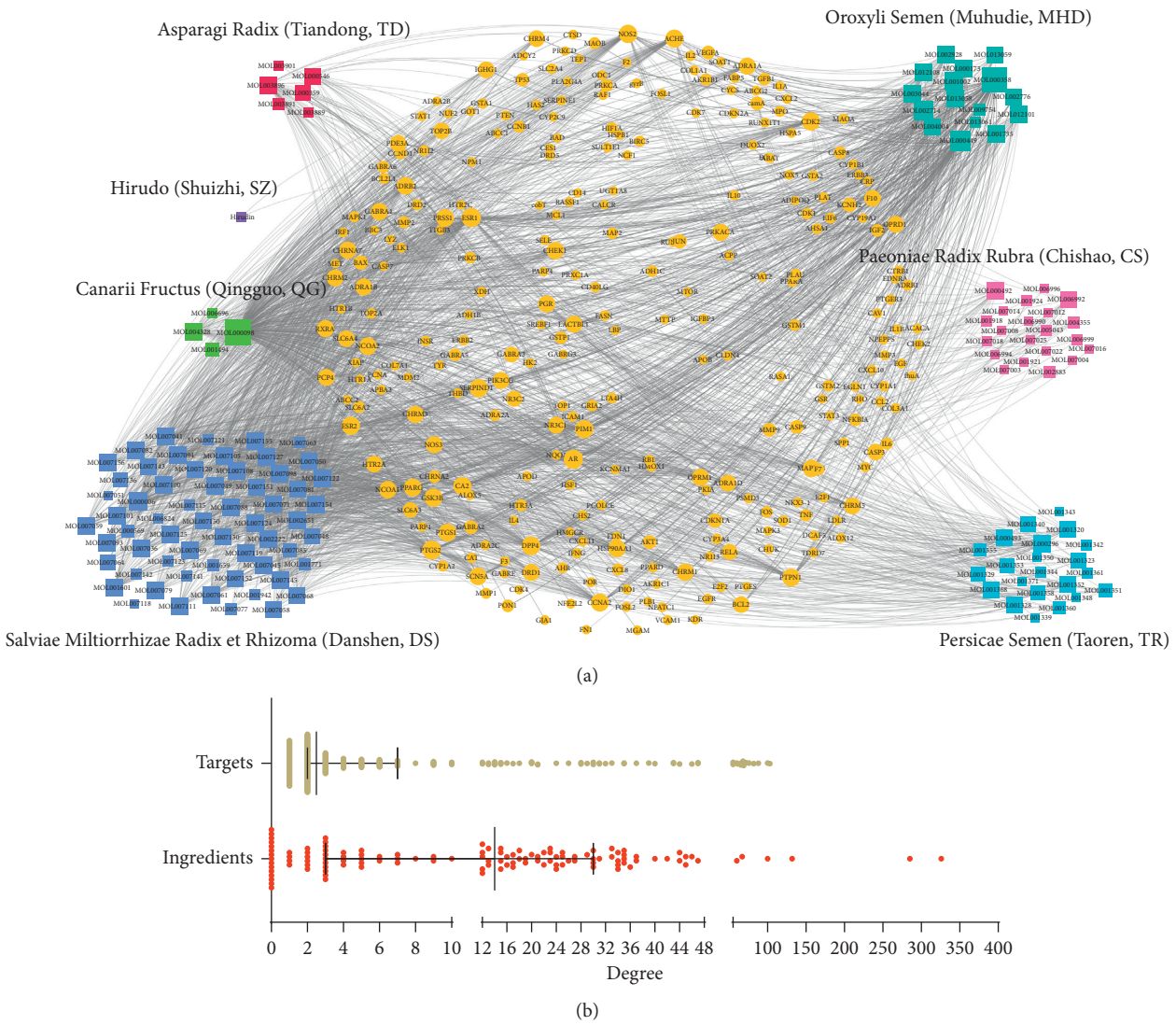
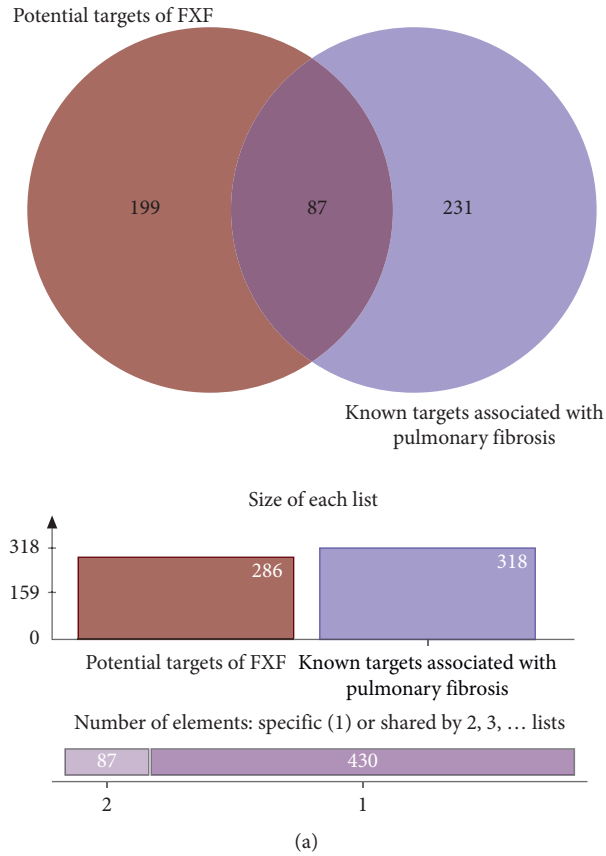


Figure 1: Establishment of the pharmacodynamic component-target network of FXF. (a) All components (compound ID) obtained from different herbs were associated with related targets for the construction of the compound-target network, in which one node represented one compound (different square colors indicated different herbs) and corresponding target (yellow circles). (b) The degree value distribution of nodes (ingredients and targets) in the network.

3.3. Compound-Target Network Validation. The relationships between components and targets were evaluated by molecular docking analysis, which helped to reduce network complexity while improving its accuracy. Thus, 10 core targets as well as 10 core compounds were discovered by molecular docking (Table 1 and Figure 3).

Thereafter, PTGS2, NOS2, CDK2, GSK3B, CCNA2, PPARG, MAPK14, CASP3, BCL2, and RELA were searched in the PDB protein database, respectively, to acquire 3D structures. It was observed from the results of binding free energy in Table 1 that most of the core compounds could tightly combine with core targets. Typically, stigmasterol is most closely bounded to BCL2 (Figure 3(i)), CASP3 (Figure 3(h)), and RELA (Figure 3(j)). In addition, cryptotanshinone was the compound with the most tight correlation with CCNA2 (Figure 3(e)), GSK3B (Figure 3(d)), PPARG (Figure 3(f)), and PTGS2 (Figure 3(a)). Meanwhile, tanshinone IIa was the compound with the lowest binding energy to CDK2 (Figure 3(c)), MAPK14 (Figure 3(g)), and NOS2 (Figure 3(b)) in this molecular docking experiment. In virtual docking (Figure 3(a) and Figure 3(d)-3(f)), cryptotanshinone formed the hydrogen bonds with ARG_{120}, ARG_{141}, MET_{210}, and SER_{342} of COX-2, GSK-3 beta, Cyclin-A2, and PPAR-gamma, respectively. Meanwhile, tanshinone IIa formed the potent hydrophobic interaction with iNOS (π-sigma bonds with TRP_{194}, Figure 3(b)), CDK2 (alkyl bonds with ALA_{31}, Figure 3(c)), and MAP kinase 14 (alkyl bonds with MET_{78}, Figure 3(g)). Typically, the stigmasterol-caspase-3 complex became stable via forming the π-sigma and π-alkyl with the PHE_{252} and PHE_{256} (Figure 3(h)). Correspondingly, the stigmasterol–Bcl-2 complex and stigmasterol-p65 complex got steady severally via forming hydrophobic interaction with MET_{115} residues (Figure 3(i)) and the hydrogen bond with PHE_{239} residues (Figure 3(j)).



(a)

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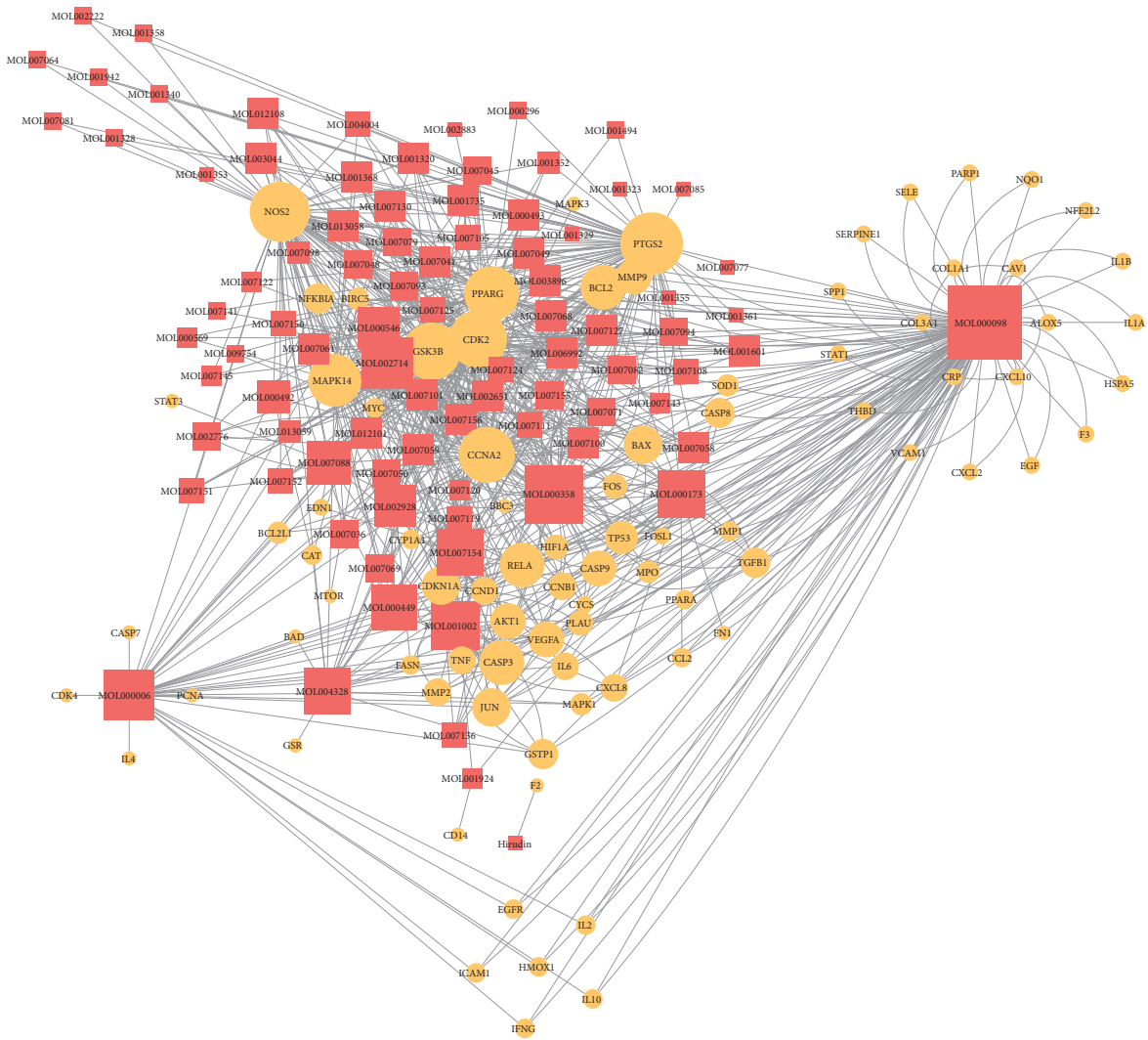

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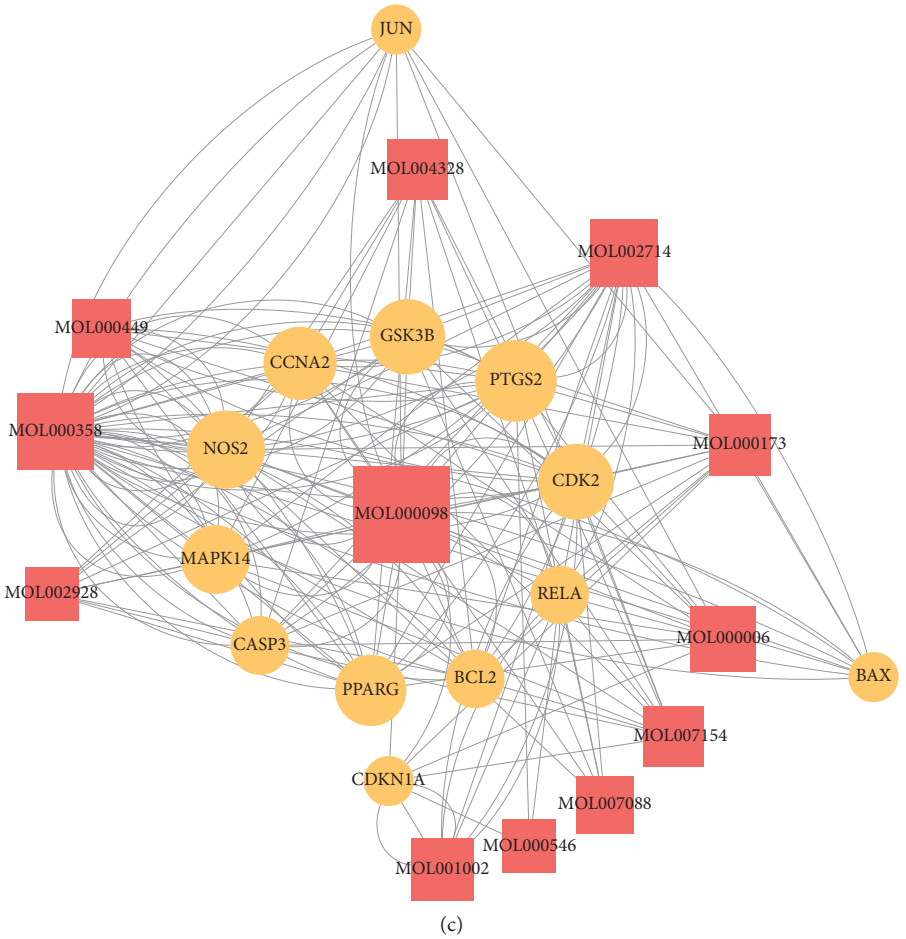
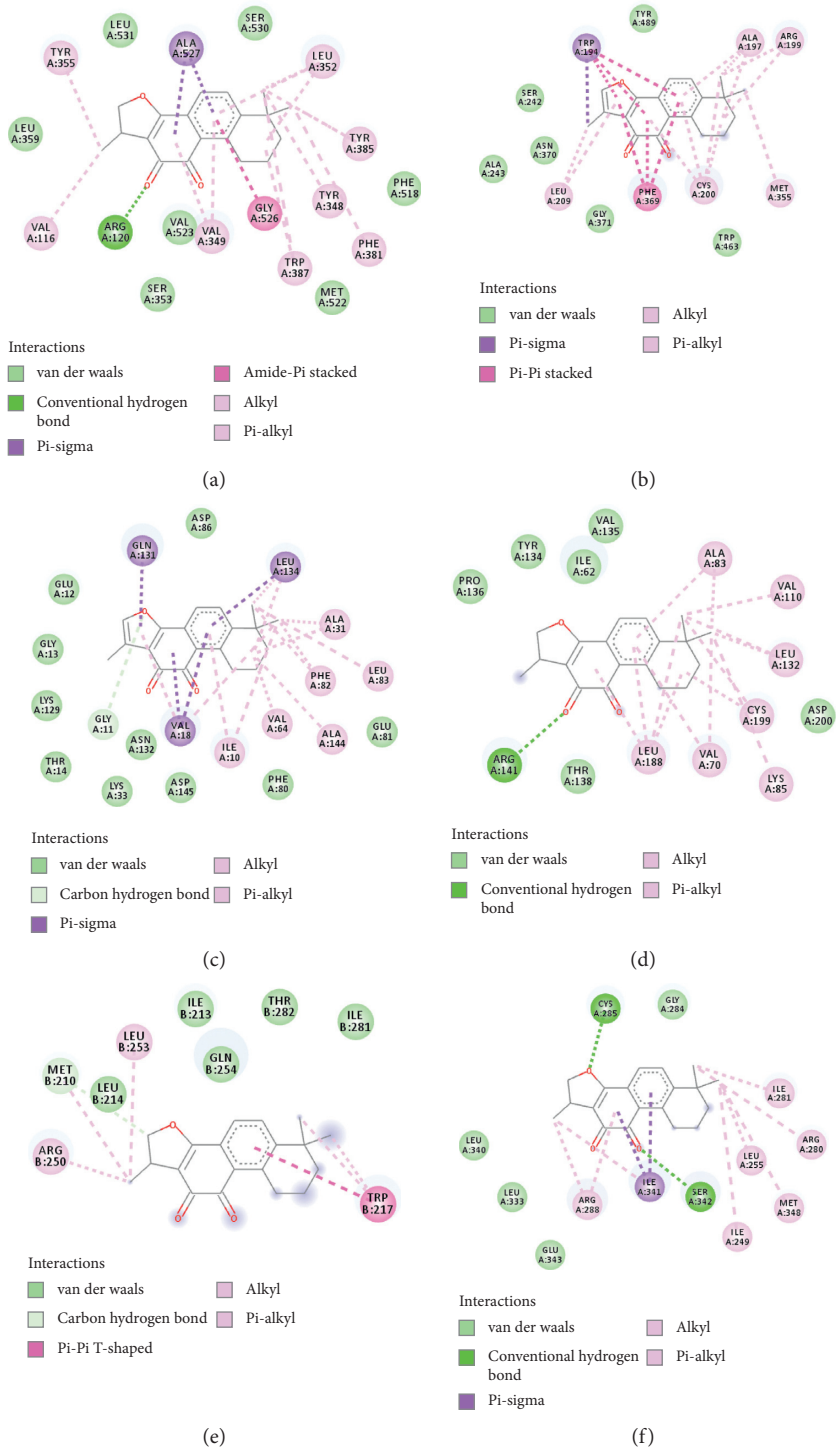


Figure 2: Screening of core targets for FXF in the treatment of pulmonary fibrosis. (a) As observed from the Venn diagram, there were 87 overlapping candidate targets between FXF and the known targets associated with pathological course in pulmonary fibrosis. (b) The candidate component-target network for FXF in the treatment of pulmonary fibrosis. (c) The core component-target network for FXF in the treatment of pulmonary fibrosis.

Table 1: Virtual docking of core components with core targets in FXF for the treatment of pulmonary fibrosis.

Core ingredients	Binding energy (kcal·mol^{-1})									
	BCL2	CASP3	CCNA2	CDK2	GSK3B	MAPK14	NOS2	PPARG	PTGS2	RELA
MOL000006	−7.2	−6.8	−6.8	−10	−8	−8.9	−8.9	−7.2	−7.1	−6.2
MOL000098	−7.2	−6.5	−6.8	−10.1	−8	−8	−8.7	−7.2	−6.6	−6.1
MOL000173	−7.3	−6.6	−6.1	−10	−7.8	−7.8	−9.9	−7.3	−6.9	−6
MOL000358	−7.6	−7.5	−6.8	−7.7	−9.1	−9.3	−9.4	−8.7	−4.5	−6.7
MOL000449	**−8**	**−8.1**	−6.6	−5	−8.9	−8.4	−9.1	−7.7	−3.2	**−7.1**
MOL001002	−6.3	−6.3	−6	−10.4	−8.4	−8.1	−8.7	−7.3	−6.8	−6.3
MOL002714	−7.1	−6.9	−6.5	−10.1	−7.9	−8.2	−9.9	−7.1	−6.9	−6.2
MOL004328	−7	−7	−6	−6.7	−7.1	−7.7	−7.7	−7.4	−5.8	−6.5
MOL007088	−7.7	−7.8	**−7**	−10.8	**−9.5**	−9.3	−10.5	**−8.8**	**−9.2**	−6.9
MOL007154	−7.7	−7.7	−6.8	**−10.9**	−9.3	**−9.4**	**−10.6**	−8.4	−8.8	−6.7

3.4. Enrichment Analysis of FXF Core Targets for the Treatment of Pulmonary Fibrosis. For better understanding the multi-target and multi-pathway mechanism by which FXF treated pulmonary fibrosis, the OmicShare online approach was used to carry out GO as well as KEGG analysis on the thirteen core targets, to identify the biological processes/molecular functions along with signal transduction pathways in FXF for the treatment of pulmonary fibrosis ($p < 0.05$, FDR < 0.05). As shown, external stimulus (from oxidative stress, radiation, UV, chemical substances, and virus infection), apoptosis, cell cycle, aging, immune process, and protein metabolism were the mostly



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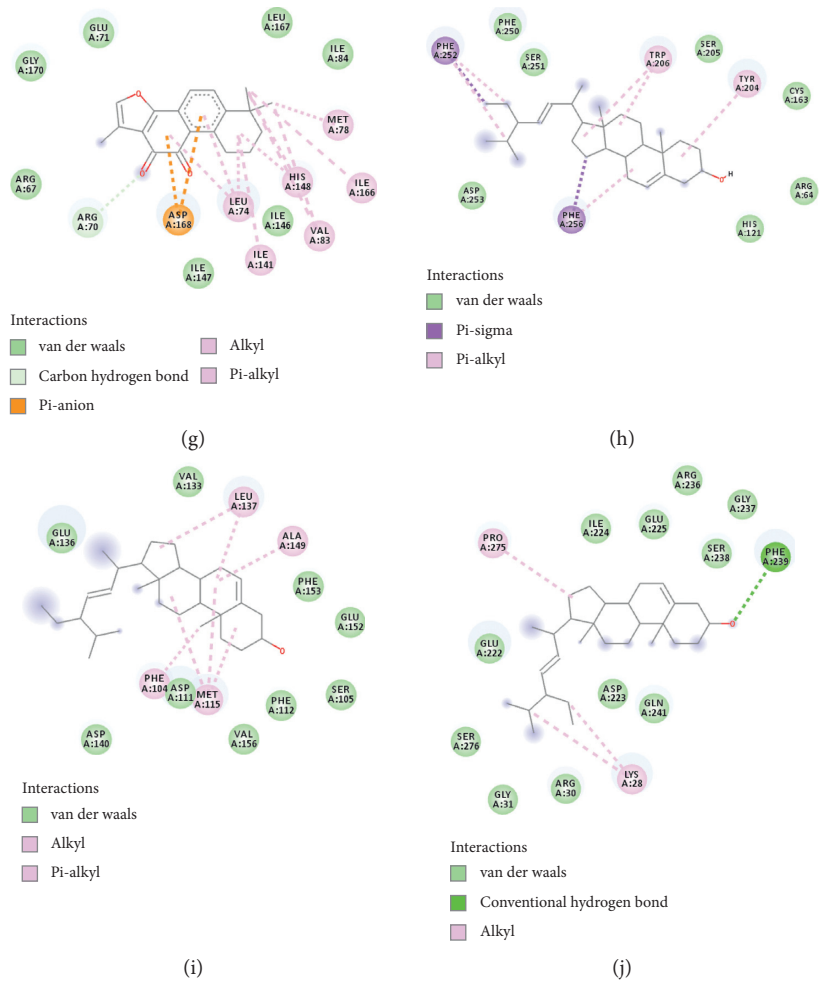

Figure 3: Virtual docking for core components and core targets in FXF for the treatment of pulmonary fibrosis. Virtual docking of cryptotanshinone with COX-2 (a), GSK-3 beta (d), Cyclin-A2 (e), and PPAR-gamma (f). Virtual docking of tanshinone IIa with iNOS (b), CDK2 (c), and MAPK14 (g). Virtual docking of stigmasterol with Caspase-3 (h), Bcl-2 (i), and p65 (j).

concentrated GO-BP terms (Figures 4(a)–4(c)). These core targets were mostly concentrated on several KEGG pathways, like IL-17, AGE-RAGE, TNF, HIF-1, PI3K-AKT, NOD-like receptor, T/B cell receptor, and virus infection-related pathways, indicating their important parts in the treatment of pulmonary fibrosis.

4. Discussion

Pulmonary fibrosis, an interstitial lung disease featured by the fibrotic, chronic, and progressive nature, is an uncommon disorder generally seen among the old people [44]. Its incidence in the North American and European countries is reported to be 2.8–9.3/100,000 persons. At present, few epidemiological data are available in China, yet the incidence of pulmonary fibrosis shows an increasing trend recently [45]. Usual interstitial pneumonia (UIP) is characteristic of pulmonary fibrosis on high-resolution chest CT or in patients with a pulmonary fibrosis history. As for the major clinical manifestations, pulmonary fibrosis mainly manifests as progressive dyspnea, hypoxia, restricted ventilation impairment, gas exchange disturbance, and respiratory failure [46]. For the time being, there is no radical treatment for pulmonary fibrosis, and treatment is mainly conducted for delaying disease progression, improving patient quality of life, and prolonging patient survival. Pirfenidone and nintedanib are the anti-fibrotic drugs adopted to treat pulmonary fibrosis, which are effective to some extent; however, their high prices and adverse reactions have restricted their application. Recently, Chinese herbs have exerted vital parts in treating pulmonary fibrosis. It is reported in numerous articles that Chinese herbs alleviate clinical symptoms, improve patient quality of life, and boost the exercise tolerance while delaying lung function decline among pulmonary fibrosis patients [47, 48].

Chinese herbs represent an important TCM means to treat diseases, which has been utilized due to the complicated pulmonary fibrosis pathogenesis as well as the common patient syndrome [49]. Pulmonary fibrosis can be classified as Yin deficiency and internal heat syndrome, lung and kidney qi deficiency syndrome, phlegm turbid/heat obstruction of lung syndrome, and blood stasis syndrome, based on the diffuse interstitial lung disease diagnostic criteria in TCM (2012 Edition).

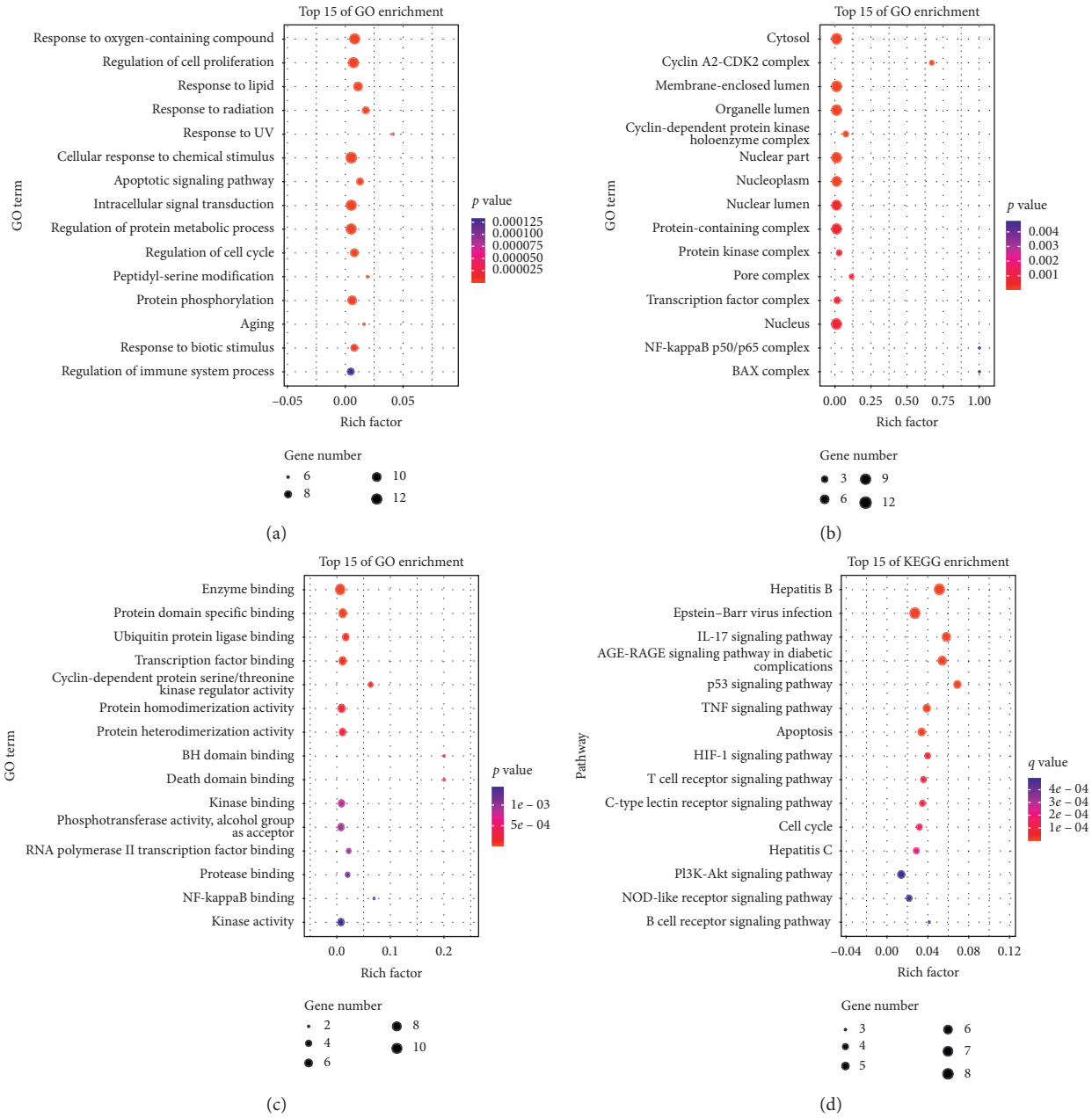

Figure 4: Enrichment analysis of core targets for FXF on the treatment of pulmonary fibrosis through OmicShare: (a) The top fifteen enriched GO-biological process; (b) GO-molecular functions; (c) GO-cellular components terms; and (d) KEGG pathways. The abscissa shows the enrichment factor, and the ordinate shows the GO terms or KEGG pathways. The color of the dot represents the adjusted p-value/q-value, and the size of the dot represents the number of core targets mapped to the reference GO terms or pathways.

Typically, clinical medication mainly aims to promote qi, accelerate blood circulation, eliminate blood stasis, and reinforce Yin. Therefore, FXF, which has the effect of nourishing Yin and removing blood stasis, is used as a cipher prescription in our hospital for the treatment of pulmonary fibrosis.

Herbal medicine has different and complicated components. Previous research on herbal medicine is still encountered with certain problems, like the complicated composition and the unknown mechanism. Network pharmacological analysis has been extensively adopted to investigate the pharmacological mechanisms of TCM currently. As a result, the holistic view in TCM was used in combination with network pharmacology analysis and the syndrome differentiation science system in this study to identify core pharmacodynamic material basis and targets. Then, molecular docking study was carried out on those core active ingredients together with the core targets using AutoDock Vina for verifying results of network pharmacology analysis. Finally, these core targets were subjected to GO and pathway enrichment analysis.

As revealed in this work, quercetin, beta-sitosterol, baicalein, luteolin, ellagic acid, wogonin, naringenin, tanshinone IIa, stigmasterol, and cryptotanshinone were identified as the core compounds for treating pulmonary fibrosis. Some studies demonstrate the effect of quercetin, baicalein, tanshinone IIA, and cryptotanshinone on suppressing experimental pulmonary fibrosis induced by bleomycin or silica through promoting lung tissue self-healing, modulating disturbed redox-balance in lung tissue, alleviating local inflammation, reducing collagen deposition, promoting ECM degradation, inducing pulmonary fibrosis cell apoptosis, reversing angiotensin production, and regulating multiple signaling molecules and pathways (such as TGF-beta, sphingosine kinase, SMAD, Nrf2, STAT3) [41, 42, 50–54]. Our research results were consistent with those in previous study, indicating that these compounds were the core effective components of FXF to treat pulmonary fibrosis.

According to the node degrees in our constructed core component-target network, PTGS2, NOS2, CDK2, GSK3B, CCNA2, PPARG, MAPK14, CASP3, BCL2, and RELA were suggested as core targets of FXF for the treatment of pulmonary fibrosis. In addition, molecular docking was conducted to validate the associations of core components with core targets. Upon GO-BP annotation analysis, the core targets exerted vital parts in external stimulus (from oxidative stress, radiation, UV, chemical substances, and virus infection), apoptosis, cell cycle, aging, immune process, and protein metabolism. As revealed by KEGG pathway analysis, numerous pathways were tightly associated with the pulmonary fibrosis pathogenic mechanism. Typically, IL-17, AGE-RAGE, TNF, HIF-1, PI3K-AKT, NOD-like receptor, T/B cell receptor, and virus (EBV and hepatitis virus) infection-related pathways were the most significant pathways involved. The associations of diverse pathways, including EBV and hepatitis virus infections, with pulmonary fibrosis are identified through fundamental clinical studies to induce pulmonary fibrosis [55–57]. Meanwhile, epithelial-mesenchymal transition (EMT) in pulmonary fibrosis may also be related to activating the NOD-like receptor protein (NLRP) 1 and NLRP3 inflammatory pathways [58]. The advanced glycation end products (AGE) may be obtained from non-enzymatic reaction of proteins and lipids with various oxidants in the process of aging. In addition, the receptor for AGE (RAGE) has been demonstrated to be associated with alveolar homeostasis as well as pulmonary fibrosis. The aberrant epithelial stromal repair capacity in pulmonary fibrosis is linked with this aging process. The elevated AGE-RAGE proportion in pulmonary fibrosis is correlated with epithelial stromal repair during pulmonary fibrosis [59, 60]. In the pathological process of pulmonary fibrosis, the infiltration of various immune cells and the release of immuno-inflammatory factors play an important role, which is also considered as a new therapeutic target to reverse the disease, such as IL-17 [61] and T cell receptor [62]. In addition, the mitochondria-mediated apoptosis also exerts a vital part during pulmonary fibrosis genesis and progression mediated by both mitochondria and ER stress (triggered by HIF-1alpha) [63–65].

Nonetheless, certain limitations should be noted in the present work [66], like the data-based network pharmacology, which made it impossible to examine whether all data were comprehensively collected and whether the criteria to select core components were completely accurate. Besides, the efficacy of additional components and effects of dosage should be further examined.

5. Conclusion

To sum up, the mechanism of action by which FXF treats pulmonary fibrosis may be related to the regulation of several pathways, like IL-17, AGE-RAGE, TNF, HIF-1, PI3K-AKT, NOD-like receptor, T/B cell receptor, and virus infection-related pathways.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xiao-Li Chen and Cheng Tang contributed equally to this study.

Acknowledgments

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Supplementary Materials

Table S1: all the pharmacodynamic ingredients of FXF. Table S2: all the potential pharmacodynamic targets of FXF. Table S3: known pulmonary fibrosis-related targets. Table S4: FXF shared 87 potential pharmacodynamic targets with known pulmonary fibrosis-related targets. Table S5: degree values of nodes in the candidate active ingredient-target network of FXF in treating pulmonary fibrosis. (*Supplementary Materials*)

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Review Article

The Effects of Ischemic Preconditioning Supplementation on Endothelial Function: A Systematic Review and Meta-Analysis

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Objective. Ischemic preconditioning (IPC) has gradually been promoted in clinical practice to lower the risk of cardiovascular surgery and postoperative complications. We investigated the role of IPC on vascular endothelial function and the relationship between IPC, flow-mediated dilation (FMD), and brachial artery diameter (BAD). *Methods*. Systematic searches were conducted in PubMed, Medline, Cochrane Library, Embase, and Scopus databases from their inception to March 20, 2020. This research included randomized controlled trials (RCTs) with adults, and the values of FMD and BAD were considered as the primary outcomes. Ten studies comprising 292 participants were included in the meta-analysis. *Results*. Regarding FMD, we observed beneficial effects of IPC on endothelial function (standardized mean difference (SMD): 1.82; 95% confidence interval (CI): 0.64, 3.01; $p < 0.001$; $I^2 = 89.9\%$). However, the available evidence did not indicate that IPC affected BAD (SMD: 0.08; 95% CI: −0.03, 0.18; $p > 0.05$; $I^2 = 76.5\%$). *Conclusions*. Our meta-analysis indicated a significant effect of IPC on the endothelial function of the blood vessels, affecting FMD but not BAD.

1. Introduction

Currently, approximately 120 million people in the US have different forms of cardiovascular disease (CVD), which is considered to be the leading cause of death, morbidity, and disability. In 2016, CVD accounted for about 840,000 deaths in the United States, and the number of CVD deaths increased from 2011 to 2017 by 9.7% [1]. The loss of function of the endothelium is considered to be an early pathogenic step in the development of atherosclerotic lesions and the subsequent onset of cardiovascular diseases [2]. Therefore, the endothelium has been identified as a tractable physiological target for therapeutic interventions to reduce the risk of CVDs such as coronary heart disease, stroke, or atherosclerosis [3]. Endothelium, which lines the inside of the blood vessels, regulates vascular integrity, reduces thrombosis, decreases vascular tone, improves vascular wall function, and promotes angiogenesis by releasing distinct signaling molecules [4, 5]. Moreover, the dysfunction of endothelium causes multiple diseases such as pathogenesis and progression of atherosclerosis, cerebrovascular disease, and inflammatory diseases [6]. Thus, the assessment of endothelial function could predict future cardiovascular events and provide an appropriate marker for blood vessels.

Nowadays, some noninvasive techniques have been developed to assess endothelial functions, such as FMD, which represents an endothelium-dependent, primarily nitric oxide- (NO-) mediated dilation of conduit arteries in response to an imposed increase in blood flow and shear stress. Moreover, impaired FMD has been associated with the predisposition to atherosclerosis and CVD and represents an early process in the development of target organ damage and clinical events [7]. In the 1990s, high-frequency ultrasonographic imaging of the brachial artery was developed to assess endothelium-dependent FMD. This technique stimulates the release of nitric oxide, resulting in vasodilation that can be quantitated as an index of vasomotor function [8].

IPC, originally proposed by Murry and his group in 1986, is associated with the ability of endogenous mechanisms to produce strong resistance to ischemic damage shortly after nonlethal mild ischemia or reperfusion treatment [9]. Animal studies [10, 11] have confirmed that IPC reduces ischemia-reperfusion injury in multiple organs. Much time and effort have been devoted to exploring the underlying molecular mechanisms of IPC. The most noteworthy result was that clinical research paid more attention to applying IPC for preventing distal organ damage. IPC was shown to limit the deleterious effects of prolonged ischemia or ischemia/reperfusion (IR), such as complex cardiac surgery, resection of abdominal aortic aneurysm, and kidney transplant operation, particularly in high-risk surgical patients. Many clinical studies have shown that IPC provided significant protection to the cardiac and vascular systems, improving the microcirculation state of blood vessels and maintaining endothelial function. Thus, IPC is expected to be an important therapeutic strategy to alleviate IR injury of vital organs in the future. However, FMD was calculated as a relative percentage change in the baseline BAD during reactive hyperemia; baseline BAD was also regarded as an important determinant of FMD [12].

Many clinical studies have analyzed the effects of IPC on endothelial function. Remote IPC before cardiac surgery increased myocardial salvage and protected endothelial function; additionally, it was safer and more economical than other alternatives [13]. Several studies have shown that IPC by transient limb ischemia reduces myocardial IPC injury in patients [13–17]. However, few studies presented protective effects of IPC against endothelial IR injury in patients who had suffered heart failure [18].

This study was conducted to systematically summarize the pieces of evidence for the effects of IPC on endothelial function and conduct a meta-analysis. In this study, two key indicators, FMD and BAD, were evaluated before and after ischemic treatment, and the effects of IPC on endothelial function were systematically examined for the first time in this study. We also evaluated FMD and BAD to further determine the interrelationships between the brachial artery variables and the cardiovascular risk events in a large well-characterized population.

2. Methods

Our systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analyses (Supplementary Table: PRISMA-P) [19], and the study protocol was registered with the identification code PROSPERO:CRD42020176093.

2.1. Data Sources and Search Strategy. Five databases (PubMed, Medline, Embase, Cochrane, and Scopus) were used to search for articles from inception until March 20, 2020. Additionally, a manual search of the list of references was performed for the relevant reviews and articles included in the systematic review. A search strategy was developed using the following MeSH and text keywords: intervention ("ischemic preconditioning" or "remote ischemic preconditioning" or "ischemic") and outcomes ("FMD" or "BAD" or "resting diameter" or "brachial artery flow-mediated dilation" or "vasodilation" or "vascular reactivity" and "endothelial function").

2.2. Literature Selection. Original studies were included if they met the following inclusion criteria: (1) relevant human intervention studies (subjects ≥18 years old), (2) performed IPC, (3) had a control group, and (4) measured endothelial function, including FMD and BAD.

Studies were excluded when (1) they had no information on the intervention or a control group, (2) duplicate publications or substudies of the RCTs were selected, (3) the studies were observational with cross-sectional, case-control, or cohort design, (4) the studies lacked sufficient BAD or FMD information, baseline, or follow-up, and (5) the studies were published in languages other than English.

2.3. Data Extraction. Two independent researchers screened the retrieved articles for eligibility. First, the title and abstract of all the studies were reviewed. Then, the full text of relevant studies was retrieved and assessed to ascertain the suitability of the study for inclusion in the meta-analysis. Any disagreement was discussed and resolved by the third researcher. After data extraction, the following information was recorded in a database: first author's name, publication year, sex, sample size, study design, intervention, duration of the study, and the mean and standard deviation for FMD and BAD in every intervention group and control group. Then, a random-effects meta-analysis was conducted, followed by meta-regression and subgroup analyses to determine whether the effects were modified by health status (i.e., healthy participants versus participants with other diseases), age, gender, ethnicity, and treatment duration.

2.4. Quality Assessment. The quality of the studies was assessed by two independent investigators using the Cochrane Collaboration risk of bias tool and met the following criteria: "random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias." Based on the recommendations of the Cochrane Handbook, a decision of "yes" indicated a low risk of bias, while "no" indicated a high risk of bias. Labeling an item as "unclear" suggested an unclear or unknown risk of bias [19, 20]. Any disagreement was discussed and resolved by the third investigator.

2.5. Statistical Analysis. All statistical analyses for our meta-analysis were performed by the open-source statistical software Review Manager (RevMan, Version 5.3.5; The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and Stata version 15 (Stata Corp LLC, Texas, USA). Inverse variance weighting was used to pool the different studies [21]. Potential sources of heterogeneity were investigated by stratified meta-analyses

according to various study characteristics defined a priori: year, characteristics, different interventions, and duration of intervention. Heterogeneity in the results was quantified by the I^2 statistic [22]. Sensitivity analyses were performed to assess the robustness of the meta-analysis by removing one study at a time. Publication bias was assessed by visual inspection of the funnel plot [23] and Egger's regression test. The "trim and fill" method by Duval and Tweedie was used to adjust the analysis for the effects of publication bias [24].

3. Results

3.1. Study Characteristics. The literature search identified 7,038 articles. Ultimately, ten studies with 12 trials were included in our research. A flow diagram of the process of selecting the studies is shown in Figure 1, and the details of the included studies are presented in Table 1.

In total, 292 participants were listed. The studies included were conducted in America, Europe, and Asia. The intervention period in these studies ranged between one day and eight weeks. The values of FMD and BAD were considered as outcomes.

3.2. Quality Assessment and Potential Bias. The quality score and risk of bias for each study are shown in Figure 2. The outcome of the quality assessment is provided in Table 1. All studies were randomized [18, 25–33], and four of the studies had additionally conducted before and after randomized trials [18, 33]. While it was hard to blind researchers and participants to the IPC protocol order, blinding the assessment of outcomes was performed in five studies [26–30]. Distribution concealment and reporting bias were not mentioned in any of the studies, which might have produced certain types of bias.

3.3. Effects of IPC on FMD. Meta-analysis of the 12 sets of independent results showed that IPC improved endothelial function (SMD: 1.824; 95% CI: 0.64, 3.01; $p < 0.05$, shown in Figure 3). Heterogeneity between studies was significant ($Q = 99.13$; $I^2 = 89.9\%$; $p < 0.05$). The results indicated that IPC had a positive effect on the improvement of vascular function. The IPC group increased FMD by 1.82 compared to the FMD in the untreated group. Given that the methods for testing macrovascular and microvascular endothelium-independent reactivity remained largely unstandardized, meta-regression was only performed on the FMD data. Then, we performed subgroup analysis. The remaining heterogeneity after subgroup meta-analyses (Table 2) showed that age, gender, health status, ethnicity, and treatment duration might be responsible for the substantial amount of heterogeneity among the studies.

3.4. Effects of IPC on BAD. Meta-analysis of the eight sets of independent results showed that IPC did not affect changes in BAD (SMD: 0.08; 95% CI: −0.03, 0.18; $p = 0.148$, shown in Figure 4). Heterogeneity between studies was significant ($Q = 29.73$; $I^2 = 76.5\%$, $p < 0.001$), which was due to the variations in the characteristics of the populations (age, gender, or health status), different procedures and methods used (measurements of vascular function and intervention duration), and differences in the study design and quality of research. However, the sources of heterogeneity could not be fully determined since the number of included studies and the sample size of the majority of the studies were relatively small.

3.5. Sensitivity Analyses and Publication Bias. The sensitivity analyses of FMD with the random-effects models are shown in Figure 5. Removing studies individually did not substantially modify the differences in the effect on FMD. Visual inspection of the funnel plot (Figure 6) suggested that, overall, there was no evidence of publication bias, which was also confirmed by Egger's Regression test ($p = 0.813$; Figure 7) and Beeg's test ($p = 0.35$).

4. Discussion

Overall, the results of the meta-analysis demonstrated that IPC protected endothelial function and improved FMD. However, it did not show any significant increase in BAD following the application of IPC.

Murry et al. first described the phenomenon of IPC [9]; several studies had demonstrated that IPC could reliably provide myocardial protection [34, 35]. The breakthrough in the clinical applicability of preconditioning protection came with the discovery by Kharbanda et al. that transient limb ischemia provided cardiovascular protection in humans and animals [36, 37]. Besides, IPC used as an adjunct to primary percutaneous coronary intervention in patients with ST-elevation myocardial infarction improved long-term clinical outcomes [17].

Endothelial dysfunction is involved in the development of atherosclerosis, which precedes asymptomatic structural vascular alterations, as well as clinical manifestations of CVD. Endothelial function can be assessed noninvasively using the FMD technique. Therefore, we can improve FMD through IPC and indirectly reduce the risk of related diseases. As an emerging detection indicator, FMD is closely related to the occurrence and development of many diseases. Moreover, brachial artery FMD has been used independently to predict long-term adverse CV events in healthy subjects with no apparent heart disease in addition to being used for assessing some traditional risk factors [38]. Besides, Perri et al. [39] found that AF patients with low FMD were associated with an increased risk of CVE (cardiovascular events), suggesting that impaired artery dilation predisposes to atherosclerotic complications. Additionally, some researchers suggested that the combination of FMD and nitroglycerine-induced vasodilation measurements could more accurately predict cardiovascular events than by measuring vasodilation with nitroglycerine only [40]. One study on systemic lupus erythematosus showed that the accumulation of damage in patients was associated with a progressive loss of FMD, with preserved endothelium-independent vasodilation [41]. Han et al. [42] found that low

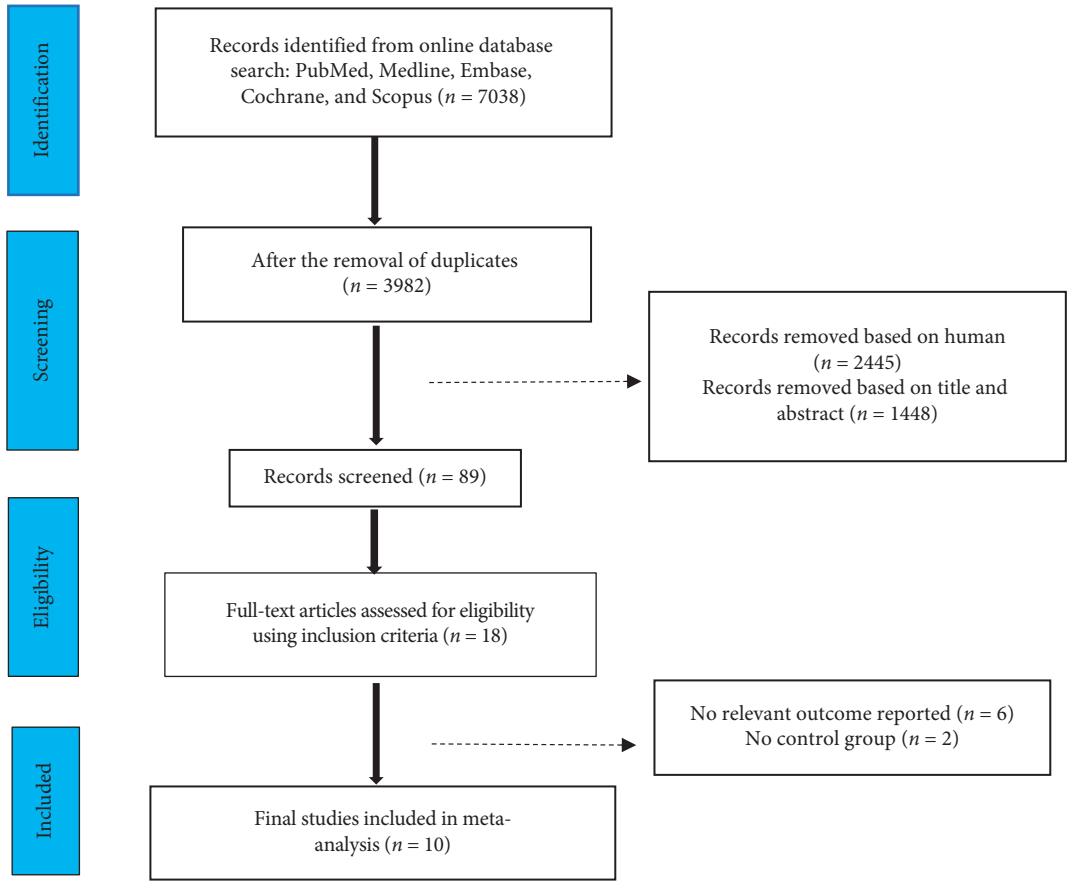

Figure 1: Flowchart showing the literature searched and reviewed for the selection of the studies.

Table 1: Details of the included studies.

Author	Publication year	Male	Country	Sample size (control/intervention)	Age	Intervention	Duration	Presented data
Jones et al. [25]	2014	16	England	8/8	24	IPC or not	8 weeks	FMD, BAD
Liuni et al. [26]	2010	20	America	10/10	18–33	IPC or not	5 days	FMD, BAD
Liuni et al. [27]	2011	18	America	9/9	18–29	IPC or not	20 days	FMD, BAD
Seeger et al. [18]	2014	15	The Netherlands	15/15	67	IPC or not	7 days	FMD, BAD
Seeger et al.* [18]	2014	15	The Netherlands	15/15	65	IPC or not	7 days	FMD, BAD
Verouhis et al. [28]	2019	4	The Swedish	4/4	30.5	IPC or not	6 days	FMD, BAD
Bailey et al. [29]	2012	11	England	11/11	25	IPC or not	Immediate	FMD, BAD
Luca et al. [30]	2013	15	Canada	15/15	20–31	IPC or not	1 day	FMD, BAD
Liang et al. [31]	2015	20	China	20/20	64	IPC or not	20 days	FMD
Manchurov et al. [32]	2014	26	Russia	25/23	62	IPC or not	7 days	FMD
Munckhof et al. [33]	2013	15	The Netherlands	15/15	72	IPC or not	7 days	FMD
Munckhof et al.* [33]	2013	15	The Netherlands	15/15	22	IPC or not	7 days	FMD

*From the same article.

baseline FMD in hyperuricemia patients was associated with a significantly increased risk of incident hypertension and that FMD could be used as one of the predictive factors of the risk of diseases.

There are several mechanisms through which FMD could improve endothelial function and might account for the beneficial effects observed in this study. First, cardiovascular protection provided by the early phase of IPC is mediated by the stimulation of receptors linked to protein kinase C (PKC) activation by adenosine, bradykinin, NO, and free radicals [43–45]. Recently, Kharbanda et al. [36] reported that IPC might help to reduce endothelial injury during ischemic reperfusion in humans. Subsequent studies in humans have confirmed that IPC decreased inflammatory

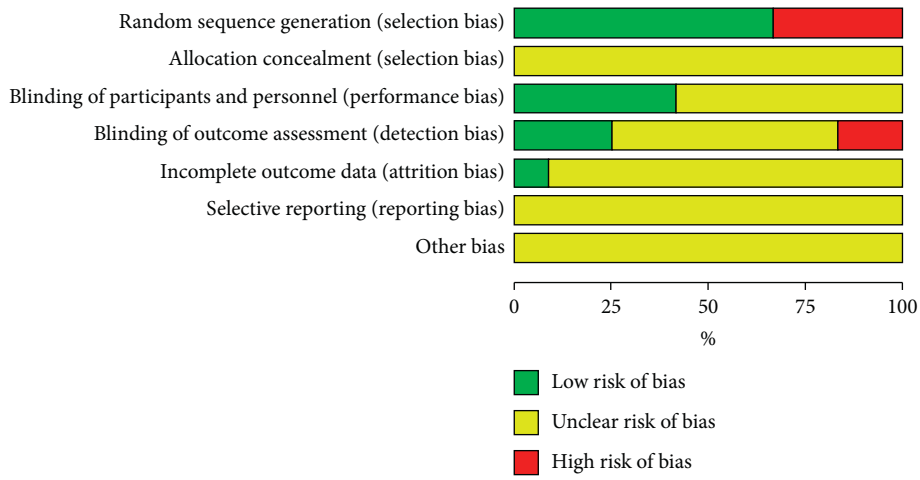

Figure 2: Summary of the risk of bias.

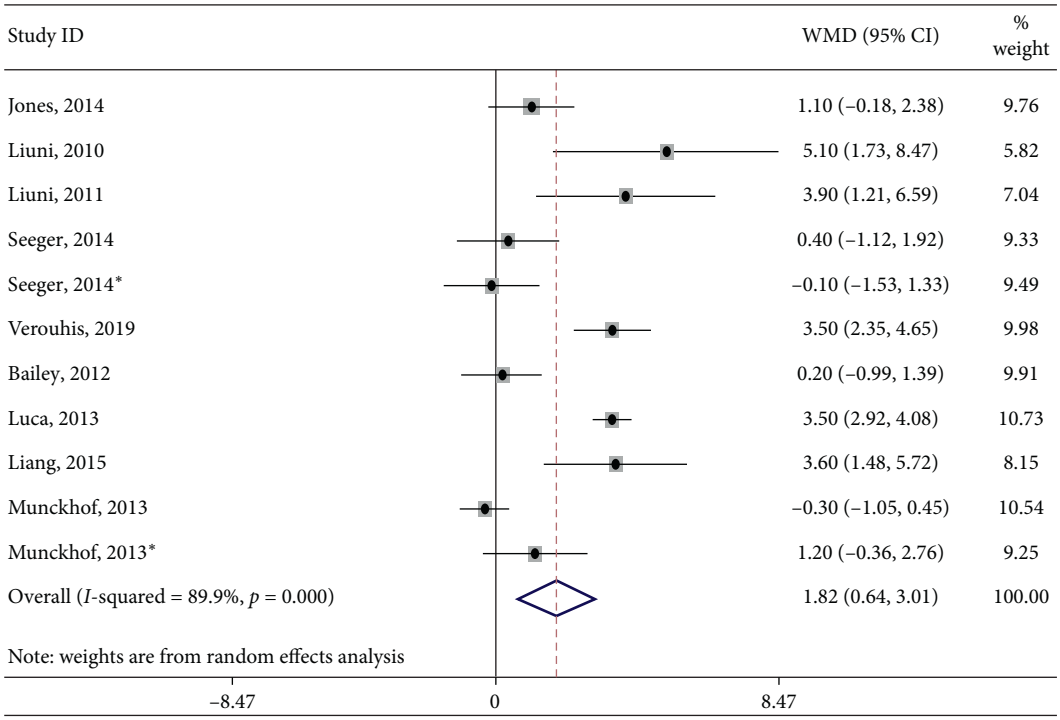

Figure 3: Forest plot showing the overall effect of IPC on flow-mediated dilation in adults (expressed as a percentage change). Data are shown as the percentage differences in means. Horizontal lines denote 95% CI. The size of the boxes is proportionally scaled to the effect size for each study.

reaction and improved endothelial function by these humoral mediators [46–48]. One possible mechanism by which repetition of IPC augments endothelial function is by stepping up the vascular shear stress resulting from increased blood flow. Acute or chronic increase in the shear stress stimulates the release of NO in the blood vessels [49]. Additionally, a steady increase in shear stress has been shown to cause functional and histological alterations of the vascular endothelium, resulting in enhanced vascular structure and function [50]. This beneficial change in the endothelium after the repetition of IPC also might contribute to the augmented forearm vascular response to ACh (acetylcholine) and the ACh-stimulated NO release. On the other hand, preconditioning stimulus did not directly alter the endothelial function but avoided endothelial dysfunction in both the conduit and resistance vessels in response to IR.

In conclusion, our findings indicated that IPC augmented the endothelial function through an increase in FMD. It is important to select an appropriate intervention that is effective in improving or augmenting endothelial

Table 2: Subgroup analyses for the effects of FMD on the markers of endothelial function.

Trial characteristic	Meta-regression analysis		Subgroup analysis					
	P value	95% CI	Stratification variable	Number of effect sizes	Pooled WMD	95% CI	P value within subgroups	I^2 (%)
Age	0.16	(−5.29, 1.14)	≤35 y	7	2.41	(1.15, 3.68)	<0.001	84.4
			≥60 y	5	0.66	(−0.70, 2.01)	0.008	74.5
Gender	0.42	(−3.70, 1.82)	>50% male	10	2.20	(0.83, 3.56)	<0.001	90.8
			≤50% male	2	0.28	(−0.66, 1.21)	0.839	0
Health status	0.86	(−4.25, 4.90)	Asymptomatic	9	1.82	(0.47, 3.16)	<0.001	91.4
			Diseased	3	1.91	(−1.22, 5.04)	0.016	82.7
Ethnicity	0.05	(−0.45, 3.62)	American	3	3.56	(3.00, 4.12)	0.64	0
			European	8	0.85	(−0.21, 1.92)	<0.001	81.5
			Asian	1	3.60	(1.48, 5.72)		
Treatment duration	0.68	(−1.99, 1.40)	5 min	2	0.14	(−0.91, 1.18)	0.64	0
			15 min	3	3.56	(3.00, 4.12)	0.64	0
			20 min	7	1.45	(0.08, 2.83)	<0.001	86.6

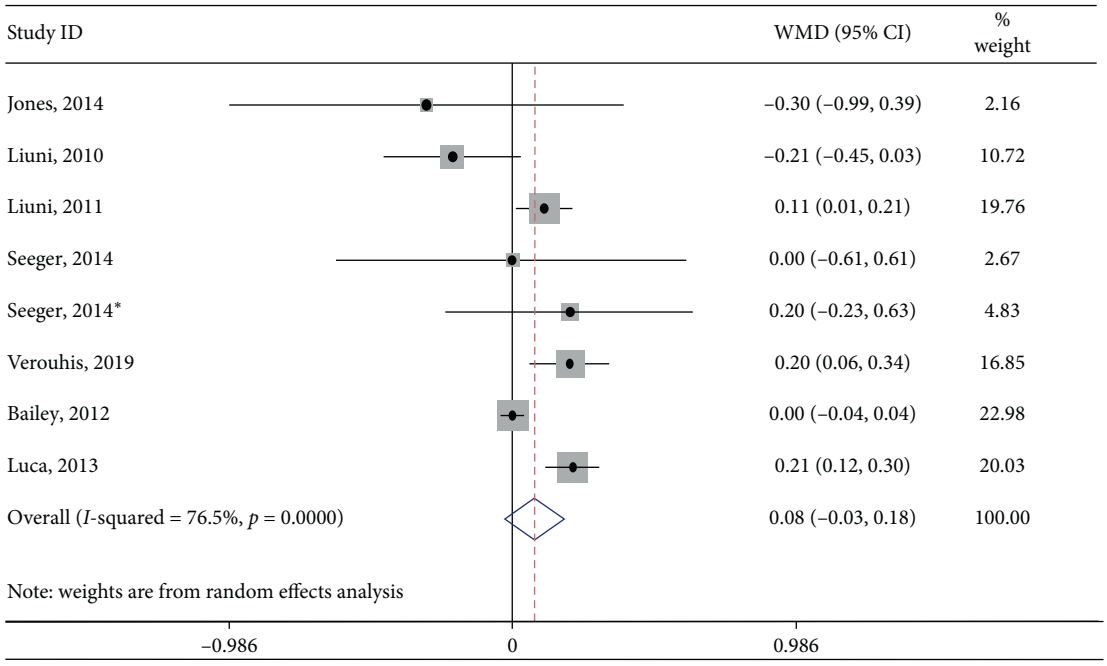

Figure 4: Forest plot showing the overall effect of IPC on BAD. Horizontal lines denote 95% CI. The size of the boxes is proportionally scaled to the effect size for each study.

function. IPC has the potential for improving endothelial function as a novel method for predicting and preventing cardiovascular diseases associated with endothelial dysfunction. Besides, the idea of providing significant myocardial protection with transient limb ischemia is highly attractive to clinicians because it only requires a blood pressure cuff [16]. As a simple, safe, and feasible therapeutic technique, which can easily be applied in the preoperative setting to patients with acute cardiac events, IPC may have the potential to reduce mortality. Although IPC is a part of the most powerful and reproducible phenomenon in cardioprotection, it has not been readily translated into routine clinical use because of methodological hurdles and limitations. Overall, our findings need to be demonstrated in a larger multicenter trial before IPC can be implemented extensively as adjunctive therapy in clinical settings [17].

5. Limitations

The overall quality of the studies included was the modest. The majority of the investigations did not allow blind participants to the intervention arm, and no study reported methods of allocation concealment. Additionally, given the small number of studies included in this review, our analysis might have been underpowered to detect differences in the effectiveness of interventions based on health status, type of

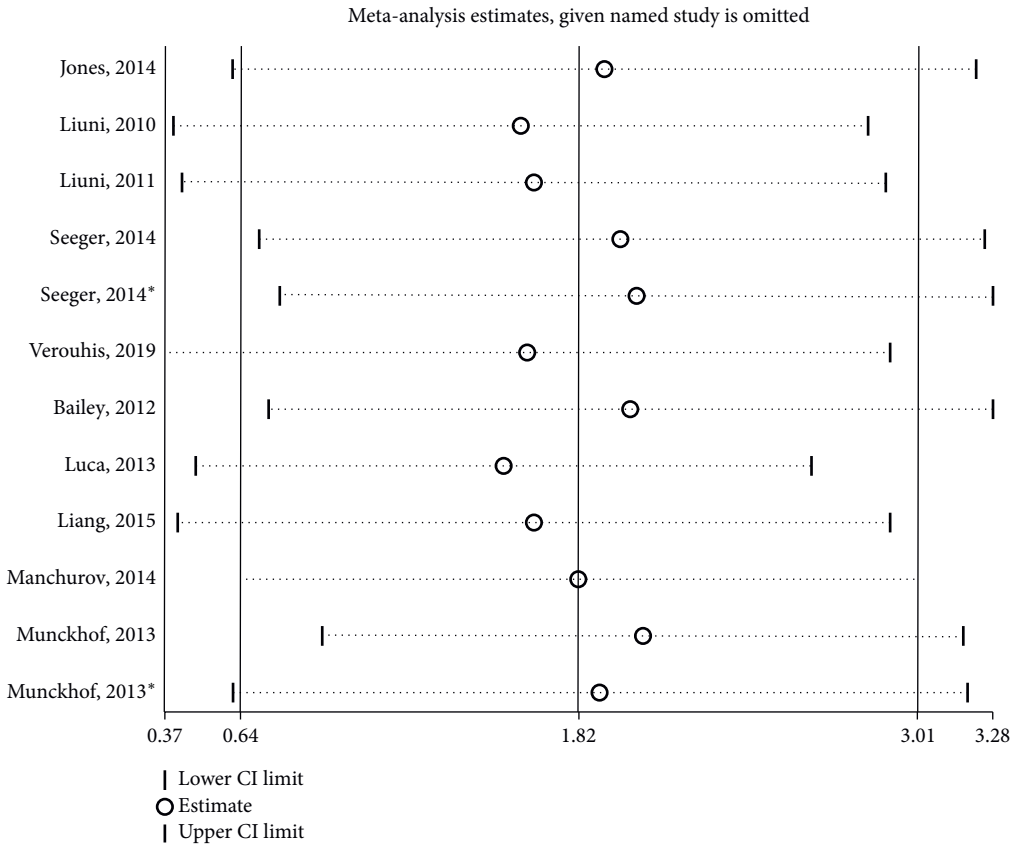

Figure 5: Sensitivity analyses of FMD.

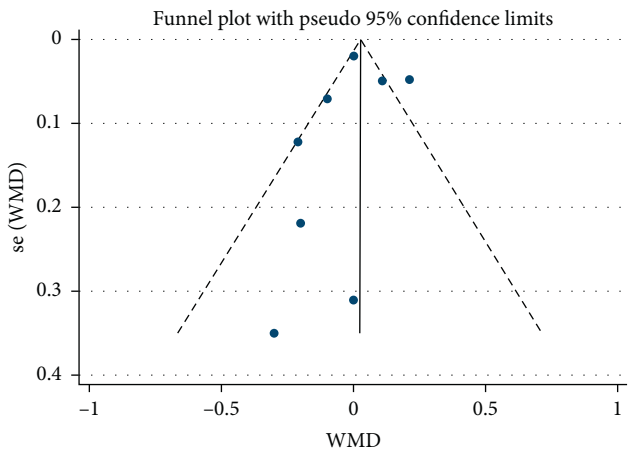

Figure 6: Funnel plot used to evaluate publication bias.

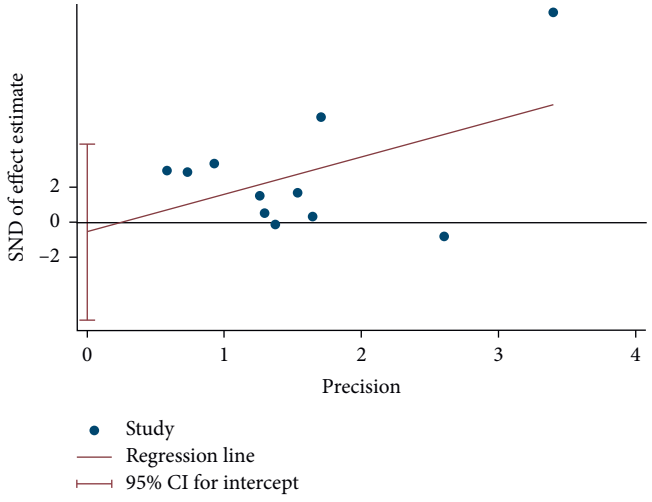

Figure 7: Egger's test used to evaluate publication bias.

measurement, and study design. Differences in the effect size among the included studies could have been affected by the coexistence of traditional CVD risk factors (e.g., hypertension, smoking habit, diabetes mellitus, obesity, and hyperlipidemia) and the concomitant treatments. Further investigation is needed to establish the applicability and safety of IPC in clinical populations. The consideration of the other factors related to changes in cardiovascular risk is also warranted.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xufang Gu and Zhichao Liu contributed equally to the work. Gu is the guarantor. Liu drafted the manuscript. Wang, Gao,

and Chen collected and assessed data. Wang and Gao collaborated with Liu and revised the study. Li and Gu developed search strategy. Two independent researchers (Liu and Chen) assessed the quality of all studies; if there is a difference, a third researcher (Li) was invited to solve. All authors contributed to the development of the selection criteria, the risk of a bias assessment strategy, and data extraction criteria. They also read, provided feedback, and approved the final draft of the manuscript.

Acknowledgments

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Supplementary Materials

Supplemental Table: PRISMA-P. (*Supplementary Materials*)

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Research Article

Design and Evaluation of a Prescription Drug Monitoring Program for Chinese Patent Medicine based on Knowledge Graph

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Background. Chinese patent medicines are increasingly used clinically, and the prescription drug monitoring program is an effective tool to promote drug safety and maintain health. *Methods*. We constructed a prescription drug monitoring program for Chinese patent medicines based on knowledge graphs. First, we extracted the key information of Chinese patent medicines, diseases, and symptoms from the domain-specific corpus by the information extraction. Second, based on the extracted entities and relationships, a knowledge graph was constructed to form a rule base for the monitoring of data. Then, the named entity recognition model extracted the key information from the electronic medical record to be monitored and matched the knowledge graph to realize the monitoring of the Chinese patent medicines in the prescription. *Results*. Named entity recognition based on the pretrained model achieved an F1 value of 83.3% on the Chinese patent medicines dataset. On the basis of entity recognition technology and knowledge graph, we implemented a prescription drug monitoring program for Chinese patent medicines. The accuracy rate of combined medication monitoring of three or more drugs of the program increased from 68% to 86.4%. The accuracy rate of drug control monitoring increased from 70% to 97%. The response time for conflicting prescriptions with two drugs was shortened from 1.3S to 0.8S. The response time for conflicting prescriptions with three or more drugs was shortened from 5.2S to 1.4S. *Conclusions*. The program constructed in this study can respond quickly and improve the efficiency of monitoring prescriptions. It is of great significance to ensure the safety of patients' medication.

1. Background

Drug safety is an important livelihood issue of concern to countries all over the world. According to the data of WHO [1], one in seven hospitalized patients worldwide each year is due to prescription safety issues. Hospitalizations for drug reactions account for nearly 30% of all hospitalizations in the United States, with approximately 6% of their deaths [2, 3]. At least one out of every eight hospitalized patients in the UK is caused by the wrong medication or problems with the medication itself [4, 5]. Approximately 5% of hospital admissions in developing countries are due to adverse drug reactions, and another 10–20% of hospitalized patients had adverse drug reactions [6, 7]. The phenomenon of irrational drug use in China also cannot be ignored. With the continuous improvement of the level of medicine, the variety and quantity of clinical drugs are increasing, and the probability of drug combination is rapidly increasing. Drug-induced diseases are serious due to the neglect of the rational use and interaction of drugs [8].

Chinese patent medicines (CPM) are widely used because of their stable properties, definite curative effects, relatively small side effects, and convenient administration. However, there are many problems in clinical prescribing [9, 10]. These problems include irregularities in prescribing behavior or repeated use of drugs when patients are treated

in different departments and hospitals. When prescribing CPM, doctors ignore the diagnosis and treatment of Chinese medicine. They do not consider the physical characteristics of special populations, including the elderly, children, pregnant, and lactating women, etc., or do not consider the damage to the patients' liver and kidney function caused by the amount of CPM. Doctors ignore the contraindications of CPM and the interaction between medicines.

Resolving the current irrational use of drugs through the development of a safe drug monitoring program has become an effective method for modern pharmaceutical information services [11–13]. This not only helps clinical professionals obtain pharmaceutical information but also simulates the prescription review process and automatically monitors the prescription. This can effectively prevent the occurrence of adverse drug events and promote safe medication use.

2. Related Work

Developed countries in Europe and the US first embed prescription drug monitoring programs into electronic prescription systems for real-time regulatory control. European countries have established the European Antimicrobial Resistance Surveillance System (EARSS) and the European Surveillance of Antimicrobial Consumption (ESAC). Boston Hospital introduced the experience and results of clinical pathways into the Prescription Automatic Screening System (PASS). First DataBank, the world's largest drug information database development center, provides comprehensive technical support and data sources for the PASS [14, 15]. The main prescription monitoring systems applied in China are Sichuan Meikang Pharmaceutical's PASS rational drug use monitoring system and Shanghai Datong Pharmaceutical Information Technology Co. This system monitors medication dosage, drug contraindications, interactions, and other factors that may cause physical harm to patients. Real-time monitoring and reminding are performed to avoid medical accidents [16–18].

TCM has been developed for thousands of years, and the knowledge of TCM is constantly emerging. However, it lacks a unified description and completeness of the knowledge system, which makes it difficult to use and share information. In particular, the adverse reactions of CPM have the characteristics of many kinds of drugs, a wide application range, complex components, inconsistent understanding, and nonstandardized naming, etc. [19, 20]. The current basic rule database used in the monitoring framework is mainly for western medicine [21, 22]. There is a lack of standardized data rule bases such as contraindications to the combined use of CPM and evidence-based treatment. Therefore, the establishment of a complex rule base for monitoring the CPM in prescription urgently needs the support of an information method system adapted to its characteristics.

The knowledge graph is a visual representation of the core structure, frontier fields, and overall knowledge structure and is a method system to achieve the goal of multidisciplinary integration [23–25]. It meets the requirements for a unified description of TCM knowledge and multiscale incomplete information integration and can provide technical support for the monitoring of the rational use of CPM. In recent years, scholars have made attempts and explorations in the construction methods and standardization processes of TCM knowledge graph. Yu and Liu [26] proposed the concept of constructing a large-scale knowledge graph based on the TCM Language System (TCMLS) as a framework, and the existing terminology and database resources in the field of TCM as the content and carried out exploration and practices. However, the effective integration of knowledge resources of traditional Chinese medicine has not been realized, and comprehensive, timely, and reliable knowledge services cannot be provided. Tong et al. [27] proposed a semiautomated construction process of knowledge graphs in the field of Chinese medicine knowledge question and answer and auxiliary prescription based on text extraction, relational data conversion, and data fusion technologies.

Zhang et al. [28] proposed an ontology-based representation of the core knowledge graph of Chinese medicine and its construction method. They explored the mapping method between the ontology of Chinese medicine and the knowledge graph and provided a more systematic method and process for the construction of the Chinese medicine knowledge graph. However, the research on the acquisition technology of multisource data and the actual clinical diagnosis and treatment data of traditional Chinese medicine doctors is not in-depth research. Wang et al. [29] took the visualization of chronic gastritis data of traditional Chinese medicine as an example and introduced the random forest technology to visualize the previsual data preprocessing. In general, the knowledge graph theory in TCM is still at the stage of the macro overview on the structure of each discipline. It is urgent to solve the strategy and technology of knowledge graph modeling for the deep integration of multilayer information.

In this paper, we formed a rule base for monitoring CPM by associating disease entities, disease entities, and CPM drug entities through knowledge graphs. The program extracted key information on symptoms, diseases, and medicines in prescriptions through named entity recognition technology and matched them with the existing knowledge base in the knowledge graph. This program can monitor five aspects of prescriptions involving the combined use of CPM, repeated use of medicines, medicines and diseases, the dosage of medicines, and evidence-based treatment.

3. Methods

The overall design of this paper is based on a knowledge graph-based rational drug use rule base library and builds a prescription drug monitoring program for CPM. The method is to extract information on CPM, diseases, and conditions through information extraction techniques [30, 31] from the national pharmacopoeia, authoritative data, and high-quality electronic medical record groups. According to the entities and relations of CPM, a knowledge graph was constructed to form a rule base for monitoring the use of CPM.

For the electronic medical records to be monitored, the information related to CPM in the medical records is identified through BERT [32] pretraining model word segmentation [33] and entity recognition. The obtained information is matched with the constructed knowledge graph to monitor the combined use of CPM, repeated use of medicines, medicine and disease, medicine dosage, and evidence-based treatment. The framework design of the prescription drug monitoring program for CPM is shown in Figure 1.

3.1. Knowledge Graph. In the knowledge graph, entities are used to represent nodes in the graph, and relations are used to represent edges. In the fields related to Chinese medicine, CPM, diseases, and symptoms can be used as entities, and the relationships between them can connect the corresponding entities to form a relational network library.

The construction and application of a certain scale of knowledge base or rule base require a variety of intelligent information processing technical support. After entity extraction, relationship extraction, knowledge representation, knowledge fusion, etc., the professional domain knowledge graph is formed. In this study, the knowledge base construction work focuses on knowledge extraction, using relational extraction techniques to extract key information of CPM, diseases, and symptoms from the corpus. Finally, it is corrected into a knowledge base and a rule base through manual assistance to serve the prescription drug monitoring program of CPM.

3.2. Preprocessing of Medical Records to Be Monitored Based on the BERT Model. This paper mainly analyzed a large number of medical records with CPM prescriptions from local medical institutions in the past five years. There was little medical information in the outpatient medical records and patient's treatment results. Therefore, we mainly monitored the course records and discharge summary in the hospitalized medical records. This was also the main data object for word segmentation of electronic medical records and extraction of medical entities in this paper. In addition, there is no uniform standard for the description of medical records, which is strongly personalized by physicians. Therefore, it is urgent to develop an effective and appropriate method for extracting words and entities.

The BERT model launched by Google in 2018 is a bidirectional encoder representation based on Transformer [32]. When the bidirectional representation model processes a word, it can simultaneously use both the information of the preceding word and the following word. This bidirectional encoding of information makes BERT more suitable than other language models for monitoring a large number of electronic medical records and complex semantics.

Based on the pretraining model, the uploaded electronic medical records are processed for language segmentation and entity extraction. First, based on the maximum probability path of word frequency, the professional dictionary (symptoms, diseases, CPM) was organized to improve the accuracy of word segmentation. Further data screening and entity extraction are required after the word segmentation. We extracted the entities in the electronic medical record and stored them in an array. The values of the array were passed to the constructed rule base module for a matching search of the rule base to achieve the effect of monitoring the CPM in the prescription.

4. Results

4.1. Knowledge Graph Construction

4.1.1. Knowledge Graph of the Rational Use of CPM. The core of rational use of CPM is "eighteen contraindications and nineteen fears." The Pharmacopoeia of the People's Republic of China clearly indicates that the varieties in the "Eighteen Antibodies" and "Nineteen Fears" are taboos. We have sorted out the contents of the eighteen contraindications and nineteenth fears in the book written by Pang Chunyan. Combining the pharmacopoeia data, it is compiled into a knowledge graph of the rational use of CPM. The drug relationship of some CPM is shown in Figure 2.

4.1.2. Knowledge Graph of Repeated Efficacy Medication. Repeated efficacy medication refers to the simultaneous use of different CPM with the same active ingredients and whether the combination of Chinese and Western medicines is reasonable.

The project designed in this paper mainly adopted the National Essential Medicine (Chinese Medicine) Clinical Guide, Zhang Hongchun's Chinese Medicine Clinical Application Guide-Respiratory Diseases Volume, and the data obtained by crawlers to sort out the results of the knowledge graph.

4.1.3. Knowledge Graph of Disease Symptoms. There are many kinds of Chinese patent medicines and diseases, which cause obstacles for doctors to use medicines. The symptoms corresponding to different diseases are different, and the relationship between the two is many-to-many. We transformed the original unstructured data into structured data and showed the connection between symptoms and diseases by means of knowledge graphs. This can more accurately determine the connection between diseases and symptoms. The knowledge graph shows a wide variety of symptoms, and one disease corresponds to dozens of symptoms. Figure 3 shows the relationship between stroke, symptoms, and contributing factors in the disease knowledge gap.

4.2. Electronic Medical Record Word Segmentation and Entity Recognition. There is more word segmentation in professional fields than in general fields. In order to label the CPM words more accurately, it was necessary to use a self-built dictionary for word segmentation. Then, when labeling the data of CPM, three entity categories were defined.

After defining the entity categories, the National Essential Drug Clinical Application Guide was selected as the original data source. Then, the raw data was turned into word annotated form according to BIO rules for model training. The entity label starts with B, ends with I, and

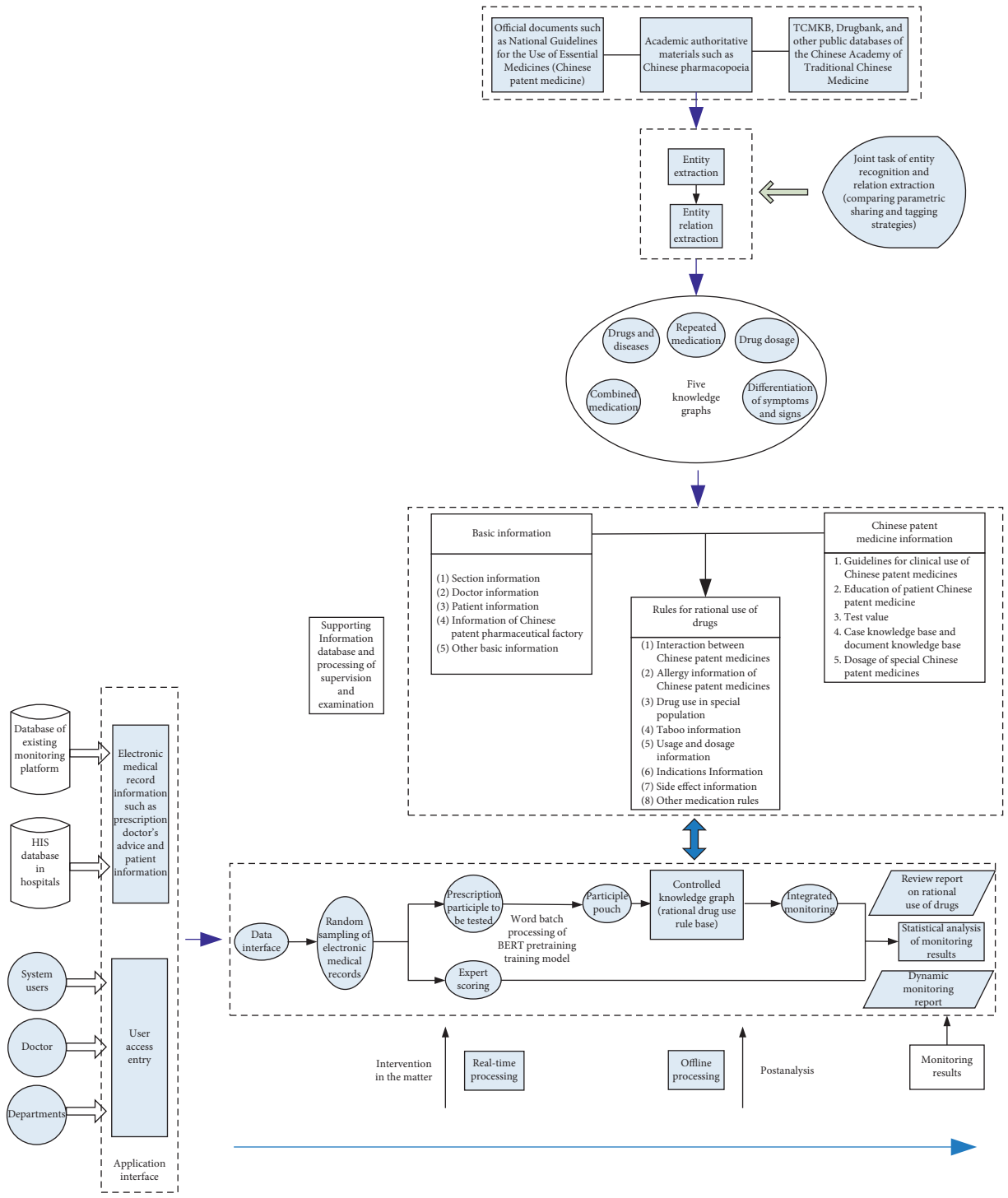


Figure 1: Design of prescription drug monitoring program for CPM.

irrelevant text is marked as O. According to the labeling strategy, there were more than 1.7 million characters in total.

We used the BERT pretraining model for named entity recognition training. The evaluation metrics of the model consisted of precision, recall, and F1. The results are shown in Table 1.

For the electronic medical records to be monitored, the model files obtained through training were used to predict the entities in the electronic medical records related to CPM, diseases, and symptoms. These entities were retrieved with the matching of the rule base to achieve the monitoring of CPM in prescriptions.

For the electronic medical records to be monitored, predictions will be made using the model files obtained from the training to obtain the entities in the electronic medical records related to CPM, diseases, and symptoms, which will

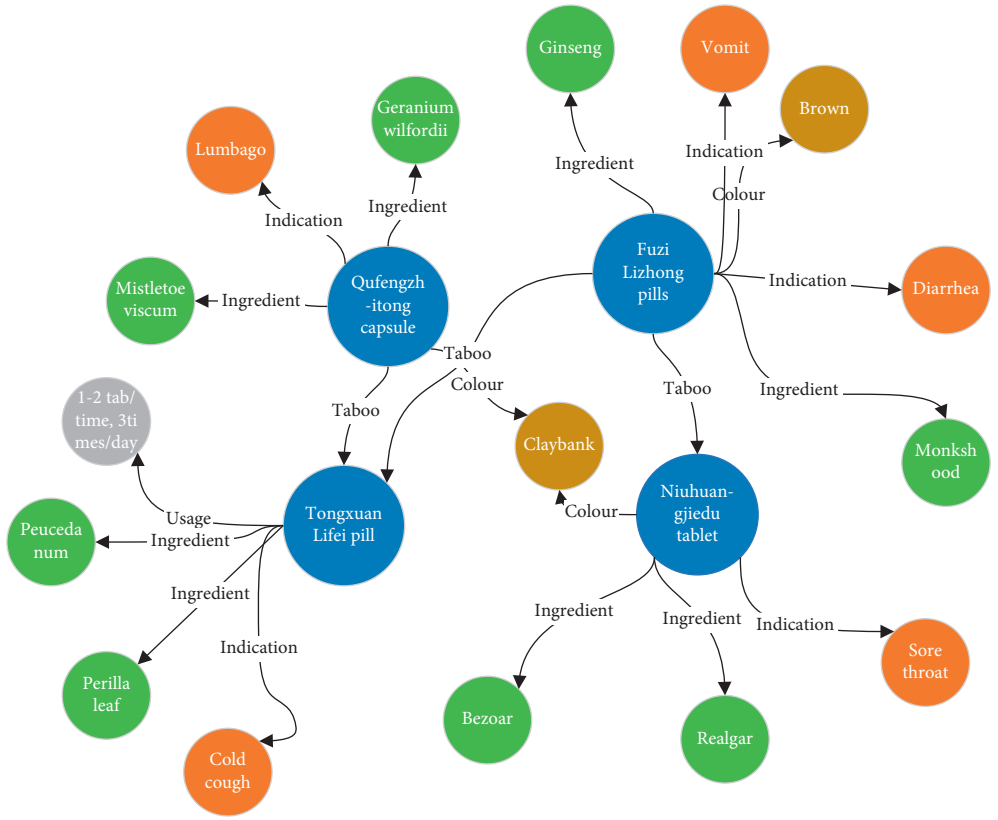


Figure 2: Knowledge graph of rational use of CPM.

then be retrieved with the matching of the rule base to achieve the monitoring of CPM in prescriptions.

4.3. Framework Applications. The program mainly includes functional modules, such as rational drug knowledge base, rational drug use review, drug dynamic monitoring, and expert prescription review.

The rational drug knowledge base is to retrieve the drug data in the database and display basic information on the interface. Rational drug dynamic monitoring is introduced to the prescription document to judge whether the prescription is reasonable through monitoring of all aspects. The results are reviewed and confirmed by experts. The program application is shown in Figure 4.

4.4. Program Evaluation. To verify the usefulness of the prescription drug monitoring program for CPM, a total of 3,000 electronic medical records involving CPM were monitored and analyzed in 150 sessions in respiratory medicine and pediatrics. Comparing with the indicators of the traditional monitoring program, the indicators of the electronic medical record in this paper were processing response time, offline monitoring of the number of one-time electronic medical records and the accuracy of combined medication monitoring, symptomatic drug administration monitoring, repeat efficacy medication monitoring, evidence-based treatment monitoring, and medication dosage monitoring of CPM. The results are shown in Table 2.

In Table 2, there is no big difference in the accuracy rate of the combined drug monitoring of the two drugs, but the accuracy rate of the combined drug monitoring of three or more drugs is significantly greater than that of the relational database, from 68% to 86.4%. For example, when multidrug combination monitoring is performed in a relational database, multiple tables are associated through fields, which involve the combination of ingredients, toxic ingredients, drug master signs and symptoms contraindications, drug chemical reactions, and other aspects of the synthesis, and then the final monitoring results are obtained. In the association process, the amount of data corresponding to each drug is inconsistent, leading to gaps in the final data missing, which affects the accuracy of monitoring. The graphical database integrates multiscale information together, which can well handle complex and diverse association analysis and meet the analysis and monitoring of the relationship between the combined use of CPM.

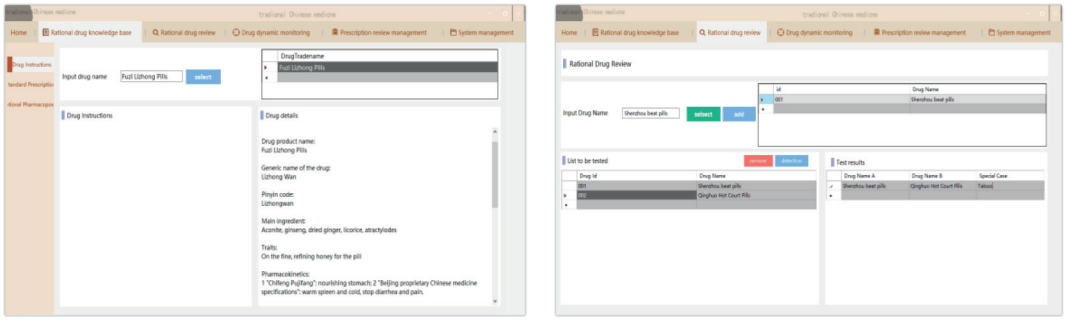
Dosage monitoring of CPM should be integrated with diseases and populations, etc. The interactive exploratory analysis based on the knowledge graph can simulate the human thinking process to discover and verify the dosage of CPM in prescriptions. Its accuracy rate has increased from 70% for the traditional program to 97% for the program in this study.

In addition, online prescribing of three or more drugs with conflicting response times of 1.4 seconds in this paper is quicker than that of 5.1 seconds in a relational database. Compared with the traditional storage method, the graphical

Figure 3: Knowledge graph of disease symptoms.

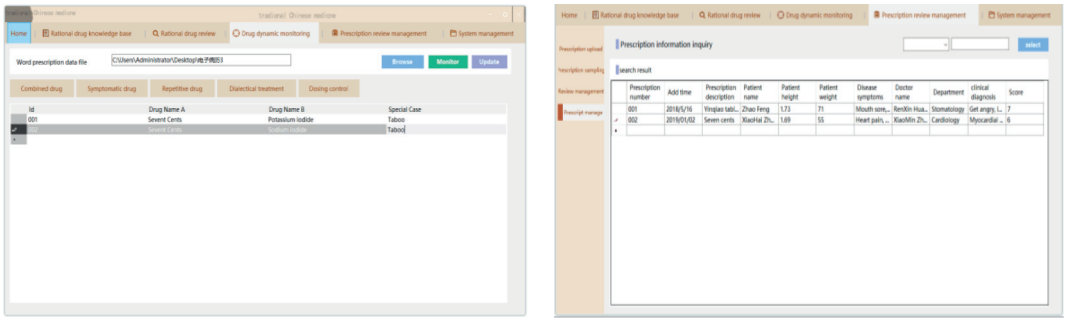
Table 1: Metrics of entity recognition.

	DIS (disease)	SYM (dymptom)	ZCY (CPM)	Average
Precision	78.98	82.82	86.57	82.79
Recall	82.26	81.56	87.04	83.62
*F*1	80.58	82.09	86.80	83.16



(a) (b)

Figure 4: Continued.



(c) (d)

Figure 4: Program applications.

Table 2: Operating effect comparison.

Contrast indicators	Knowledge graph monitoring program	Relational database monitoring program
Accuracy of monitoring combined use of two drugs	95.1%	91.3%
Accuracy of monitoring combined use of three or more drugs	86.4%	68%
Accuracy of monitoring symptomatic administration	92%	No such function
Accuracy of repetitive efficacy medication monitoring	100%	100%
Accuracy rate of monitoring syndrome differentiation and treatment	83.1%	80%
Accuracy of dosage monitoring	97%	70%
Simultaneous monitoring of the number of electronic medical records	200	1
Conflict response time of two drugs prescribed online	0.8 S	1.3 S
Conflict response time of three or more drugs prescribed online	1.4 S	5.1 S
Background support for the number of Chinese patent medicines	Up to 20,000 species	8,000 species

data storage is quicker in data retrieval and enables real-time decision making in the process of monitoring CPM in prescriptions. Moreover, the query of the relational database is complicated, slow, and beyond expectations, and whose support for joins between nodes is not very friendly. The graphical database represented by the knowledge graph can meet the design requirements based on the characteristics of CPM.

5. Conclusions

The prescription drug monitoring program for CPM is based on the basic features and requirements of CPM clinical safety with knowledge graph technology to standardize information on scientific, authoritative, and updated medical and pharmacological knowledge. According to the uploaded electronic medical records, a number of basic reviews of doctors' prescriptions are carried out to realize prescription monitoring and ensure safe medication. The program can effectively regulate the prescribing behavior of physicians and reduce the incidence of irrational use of CPM. This is of great significance to the rational use of drugs in clinical practice and the improvement of medication safety.

In the future work, we will further improve the rational drug use knowledge base and rule base in this paper, including full species drug interaction rule base, drug-food interaction rule base, population contraindication rule base, etc. In addition, an automatic retrieval method for unreasonable drug prescriptions, including prescription medical information, electronic medical records, medical subject thesaurus, and other information, will be established. It can realize active search to locate adverse drug reaction events, and unreasonable drugs use information.

Abbreviations

CPM: Chinese patent medicines
BERT: Bidirectional encoder representations from transformers
TCM: Traditional Chinese medicine.

Data Availability

Datasets generated during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Pharmacological Mechanisms Underlying the Hepatoprotective Effects of *Ecliptae herba* on Hepatocellular Carcinoma

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Background. The number of hepatocellular carcinoma (HCC) cases worldwide has increased significantly. As a traditional Chinese medicine (TCM) with a long history, *Ecliptae herba* (EH) has been widely used in HCC patients in China, but its hepatoprotective mechanism is still unclear. *Methods*. In this study, we applied a network pharmacology-based strategy and experimental verification to systematically unravel the underlying mechanisms of EH against HCC. First, six active ingredients of EH were screened from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) by the ADME method. Subsequently, 52 potential targets of 6 active ingredients acting on HCC were screened from various databases, including TCMSP, DGIdb, SwissTargetPrediction, CTD, and GeneCards. Then, by constructing protein-protein interaction (PPI) network from STRING, we displayed the intricate connections among these 52 targets through Cytoscape software. We also applied enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, to provide an outline and set of concepts for describing gene functions and the advanced functions of biological systems of these 52 targets from genomic and molecular level information. Finally, molecular docking and biological experiments were used to reconfirm these results. *Results*. We hypothesized that EH might exert anti-HCC activity by acting on hub genes, including RELA, MMP9, PTGS2, ESR1, EGFR, AR, AKT1, HIF1A, AHR, CYP3A4, ABCG2, and MMP2. Moreover, based on GO and KEGG analysis, we speculated that EH may exert hepatoprotective effects on HCC through the following mechanisms: regulation of the PI3K-AKT signaling pathway to promote apoptosis and inhibit the abnormal proliferation of HCC, downregulation of HIF-1A expression by activating the HIF-1 signaling pathway, prevention of HCC by regulating lipid metabolism, and inhibition of nonalcoholic fatty liver disease (NAFLD) by the cytochrome P450 subfamily. Subsequent biological experiments verified that EH inhibits the PI3K-AKT signaling pathway through its active ingredients, quercetin, and wedelolactone, thereby inhibiting the proliferation of HCC cells and promoting the apoptosis of HCC cells. *Conclusions*. The network pharmacological strategy provides an efficient method to systematically explore the pharmacological mechanism of EH in HCC. Our study demonstrated that the anti-HCC proliferation activity of EH is mainly exerted by two active ingredients (quercetin and wedelolactone), which inhibit the proliferation of HCC cells (HepG2 and Huh-7) by inhibiting PI3K-AKT signaling.

1. Background

Cancer statistics show that liver cancer is the fourth most common cause of cancer-related death globally, with the World Health Organization estimating that more than 1 million people will die from liver cancer by 2030 [1]. More than 80%–90% of cases of liver cancer are classified as hepatocellular carcinoma (HCC), which is the most common primary liver cancer [2]. Hepatocellular carcinoma typically develops on the background of chronic liver disease, mostly as a result of hepatitis B (HBV) or hepatitis C (HBC) virus infection, alcohol abuse, and nonalcoholic fatty liver disease [3]. HCC is not caused by a single gene, but by an accumulation of multiple genetic defects and mutations.

The pathogenesis of HCC is complex and includes multiple biological mechanisms such as epithelial-mesenchymal transition (EMT), the tumor microenvironment, tumor-stromal interactions, immune mechanisms, and well-known signaling pathways [4]. There are several treatments for HCC, including curative treatment options for early-stage HCC (surgical options, ablative electrochemical therapies, chemoembolization, radioembolization, and liver transplantation) and systemic therapy (targeted therapy and immunotherapy) for those patients who have failed locoregional therapy [1, 5–7]. However, the main drawbacks of curative therapies are recurrence of HCC and lower survival benefits than systemic chemotherapies. Although these therapeutics have greatly prolonged the survival of some patients with HCC, more effective medications and therapies are urgently needed.

Traditional Chinese medicine, which originated in China, has been practiced by Chinese people for thousands of years because of its satisfactory therapeutic effect and few side effects [8–10]. It has played an important role in the health care of Asians. *Ecliptae herba* (EH), known as "Mo-Han-Lian" in China, has been used as a "liver-nourishing" treatment in traditional Chinese medicine (TCM) for several thousand years [11]. EH has a broad range of pharmacological properties including antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, hepatoprotective, and hypolipidemic effects [11–17]. Ethanolic extract of EH has been shown to have hepatoprotective effects on damaged livers in rats and mice [18, 19]. Its extracts include a variety of natural products, such as triterpenoid saponins, coumarins, and flavonoids [11]. Although several *in vitro* and *in vivo* experiments have shown that EH has hepatoprotective and antihepatotoxic effects in recent years, the detailed therapeutic targets and molecular mechanisms of EH against HCC have not been systematically elucidated.

Network pharmacology is an innovative method to investigate the interrelationship between drugs and diseases by integrating multiple sources of information which is an efficient method for understanding the effects of EH in HCC treatment. The strategy is based on the concept of "Medicine-Target-Gene-Disease," which can illuminate intricate drug-disease interactions, and is considered to be very suitable for studying the molecular mechanisms of drugs and drug discovery [20–22].

In this study, we performed an integrated strategy, including network pharmacology-based analysis, molecular docking, and biological experimental verification, to identify the potential therapeutic targets and the associated molecular mechanisms of EH against HCC.

2. Methods

2.1. Data Preparation

2.1.1. Screening the Active Ingredients of EH. We collected the ingredients of EH from the Traditional Chinese Medicine System Pharmacology Database (TCMSP, https://tcmspw.com/tcmsp.php). In this database, the Chinese characters of "Mo-Han-Lian" were input to identify related ingredients and their pharmacokinetic property data.

2.1.2. Screening the Targets of EH Active Ingredients, HCC Disease Targets, and EH Treatment Targets in HCC. We collected relevant targets of EH active ingredients by screening different databases. These databases include the TCMSP, the Drug Gene Interaction Database (DGIdb, https://dgidb.genome.wustl.edu/), and the SwissTargetPrediction web server (http://www.swisstargetprediction.ch/).

Related targets of HCC were collected through the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/) and the GeneCards web server (https://www.genecards.org/). We searched for the keyword "hepatocellular carcinoma" on these web servers, and the species was restricted to "Homo sapiens." Then, we used an open web tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) to filter the common targets of different databases to improve the reliability of the data obtained. We used this web tool to match the predicted target of EH active ingredients with the relevant target of HCC to obtain the overlapping targets. These overlapping targets are the targets that EH may play a role in the treatment of HCC.

2.2. Network Construction and Topology Analysis. In this process, a compound-target-disease network (C-T-D network), EH target-HCC target network (E-H network), compound-target-pathway network (C-T-P network), and hub gene network were constructed to reveal the pharmacological mechanism and therapeutic target of EH in the treatment of HCC. The graphical interactions in the C-T-D network were visualized by Cytoscape software (version 3.7.2). The E-H network was obtained by uploading the overlapping targets to the STRING (https://string-db.org/) web server. On this database, the species option was "Homo sapiens," protein interactions had a confidence score >0.4, and the disconnected nodes were hidden in this network. The resulting data were imported into the visualization software Cytoscape to establish protein-protein interaction networks of EH against HCC. Based on this E-H network, the hub gene network was obtained through the CytoHubba plugin in Cytoscape software. In addition, we constructed the C-T-P network from the results of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. For each network, we selected five parameters to evaluate its topological characteristics, which were calculated by the Cytoscape plugin, Network Analyzer. These topological parameters were "Degree," "Betweenness Centrality," "Closeness Centrality," "Clustering Coefficient," and "Topological Coefficient."

2.3. Enrichment Analysis. In this study, we performed Gene Ontology (GO) analysis of the overlapping targets through the WEB-based Gene SeT AnaLysis Toolkit (WebGestalt, http://www.webgestalt.org/). The organism of interest was "Homo sapiens," the method of interest was "Over-Representation Analysis," and the functional database was "Gene

Ontology." Then, the overlapping gene list was uploaded to the database to obtain GO enrichment analysis results, which included three parts, biological process (BP), cellular component (CC), and molecular function (MF). Then, we constructed a network of enriched GO terms through WebGestalt analysis of the overlapping genes and TCGA RNASeq liver hepatocellular carcinoma (LIHC) database, with the option of method of interest selected as "Network Topology-based Analysis," and the option of functional database selected as "TCGA RNASeq LIHC."

In addition, we calculated and evaluated significant pathways using KEGG (http://www.kegg.jp/) data obtained from WebGestalt, with the option of method of interest being "Over-Representation Analysis," and the functional database being "pathway."

2.4. Molecular Docking. In this study, we performed molecular docking to further reveal the binding pattern of EH and hub genes. The X-ray crystal structures of hub genes were obtained from the Protein Data Bank (PDB, https://www.rcsb.org/). AutoDock Vina and PyMOL 1.8 software were utilized to perform docking studies for EH and selected genes. First, the 2D chemical structures of the active ingredients of EH were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) with the format for SDF and converted to a .pdb format by PyMOL 1.8. For protein preparation, the protein was input into the PyMOL 1.8 to remove the water molecules and heteroatoms and saved in a .pdb format. Second, the .pdb format of the protein and active ingredients was converted to the .pdbqt format via AutoDockTools (version 1.5.6) and used to perform a series of operations, including adding hydrogen atoms and calculating and adding Gasteiger charges. Third, we defined the specific pocket for active ingredients of EH binding with the hub genes using the grid box function of AutoDockTools. We used the command prompt for molecule docking analysis and the output results were displayed by PyMOL.

2.5. Cell Culture and Reagents. Hepatocellular carcinoma cell lines (HepG2 and Huh-7) were obtained from South Medical University Affiliated Maternal & Child Health Hospital of Foshan (Foshan, China). HepG2 cells were cultured with DMEM (Gibco, NY, USA) containing 1% penicillin-streptomycin (Sigma, MO, USA) and 10% fetal bovine serum (FBS; Gibco, NY, USA) under 5% CO_2 at 37°C. Huh-7 cells were cultured with RPMI 1640 (Gibco, NY, USA) medium containing 1% penicillin-streptomycin (Sigma, MO, USA) and 10% fetal bovine serum (FBS; Gibco, NY, USA) under 5% CO_2 at 37°C.

Wedelolactone was purchased from MedChemExpress (Monmouth Junction, USA), and quercetin was purchased from Shanghai Fushen Biotechnology Co., Ltd. (Shanghai, China). Raw EH was purchased from Foshan Zhongtian Chinese Medicine Pieces Co., Ltd. (Foshan, China). The raw EH was authenticated by Deng Dongmei from the Department of TCM of the South Medical University Affiliated Maternal and Child Health Hospital of Foshan. The extraction step of EH was carried out using a previously reported protocol [23]. The final extract of EH was redissolved at a concentration of 0.15 g/mL, filtered through a 0.22μ membrane, and then stored at −20°C.

2.6. Cell Viability Assay. HepG2 or Huh-7 cells (3,500) were transplanted into each well of a 96-well plate and treated with different concentrations of EH, wedelolactone, and quercetin for 48 hours. Then, the proliferation of HCC cells was measured by CCK-8 assays (Beyotime, Shanghai, China). The cell viability of the sample was calculated by the OD value.

2.7. Flow Cytometry Analysis for Detection of Cell Apoptosis. HepG2 or Huh-7 cells were treated with 150 μg/L EH, 8 μg/mL wedelolactone, and 8 μg/mL quercetin for 48 hours, according to the Annexin FITC/propidium iodide (PI) cell apoptosis kit (KeyGEN BioTECH, Nanjing, China) instructions for the experimental procedures. FACSCanto II (Becton, Dickinson and Company, New Jersey, USA) was used for the analysis, and the results were analyzed by FlowJo V10 software.

2.8. Western Blot Analysis. After treatment of HepG2 or Huh-7, cells were treated with 150 μg/mL EH, 8 μg/mL wedelolactone, and 8 μg/mL quercetin for 48 hours, western blotting was performed using previously reported protocol standards [24]. The antibodies used in this study included the following: PI3K, Akt, p-Akt (Ser473), and beta-actin, and horseradish peroxidase-conjugated secondary antibodies were purchased from CST (Cell Signaling Technology, Danvers, MA, USA). The p-PI3K p85 (Tyr467)/p55 (Tyr199) antibody was purchased from Affinity Biosciences Ltd. (Jiangsu, China). Band intensity was analyzed by ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.9. Statistical Analysis. Each experiment was repeated three times independently. Data are presented as the mean ± standard error of the mean (SEM) and analyzed using GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) was used, and the difference was considered significant when P value < 0.05.

3. Results

3.1. Active Ingredients of Ecliptae herba. A total of 48 chemical ingredients of EH were obtained from TCMSP (Supplementary File S1). ADME-related properties are an important component of pharmaceutical R&D, including Lipinski's "Rule of Five," which is often referred to as a guideline for drug lead optimization [25–27]. These properties are oral bioavailability (OB), drug-likeness (DL), half-life (HL), blood-brain barrier (BBB) permeability, Caco-2 permeability, molecular weight (MW), logarithm of the 1-octanol/water partition coefficient (Log P), hydrogen-bond donors (Hdon), and hydrogen-bond acceptors (Hacc).

According to the ADME thresholds of DL > 0.18, OB > 30%, Caco-2 > 0, BBB permeability < −0.3, MW < 500 Da, Log P < 3, Hdon < 5, and Hacc < 10, 6 active ingredients were selected out [28, 29]. These active ingredients were luteolin, quercetin, wedelolactone, 3′-O-methylorobol, demethylwedelolactone, and butin. Their pharmacokinetic parameters are shown in Table 1 and their chemical structures are shown in Figure 1.

3.2. Target Screening and Analysis. In this study, we identified 426 related targets of 6 active ingredients of EH from 3 different databases, TCMSP, DGIdb, and SwissTargetPrediction (Figure 2(a), and Supplementary File S2). Luteolin had 50 targets from TCMSP, 32 targets from DGIdb, and 100 targets from SwissTargetPrediction. Quercetin had 135 targets from TCMSP, 105 targets from DGIdb, and 100 targets from SwissTargetPrediction. Demethylwedelolactone had 3 targets from TCMSP, 5 targets from DGIdb, and 100 targets from SwissTargetPrediction. Wedelolactone had 6 targets from TCMSP, 11 targets from DGIdb, and 100 targets from SwissTargetPrediction, and 3′-O-methylorobol had 13 targets from TCMSP, 0 targets from DGIdb, and 100 targets from SwissTargetPrediction. Butin obtained 3 targets from TCMSP, 0 targets from DGIdb, and 100 targets from SwissTargetPrediction. To improve the credibility of the potential targets of these 6 active ingredients of EH, we selected the overlapping targets in these databases as the objects of the next study. A total of 56 overlapping targets were collected, including 14 overlapping targets of luteolin, 44 overlapping targets of quercetin, 3 overlapping targets of demethylwedelolactone, 4 overlapping targets of wedelolactone, 5 overlapping targets of 3′-O-methylorobol, and 2 overlapping targets of butin.

For the pathogenetic targets of HCC, 33530 and 7392 were obtained from CTD and GeneCards, respectively, with 6935 overlapping targets screened from these two databases (Figure 2(b), and Supplementary File S3). By calculating the overlapping targets of 56 targets of 6 active ingredients and 6935 overlapping HCC targets, we obtained 52 therapeutic targets for EH in the treatment of HCC, and the detailed information is shown in Figure 2(c) and Table 2.

3.3. Network Construction and Topology Analysis. To clarify the potential mechanism of EH on HCC, we constructed the C-T-D network, E-H network, and hub gene network based on these 52 therapeutic targets of EH in the treatment of HCC. The C-T-D network was composed of 59 nodes and 118 edges, and quercetin had more edges, which suggests that it plays a more complex biological role in the anti-HCC function of EH (Figure 3(a)).

The PPI network can expand the understanding of protein function, so we generated an E-H network to systematically study the biological function and mechanism of these 52 potential therapeutic targets of EH against HCC (Supplementary File S4). There were a total of 52 nodes and 292 edges in this E-H network, and as shown in Figure 3(b), AKT1, EGFR, and ESR1 had more edges among these genes. To further reveal the interaction of this E-H network, we adopted the plugin of Cytoscape, Network Analyzer, to calculate the topological parameters of these hub genes. The main topological parameters used in this study were "Degree," "Betweenness Centrality," "Closeness Centrality," "Clustering Coefficient," and "Topological Coefficient." As shown in Table 3, AKT1 (degree = 35), EGFR (degree = 29), ESR1 (degree = 27), PTGS2 (degree = 25), AR (degree = 22), MMP9 (degree = 19), RELA (degree = 19), ABCG2 (degree = 16), AHR (degree = 16), and CYP3A4 (degree = 16) have high degrees, indicating that they play a core role in the E-H network (Supplementary File S5). Moreover, the genes with higher values of "Betweenness Centrality," "Closeness Centrality," "Clustering Coefficient," and "Topological Coefficient" were identified as essential proteins with significant centrality values based on the network topology analysis of 52 genes.

Furthermore, the top 10 hub genes in the E-H network were calculated using different methods through the plugin CytoHubba, and the networks were displayed by Cytoscape. These calculation methods include the degree method, maximum neighborhood component (MNC), and maximal clique centrality (MCC). As shown in Figure 3(c), the top 10 hub genes of the MCC calculation were RELA, MMP9, PTGS2, ESR1, HIF1A, EGFR, AHR, AKT1, AR, and MMP2. For the MNC method, RELA, MMP9, PTGS2, ESR1, HIF1A, EGFR, AKT1, ABCG2, AR, and CYP3A4 were obtained as the top 10 hub genes in this network (Figure 3(d)). For the degree method, RELA, MMP9, PTGS2, ESR1, EGFR, AHR, AKT1, ABCG2, CYP3A4, and AR were collected (Figure 3(e)). These results suggest that these genes, such as RELA, MMP9, PTGS2, ESR1, EGFR, AR, AKT1, HIF1A, AHR, CYP3A4, ABCG2, and MMP2, play a very important role in this E-H network.

3.4. GO and KEGG Pathway Enrichment Analysis. After uploading 52 therapeutic targets of EH in the treatment of HCC in WebGestalt, we obtained the results of the top 10 GO enrichment analyses, which were divided into biological processes, molecular functions, and cellular components (Figures 4(a)–4(c), and Supplementary File S6). For this study, only processes or pathways with a P value < 0.05 were considered significant pathways. The top 10 biological processes were mainly involved in proliferation, cell death, cell communication, and cell apoptosis. The biological processes included the apoptotic process (GO:0006915), regulation of the apoptotic process (GO:0042981), regulation of cell death (GO:0010941), regulation of programmed cell death (GO:0043067), positive regulation of cell communication (GO:0010647), and cell proliferation (GO:0008283). These results suggest that EH may induce anti-HCC activity by regulating cell proliferation, death, and apoptosis. In molecular function analysis, EH exerted anti-HCC activity mainly through the following processes: transition metal ion binding (GO:0046914), identical protein-binding (GO:0042802), protein dimerization activity (GO:0046983), oxidoreductase activity (GO:0016491), heme binding (GO:0020037), and transcription factor binding

Table 1: Pharmacokinetic parameters of 6 active ingredients of *Ecliptae herba*.

Molecule name	MW	ALog P	Hdon	Hacc	OB (%)	Caco-2	BBB	DL	HL
Luteolin	286.25	2.07	4	6	36.16	0.19	−0.84	0.25	15.94
Quercetin	302.25	1.50	5	7	46.43	0.05	−0.77	0.28	14.40
Wedelolactone	314.26	2.73	3	7	49.60	0.32	−0.45	0.48	9.61
3′-O-Methylorobol	300.28	2.05	3	6	57.41	0.45	−0.38	0.27	17.31
Butin	272.27	2.30	3	5	69.94	0.30	−0.40	0.21	16.80
Demethylwedelolactone	302.25	1.10	4	7	72.13	0.04	−0.69	0.43	9.17

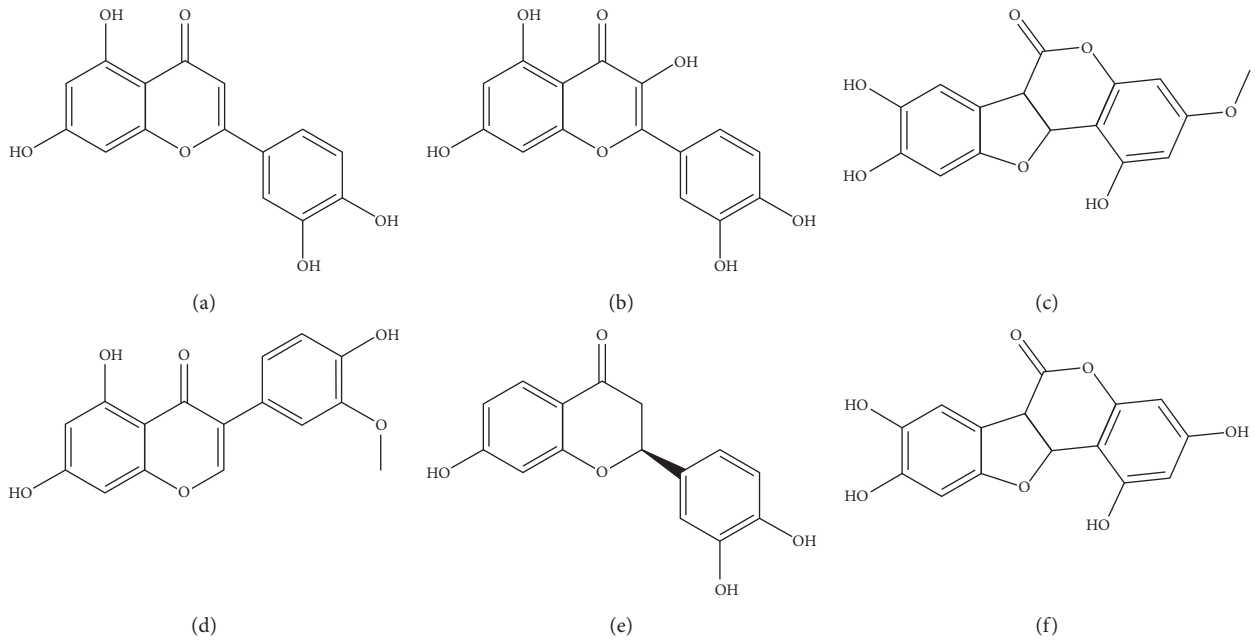


Figure 1: The chemical structures of 6 active ingredients of *Ecliptae herba*. (a) Luteolin. (b) Quercetin. (c) Wedelolactone. (d) 3′-O-Methylorobol. (e) Butin. (f) Demethylwedelolactone.

(GO:0004879). In addition, the cellular components of the 52 targets of EH in the treatment of HCC were mainly the nuclear chromosome (GO:0000228), transcription factor complex (GO:0005667), nuclear chromosome part (GO:0044454), chromosome (GO:0005694), and chromosomal part (GO:0044427).

To further reveal the mechanism of action of EH in the treatment of HCC, we built a GO network in the TCGA RNASeq LIHC database through the Network Topology-based Analysis (NTA) method on the WebGestalt web server (Supplementary File S7). As shown in Figure 5(a), the top 10 enriched GO terms were mainly involved in metabolic process, biosynthetic process, and catabolic process. For metabolic process, the GO network mainly included long-chain fatty acid metabolic process (GO:0001676), lipid metabolic process (GO:0006629), cellular lipid metabolic process (GO:0044255), hormone metabolic process (GO:0042445), eicosanoid metabolic process (GO:0006690), and cellular hormone metabolic process (GO:0034754). Moreover, through TCGA RNASeq LIHC database analysis, we obtained key genes from the subnetwork showing the anti-HCC activity of EH, such as CYP1A2, CYP3A4, ABCG2, CYP19A1, ALOX5, PTGS1, PRKCA, CYP1A1, ALOX15, HSPB1, MET, ACHE, and PIM1 (Figure 5(b)).

Then, we performed KEGG pathway enrichment analysis to further determine the functions and signaling pathways involved in the 52 therapeutic targets of EH against HCC. As shown in Figure 6(a), the molecular signaling pathway of EH against HCC was related to various pathways, including "pathways in cancer," "non-small cell lung cancer," "prostate cancer," "endocrine resistance," "EGFR tyrosine kinase inhibitor resistance," "AGE-RAGE signaling pathway in diabetic complications," "HIF-1 signaling pathway," "glioma," "proteoglycans in cancer," "ovarian steroidogenesis," "thyroid hormone signaling pathway," "hepatocellular carcinoma," "human cytomegalovirus infection," "VEGF signaling pathway," "small cell lung cancer," "Kaposi sarcoma-associated herpesvirus infection," "hepatitis B," "breast cancer," "gastric cancer," and "prolactin signaling pathway." The details of the KEGG pathway enrichment information are shown in Supplementary File S8.

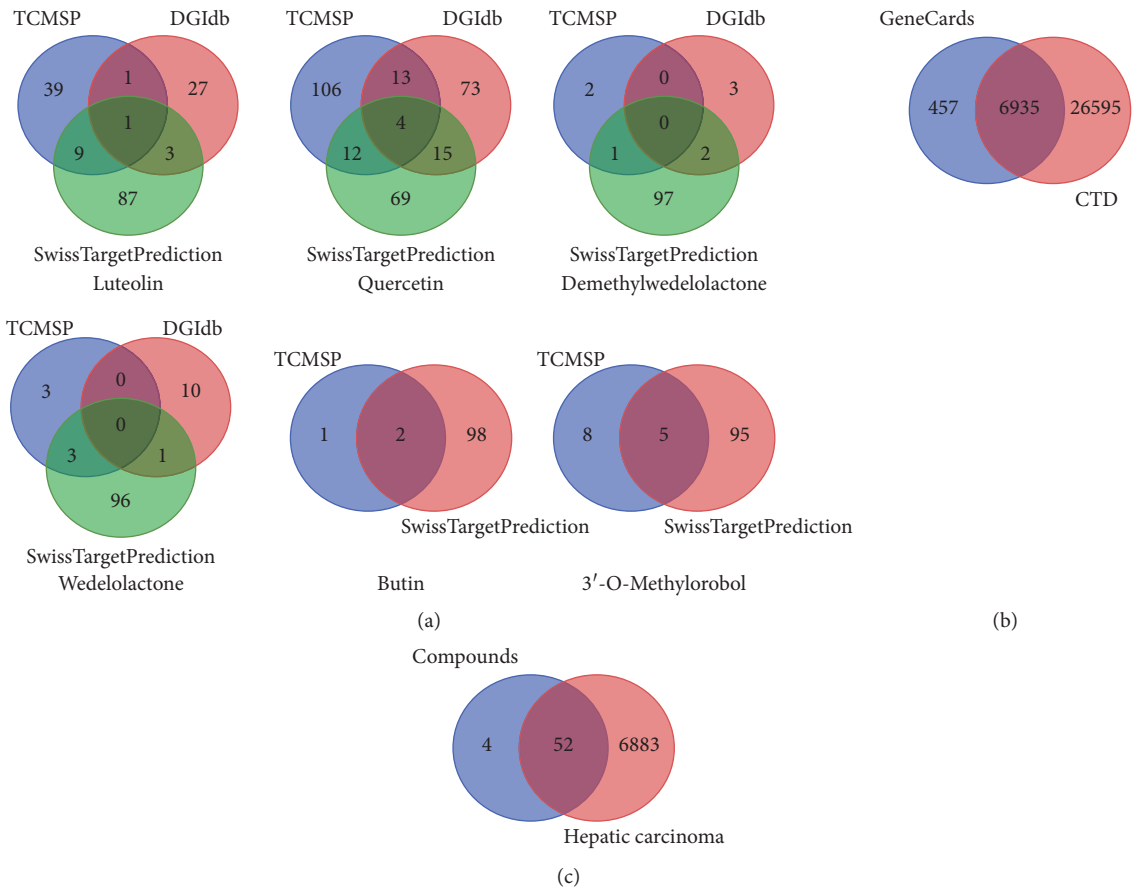


Figure 2: Screening the potential targets of EH in the treatment of HCC. (a) Venn diagram of targets of 6 active ingredients of EH collected from different databases. (b) Venn diagram of targets of HCC collected from GeneCards and CTD databases. (c) Venn diagram of targets of EH against HCC.

3.5. Compound-Target-Pathway (C-T-P) Network Analysis. In this study, we tried to further analyze the interactions among 6 active components, 52 targets, and the top 20 KEGG enrichment pathways in EH treatment of HCC, and the compound-target-pathway network could be used to analyze the relationships among these factors. As shown in Figure 6(d), the C-T-P network was comprised of 77 nodes (20 pathways, 6 compounds, and 51 targets) and 384 edges, indicating that EH plays an anti-HCC role through a complex mechanism. There were 51 targets involved in the top 20 KEGG pathways associated with HCC, and each pathway contained more than 6 targets. Among these 20 pathways, the pathways in cancer were enriched by 19 proteins, with the highest degree of enrichment. For the targets, AKT1 was enriched by 19 pathways and 2 compounds, and the enrichment was the highest among the targets. In addition, quercetin was enriched by 40 targets in this network, and these targets were widely enriched in the top 20 KEGG pathways. We speculated that EH was effective for the treatment of HCC. Detailed information is provided in Supplementary File S9.

3.6. Molecular Docking Verification. In this study, a molecular docking strategy was used to reveal the binding pattern between the active ingredients of EH and the hub genes. According to the prediction results of molecular docking, quercetin, luteolin, wedelolactone, demethylwedelolactone, and 3′-O-methylorobol bind these hub targets well with low binding energy (Table 4). This result may be because these active ingredients have multiple hydroxyl groups, making them good hydrogen-bond donors or acceptors.

As shown in Figure 7, docking results predicted that active ingredients of EH can form stable noncovalent interactions with the top 10 hub genes. In the protein-binding pocket of MMP9, luteolin can form 5 H-bond interactions (VAL-223, ALA-189, MET-247), quercetin forms 5 H-bond interactions (LEU-243, GLN-227, ALA-189), and wedelolactone forms 2 H-bond interactions (GLN-227). In the ATP-binding pocket of EGFR, luteolin can form 2 H-bond interactions (LYS-745, MET-793), and quercetin can form 3 H-bond interactions (LEU-788, LYS-745, MET-793). The predicted binding patterns of luteolin and quercetin with RELA showed that they formed 2 H-bonds (TYR-285, HIS-252) and 5 H-bonds (HIS-252, TYR-285, TYR-223, TYR-297), respectively. Similarly, luteolin and quercetin could form 4 H-bonds (ASP-292, LYS-179, ALA-230) and 4 H-bonds (ASP-292, LYS-179, ALA-230) with the binding pocket of AKT1, respectively. In addition,

Table 2: Detailed information on 52 targets of 6 active ingredients of EH in the treatment of HCC.

Name	Count	Gene symbol	Total
Luteolin, quercetin, wedelolactone, and HC	2	MMP2\MMP9	52
Quercetin, demethylwedelolactone, 3′-O-methylorobol, and HC	1	ESR2	
Luteolin, quercetin, and HC	6	EGFR\ALOX15\RELA\AKT1\GPR35\TOP1	
Demethylwedelolactone, wedelolactone, and HC	1	GSK3B	
Demethylwedelolactone, 3′-O-methylorobol, and HC	1	ESR1	
Butin, 3′-O-methylorobol, and HC	1	PTGS1	
Luteolin and HC	5	MET\TYR\AR\APP\PTGS2	
Quercetin and HC	31	XDH\PRKCB\CYP1A1\AHR\CYP19A1\MMP3\PIK3R1\CSNK2A1\PON1\MPO\ACHE\ABCG2 CYP1A2\PIK3CG\TOP2A\HIF1A\INSR\NR1I2\YP3A4\HSPB1\F2\APEX1\AKR1B1\CYP1B1\ ALOX5\E2F1\PIM1\PARP1\HSF1\BAX\PRKCA	
Demethylwedelolactone and HC	1	CBR1	
Butin and HC	1	RXRA	
3′-O-Methylorobol and HC	2	CHEK1\PRSS1	

demethylwedelolactone and 3′-O-methylorobol could bind with ESR1 to form 3 H-bonds (HIS-476, ASN-455, LEU-508) and 1 H-bonds (LEU-479), respectively. Luteolin can bind to AR (GLN-711, HIS-808) and PTGS2 (HIS-39, GLU-465) at very low binding energies, forming two H bonds. Quercetin can bind to the protein pockets of AHR (GLN-150, PHE-136), ABCG2 (THR-435), and CYP3A4 (ARG-372, ARG-106) to form 2, 1, and 3 H-bonds with very low binding energies, respectively. Analysis of the interaction mode between protein and ligand indicates that active ingredients may bind to these hub targets well and have lower binding energy primarily by forming multiple hydrogen bonds. Furthermore, the model of molecular docking provides evidence for how EH acts on these targets to inhibit HCC.

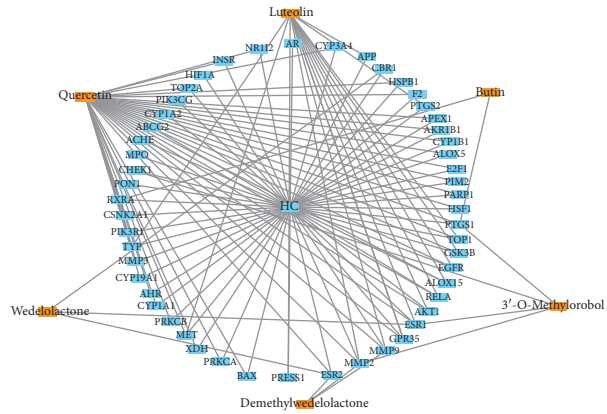
3.7. Ecliptae herba, Wedelolactone, and Quercetin Inhibit the Growth of HepG2 and Huh-7 Cells. Although network pharmacology results indicate that EH could inhibit HCC through multiple active ingredients and pathway mechanisms, biological experiments are still needed to further verify the current results. The foregoing C-T-D network results indicate that quercetin plays a complex biological role in the anti-HCC function of EH. In addition, the aforementioned study showed that wedelolactone may be an important compound for EH to exert biological activity. Therefore, we used these two active ingredients for subsequent biological research. First, the results of the CCK-8 assay showed that EH, wedelolactone, and quercetin could effectively inhibit the proliferation of the HCC cell lines HepG2 and Huh-7 (Figures 8(a)–8(c)). Treatment with the same concentrations of EH, wedelolactone, and quercetin more effectively inhibited the proliferation of Huh-7 cells than HepG2 cells.

Next, to investigate whether EH, wedelolactone, and quercetin decrease the viability of HCC cell lines by inducing apoptosis, we used Annexin V and PI staining to calculate apoptosis by flow cytometry. As shown in Figures 8(d) and 8(e), treatment of HepG2 and Huh-7 cells with 150 μg/mL EH, 8 μg/mL wedelolactone, and 8 μg/mL quercetin for 48 hours significantly promoted HCC cell apoptosis.

3.8. Wedelolactone and Quercetin Inhibit the PI3K/Akt Signaling Pathway in HepG2 and Huh-7 Cells. The results of the aforementioned KEGG enrichment analysis showed that 19 of the top 20 KEGG pathways were involved in PI3K-AKT signal transduction. Combined with the GO results, these findings indicated that EH can inhibit the abnormal proliferation of HCC and promote cell apoptosis by acting on the PI3K-AKT signaling pathway. Further western blot assays confirmed that 8 μg/mL wedelolactone and 8 μg/mL quercetin significantly inhibited the expression of p-PI3K p85 (Tyr467), p-PI3K p55 (Tyr199), and p-Akt (Ser473) in HepG2 (Figure 9(a)) and Huh-7 cells (Figure 9(b)).

4. Discussion

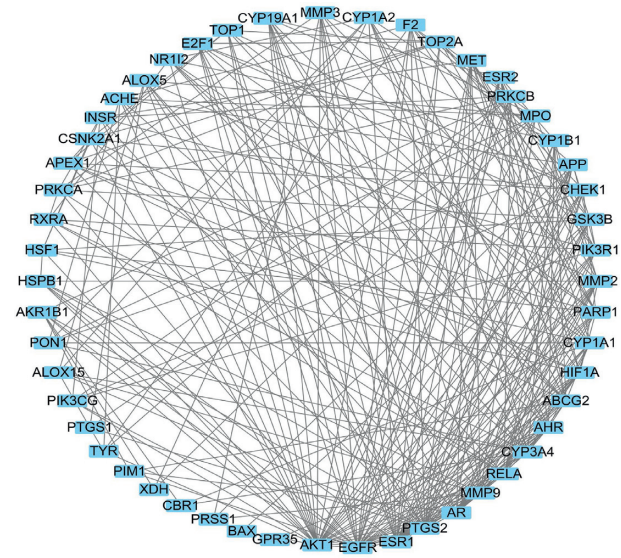
As an empirical herbal medicine widely used in China, *Ecliptae herba* has been proven to have hepatoprotective effects on damaged livers in rats and mice, but the mechanism of action and potential therapeutic targets of EH in the treatment of HCC have not been thoroughly studied [11]. Additionally, the pathological mechanism of HCC is very complicated and involves multiple targets and signaling pathways during progression. Therefore, we attempted to explore the potential mechanism of EH in the treatment of HCC through a network pharmacology-based strategy. In this study, we screened 6 active ingredients of EH: luteolin, quercetin, wedelolactone, 3′-O-methylorobol, demethylwedelolactone, and butin. These 6 active ingredients inhibit HCC by acting on 52 targets. Among these targets, some targets with high topological parameters were defined as hub genes, including RELA, MMP9, PTGS2, ESR1, EGFR, AR, AKT1, HIF1A, AHR, CYP3A4, ABCG2, and MMP2, obtained by the EH-HCC network. Additionally, by analyzing the C-T-D network, we found that quercetin plays a more complex biological function in the anti-HCC function of EH among the 6 active ingredients. Moreover, as the major component of EH, wedelolactone has been reported to have hepatoprotective effects [11]. In addition, through UPLC analysis, the content of wedelolactone in EH was shown to be 25.65% [23]. These results indicate that EH mainly exerts anti-HCC effects through quercetin and wedelolactone.



Clustering coefficient: 0.0
Connected components: 1
Network diameter: 4
Network radius: 2
Network centralization: 0.857
Shortest paths: 3422 (100%)
Characteristic path length: 2.083
Avg. number of neighbors: 4.0

Number of nodes: 59
Network density: 0.069
Network heterogeneity: 2.022
Isolated nodes: 0
Number of self-loops: 0
Multiedge node pairs: 0
Analysis time (sec): 0.031

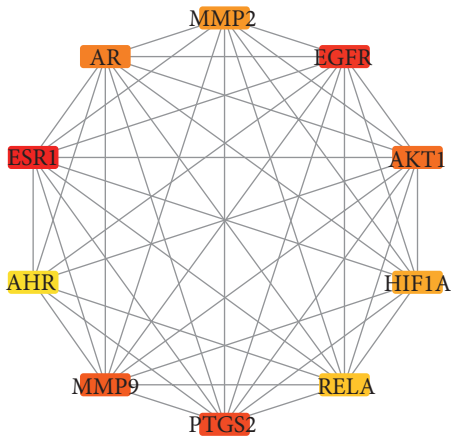
(a)



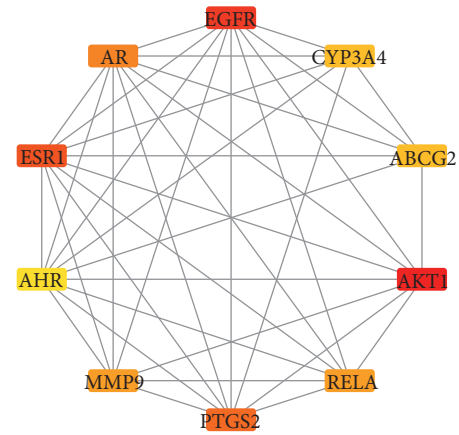
Clustering coefficient: 0.496
Connected components: 1
Network diameter: 4
Network radius: 2
Network centralization: 0.485
Shortest paths: 2652 (100%)
Characteristic path length: 1.934
Avg. number of neighbors: 11.231

Number of nodes: 52
Network density: 0.220
Network heterogeneity: 0.609
Isolated nodes: 0
Number of self-loops: 0
Multiedge node pairs: 0
Analysis time (sec): 0.031

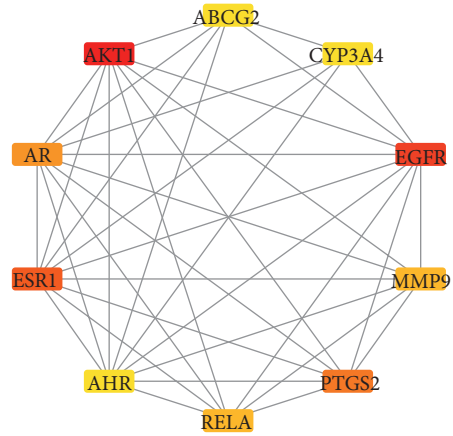
(b)



(c)



(d)



(e)

Figure 3: The network was constructed based on 52 therapeutic targets of EH in the treatment of HCC and the top 10 hub genes were analyzed in this network. (a) Compound-target-disease (C-T-D) network. (b) EH target-HCC target (E-H) network. (c) Top 10 hub genes from the maximum neighborhood component (MNC) method. (d) Top 10 hub genes from the maximal clique centrality (MCC) method. (e) Top 10 hub genes from the degree method.

Table 3: Topological parameters of the top 10 hub genes of the E-H network ranked by degree.

Gene symbol	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Topological Coefficient	Degree
AKT1	0.206016	0.761194	0.258824	0.240336	35
EGFR	0.089246	0.69863	0.337438	0.270453	29
ESR1	0.067784	0.68	0.39886	0.292665	27
PTGS2	0.085964	0.662338	0.346667	0.276078	25
AR	0.041309	0.62963	0.424242	0.313636	22
MMP9	0.040523	0.607143	0.45614	0.308421	19
RELA	0.027075	0.6	0.450292	0.330827	19
ABCG2	0.028045	0.579545	0.433333	0.306122	16
AHR	0.048945	0.593023	0.55	0.34	16
CYP3A4	0.038392	0.566667	0.358333	0.271277	16

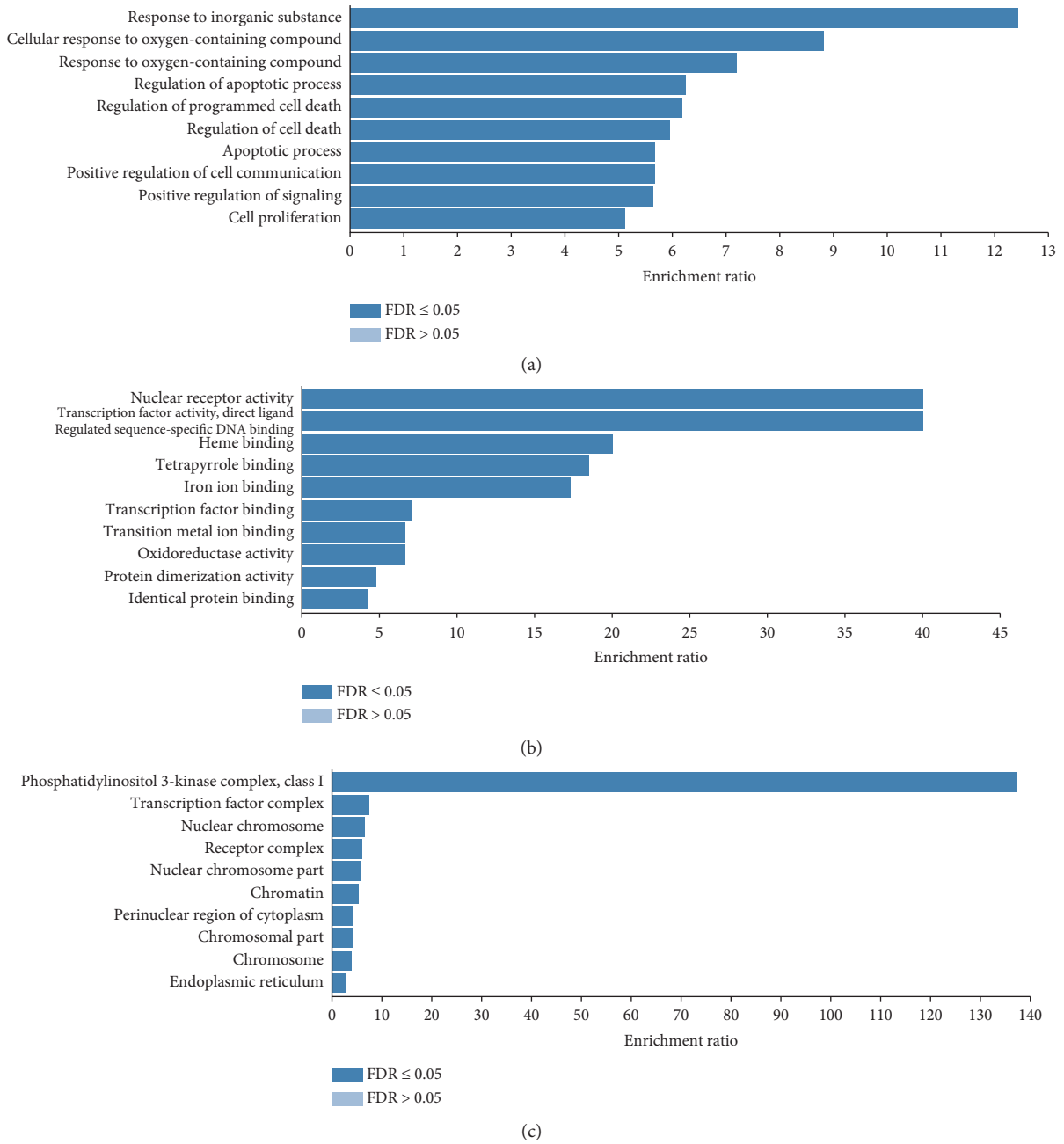


Figure 4: Gene Ontology (GO) enrichment analysis of 52 targets of EH in the treatment of HCC analyzed through WebGestalt. (a) The top 10 biological processes. (b) The top 10 molecular functions. (c) The top 10 cellular components.

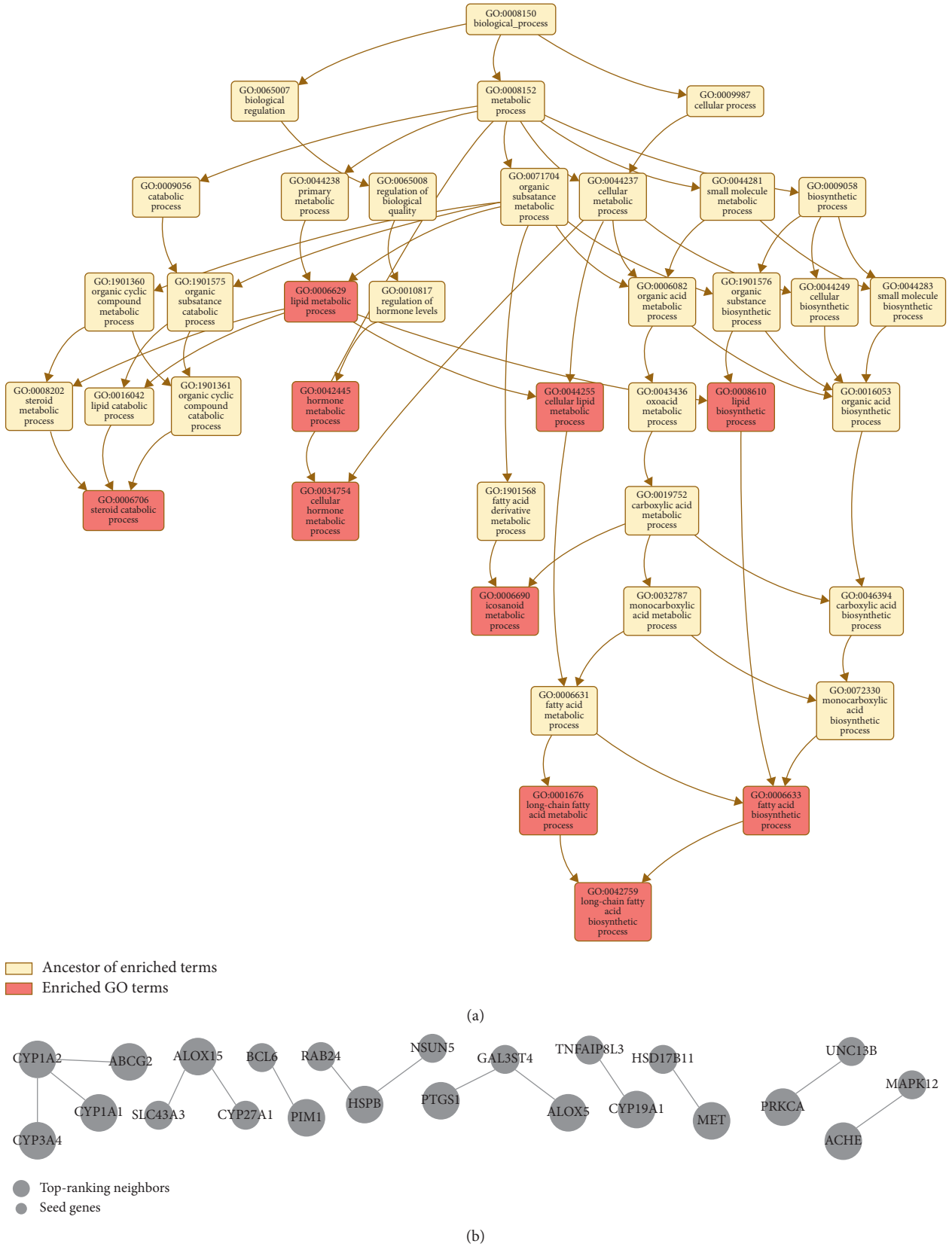
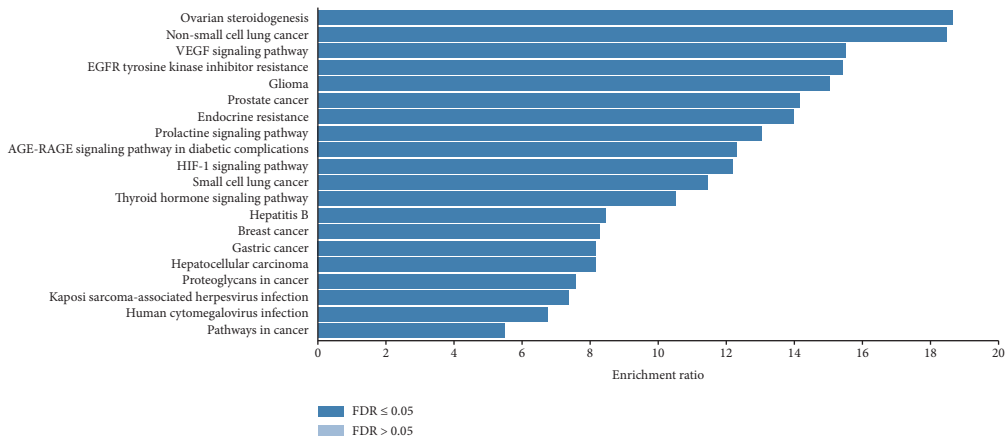
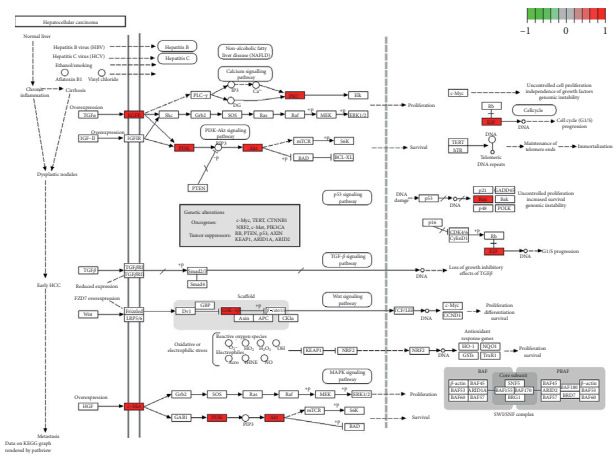


Figure 5: Gene Ontology (GO) network. (a) GO network in the TCGA RNASeq LIHC database through the Network Topology-based Analysis (NTA) method in WebGestalt. (b) The subnetwork of the GO network.



(a)



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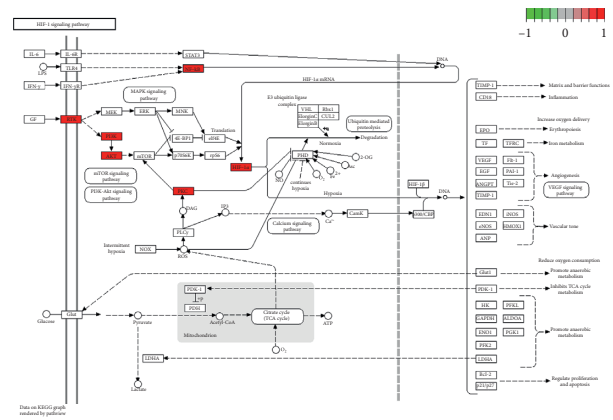
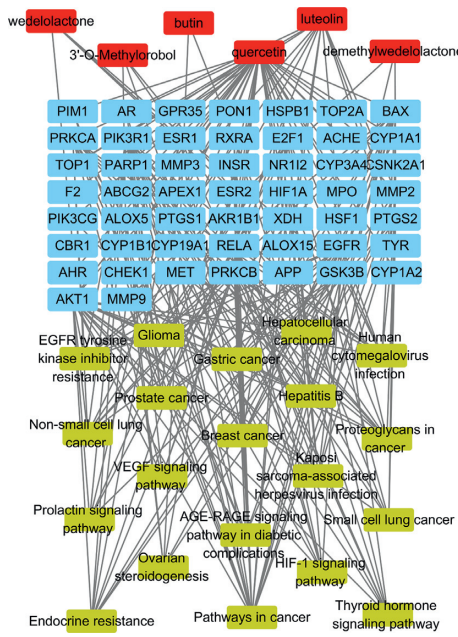


(c)



(d)

Figure 6: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and C-T-P network analysis. (a) Bar chart of the top 20 KEGG pathways. (b) Pathway diagram of EH acting on HCC through the hepatocellular carcinoma pathway. The nodes in red represent targets that EH may regulate in this pathway. (c) Pathway diagram of EH acting on HCC through the HIF-1 signaling pathway. The nodes in red represent targets that EH may regulate in this pathway. (d) C-T-P network. Nodes represent the compounds (red), the targets (blue), and the top 20 KEGG pathways (yellow). The edges represent compound-target and target-pathway interactions.

Table 4: Energy and RMSD values of the compounds binding to the target obtained in molecular docking analysis.

Targets (PDB ID)	Compound	Binding energy (kcal/mol)	RMSD/LB	RMSD/UB
MMP9 (6ESM)	Luteolin	−10.6	0	0
MMP9 (6ESM)	Quercetin	−10.4	0	0
MMP9 (6ESM)	Wedelolactone	−8.8	0	0
EGFR (3W2S)	Luteolin	−8.9	0	0
EGFR (3W2S)	Quercetin	−8.8	0	0
RELA (3QXY)	Luteolin	−8.9	0	0
RELA (3QXY)	Quercetin	−8.9	0	0
AKT1 (4EKL)	Luteolin	−8.5	0	0
AKT1 (4EKL)	Quercetin	−8.7	0	0
ESR1 (4XI3)	Demethylwedelolactone	−6.8	0	0
ESR1 (4XI3)	3′-O-Methylorobol	−6.6	0	0
AR (1XJ7)	Luteolin	−8.3	0	0
PTGS2 (5IKT)	Luteolin	−9.5	0	0
AHR (5NJ8)	Quercetin	−7.4	0	0
ABCG2 (6ETI)	Quercetin	−9.1	0	0
CYP3A4 (4D75)	Quercetin	−8.9	0	0

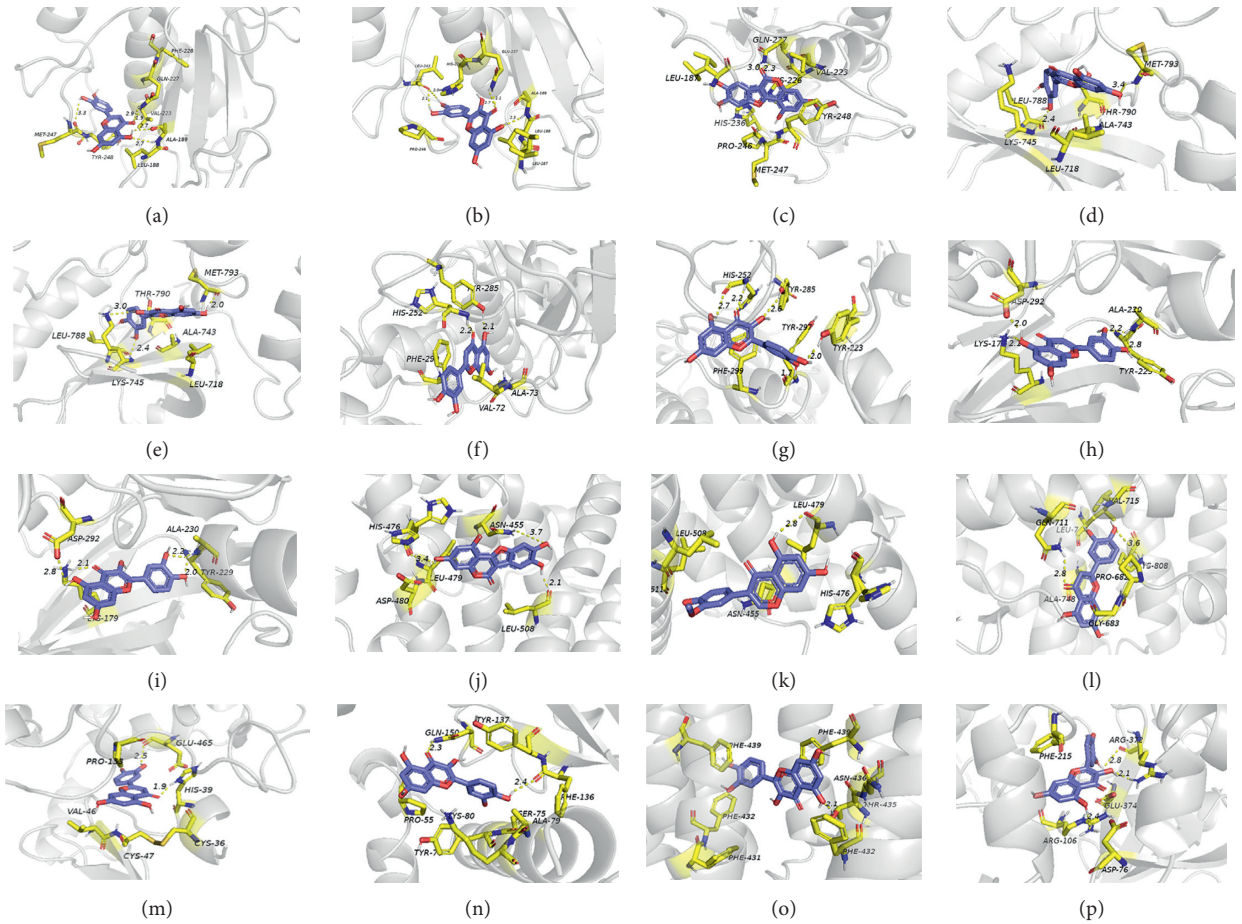


Figure 7: The 3D interaction diagram of the compound and the target was analyzed by the molecular docking method and displayed by PyMoL 1.8. Compounds (purple), residue (yellow), hydrogen bonding (dotted line). (a) Luteolin-MMP9. (b) Quercetin-MMP9. (c) Wedelolactone-MMP9. (d) Luteolin-EGFR. (e) Quercetin-EGFR. (f) Luteolin-RELA. (g) Quercetin-RELA. (h) Luteolin-AKT1. (i) Quercetin-AKT1. (j) Demethylwedelolactone-ESR1. (k) 3′-O-Methylorobol-ESR1. (l) Luteolin-AR. (m) Luteolin-PTGS2. (n) Quercetin-AHR. (o) Quercetin-ABCG2. (p) Quercetin-CYP3A4.

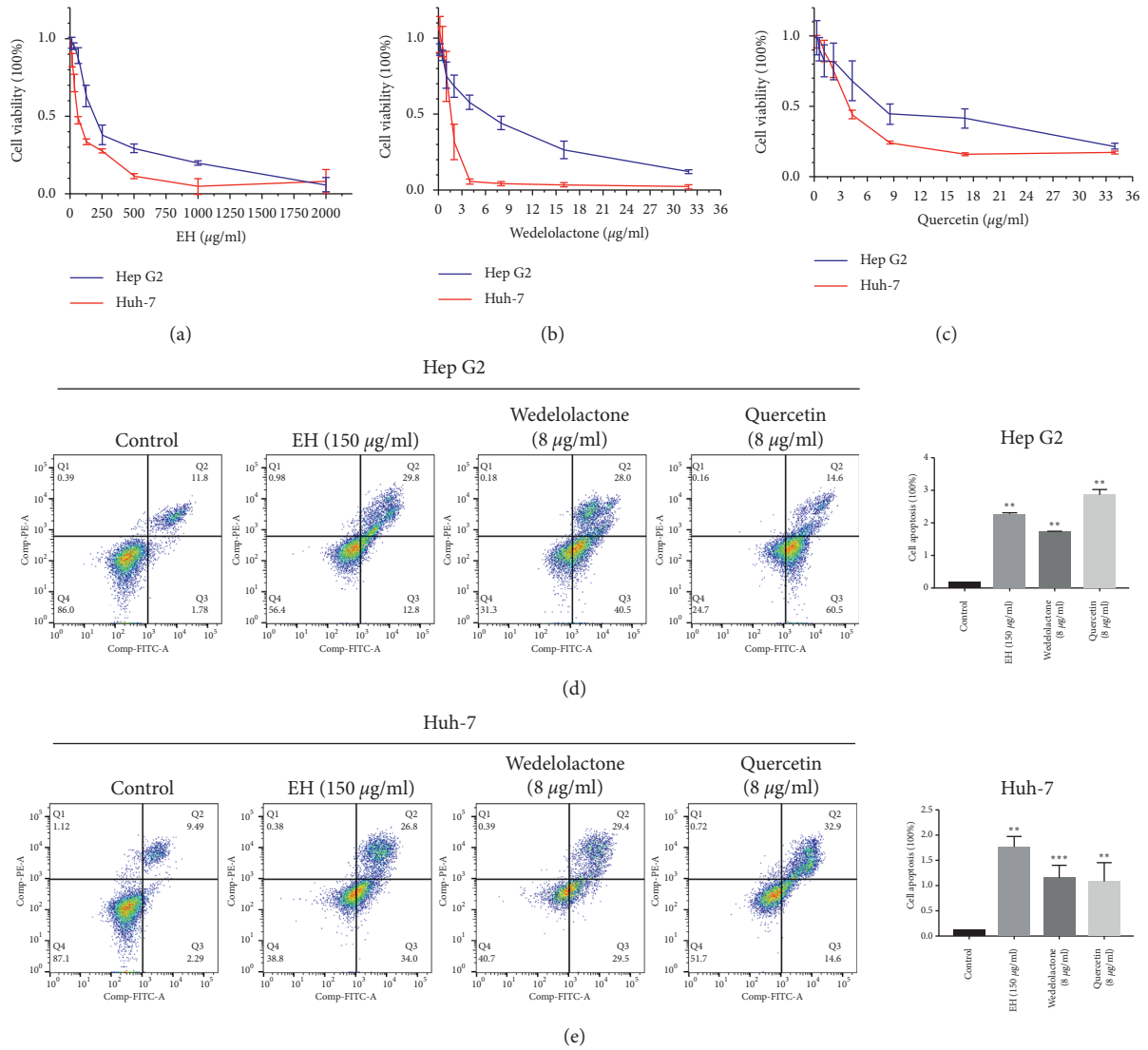


Figure 8: *Ecliptae herba*, wedelolactone, and quercetin inhibited proliferation and promoted apoptosis in hepatocellular carcinoma cell lines. (a) The CCK-8 assay detected the proliferation of HepG2 and Huh-7 cells treated with different concentrations of Ecliptae herba for 48 hours. (b) The CCK-8 assay detected the proliferation of HepG2 and Huh-7 cells treated with different concentrations of wedelolactone for 48 hours. (c) The CCK-8 assay detected the proliferation of HepG2 and Huh-7 cells treated with different concentrations of quercetin for 48 hours. (d) Flow cytometry detected the apoptosis of HepG2 cells with 150 μg/mL EH, 8 μg/mL wedelolactone, and 8 μg/mL quercetin treatment after Annexin-V-FITC and PI staining. (e) Flow cytometry detected the apoptosis of Huh-7 cells with 150 μg/mL EH, 8 μg/mL wedelolactone, and 8 μg/mL quercetin treatment after Annexin-V-FITC and PI staining. The data are expressed as the mean ± standard deviation values; $n = 3$. ** indicates $P < 0.01$ and *** indicates $P < 0.001$ compared to the control group.

To better reveal the molecular mechanism of EH treatment of HCC, we performed enrichment analysis in this study. Through GO enrichment analysis, we found that EH produced anti-HCC activity by regulating cell proliferation (GO:0008283) and cell apoptosis (GO:0006915, GO:0042981). We concluded that the regulation of cell proliferation and apoptosis plays a vital role in the development of HCC. The effect of EH on HCC through cell proliferation and apoptotic processes involves 29 targets and 31 targets in these biological processes, respectively. These targets include AKT1, EGFR, PI3K, GSK3B, and MET. Additionally, KEGG results showed that 19 of the top 20 KEGG pathways were involved in the PI3K-AKT signaling pathway, including "hepatocellular carcinoma (hsa05225)" (Figure 6(b)). This finding suggests that EH may inhibit the abnormal proliferation of HCC and promote apoptosis by acting on the PI3K-AKT signal transduction axis. PI3K-AKT signaling is one of the key pathways in the occurrence and development of HCC, and dysregulation of this signaling pathway can lead to reduced cell proliferation and apoptosis and inhibition of tumor development [30–32]. In this pathway, EH may activate the PI3K-AKT pathway by acting on some upstream receptors, such as EGFR and MET. The PI3K-AKT pathway can affect the biological functions of HCC by mediating RELA, GSK3B, MMP, and other downstream factors. In addition,

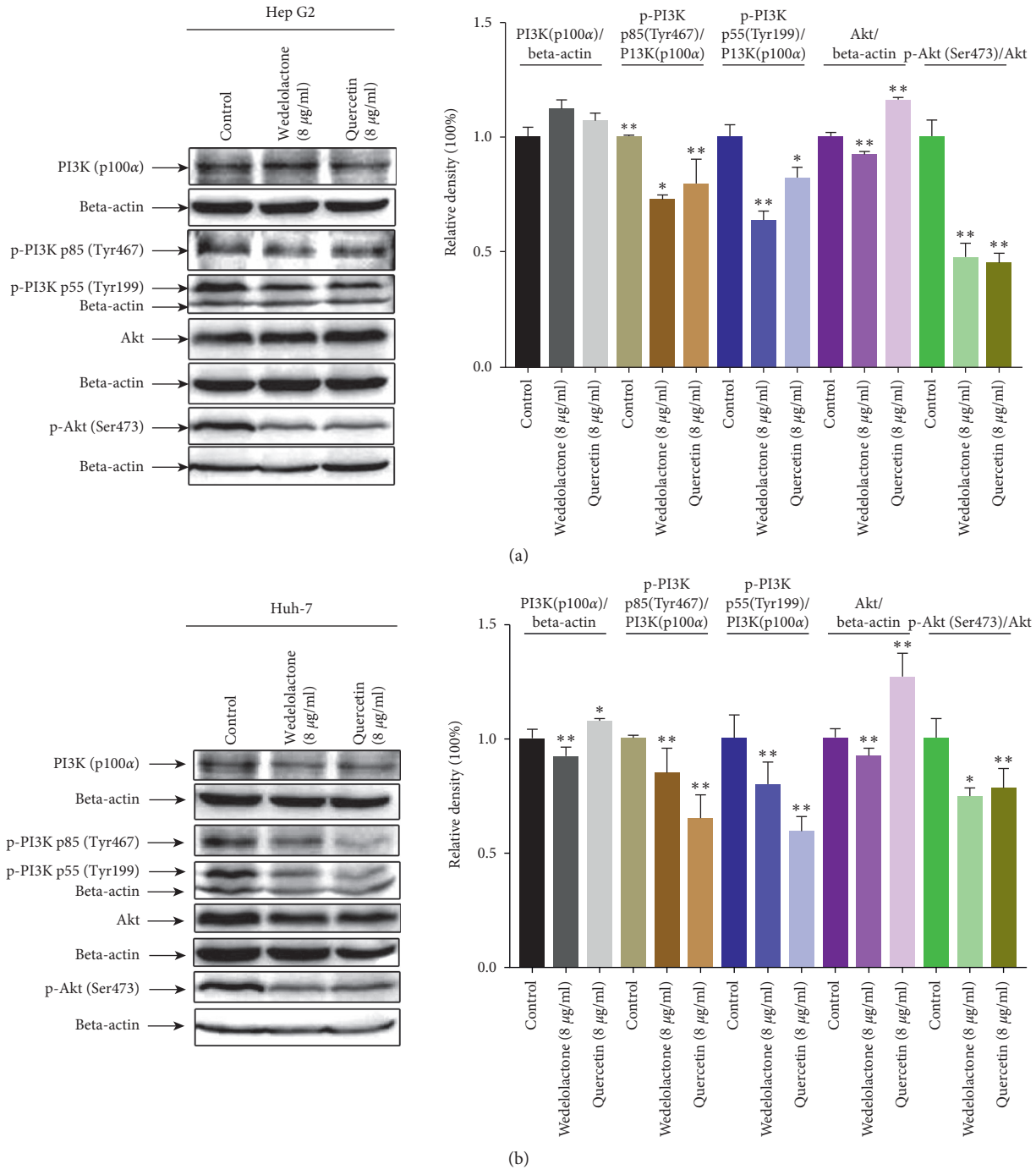


Figure 9: Wedelolactone and quercetin inhibited the PI3K/AKT pathway in hepatocellular carcinoma cell lines. (a) Western blot analysis showed that wedelolactone and quercetin inhibited the protein expression of p-PI3K p85 (Tyr467), p-PI3K p55 (Tyr199), and p-Akt (Ser473) in HepG2 cells. (b) Western blot analysis showed that wedelolactone and quercetin inhibited the protein expression of p-PI3K p85 (Tyr467), p-PI3K p55 (Tyr199), and p-Akt (Ser473) in Huh-7 cells. The data are expressed as the mean ± standard deviation values; $n = 3$. * indicates $P < 0.05$ and ** indicates $P < 0.01$ compared to the control group.

KEGG results showed that EH may inhibit the development of HCC by regulating the HIF-1 signaling pathway (hsa04066) (Figure 6(c)). Many studies have reported that inhibition of HIF1A can significantly inhibit the growth of tumors, including HCC [33, 34]. Quercetin, as an effective flavonoid component in EH, combined with specific anticancer drugs can increase the expression of p53 by downregulating the expression of HIF1A, thus inducing an increase in the proapoptotic process of liver cancer cells [35]. In addition, the expression of HIF1A can be regulated by activation of the PI3K-AKT pathway, which can be activated by RTK [36, 37].

The liver plays an important role in lipid metabolism, and excessive lipid accumulation in hepatocytes can lead to nonalcoholic fatty liver disease, which is a chronic liver disease that has emerged as a major cause of liver cancer in Western countries in recent years [38, 39]. Cytochrome P450 of the liver plays a pivotal role in the process of lipid metabolism and lipid biosynthesis [40]. Several studies have shown that regulating the activity of cytochrome P450 can affect the development of NAFLD through different mechanisms, such as oxidative stress [41–43]. By analyzing the TCGA RNASeq LIHC database and 52 targets of EH against HCC, we obtained an enriched GO term network. This GO network reveals that EH may play a role in HCC by regulating lipid metabolic process (GO:0006629), lipid biosynthetic process (GO:0008610), and cellular lipid metabolic process (GO:0044255), involving the CYP1A1, CYP1A2, and CYP3A4 gene subnetworks. These results suggest that EH may inhibit the occurrence of NAFLD by regulating the lipid metabolism process and produce hepatoprotective activity in the liver to prevent HCC.

In addition, we simulated the interaction pattern of these 6 active ingredients of EH with the hub genes by molecular docking technology, and the results showed that these molecules effectively bind to the binding pocket of these genes by forming several hydrogen bonds. However, this phenomenon does not allow them to selectively interact with specific proteins. Therefore, it is necessary to improve the selectivity of these compounds through subsequent structural modification.

Although network pharmacology has revealed the potential mechanism by which EH inhibits HCC, validation experiments are still needed to verify the above results. CCK-8 and cell apoptosis experiments confirmed that EH and its main active ingredients, quercetin, and wedelolactone, can significantly inhibit the proliferation of HCC and promote apoptosis, which is consistent with the GO analysis. To further clarify the molecular mechanism by which EH inhibits HCC, we confirmed through western blot assay that the active components of EH, quercetin, and wedelolactone can inhibit the PI3K-AKT signaling pathway in the HCC cell line. These results suggest that the pharmacological effects of EH on the inhibition of the abnormal proliferation of HCC and the promotion of HCC cell apoptosis are achieved by regulating the PI3K-AKT signaling pathway.

5. Conclusions

Overall, this study explored the underlying mechanism of EH against HCC based on the strategies of network pharmacology and experimental verification. These mechanisms of network pharmacology prediction mainly involve the following aspects: regulation of the PI3K-AKT signaling pathway promotes apoptosis and inhibits abnormal proliferation of HCC cells, downregulation of HIF-1A expression is mediated by activating the HIF-1 signaling pathway, and prevention of HCC is induced by regulating lipid metabolism and inhibiting NAFLD by the cytochrome P450 subfamily. Moreover, we verified through experiments that EH inhibits the PI3K-AKT signaling pathway through its active ingredients, quercetin, and wedelolactone, thereby inhibiting the proliferation of HCC cells and promoting apoptosis of HCC cells. Although the network pharmacology method can be a good solution to this issue, further experimental verification is necessary.

Abbreviations

EH: *Ecliptae herba*
HCC: Hepatocellular carcinoma
TCM: Traditional Chinese medicine
TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform
ADME: Absorption, distribution, metabolism, and excretion
DGIdb: The Drug Gene Interaction Database
CTD: Comparative Toxicogenomics Database
PPI: Protein-protein interaction
NAFLD: Nonalcoholic fatty liver disease
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
HBV: Hepatitis B
HCV: Hepatitis C
EMT: Epithelial-mesenchymal transition
OB: Oral bioavailability
MW: Molecular weight
Log *P*: The logarithm of 1-octanol/water partition coefficient
Hdon: Hydrogen-bond donors
Hacc: Hydrogen-bond acceptors
DL: Drug similarity
HL: Half-life
BBB: Blood-brain barrier permeability
C-T-D: Compound-target-disease
E-H: EH target-HCC target
C-T-P: Compound-target-pathway
BP: Biological process
CC: Cellular component
MF: Molecular function
LIHC: Liver hepatocellular carcinoma
TCGA: The Cancer Genome Atlas
PDB: Protein Data Bank.

Data Availability

The data used in the article comes from public databases, all of which are described in the Methods section of the article. Besides, the rest of them used to support the findings of this study are included within the supplementary information files.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

CX and ZL reviewed and revised the manuscript. BP performed all the experiments and drafted and reviewed the

manuscript. WP contributed to data collection and statistical analysis. All the authors reviewed the manuscript.

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Supplementary Materials

Supplementary File S1: a total of 48 chemical ingredients of EH were obtained from TCMSP. Supplementary File S2: detailed information of the targets of 6 active ingredients in EH was extracted from three databases, TCMSP, DGIDB, and SwissTargetPrediction. Supplementary File S3: detailed information on HCC-related targets was extracted from GeneCards and CTD. Supplementary File S4: detailed information on the PPI network of 52 potential therapeutic targets for HCC was obtained from the STRING platform. Supplementary File S5: topological parameters of nodes in the E-H network obtained from Cytoscape. Supplementary File S6: detailed information on GO enrichment analysis obtained from WebGestalt. Supplementary File S7: detailed information on the top 10 GO terms of the GO network in the TCGA RNASeq LIHC database through Network Topology-based Analysis obtained from WebGestalt. Supplementary File S8: detailed information on the top 20 KEGG enrichment pathways obtained from the WebGestalt. Supplementary File S9: detailed information on the C-T-P network obtained from Cytoscape. (*Supplementary Materials*)

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Research Article

The Combination of Electroacupuncture and Massage Therapy Alleviates Myofibroblast Transdifferentiation and Extracellular Matrix Production in Blunt Trauma-Induced Skeletal Muscle Fibrosis

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Complementary therapies, such as acupuncture and massage, had been previously reported to have therapeutic effects on skeletal muscle contusions. However, the recovery mechanisms on skeletal muscles after blunt trauma via the combination of electroacupuncture (EA) and massage therapy remain unclear. In the present study, a rat model of the skeletal muscle fibrosis following blunt trauma to rat skeletal muscle was established, and the potential molecular mechanisms of EA + massage therapy on the skeletal muscle fibrosis were investigated. The results suggested that EA + massage therapy could significantly decrease inflammatory cells infiltration and collagenous fiber content and ameliorate the disarrangement of sarcomeres within myofibrils compared to the model group. Further analysis revealed that EA + massage therapy could reduce the degree of fibrosis and increase the degree of myofibroblast apoptosis by downregulating the mRNA and protein expression of transforming growth factor- (TGF-) β1 and connective tissue growth factor (CTGF). Furthermore, the fibrosis of injured skeletal muscle was inhibited after treatment through the normalization of balance between matrix metalloproteinase- (MMP-) 1 and tissue inhibitor of matrix metalloproteinase (TIMP). These findings suggested that the combination of electroacupuncture and massage therapy could alleviate the fibrotic process by regulating TGF β1-CTGF-induced myofibroblast transdifferentiation and MMP-1/TIMP-1 balance for extracellular matrix production.

1. Introduction

Skeletal muscle injury is one of the common types of exercise-related injuries in sports medicine, and its effective treatment is rather challenging. The regeneration process of skeletal muscle injury is similar in most types of muscle injuries. However, it is usually hard to achieve complete recovery from the injury because of the development of fibrosis in the second week after the injury. The formed scar tissue is usually difficult to exert the normal muscle fiber functions and more susceptible to be reinjured [1]. Surgery is a treatment option only implemented in certain specific conditions, such as the presence of a large intramuscular hematoma, a complete strain or tear of a muscle with few agonist muscles, or a partial strain with persistent extension pain (>4–6 months) [2]. Conservative treatment strategies are frequently used for acute and chronic skeletal muscle injuries [3], which aim to minimize further damage, relieve pain, reduce hemorrhage and edema, and promote healing. For example, physical therapy approaches such as limb elevation and local cooling had been used to improve muscle repair [4]. Another alternative treatment of enhancing muscle healing includes pharmacological therapies with nonsteroidal anti-inflammatory drugs (NSAIDs) being the

most common drug [5]. Although the NSAIDs could reduce the early inflammatory responses, there were some side effects such as hypertension, gastrointestinal disorders, and renal toxicity. Recently, complementary therapies such as acupuncture and massage therapy had gained increasing attention for the treatment of skeletal muscle injuries [6].

Acupuncture, one branch of traditional medicine, is widely used to treat various diseases in clinics [7]. The World Health Organization (WHO) had issued a consensus statement that current data supported the use of acupuncture to treat certain diseases, such as the stroke rehabilitation, low back pain, headache, carpal tunnel syndrome, osteoarthritis, asthma, and other conditions [8]. Furthermore, electroacupuncture (EA) is usually used to recover musculoskeletal disorders and skeletal muscle fatigue [9, 10]. Massage therapy mainly involves topical stroking and kneading of the skin and underlying musculature to produce pressure and muscle distension. Massage is widely used to treat moderate muscle injuries for reducing muscle soreness and enhancing postexercise muscle recovery [11]. Although massage has become a common complementary treatment approach in muscle repair, its potential mechanisms are still unclear.

Skeletal muscle injury contains three main phases including inflammation, regeneration, and fibrosis [12]. Skeletal muscle fibrosis is characterized by the redundant accumulation of matrix, unbalance of synthesis, and the degradation of matrix proteins. Transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) are two potent profibrotic growth factors that induce the matrix production and accumulation. Moreover, CTGF is the downstream effector of TGF-β1 [13]. Matrix metalloproteinases (MMPs) played vital roles in maintaining the functional integrity of myofiber. The extracellular matrix (ECM) can be broken down by MMPs to allow cell growth, and skeletal muscle cell migration and differentiation are also regulated by MMPs [14]. The MMP collagenases, such as MMP-1, -8, and -13, show the ability to clear the interstitial collagen types I, II, and III, and MMP-2 and -9 can degrade the denatured collagen [15].

In this study, a rat model of the skeletal muscle fibrosis following blunt trauma to rat skeletal muscle was established, and the effects of the combination of EA and MST and the possible mechanisms on the skeletal muscle fibrosis were investigated.

2. Materials and Methods

2.1. Reagents. PrimeScript™ RT Master Mix kit was bought from TaKaRa Co. (Kyoto, Japan). The cell lysis kit, RNA extraction kit, and anti-GAPDH were purchased from Sangon Biotech (China). Anti-TGF-β1, anti-CTGF, and anti-TIMP-1 were obtained from Abcam (United Kingdom). Anti-Smad 2 and anti-Smad 3 were obtained from CST (USA). Anti-MMP-1 was obtained from Thermo Fisher Scientific (USA).

Sprague-Dawley (SD) rats (male, 180–220 g) were obtained from Beijing Vital River Laboratories Animal Technology Co., Ltd. Experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institute Ethics Committee (TCM-2019-013-E04, Tianjin University of Traditional Chinese Medicine).

2.2. Model of Skeletal Muscle Fibrosis and Treatment in Rats. A rat model of the skeletal muscle fibrosis following blunt trauma to rat skeletal muscle was established by the method of Kami et al. [16] with minor modifications. The rats were allocated into 5 groups randomly ($n = 16$/group): control group, skeletal muscle fibrosis model group, skeletal muscle fibrosis model group + treatment with massage therapy group, skeletal muscle fibrosis model group + treatment with electroacupuncture therapy group, and skeletal muscle fibrosis model group + treatment with the combination of massage and electroacupuncture therapy. The massage therapy was performed based on our previous study [17]. In brief, the massage was performed with the massage manipulation simulator on the damaged tissue for 10 minutes per day and continued with a total intervention time of 25 days. EA was performed based on the previous method [18]. Rats were performed with EA at Zusanli with synchronously stimulated identical parameters for 15 min, which was continued for a total of 25 days.

2.3. Histological Assessment and Masson's Trichrome Stain. After the treatment of massage therapy and electroacupuncture therapy, rats were sacrificed by anaesthetization with pentobarbital sodium. The skeletal muscle was then separated quickly on ice. The samples were fixed in 10% buffered formalin solution and dehydrated by ethanol and xylene. After fixation and dehydration, the samples were then embedded in paraffin and cut into sections with 5 μm thickness. The obtained sections were stained with H&E and Masson's trichrome stain and observed under the light microscope.

2.4. Immunofluorescence of α-SMA and TUNEL Staining. The expression of α-SMA was analyzed by the immunofluorescence staining using the α-SMA antibody and the immunofluorescence detection kit. In brief, slides of skeletal muscle were deparaffinized and rehydrated by using xylene, ethanol, and water, sequentially. After pairing the retrieval antigen and blocking the nonspecific antigen in skeletal muscle tissue, the tissues were incubated overnight with a dilution of anti-α-SMA antibody at 4°C. The tissues were then incubated with diluted fluorescent-conjugated secondary antibody for 1 h at room temperature. After incubation, the secondary antibody was washed off, and TUNEL staining was performed based on the manufacturer's instructions. Finally, tissues were incubated with DAPI staining solution for 5 minutes. Fluorescence was observed using a laser confocal microscope.

2.5. Preparation of Muscle Samples for Transmission Electron Microscopy (TEM) Analysis. For the TEM analysis, the muscle samples were cut into 3 mm cubes and fixed for 4 h in cold 2.5% glutaraldehyde at 4°C. The muscle tissues were

then dehydrated with ethanol and acetone. The dehydrated tissues were embedded in epoxy resin and cut into sections with 2 μm thickness. The toluidine blue was used to stain the specific area, which was further cut into 50 nm thickness collected to a copper grid. The ultrathin section was stained with uranyl acetate to acquire the ultrastructure using the Hitachi TEM system (Tokyo, Japan).

2.6. Real-Time PCR. RNA from muscle tissues was extracted using a Trizol extraction kit. The reverse transcription was performed with PrimeScript RT Master Mix at 37°C for 15 min, 85°C for 5 sec, 4°C. Finally, PCR amplification was performed using TB Green™ Premix Ex Taq™ II. The oligonucleotide primers are shown in Table S1. The amplification was carried out by a 7500 real-time PCR system (ABI Prism) with protocols of TB Green™ Premix Ex Taq™ kits. At the end of each experiment, a melt curve analysis was performed. The mRNA expression was analyzed using the $2^{-\Delta\Delta CT}$ method [19, 20].

2.7. Western Blot. The protein expression levels of TGF-β, CTGF, MMP-1, and TIMP-1 were investigated using the western blot method. The total protein was extracted and separated on a 10% SDS-polyacrylamide gel and electroblotted to a PVDF membrane. The PVDF membrane was firstly incubated with 5% skim milk for 2 h, and then the PVDF membrane was incubated with anti-TGF-β1, anti-CTGF, anti-MMP-1, anti-TIMP-1, and anti-GAPDH antibody overnight at 4°C. The obtained blots were washed with TBS and incubated with horseradish peroxidase-conjugated secondary antibody. Then, the blots were washed with TBS again and incubated with horseradish peroxidase substrate for 5 minutes. At last, the protein expression was obtained on a ChemiScope 6200 Chemiluminescence imaging system.

2.8. Statistical Analysis. The data were analyzed with Origin Pro 9.0 software. The results are presented as the mean ± SD. Statistically significant differences between values of different groups were determined with one-way ANOVA following by Scheffe's multiple range test. The value of $P < 0.05$ was considered statistically significant.

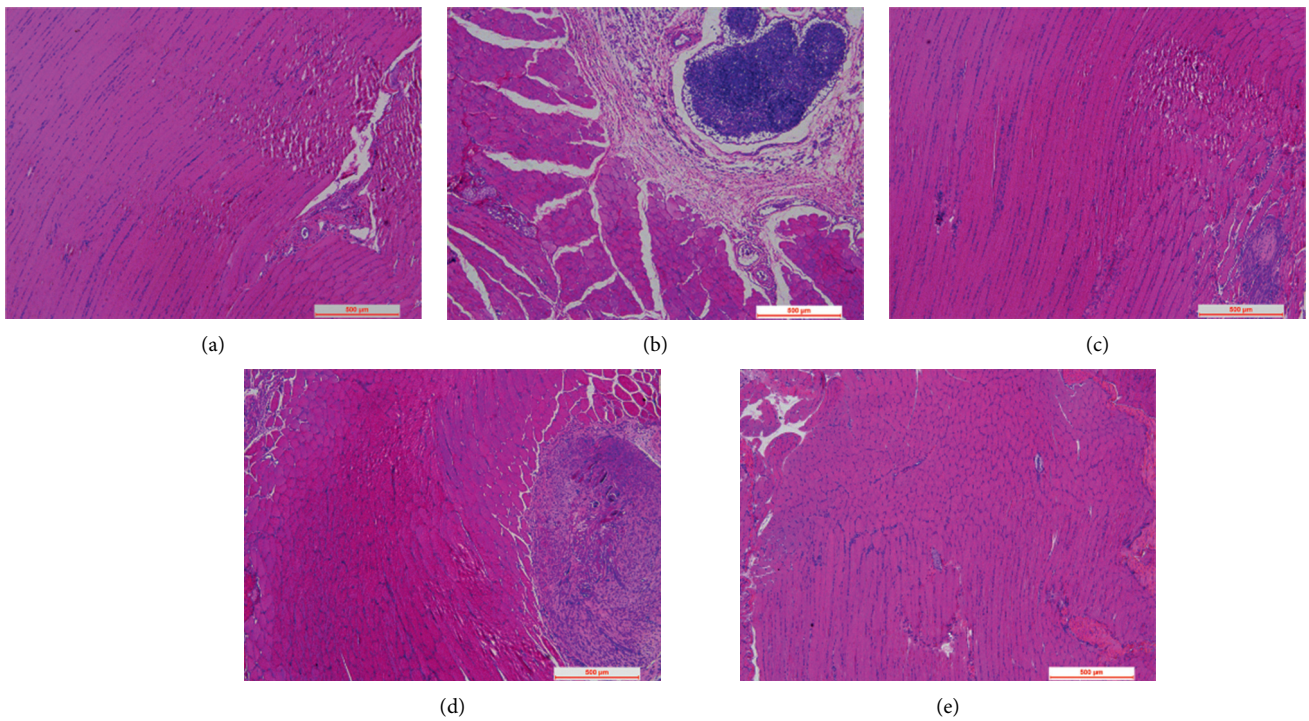
3. Results

3.1. Effect of the Combination of Massage and Electroacupuncture Therapy on Histological Change and the Degree of Fibrosis in the Skeletal Muscle Fibrosis Rats. The results of H&E staining are shown in Figure 1. The myofibrous tissue of normal rats showed polygonal, regular, and tight distribution, and no edema, hyperaemia, and inflammation were observed (Figure 1(a)). In the model of the skeletal muscle fibrosis group, the skeletal muscle cells tend to be round, and there were more inflammatory cells infiltrating in damaged skeletal muscle than those in the normal cells (Figure 1(b)). The condition was improved by continuous massage therapy or electroacupuncture therapy with fewer inflammatory cells surrounding the damaged skeletal muscle tissue (Figures 1(c) and 1(d)). No inflammatory cell infiltration was observed in the treatment group with the combination of continuous massage and electroacupuncture therapy, and the shape of skeletal muscle cells was similar to the control group (Figure 1(e)). As shown in Figure 2(f), collagenous fiber volume fraction of the massage therapy group, electroacupuncture therapy group, and the combination treatment group significantly reduced than the model group ($P < 0.01$). Among three groups, the combination treatment group is the least ($P < 0.05$ or $P < 0.01$). Collagenous fibers are a major component of the ECM. Also, they are the most abundant protein within the body. Therefore, the result indicated the combination treatment group is effective to reduce ECM production in blunt trauma-induced skeletal muscle fibrosis.

Masson's trichrome stain was used for evaluating the degree of fibrosis for the skeletal muscle (Figure 2). There was little collagenous fiber in the control group (Figure 2(a)), but the content of collagenous fiber increased significantly in the model of the skeletal muscle fibrosis group (Figure 2(b)). The content of collagenous fiber decreased after continuous massage therapy or electroacupuncture therapy (Figures 2(c) and 2(d)), and the combination of continuous massage and electroacupuncture therapy showed least content of collagenous fiber (Figures 2(e) and 2(f)).

3.2. Effect of the Combination of Massage and Electroacupuncture Therapy on Apoptosis of Myofibroblast in the Skeletal Muscle Fibrosis Rats. The apoptosis of myofibroblast was investigated by immunofluorescent staining (Figure 3). The myofibroblast was firstly marked with α-SMA (red), and the TUNEL was used to examine the apoptosis of myofibroblast (green). Compared with the control group, the fluorescence intensity of α-SMA (red) was increased, but the apoptosis of myofibroblast (green) was not increased. After the continuous massage therapy or electroacupuncture therapy, the positive fluorescence intensity (red) of α-SMA was decreased. However, the apoptosis of myofibroblast (green) was increased relatively compared to the model group. The combination of continuous massage and electroacupuncture therapy showed the most degree of apoptosis of myofibroblasts (green).

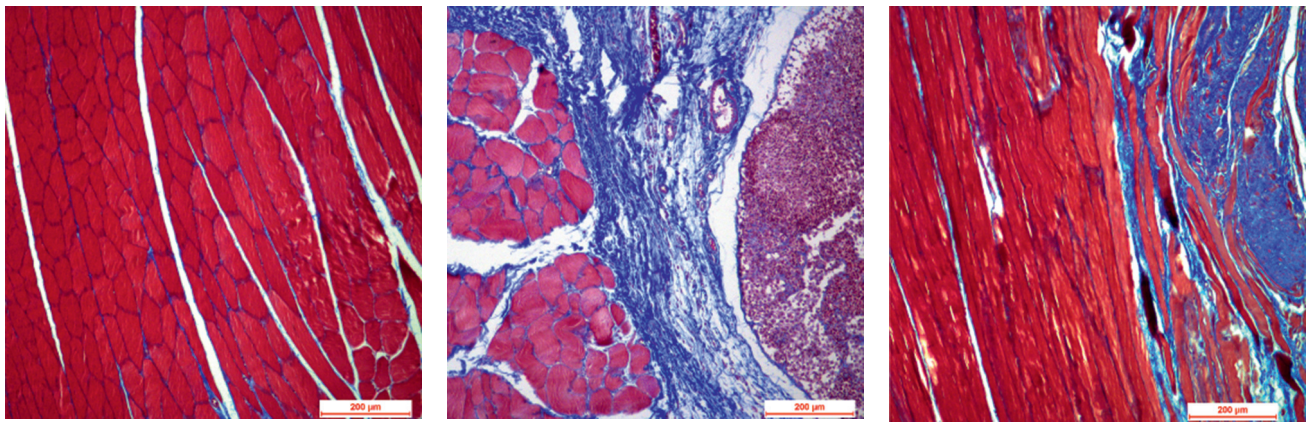
3.3. Effect of the Combination of Massage and Electroacupuncture Therapy on the Ultrastructure Alteration of Skeletal Muscle in the Skeletal Muscle Fibrosis Rats. The ultrastructure alteration of skeletal muscle was observed by transmission electron microscopy. In the control group, the shape of mitochondria was round and oval, and the arrangement of sarcomeres in one myofibril was relatively regular (black arrow) (Figure 4(a)). In the rat model of the skeletal muscle fibrosis, some myofibrils in the muscle were ruptured and interlaced, and the mitochondria were swollen (red arrow). Furthermore, the direction of myofilaments in one myofibril was changed (Figure 4(b)). The continuous massage therapy or electroacupuncture therapy could improve the



(a) (b) (c)

(d) (e)

Figure 1: Histological recovery of skeletal muscle of a rat model with the skeletal muscle fibrosis. Histological sections with hematoxylin and eosin (HE) staining of skeletal muscle in rat. (a) Rat treated without blunt trauma to skeletal muscle (control group). (b) Rat treated after blunt trauma to skeletal muscle (model group). (c) Rat treated with massage therapy after blunt trauma to skeletal muscle (massage therapy group). (d) Rat treated with electroacupuncture therapy after blunt trauma to skeletal muscle (electroacupuncture therapy group). (e) Rat treated with the combination of continuous massage and electroacupuncture therapy after blunt trauma to skeletal muscle (the combination treatment group). Scale bars, 500 μm.



(a) (b) (c)

Figure 2: Continued.

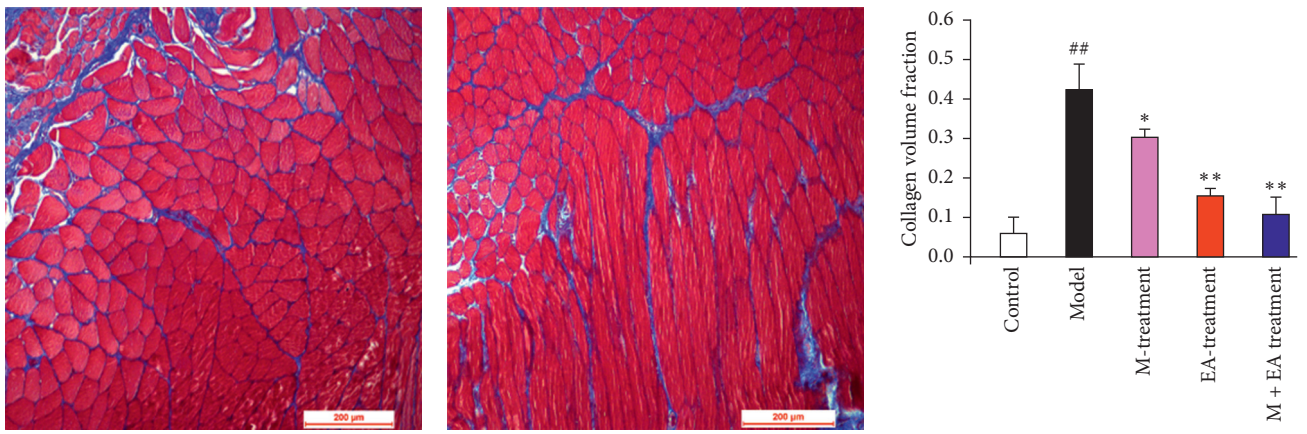


FIGURE 2: Fibrosis in skeletal muscle of blunt trauma-induced rats. Histological sections with Masson's trichrome staining of skeletal muscle in rats. (a) Rat treated without blunt trauma to skeletal muscle (control group). (b) Rat treated after blunt trauma to skeletal muscle (model group). (c) Rat treated with massage therapy after blunt trauma to skeletal muscle (massage therapy group). (d) Rat treated with electroacupuncture therapy after blunt trauma to skeletal muscle (electroacupuncture therapy group). (e) Rat treated with the combination of continuous massage and electroacupuncture therapy after blunt trauma to skeletal muscle (the combination treatment group). Scale bars, 200 μm. (f) Collagen volume fraction of the skeletal muscle. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control. $^{*}P < 0.05$ and $^{**}P < 0.01$ ($n = 4$) vs. model.

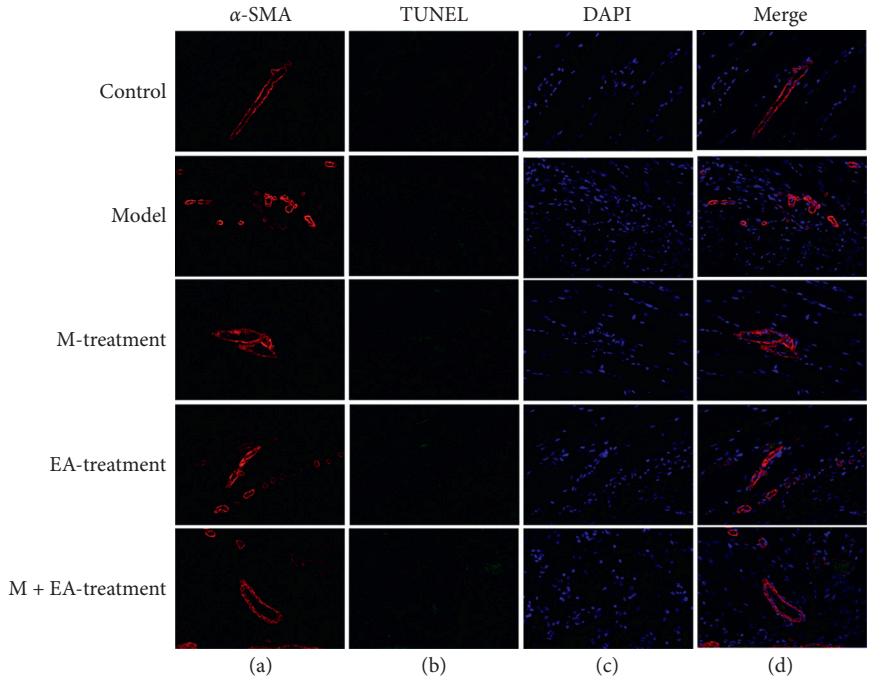


FIGURE 3: The apoptosis of myofibroblast in the skeletal muscle fibrosis rats. (a) Rat treated without blunt trauma to skeletal muscle (control group). (b) Rat treated after blunt trauma to skeletal muscle (model group). (c) Rat treated with massage therapy after blunt trauma to skeletal muscle (massage therapy group). (d) Rat treated with electroacupuncture therapy after blunt trauma to skeletal muscle (electroacupuncture therapy group). (e) Rat treated with the combination of continuous massage and electroacupuncture therapy after blunt trauma to skeletal muscle (the combination treatment group). The orange was α-SMA, and the DAPI (blue) was used to stain the cell nucleus. The apoptosis of myofibroblast was observed with the TUNEL assay kit (green).

condition compared to the model group (Figures 4(c) and 4(d)). Furthermore, after the combination of continuous massage and electroacupuncture therapy, mitochondria were shown with round and oval shape, and the arrangement of sarcomeres within one myofibril was relative orderliness, which was close to the control group (Figure 4(e)).

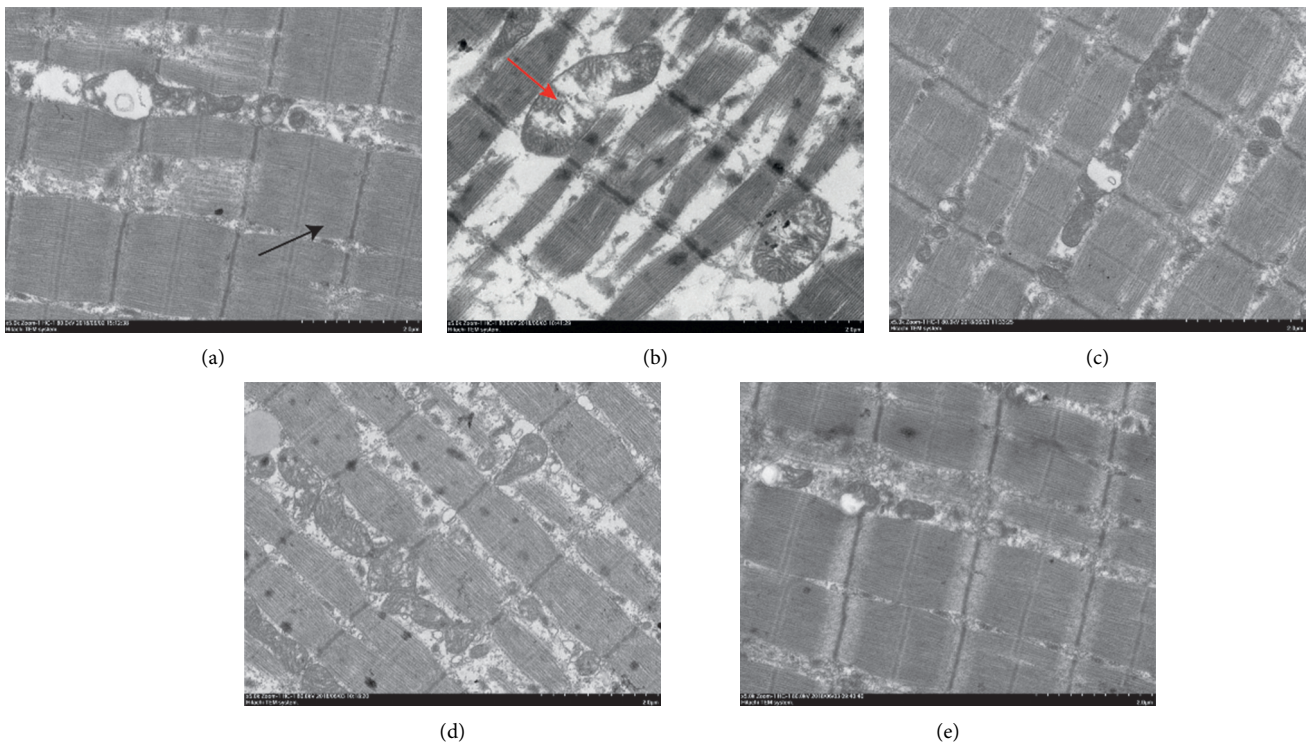


Figure 4: The ultrastructure alteration of skeletal muscle in the skeletal muscle fibrosis rats. (a) Rat treated without blunt trauma to skeletal muscle (control group). (b) Rat treated after blunt trauma to skeletal muscle (model group). (c) Rat treated with massage therapy after blunt trauma to skeletal muscle (massage therapy group). (d) Rat treated with electroacupuncture therapy after blunt trauma to skeletal muscle (electroacupuncture therapy group). (e) Rat treated with the combination of continuous massage and electroacupuncture therapy after blunt trauma to skeletal muscle (the combination treatment group). The sarcomeres were marked with black arrows, and the mitochondria were marked with red arrows.

3.4. Effect of the Combination of Massage and Electroacupuncture Therapy on mRNA Expression of TGF-β1, CTGF, MMP-1, and TIMP-1 in the Skeletal Muscle Fibrosis Rats. For the purpose of investigating the possible mechanism of combination of massage and electroacupuncture therapy on regulating skeletal muscle fibrosis, the mRNA expressions of TGF-β1, CTGF, MMP-1, and TIMP-1 were firstly detected by real-time PCR (Figure 5). Compared with the control group, the mRNA expressions of TGF-β1 and CTGF were upregulated significantly in the model group, and the continuous massage therapy or electroacupuncture therapy could significantly downregulate the mRNA expression of TGF-β1 and CTGF ($P < 0.01$) (Figures 5(a) and 5(d)). The combination of continuous massage and electroacupuncture therapy had the strongest downregulation effect on the mRNA expression of TGF-β1 and CTGF. The mRNA expression of MMP-1 was significantly downregulated in the model group compared with the control group ($P < 0.01$), and the continuous massage therapy or electroacupuncture therapy and the combination of continuous massage and electroacupuncture therapy could upregulate the mRNA expression of MMP-1 significantly ($P < 0.05$ or $P < 0.01$) (Figure 5(c)). Moreover, the continuous massage therapy or electroacupuncture therapy could significantly downregulate the mRNA expression of TIMP-1 compared with the model group ($P < 0.05$ or $P < 0.01$), and the combination of continuous massage and electroacupuncture therapy exhibited the strongest downregulation effect on the mRNA expression of TIMP-1 (Figure 5(d)).

3.5. Effect of the Combination of Massage and Electroacupuncture Therapy on Protein Expression of TGF-β1, CTGF, MMP-1, and TIMP-1 in the Skeletal Muscle Fibrosis Rats. The balance between ECM production and degradation is mediated by MMPs and TIMPs [21]. MMP collagenases, such as MMP-1, -8, and -13, show the ability to clear the interstitial collagen types I, II, and III. TIMP-1 is an important regulator in the synthesis and degradation of ECM. The protein expressions of TGF-β1, CTGF, MMP-1, and TIMP-1 were investigated by western blot (Figure 6(a)). The density analysis of TGF-β1, CTGF, MMP-1, and TIMP-1 was obtained in Figures 6(b)–6(e). Compared with the model group, continuous massage therapy or electroacupuncture therapy and the combination of continuous massage and electroacupuncture therapy could downregulate the protein expressions of TGF-β1, CTGF, and TIMP-1 and significantly upregulated the protein expression of MMP-1 ($P < 0.01$). The combination of continuous massage and electroacupuncture therapy showed the strongest downregulation effect on protein expressions of TGF-β1, CTGF, and TIMP-1. Therefore, those results

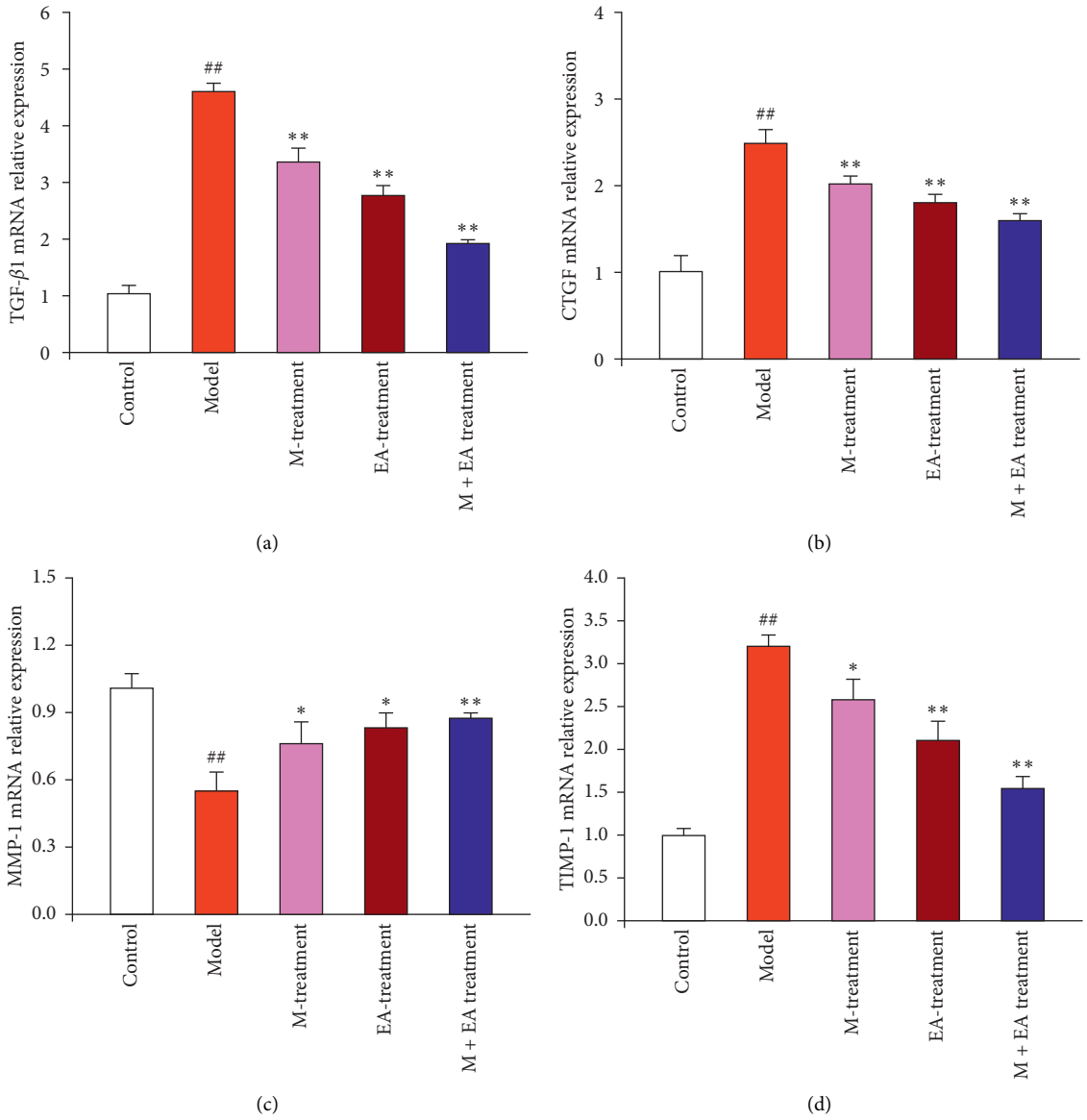


Figure 5: Effect of different treatments on mRNA expression of TGF-β1, CTGF, MMP-1, and TIMP-1 in the skeletal muscle fibrosis rats. The mRNA expression of TGF-β1 (a), CTGF (b), MMP-1 (c), and TIMP-1 (d) was analyzed by real-time PCR normalized to GAPDH. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control. $^{*}P < 0.05$ and $^{**}P < 0.01$ ($n = 4$) vs. model.

indicated the combination treatment group is effective to reduce ECM production in blunt trauma-induced skeletal muscle fibrosis by downregulating the expression of TGF-β1, CTGF and TIMP-1 and upregulating the expression of MMP-1.

4. Discussion

Previous evidence had showed that electroacupuncture treatment is safe and effective for certain diseases such as knee pain, whiplash injury, tendinitis, and dysmenorrhea [22–25]. As another alternative therapy, massage was also a widely used treatment solution for moderate muscle injuries and muscle recovery [26]. Although he effect of electroacupuncture therapy or massage therapy on contusion injury in skeletal muscle had been reported previously, the possible mechanisms of the combination of massage and electroacupuncture therapy in the recovery of skeletal muscles were still unclear.

In the present study, the combination of massage and electroacupuncture therapy was performed to treat blunt trauma in skeletal muscles. We firstly investigated the effects of the combination therapy on histological changes and fibrosis in a rat model of skeletal muscle fibrosis. Our results suggested that there was no inflammatory cell infiltration, and the shape of skeletal muscle cells was similar to the control group with the combination of massage and electroacupuncture therapy. Moreover, the combination of continuous massage and electroacupuncture therapy showed the least content of collagenous fiber compared with

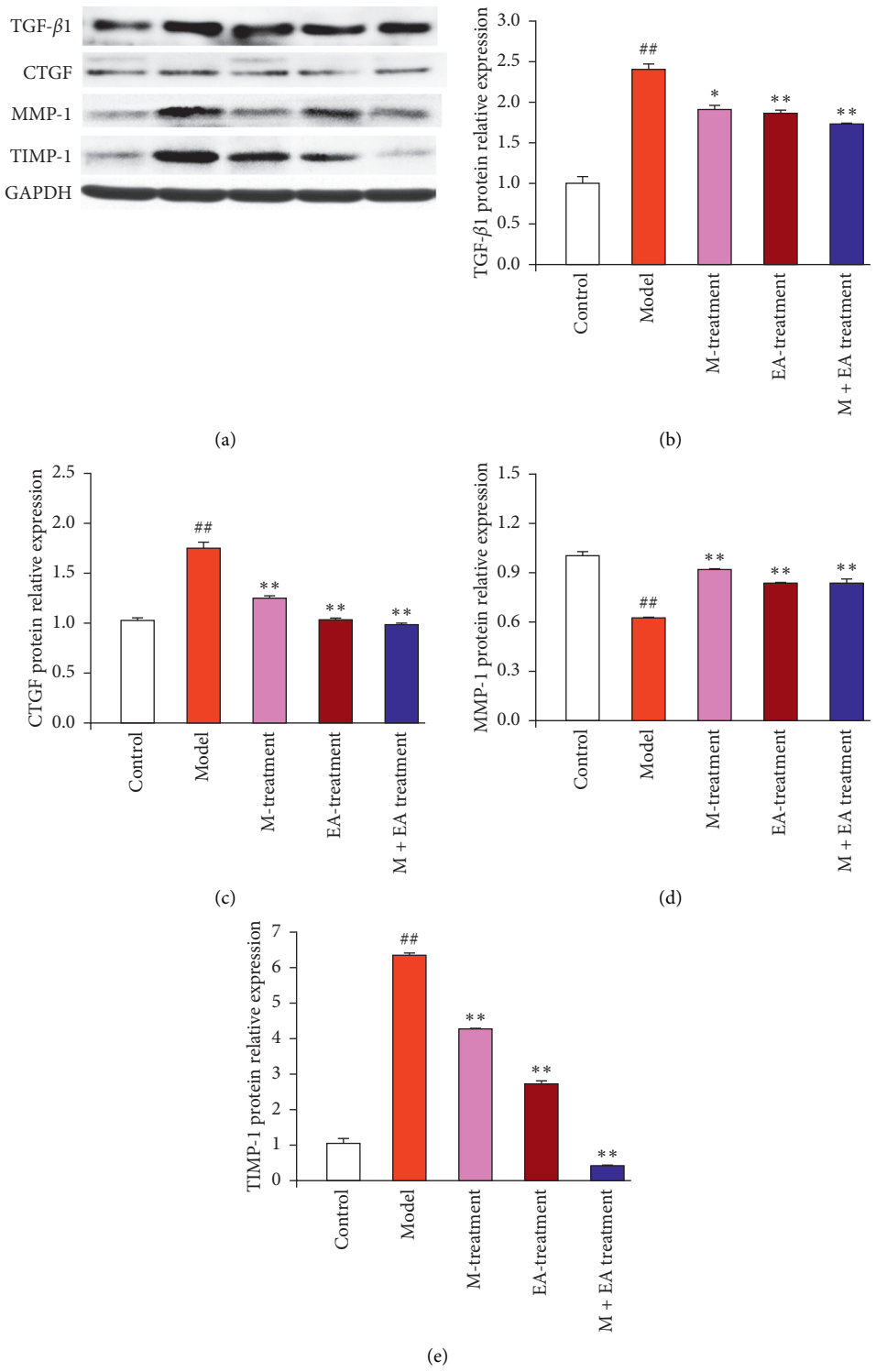


Figure 6: Effect of different treatments on protein expression of TGF-β1, CTGF, MMP-1, and TIMP-1 in the skeletal muscle fibrosis rats. The protein expression of TGF-β1, CTGF, MMP-1, and TIMP-1 (a) was investigated by western blot. The intensity (b–e) was quantified by ImageJ version 1.51n. $^{\#\#}P < 0.01$ vs. control. $^{*}P < 0.05$ and $^{**}P < 0.01$ ($n = 4$) vs. model.

massage therapy or electroacupuncture therapy. The results suggested that the combination of continuous massage and electroacupuncture therapy manifested the optimal effect on histological changes and fibrosis. Next, we investigated the ultrastructure alteration of skeletal muscle in skeletal muscle fibrosis rats. Mitochondria in muscle physiology were regarded as the energy source by the generation of ATP [27]. Calcium ion uptake by mitochondria played a vital role in regulating calcium ion signal for the contraction-relaxation cycle in skeletal muscle [28]. The present study suggested

that the combination of continuous massage and electroacupuncture therapy could restore the normal round or oval shape of mitochondria compared to the model group. Moreover, the combination of continuous massage and electroacupuncture therapy could also improve the arrangement of sarcomeres within one myofibril to be relative orderliness after blunt trauma to rat skeletal muscle.

Fibroblast and myofibroblast accumulation play an important role in the development of fibrosis, which can lead to produce the excessive extracellular matrix [29, 30]. The transdifferentiation of fibroblast to myofibroblast is regulated by the inflammatory factors [31]. Among the inflammatory factors, TGF-β is the primary factor that drives fibrosis in most, if not all [32]. TGF-β has three isoforms, including TGF-β1, TGF-β2, and TGF-β3. TGF-β1 was verified to induce myofibroblast differentiation and synthesis of extracellular matrix [33]. Our results suggested that either massage or electroacupuncture therapy could significantly downregulate the protein expression of TGF-β1 compared with the model group, with the strongest inhibition. Moreover, previous studies revealed that TGF-β1 could induce the expression of CTGF and α-SMA in fibroblasts [34]. CTGF acted as the important downstream factor of TGF-β1 and was an essential mediator for the composition of extracellular matrix, and α-SMA was a characteristic actin isoform expressed in myofibroblasts [35]. The combination of massage and electroacupuncture therapy could significantly downregulate the protein expression of CTGF compared with the model group. Furthermore, we found that the apoptosis of myofibroblast was induced in response to the combination of both massage and electroacupuncture. The increase of impaired matrix degradation in fibrosis can be reflected by decreased MMP-1 and increased TIMP-1, which is the inhibitor of MMP-1 [36]. The increased extracellular matrix leads to interstitial fibrosis by increasing the production of collagen type I and III. MMP-1 as the specific enzyme for collagen type I and III could help with the degradation of extracellular components. In contrast, TIMP-1, the inhibitor of MMP-1, inhibits collagen degradation. The dynamic change of MMP/TIMP is important for the degradation of extracellular matrix [37]. Our results suggested that the MMP-1 expression was downregulated, and the TIMP-1 expression was upregulated after blunt trauma to rat skeletal muscle, which resulted in downregulation of MMP-1/TIMP-1. This could destroy the balance of the synthesis and degradation for collagen type I and III and accelerate the fibrosis of injured skeletal muscle. The combination of massage and electroacupuncture therapy could reverse the unbalance of MMP-1/TIMP-1 induced by blunt trauma to rat skeletal muscle. These results suggested that the combination of massage and electroacupuncture therapy could reduce the inflammatory response and promote the MMP-1/TIMP-1 to degrade the excessive collagen type I and III and thus inhibit the fibrosis of injured skeletal muscle.

5. Conclusion

In the present study, the combination of massage and electroacupuncture therapy exhibited better effects than monotherapy on reducing skeletal muscle fibrosis in blunt trauma-induced rat skeletal muscle fibrosis. The combination of massage and electroacupuncture therapy could not only reduce the degree of fibrosis by downregulating the mRNA expressions and protein expressions of TGF-β1 and CTGF but also regulate MMP-1/TIMP-1 balance for extracellular matrix production. These results indicate that the combination of massage and electroacupuncture therapy has an additive effect on alleviating the fibrotic process.

Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Na Zhao and Bo Liu contributed equally to this work.

Acknowledgments

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Supplementary Materials

Table S1: the real-time RT-PCR oligonucleotide primers. (*Supplementary Materials*)

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Research Article

Examining the Effector Mechanisms of the Feishu Acupoint (BL13) in the Treatment of Pneumonia Based on Systematic Acupuncture and Moxibustion Research

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Background. Pneumonia is a serious global health problem. In traditional Chinese medicine, acupuncture or moxibustion is used to directly stimulate select acupoints on the surface of the human body and produce physical stimulation to further stimulate regulatory functions in the body, strengthening bodily resistance, eliminating disease, and adjusting the viscera. However, this Chinese medicine knowledge does not include the specific mechanisms of action or targets of acupoints. Therefore, an in-depth research is needed. *Methods*. An acupoint-element database was constructed, and the target elements of the Feishu point were screened. The UniProt-Swiss-Prot sublibrary was used to obtain correct gene name information. The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database and GEO2R were used to analyze differentially expressed genes in pneumonia. The STRING database was used to analyze interactions, construct a network of the Feishu point efficacy system in pneumonia, and elucidate the mechanisms of action. *Results*. The Feishu point comprises 34 elements in total. The protein interaction analysis has 38 nodes and 115 edges. The Feishu point efficacy system-pneumonia system network shows that cytokine signaling in the immune system, signaling by interleukins (ILs), IL-4 and IL-13 signaling, and the immune system may be related to immunity and inflammation. The Feishu point efficacy system regulating pneumonia showed that FCER2, IL4R, FASLG, TGFB1, IL6R, STAT6, IL1B, CASP3, IL5RA, IL2RB, MYD88, SQSTM1, IL12RB1, IFNGR1, ADAM17, and CDH1 are the main targets. *Conclusion*. From the perspective of systematic acupuncture and moxibustion, the Feishu point regulates cytokine signaling in the immune system, signaling by ILs, IL-4 and IL-13 signaling, and the immune system by targeting FCER2, IL4R, FASLG, TGFB1, IL6R, STAT6, IL1B, CASP3, IL5RA, IL2RB, MYD88, SQSTM1, IL12RB1, IFNGR1, ADAM17, and CDH1, thereby regulating pneumonia.

1. Background

The Feishu point (BL13) was first described in *Lingshu Backshu*. It belongs to the bladder meridian (BL) and is the Back-Shu point of the lung. This point is located 1.5 inches beside and below the spinous process of the third thoracic vertebra on the back. The indications for Feishu point stimulation include pulmonary symptoms such as cough, expectoration, wheezing, and shortness of breath. Acupoint massage [1], applicator therapy [2, 3], and iontophoresis [4] at the Feishu point can shorten the time to cough improvement and reduce cough symptom scores, inflammatory indicator levels, and adverse reaction rates in patients with pneumonia. Moxibustion at the Feishu point can regulate fatigue and muscle aches in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [5]. The efficacy of the Feishu point in the treatment of lung diseases has been verified for thousands of clinical applications, but studies on its specific mechanisms of action are rare. Because of the modernization and globalization of

traditional Chinese medicine, the mechanisms of action of the Feishu point must be addressed.

Investigations of the mechanisms of action of acupoints must first explain the nature of meridians and collaterals. However, controversy regarding the nature of meridians and collaterals remains. Some scholars working from the perspective of modern medicine consider meridians and collaterals to be complicated three-dimensional structures composed of nerves, blood vessels, muscles, tendons, and fasciae. Acupuncture and moxibustion stimulate acupoints to cause changes in liquid components, nerve conduction, and physical properties in local tissues, which result in the alleviation or cure of the disease condition in the human body [6, 7]. However, some scholars disagree and consider that the understanding of the nature of "meridians and collaterals" is inseparable from the specific lifestyle, cognitive orientation, and language framework of traditional Chinese medicine, namely, traditional Chinese medicine culture and theory. The evolution of modern medical explanations and research methods deviates from the original meaning of the original classics [8]. The present study considered that current explorations of the philosophical theory of traditional Chinese medicine and the development of science and technology cannot resolve the issue of the nature of meridians and collaterals. However, methods that indirectly elucidate the mechanisms of action of acupoints may be applied.

Proteins are important constituents of all cells and tissues in the human body. All substances with physiological activities in the human body, such as amines, neurotransmitters, polypeptide hormones, antibodies, enzymes, nuclear proteins, and proteins that play "carrier" roles in cell membranes and in blood, are inseparable from proteins. Proteins play very important roles in the regulation of physiological functions and the maintenance of metabolism. Acupoint stimulation produces positive and negative regulatory effects in the body. The external presentation is disease improvement and recovery, and the internal presentation is an increase or decrease in protein levels. Therefore, the direct detection of changes in protein levels in the body bypasses the "black box" of the mechanisms of action of acupoints and indirectly elucidates these mechanisms (Figure 1).

Pneumonia is a serious global health problem [9], and it is the most common cause of death from infectious diseases worldwide, causing approximately 3.5 million deaths annually [10]. South Asia and Sub-Saharan Africa have the most severe pneumonia infections because of the economy and lack of medical care. Additionally, most deaths from pneumonia occur in children because of their unique physiological and pathological factors [11]. Acupuncture and moxibustion directly stimulate select acupoints on the surface of the human body and produce physical stimulation to further stimulate regulatory functions in the body, including the dredging of meridians and collaterals, the coordination of qi and blood, the strengthening of bodily resistance, the elimination of disease, and adjustment of the viscera. Because no special apparatus or equipment is required, acupuncture and moxibustion may be used as major methods for treating pneumonia in areas with scarce resources.

In summary, this study used systematic acupuncture and moxibustion to investigate the specific mechanisms of action of the Feishu point for regulating pneumonia. The results provide new ideas and methods for the globalization and moderation of acupuncture and moxibustion.

2. Methods

2.1. Construction of the Acupoint-Element Database. We performed a massive search of the literature in the China National Knowledge Infrastructure (CNKI), the Wanfang database, the Chongqing VIP Information network, PubMed, and Web of Science and constructed an acupoint-element (target) database for the 12 main meridians, conception vessels (CVs), and governor vessels (GVs) that included a total of 361 acupoints. Dedicated personnel rechecked, debugged, double-checked, and supplemented the results.

2.2. Retrieval of Target Elements of the Feishu Point. The Feishu point was retrieved from the acupoint-element (target) database, and a total of 34 target elements were obtained. Related information can be found on the website https://www.tcmmodel.com/feishu/. These target elements were entered into the UniProt database (https://www.uniprot.org/), one at a time for correction and conversion into gene names. UniProt is a nonredundant protein sequence database with the most complete sequence data and the most abundant annotated information worldwide. The more than 500,000 sequences in its Swiss-Prot sublibrary were manually reviewed and annotated. The correction databases used in this study used the Swiss-Prot sublibrary.

2.3. The Protein-Protein Interaction (PPI) Network and the Feishu Point Efficacy System Mechanism Network. The STRING database (https://string-db.org/) was used to search for interactions between proteins (gene names may be entered). It contains direct physical interactions between proteins and indirect functional correlations between proteins. The present study used the PPI network in STRING to evaluate elemental relationships. The median interaction score was 0.4.

The interaction score was the standard for determining the connections of the PPI network. The score was obtained via examination of the predictive performance using the public reference set (KEGG database), with the real relationship as the standard. The calculation formula was as follows:

$$Q = \log\left\{\frac{\left(N_{\text{together}} \cdot N_{\text{total}}\right)}{\left[\left(N_{\text{alone}_1} + 1\right) \cdot \left(N_{\text{alone}_2} + 1\right)\right]}\right\}. \quad (1)$$

The diagram in this study concealed nodes that did not have interaction to ensure the reliability of the interactions in the PPI network.

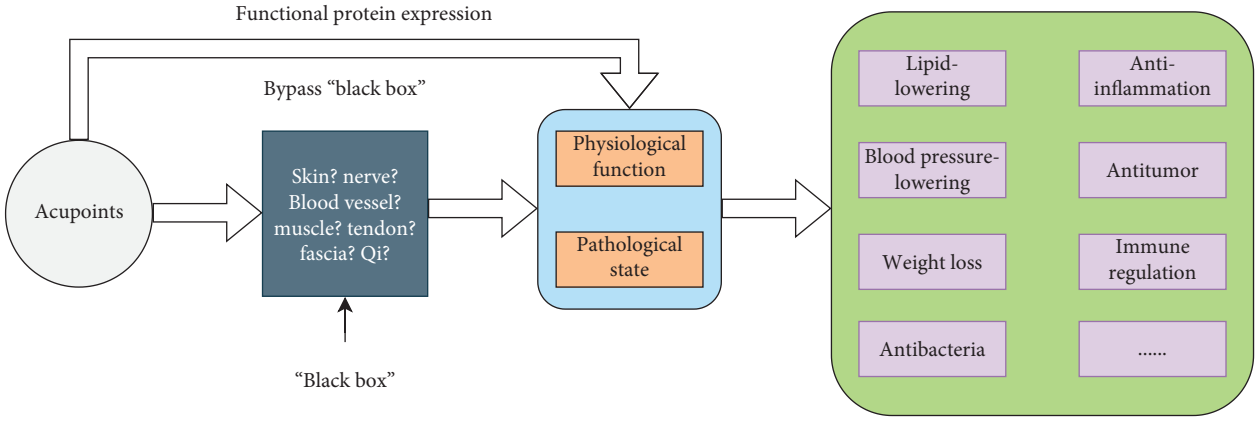

Figure 1: Road map of the mechanisms of action of acupoints.

2.4. Plotting the Network Diagram. The network of the interaction between the pneumonia efficacy system and pneumonia was plotted using Cytoscape (version 3.7.2). The degree value reflected the number of connections that a node in the network had with other nodes, which was the most intuitive parameter for determining the influence of a node. When the degree was larger, the influence was greater. Betweenness was the ratio of the closeness of nodes that passed through a certain node to the total closeness. When the betweenness was larger, the node was more important in the network. Closeness was the reciprocal of the average distance of the shortest paths between a node and other nodes in the network. Closeness considered the average length of the shortest paths between each node and other nodes. When a node was closer to other nodes, its closeness was higher.

2.5. Differentially Expressed Genes in Pneumonia. Differentially expressed genes were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/). The GEO is an online database that may be used to retrieve the gene expression data for any species. The GSE103119 data used in the present study contained the transcriptome data of 152 children with community-acquired pneumonia and 20 healthy children. The GEO2R online tool was used for analysis [12], and $|\text{LogFC}| > 1$ and $P < 0.05$ were used as the standards for screening differentially expressed genes. Children were chosen as subjects because children are the main victims of pneumonia.

2.6. Reactome Pathways. Reactome (https://reactome.org/) is a biological database of various reactions and biological pathways in the human body that has annotated more than 70% of human proteins. This study used the Reactome database for the functional analysis. The false discovery rate (FDR) was defined as the percentage of all discoveries that were false. When the FDR was smaller, the result was more reliable. The formula was as follows:

$$\text{FDR} = \frac{\text{FP}}{(\text{FP} + \text{TP})}, \tag{2}$$

where FP (false positive) indicated that the detection result of a negative experimental sample was positive and TP (true positive) indicated that the detection result of a positive experimental sample was positive.

3. Results

3.1. Identification of Target Elements in the Feishu Point Efficacy System. The targets were the focus of the construction of the Feishu point efficacy system and were considered the basic routes by which acupoints exert their effects. The targets of the Feishu point were obtained via a database search using the search term "Feishu" (Table 1).

3.2. Correction of Target Element Names in the Feishu Point Efficacy System. The target element names in the database were obtained from the literature search, and the nomenclature of targets in the literature varied slightly according to the reagent company. The target elements were entered into the UniProt database one at a time to standardize the nomenclature. All genes were uniformly expressed as gene names (Table 2).

3.3. Construction of the Structure and Relationship of the Feishu Point Efficacy System. The STRING website was used to perform a protein interaction analysis of all targets of lung acupoints. Then, the network of relationships among the elements was built, and a structure-relation network was constructed (Figure 2). There were 38 nodes and 115 edges in the network. To extract the key elements in the network, calculations were performed based on degree, betweenness, and closeness. Elements with the higher-than-average degree, betweenness, and closeness values were considered key elements in the network (Table 3).

3.4. Construction of the Feishu Point Efficacy System Network. To describe the Feishu point efficacy system network in detail, Reactome pathway enrichment was used to define the

Table 1: Target elements of the Feishu point.

Acupoint	Targets
Feishu point	LC3-II [13, 14], *p*62 [13], IL-6 [13, 15], Bax [16], Fas [16], FasL [16], Bcl-2 [16], IL-1β [17, 18], E-cad [19], MyD88 [20], caspase 3 [20], p-gp [21, 22], IL-12 [23, 24], IL-4 [15, 23, 24], IgE [23], IL-2 [24, 25], IL-5 [24], IFN-γ [15, 26, 27], TGFβ1 [25, 28], β-catenin [29], MMP-7 [29], WISP-1 [29], Wnt3a [29], Beclin-1 [14], ACTH [15], CYP3A [22], TNF-α [22], sICAM-1 [25], CCL1 [30], CCR8 [30], STAT6 [30], ChAT [31], AChE [31], and mAChRs [31]

Table 2: Correction of target elements of the Feishu point.

Acupoint	Gene name
Feishu point	MAP1LC3B, SQSTM1, IL6R, BAX, FAS, FASLG, BAD, IL1B, CDH1, MYD88, CASP3, ABCB5, IL12RB1, IL4R, FCER2, IL2RB, IL5RA, IFNGR1, TGFB1, CTNNBIP1, MMP7, CCN4, Wnt3a, BECN1, MC2R, Cyp3a2, ADAM17, sICAM-1, CCL1, CCR8, STAT6, CHAT, AChE, and SLURP2

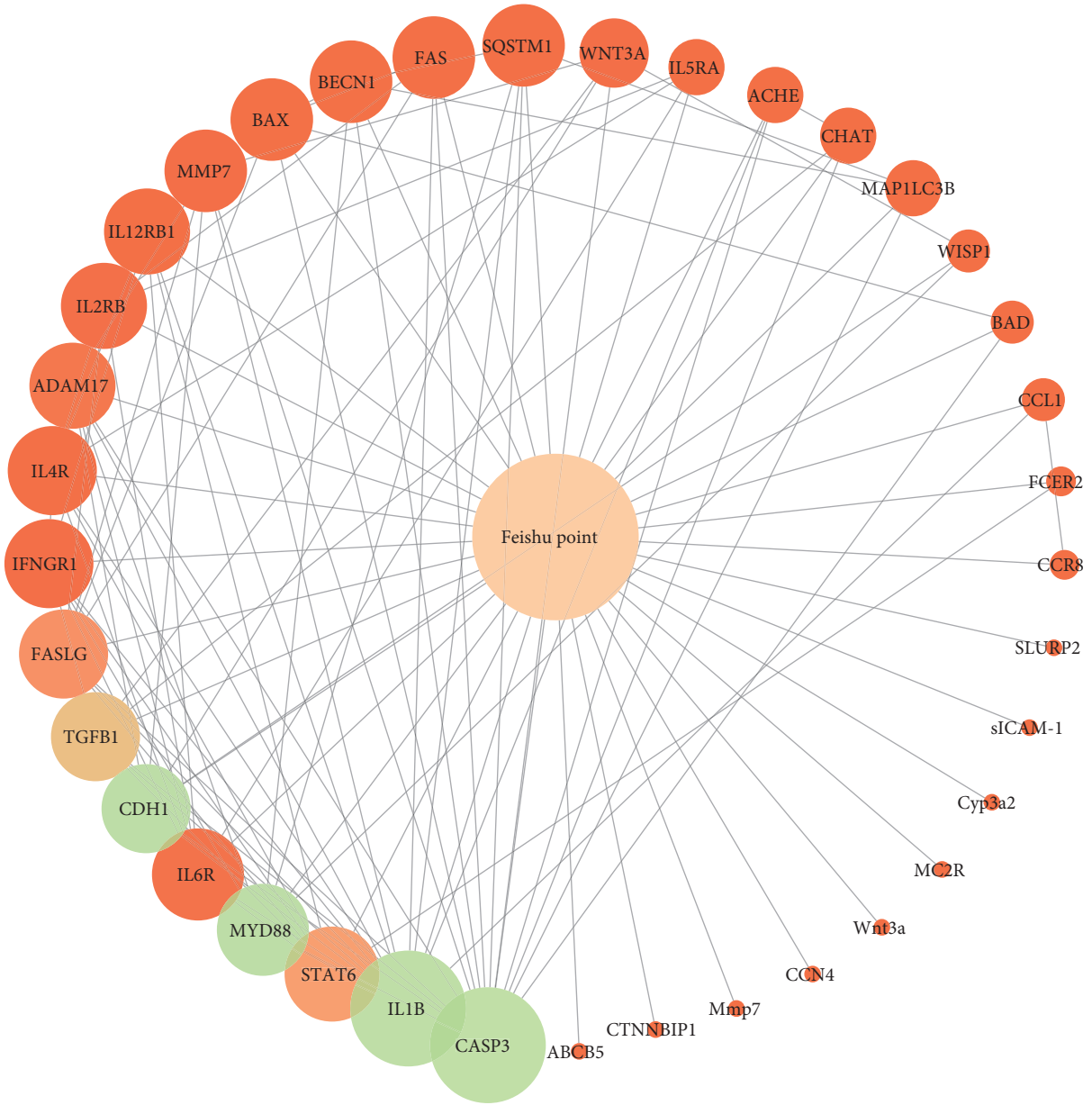

Figure 2: Relationship between the structure and efficacy of the Feishu point. The circle represents the degree, with larger circles corresponding to greater degrees. The color represents betweenness, with light green representing extensive betweenness and red representing limited betweenness.

Table 3: Key elements of the Feishu point efficacy system.

Gene name	Degree	Betweenness	Closeness
CASP3	17	0.07995257	0.64912281
IL1B	17	0.06253873	0.64912281
STAT6	10	0.01346287	0.578125
MYD88	9	0.03216609	0.56923077
IL6R	9	0.00638972	0.55223881
CDH1	8	0.03350434	0.56060606
TGFB1	8	0.01788336	0.56060606
FASLG	8	0.0114984	0.55223881
IFNGR1	8	0.00362446	0.56060606
IL4R	8	0.00296964	0.54411765
ADAM17	7	0.00740502	0.55223881
IL2RB	7	0.00467134	0.53623188
IL12RB1	7	9.26E-04	0.53623188
MMP7	6	0.00372635	0.4625
BAX	6	0.0025025	0.53623188
BECN1	6	0.00234163	0.54411765
FAS	6	0.00187688	0.53623188
SQSTM1	6	0.00146575	0.54411765

boundary of the system using the FDR parameter as the standard (Table 4).

3.5. Differentially Expressed Genes in Pneumonia. Differentially expressed genes in pneumonia were obtained from the GEO database. Differentially expressed genes were screened from healthy children and children with community-acquired pneumonia, and a total of 1074 genes were obtained (supplementary file (available here)).

3.6. Construction of the Feishu Point Efficacy System-Pneumonia System Network. Systems do not exist in isolation. Similar to elements, systems also have relationships and structures. Therefore, the Feishu point efficacy system may also have a relationship with the pneumonia system. This relationship would be the key point of interaction between the two systems, and all physiological and biological functions would be based on this relationship. The construction of the Feishu efficacy system-pneumonia system network was equivalent to artificially determining the boundary of the Feishu point efficacy system so that only the parts of the Feishu point efficacy system that were relevant to pneumonia could be studied. After establishing the correspondence between the Feishu point efficacy system and the differentially expressed targets in pneumonia, the Feishu point efficacy system-pneumonia network relationship diagram was constructed (Figure 3). The Feishu point efficacy system had 4 pathways that could function in pneumonia, including cytokine signaling in the immune system, signaling by interleukins (ILs), IL-4 and IL-13 signaling, and the immune system. These 4 pathways were all associated with immunity and inflammation.

3.7. Mechanism of Action of by which the Feishu Point Efficacy System Regulates Pneumonia. The roles of all of the proteins in the Feishu point efficacy system in the efficacy network were further examined. A median interaction score of 0.4 was used as the cutoff value for the plot shown in Figure 4. Sixteen proteins were involved in the mechanism by which the Feishu point efficacy system regulates pneumonia. FCER2, IL4R, FASLG, TGFB1, IL6R, STAT6, and IL1B were involved in the functions of all pathways, and CASP3, IL5RA, IL2RB, MYD88, SQSTM1, and IL12RB1 were involved in cytokine signaling in the immune system, IL signaling, and the immune system. IFNGR1 and ADAM17 were involved in cytokine signaling in the immune system and the immune system, and CDH1 was involved in the immune system.

4. Discussion

4.1. Theoretical Basis of Systematic Acupuncture and Moxibustion. The extensive application of network pharmacology, systems pharmacology, and integrated pharmacology fields in recent years has promoted the development of molecular biology and pharmacology-related subjects that integrate systems science theory. These methods and technologies have focused on interactions between the body and drugs at the overall level and have promoted the preliminary establishment of a theory of systematic traditional Chinese medicine [32, 33]. Against the backdrop of systematic traditional Chinese medicine theory, the present study proposed a concept of systematic acupuncture and moxibustion that could affect the mechanisms of action of acupoints, meridians, and collaterals. This concept considered systematic acupuncture and moxibustion as a multidisciplinary subject of epistemology, a methodology that uses systems science thinking and methods to understand acupoints, meridians, and collaterals. This emerging discipline uses systems engineering technology to elucidate microscopic and macroscopic structures and relationships and can be applied to the science and art of acupoints, meridians, and collaterals. It is also an interdisciplinary subject that integrates acupuncture and moxibustion with systems science to unravel the self-similarity, self-organization, and self-adaptability of systems and elucidate the inheritance, additivity, and emergence of overall functions. The basic concept of systematic acupuncture and moxibustion includes elements and the relationships among elements, structures, boundaries, and functions. In systems science, a system is a whole that interacts with the external environment and has specific capabilities [34]. Elements are basic factors that constitute a system. Relationships refer to relationships between elements at the same level. Structure refers to the relationships between elements at different levels. The boundary is the boundary between the inside and outside of a system. Functions refer to the characteristics, behavior, performance, and functions of interactions between the system and the external environment [35]. Therefore, the use of data mining technology and biological network technology to confirm elements, structures, boundaries, and functions from the perspective of systematic acupuncture and moxibustion may systematically explain the mechanisms of action of acupoints, meridians, and collaterals (Figure 5).

Table 4: Top 10 Reactome enrichment results for the Feishu point efficacy system.

Reactome ID	Reactome description	FDR	Matched element
HSA-1280215	Cytokine signaling in the immune system	$7.18E-12$	ADAM17, CASP3, FASLG, FCER2, IFNGR1, IL12RB1, IL1B, IL2RB, IL4R, IL5RA, IL6R, MYD88, SQSTM1, STAT6, and TGFB1
HSA-449147	Signaling by interleukins	$1.31E-11$	CASP3, FASLG, FCER2, IL12RB1, IL1B, IL2RB, IL4R, IL5RA, IL6R, MYD88, SQSTM1, STAT6, and TGFB1
HSA-162582	Signal transduction	$1.23E-08$	ADAM17, BAD, BAX, CASP3, CCL1, CCR8, CDH1, CTNNBIP1, FAS, FASLG, FCER2, IL2RB, IL5RA, IL6R, MC2R, MYD88, SQSTM1, STAT6, TGFB1, and WNT3A
HSA-6785807	Interleukin-4 and Interleukin-13 signaling	$3.34E-08$	FASLG, FCER2, IL1B, IL4R, IL6R, STAT6, and TGFB1
HSA-73887	Death receptor signaling	$1.62E-07$	ADAM17, BAD, CASP3, FAS, FASLG, MYD88, and SQSTM1
HSA-168256	Immune system	$4.40E-07$	ADAM17, CASP3, CDH1, FASLG, FCER2, IFNGR1, IL12RB1, IL1B, IL2RB, IL4R, IL5RA, IL6R, MYD88, SQSTM1, STAT6, and TGFB1
HSA-109581	Apoptosis	$9.96E-06$	BAD, BAX, CASP3, CDH1, FAS, and FASLG
HSA-5357801	Programmed cell death	$9.96E-06$	BAD, BAX, CASP3, CDH1, FAS, and FASLG
HSA-193704	*p*75 NTR receptor-mediated signaling	$1.68E-05$	ADAM17, BAD, CASP3, MYD88, and SQSTM1
HSA-5357769	Caspase activation via the extrinsic apoptotic signaling pathway	0.00032	CASP3, FAS, and FASLG

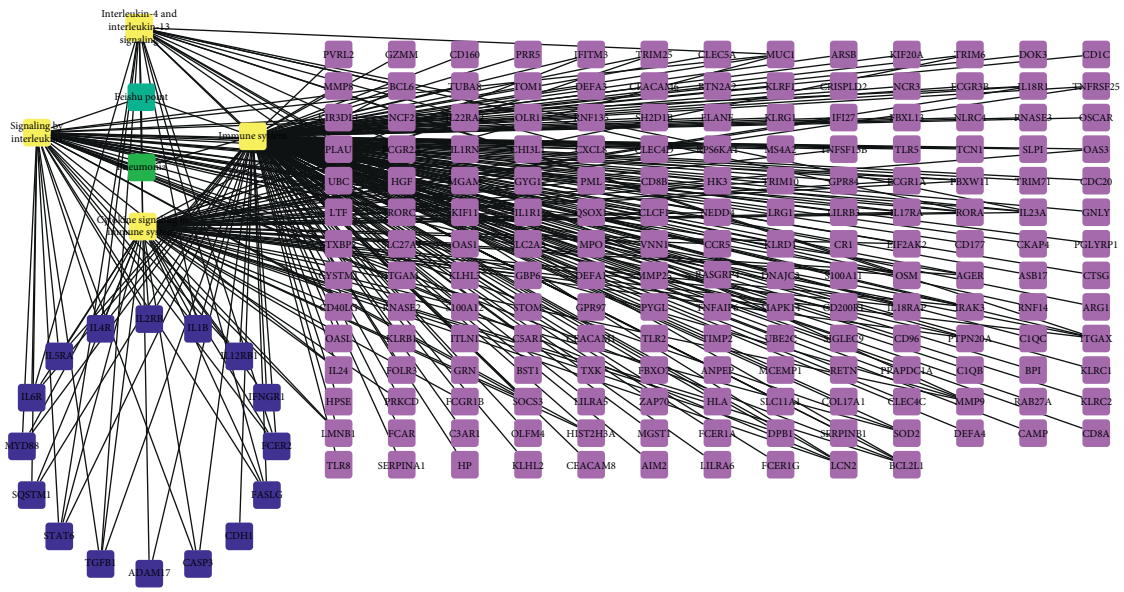

Figure 3: The Feishu point efficacy system-pneumonia system interaction network.

4.2. Investigation of the Mechanisms of Action of the Feishu Point for the Regulation of Pneumonia. The present study demonstrates that the Feishu point exerts therapeutic effects in pneumonia via cytokine signaling in the immune system, IL signaling, IL-4 and IL-13 signaling, and the immune system. Cytokines are peptide substances that are extensively present in the body. Cytokines are secreted by immune cells, such as macrophages, B lymphocytes, T lymphocytes, and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. Based on their biological functions, cytokines are divided into chemokines, interferons, ILs, lymphokines, and tumor necrosis factors (TNFs). Cytokines in body fluids or tissues regulate the development, differentiation, and function of immune cells at a lower level under normal conditions to maintain the balance of the cytokine network. Once the body encounters abnormalities, the balance between anti-inflammatory cytokines and proinflammatory cytokines is impaired, and large amounts of proinflammatory cytokines are produced. Cells and intracellular signaling pathways also change accordingly, resulting in inflammation and immune processes [36]. IL-4 is primarily produced by activated T helper 2 (Th2) cells and mast cells, and it plays a role in the production of Th2 cytokines and the immunoregulatory function of lymphocytes and macrophages. IL-4 blocks antibody-dependent cytotoxicity, inhibits the production

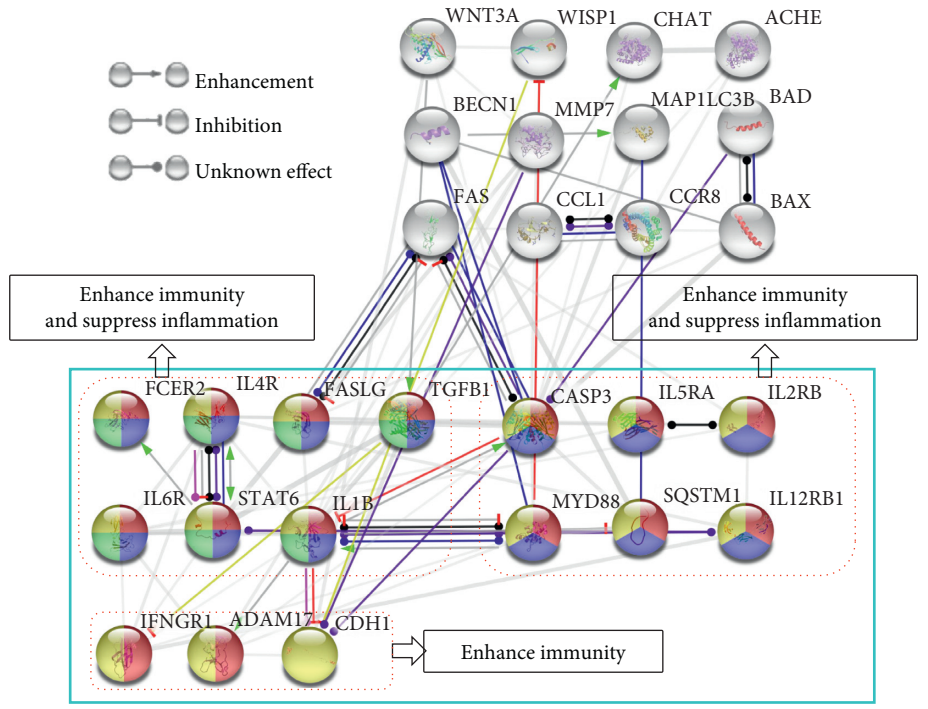

Figure 4: Diagram of the mechanisms of action of the Feishu point efficacy system in the treatment of pneumonia. Red indicates cytokine signaling in the immune system, blue indicates signaling by ILs, green indicates IL-4 and IL-13 signaling, yellow indicates the immune system, and white indicates other systems.

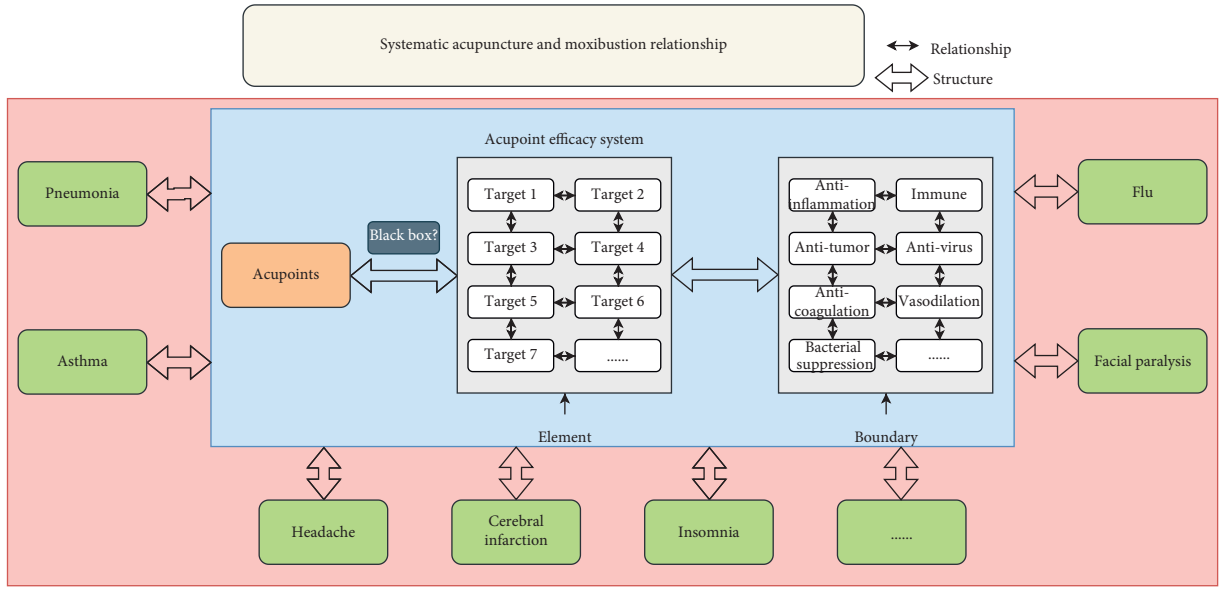

Figure 5: Systematic acupuncture and moxibustion research methods.

of IL-1β, TNF-α, prostaglandin E2, IL-6, IL-8, and nitric oxide, and induces B cells to produce IgG and IgE [37]. IL-13 also downregulates the synthesis of TNF-α, IL-1, and IL-6 and promotes IgE synthesis [38]. Overall, as important cytokines, IL-4 and IL-13 play important roles in mitigating the development of inflammatory responses and increasing immunity. At the gene level, this study demonstrates that the Feishu point regulates cytokine signaling in the immune system, signaling by ILs, IL-4 and IL-13 signaling, and the immune system via FCER2, IL4R, FASLG, TGFB1, IL6R, STAT6, IL1B, CASP3, IL5RA, IL2RB, MYD88, SQSTM1, IL12RB1, IFNGR1, ADAM17, and CDH1 gene regulation to exert effects on the regulation of inflammation and immunity (Figure 6), thereby preliminarily elucidating the mechanisms of action of the Feishu point in treating pneumonia.

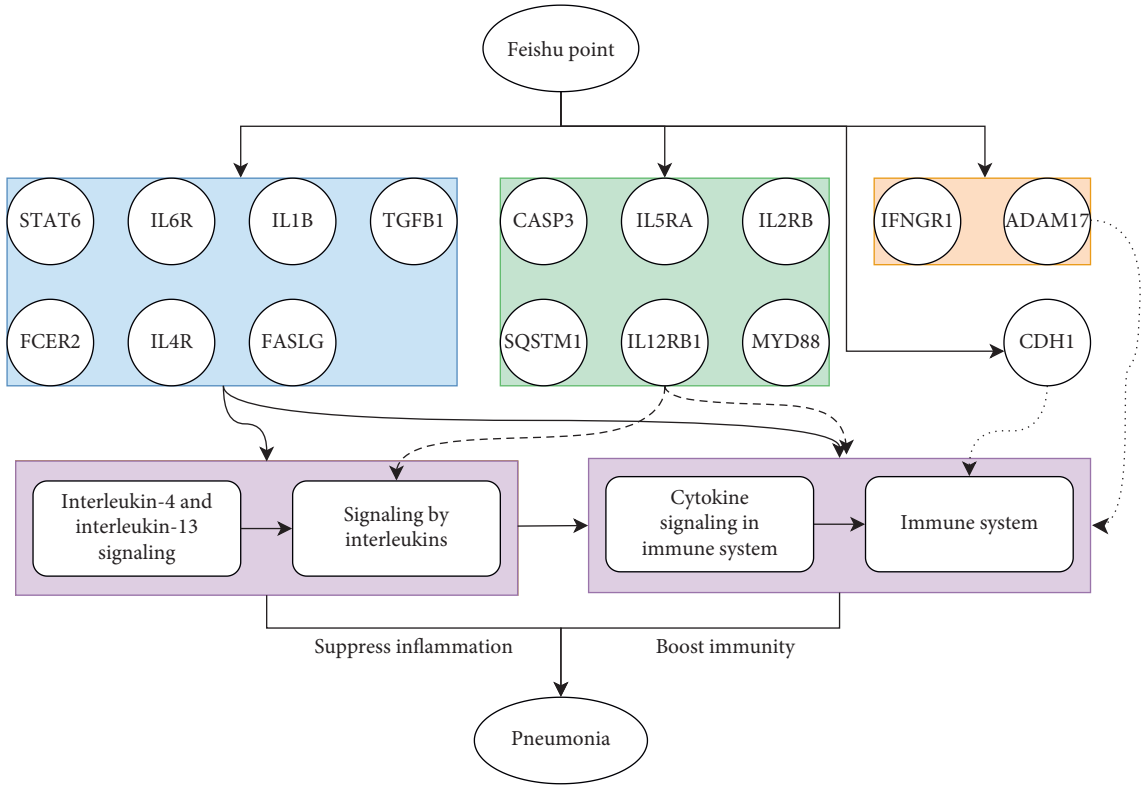

Figure 6: Mechanisms of action of the Feishu point in the regulation of pneumonia.

Our research has some clinical significance. It differs from previous clinical studies that focused on different methods, such as application [39], cupping [40], and acupoint injection [41] to stimulate the Feishu acupoint and achieve the treatment goal. Our research starts with the physiological and pathological mechanisms that occur after acupoints are stimulated and adds specific biological evidence supporting their clinical effectiveness. Furthermore, unlike the previously described single mechanism [42, 43], we present a more comprehensive description of the mechanism through which lung acupoints regulate pneumonia.

4.3. Feishu Point Treatment System. The Feishu point is a Back-Shu point on the bladder meridian (BL). It is located 1.5 inches beside and below the spinous process of the third thoracic vertebra and corresponds to the lung. The Feishu point is also the Back-Shu point of the lung. A complete review of the Feishu point treatment system based on existing studies showed very good efficacy of the Feishu point for the treatment of lung diseases, including cough [44], pneumonia with dyspnea and cough [45, 46], asthma [47], acute lung injury [48], allergic rhinitis [49], chronic obstructive pulmonary disease [50], lung cancer [51, 52], acute tonsillitis [49], and bronchiolitis [53]. The Feishu point efficacy system is also used to treat heart diseases, such as chronic heart failure [54], and skin diseases, such as chronic urticaria [55], shingles [56], abdominal urticaria [57], and acne [58]. To specifically elucidate the Feishu point efficacy system, it was considered as a whole according to systematic acupuncture and moxibustion theory and the evidence presented above [44–58] and plotted as a diagram (Figure 7). The diagram shows that the Feishu point treatment system is a complicated network that includes various diseases. This point plays a role in lung diseases, heart diseases and some skin diseases. This treatment system completely satisfies the theoretical bases of "all meridians and vessels converge in the lung to assist the heart in promoting blood circulation" and "the lung connects with the skin," and it is a specific presentation of traditional Chinese medicine theory.

In summary, the elucidation of the therapeutic effect of the Feishu point from an overall perspective via the construction of the Feishu point treatment system met the basic

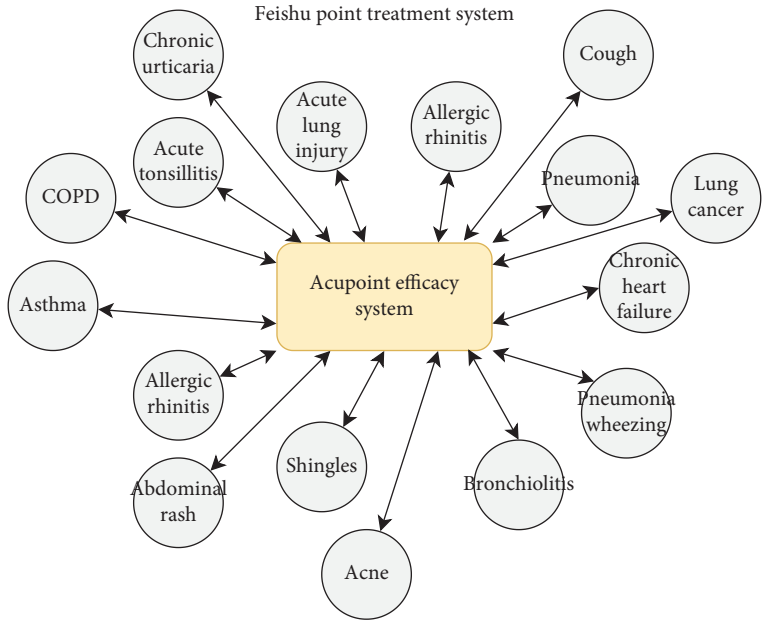

Figure 7: The Feishu point treatment system.

characteristics of traditional Chinese medicine theory and provided scientific evidence for studies on the elucidation of the systematic functions and modernization of acupoints.

5. Conclusion

(1) From the perspective of systematic acupuncture and moxibustion, this research explored the specific mechanism by which the Feishu point regulates pneumonia and provided new ideas for the development of acupuncture and moxibustion.

(2) The Feishu point regulates cytokine signaling in the immune system, signaling by ILs, IL-4, and IL-13 signaling, and the immune system by targeting FCER2, IL4R, FASLG, TGFB1, IL6R, STAT6, IL1B, CASP3, IL5RA, IL2RB, MYD88, SQSTM1, IL12RB1, IFNGR1, ADAM17, and CDH1, thereby regulating pneumonia.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Yan Xu, Jiali Cai, and Weibin Li conceived and designed the study. Xinna Wang, Hanying Xu, Jingkun Miao, Yan Mei, and Qixiong Chen collected the data. Fang Liu and Hongtao Cui wrote the manuscript. All of the authors were responsible for reviewing the data. All of the authors read and approved the final manuscript. Yan Xu, Jiali Cai, and Weibin Li-contributed equally to this work.

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Supplementary Materials

1074 differentially expressed genes in pneumonia. (*Supplementary Materials*)

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Review Article

Quality Evaluation of Randomized Controlled Trials of *Rhodiola* Species: A Systematic Review

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Background. *Rhodiola* is a worldwide used medicinal plant for its various medicinal functions, and the number of randomized controlled trials (RCTs) of *Rhodiola* is increasing in recent years. This study aims to evaluate the reporting quality and risk of bias of the current RCT reports of different *Rhodiola* species. *Methods*. Six databases including Embase, PubMed, Web of Science, the Cochrane Library, ClinicalTrial.gov, and China National Knowledge Infrastructure were searched to identify RCTs that used *Rhodiola* as a single intervention and were published in English or Chinese from inception to December 2020. The Consolidated Standards of Reporting Trials (CONSORT) 2010 statement was used as the checklist for assessment, and a scoring system was applied to the evaluation of RCTs. Score 0 represents no reporting or inadequate reporting, and score 1 represents adequate reporting. The risk of bias of the included studies was also assessed using the Cochrane Risk of Bias tool. *Results*. A total of 39 RCTs were included in this study, including 23 RCTs of *Rhodiola rosea* (*R. rosea*), 8 RCTs of *Rhodiola crenulata* (*R. crenulata*), and 8 RCTs of *Rhodiola wallichiana* (*R. wallichiana*). None of the included studies met all the CONSORT statement criteria, and the reporting quality of RCTs of the three *Rhodiola* species was all generally poor. Based on the risk of bias assessment, the majority of included studies were judged to have an unclear risk of bias in most domains due to inadequate reporting. *Conclusions*. There is inadequate reporting among the included RCTs of different *Rhodiola* species, and RCTs of *Rhodiola* with higher reporting quality and better methodological quality are needed.

1. Introduction

The *Rhodiola* genus, belonging to the Crassulaceae family, is a medicinal plant that has been traditionally used as an adaptogen and tonics, as well as in remedies of anti-inflammatory and antidepression in Europe and Asia since ancient times [1, 2]. *Rhodiola* is inclined to grow in mountainous areas of low temperature such as precipices, tundra, riverbanks, and rock ledges in the northern hemisphere including Asia, North and Central Europe, and North America [3]. It consists of more than 100 species, of which about 20 species are used as traditional medicines, including *Rhodiola rosea* (*R. rosea*), *Rhodiola crenulata* (*R. crenulata*), *Rhodiola sacra* (*R. sacra*), and *Rhodiola kirilovii* [4, 5]. Growing studies have demonstrated that *Rhodiola* possesses varieties of bioactivities such as antistress, antifatigue, antioxidant, antitumor, anti-inflammation, antiaging, antiradiation, and immunomodulatory [2, 6, 7]. Currently, apart from the traditional applications, *Rhodiola* is also used to treat bronchial asthma and coronary heart disease, to improve chronic fatigue syndrome and physical activity, and to alleviate mountain sickness syndrome in clinical practices [8, 9]. Owing to its numerous functions and economic value, *Rhodiola* has been developed into diverse products including drugs, food supplements, food additives, drinks, and cosmetics [10, 11].

In order to evaluate the claimed functions of *Rhodiola*, a randomized controlled trial (RCT) design has been

conducted since the 1960s [9]. As the "gold standard" for evaluating the efficacy of most interventions [12], RCTs are increasingly being used in traditional medicine. RCTs with high reporting quality are essential for the interpretation and reproducibility of a trial and for proper healthcare decisions [13]. On the contrary, low-quality RCTs reports may lead to distorted findings of a study, thus drawing misleading conclusions [14]. Besides, it has been previously shown that RCTs of low quality may be included in the meta-analysis, thereby biasing downstream treatment [15]. Therefore, with the necessity to evaluate the quality of RCTs, several quality assessment tools have been developed. The Consolidated Standards of Reporting Trials (CONSORT) statement [16], which was first published in 1996 and updated in 2001 and 2010, is regarded as the "gold standard" for evaluating the reporting quality of RCTs, aiming to help improve the reporting quality of RCTs [17, 18]. Another special assessment tool for methodological quality assessment, namely, the Cochrane Collaboration's Risk of Bias, has been developed to evaluate the study validity in clinical trials by the Cochrane Collaboration since 2008 [19].

In terms of the quality of *Rhodiola* RCTs, there have been several previous studies evaluating the quality of RCTs of *R. rosea* [20, 21]. However, as a globally used herbal medicine, different *Rhodiola* species are widely used, and there has been an increasing number of RCTs of *Rhodiola* species in recent years. Furthermore, there has been an increasing demand for *Rhodiola* products due to their multiple medicinal functions, while the similarities and differences between different *Rhodiola* species still need further investigation. There has been little systematic effort so far to evaluate the quality of RCTs of different *Rhodiola* species. Therefore, this study is designed to evaluate the reporting quality and methodological quality of the current RCTs of different *Rhodiola* species by using the CONSORT 2010 statement and the Cochrane Risk of Bias tool, aiming to help improve the quality of future clinical trials of *Rhodiola* species and provide useful information for the utilization and product development of *Rhodiola* species.

2. Methods

This study complied with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [22]. The reporting quality of included RCTs was assessed by utilizing the CONSORT 2010 checklist [16], and the methodological quality was evaluated in accordance with the Cochrane Risk of Bias tool [23].

2.1. Eligibility Criteria. Clinical trials with RCT design that used *Rhodiola* as intervention and were published from inception to December 2020 were included for the eligibility screening. Studies were excluded if they met the following criteria: (i) study subjects being not human, (ii) *Rhodiola* combined with other medicines as therapy intervention, (iii) protocol, (iv) not published in English or Chinese, (v) not peer-reviewed journal articles (e.g., theses or dissertations, conference abstracts), (vi) no full-text available, and (vii) no results posted.

2.2. Information Sources and Database Search. To identify the eligible studies, literature search was conducted in 6 electronic databases including Embase, PubMed, Web of Science, the Cochrane Library, ClinicalTrial.gov, and China National Knowledge Infrastructure (CNKI). Besides, the references of the final included studies and relevant reviews were also screened for additional eligible studies. Different strategies were used to search the six databases. For the Cochrane Library, "Rhodiola" was used as the search item, and the study type was limited to trials. For the ClinicalTrial.gov, "Rhodiola" was used as the search item in the search bar "Other terms". The strategies used for the other four databases are presented as follows:

Embase: ("rhodiola"/exp OR rhodiola) AND (random*: ab, ti OR ((clinical NEXT/1 trial*): de,ab,ti) OR 'health care quality'/exp)

PubMed: (Rhodiola) AND ((randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized [tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab] NOT (animals [mh] NOT humans [mh])))

Web of Science: TS = (Rhodiola AND (randomized OR randomized OR randomization OR randomization OR placebo* OR (random* AND (allocat* OR assign*)) OR (blind* AND (single OR double OR treble OR triple))))

CNKI (The search terms are in Chinese): SU = hóng jǐng tiān AND SU = lín chuáng AND FT = suí jī

2.3. Study Selection. All the search results were retrieved from the six databases for eligibility screening. After duplicates were removed, the first round of screening was conducted with the title and abstract of each study based on the eligibility criteria mentioned above. In the second round of screening, the full text of the rest of the studies in the first round was further accessed for eligibility. At last, the included studies were grouped into different *Rhodiola* species. The study selection was conducted by two authors (X. L. and W. C.) independently, and any discrepancies were discussed by S. W., X. L., and W. C. to achieve consensus.

2.4. Data Items and Extraction. The information of several descriptive characteristics, namely, nonmarketed/marketed products, formulation, focused functions, publication year, locations of RCTs, sample size, and trial length, was extracted from the full text and recorded in a standard form using Microsoft Excel 16.39. This process was performed by two authors (X. L. and W. C.) individually. Any discrepancies were discussed to resolve by S. W., X. L., and W.C. in order to reach an agreement.

2.5. CONSORT Items and Scoring System. All the items (37 subdivided items) of the CONSORT statement were included in the reporting quality assessment of RCT reports. To measure the adherence of each study to each item, a scoring system with two grades was used. The reviewers can grade the study for each item with a score 0 or 1. Score 1 indicates that the study adequately reported the item, while score 0 means that the study did not report or inadequately reported the item. In addition, if the item was not applicable for the study, the item would be excluded from the quality assessment. The scoring process was conducted by two authors (X. L. and W. C.) independently, and any discrepancies were discussed by Y. W., S. W., X. L., and W. C. to achieve consensus.

2.6. Risk of Bias Assessment. The six domains of bias in the Cochrane Risk of Bias tool were all included in the assessment: (i) selection bias (random sequence generation, allocation concealment), (ii) performance bias (blinding of participants and personnel), (iii) detection bias (blinding of outcome assessment), (iv) attrition bias (incomplete outcome data), (v) reporting bias (selective reporting), and (vi) other bias. Each domain was given a judgment of "low risk of bias", "unclear risk of bias", or "high risk of bias" in line with the criteria in the Cochrane handbook [23] by the two authors (X. L. and W. C.) independently. Any discrepancies were discussed with another author S. W. to achieve consensus.

2.7. Synthesis of Results and Statistical Methods. The number of RCTs by nonmarketed/marketed products, formulation, focused functions, publication year, and locations of RCTs was analyzed descriptively to give an overview of the characteristics of the included RCTs.

In the part of quality evaluation, with the above scoring system applying to the quality assessment, each study was given a total score. Due to the different number of applicable items of each study, an average score that ranges from 0 to 1 was finally obtained by dividing the total score by the number of items, with a higher score indicating a higher reporting quality of the study.

To identify sections in which authors could improve the reporting quality, the average CONSORT score on each grouped item (e.g., title and abstract, trial design) was synthesized. Besides, in order to assess the influence of the publication of the CONSORT statement on the reporting quality, the CONSORT scores of RCTs were analyzed by publication years. Pearson's Correlation Coefficient (Pearson's r) with 2-tailed significance between the average CONSORT score and the sample size/trial length of RCTs was also performed to explore the correlation between them.

The synthesis of results was all analyzed by grouping RCTs into different *Rhodiola* species to see their respective quality. Excel 16.39 (Microsoft, Redmond, WA, USA), Prism 9.0 (GraphPad Software, San Diego, CA, USA), SPSS 26.0 (IBM, Armonk, NY, USA), and RevMan 5.4 (The Cochrane Collaboration, 2020) were used to perform the analysis.

3. Results

3.1. Study Selection. The study selection process is presented in Figure 1. A total of 923 records were retrieved from the six databases (205 from PubMed, 176 from web of science, 243 from Embase, 23 from ClinicalTrials.gov, 101 from Cochrane Library, and 175 from CNKI), and no additional study was found through other sources. After duplicates were removed, 657 potentially relevant records were assessed for eligibility. A total of 595 of these records were excluded for the following reasons: (i) not RCT reports (403), (ii) protocol (1), (iii) no result presented (13), (iv) not published in English or Chinese (5), (v) not involving *Rhodiola* in the trial intervention (7), and (vi) *Rhodiola* combined with other medicine as therapy intervention (167). The remaining 62 records were subsequently screened with the full text for further exclusion. An additional 22 records were excluded then, including 8 records of which full text was not available, 2 records that were not randomized, and 12 records that involved combination intervention. The remaining 40 records were included after the full-text screening and were grouped into four *Rhodiola* species. As a result, there are 23 records (22 published in English, 1 published in Chinese) of *R. rosea*, 8 records of *R. crenulata* (2 published in English, 6 published in Chinese), 8 records (all published in Chinese) of *Rhodiola wallichiana* (*R. wallichiana*), and 1 record of *R. sacra*. Considering that only 1 record of *R. sacra* being evaluated is not significant, 1 record of *R. sacra* is not included in our quality assessment

3.2. Study Characteristics. As illustrated in Figure 2(a), the included 39 RCTs investigated several conditions regarding physical capacity and exercise-induced damage, mental performance and disorder, cerebro-cardiovascular disease, hypoxemia, and others. Among these three *Rhodiola* species, RCTs of *R. rosea* mainly focus on physical capacity and exercise-induced damage ($n = 13$), as well as mental performance and disorder ($n = 11$). 4 studies of *R. rosea* investigated conditions of both physical capacity and exercise-induced damage and mental performance and disorder, and thus, the total number of *R. rosea* studies here in this result is 4 more than the previous 23 studies. As for RCTs of *R. wallichiana*, they mainly focus on cerebro-cardiovascular disease ($n = 6$). While for *R. crenulata*, except for the 4 conditions mentioned above, RCTs of it also investigated conditions regarding chronic obstructive pulmonary disease (COPD), immunity, and highland alopecia, there is not a focused condition among the above.

The characteristics of the trial interventions of the three *Rhodiola* species were also summarized. In process of collecting data about whether a trial used marketed or nonmarketed products, 1 study of *R. rosea* and 3 studies of *R. crenulata* were excluded from the analysis due to their missing data regarding the intervention products. As shown in Figure 2(b), for *R. rosea* and *R. wallichiana*, more than half of the RCTs used marketed *Rhodiola* products (*R. rosea* = 12, *R. wallichiana* = 5) as the interventions. It is worth noting that the marketed products used in the included RCTs of *R.*

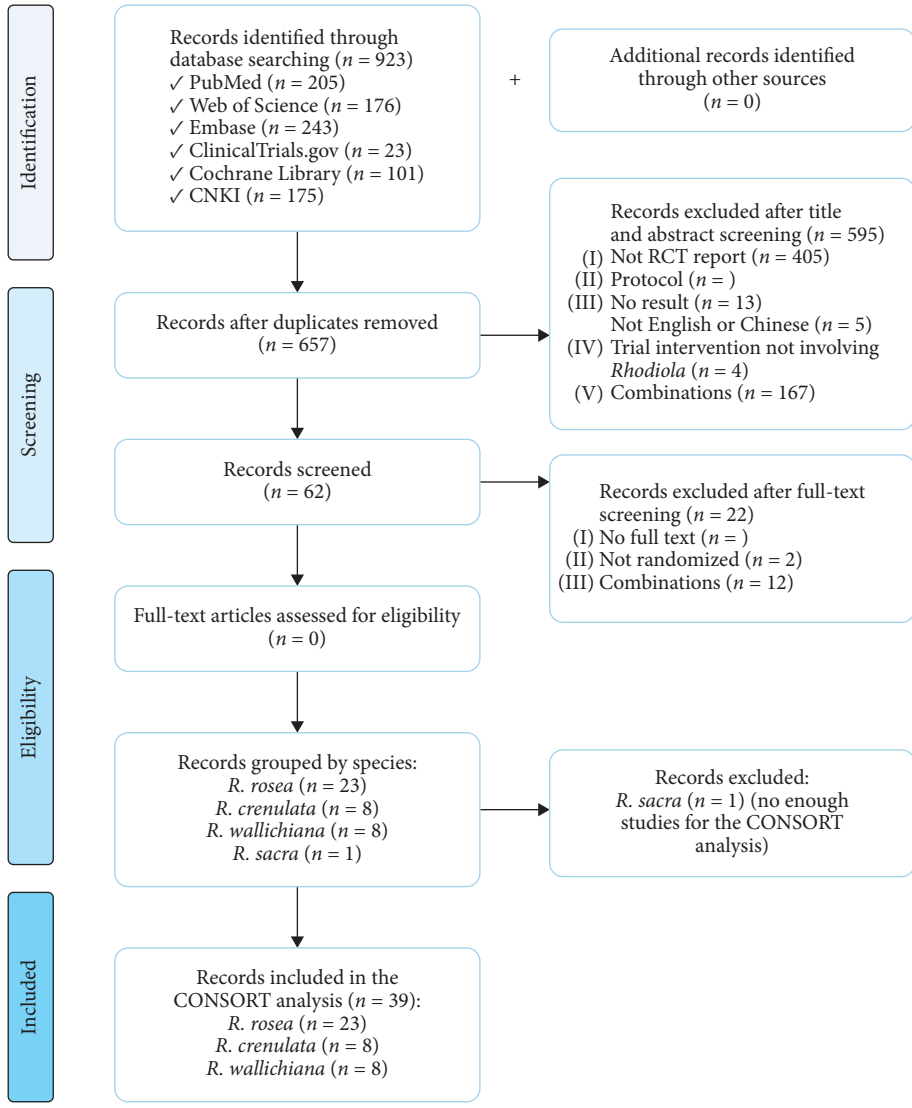


Figure 1: PRISMA flow diagram of study selection.

rosea are all health food, while for those of *R. wallichiana*, they are all approved drugs. As for the included five RCTs of *R. crenulata*, the intervention products used were all nonmarketed.

Another characteristic of the trial interventions we looked into is the product formulations. As seen in Figure 2(c), the formulations of the intervention products range from the capsule, tablet, oral solution to injection. Among RCTs of *R. rosea*, capsule ($n = 10$) and tablet ($n = 8$) are the top two formulations of intervention products. Notably, products of the tablet are only adopted in RCTs of *R. rosea*, while products of the capsule are adopted in all three species and are the most commonly used formulation among RCTs of *R. crenulata* ($n = 7$). In regard to oral solution, there are only 3 RCTs (*R. rosea* = 2, *R. crenulata* = 1) that chose the oral solution products as the interventions. In particular, products of injection are only used in RCTs of *R. wallichiana* and they account for 75% of the *R. wallichiana* RCTs.

A landscape regarding the number of the three *Rhodiola* species RCTs grouped by the publication year is presented in Figure 2(d). Among the included 39 RCTs, an RCT of *R. crenulata* published in 1998 is the earliest published, followed by *R. rosea* (in 2000) and *R. wallichiana* (in 2005). As can be seen from the curve of the number of RCTs cumulated by publication year, the rising trend of RCTs of *R. rosea* is the most rapid, while the rising trend of RCTs of the other two species is relatively much slow. The RCTs of *R. rosea* were published the most in 2009, with 4 studies. But overall, they were published most intensively from 2013 to 2019, with 11 studies being published in total. Regarding the RCTs of *R. wallichiana*, they were published the most in 2016, with 4 studies, while the publication years of RCTs of *R. crenulata* were relatively scattered, with the most annual publication of 2 studies in 2015.

A world map in Figure 2(e) presents the trial locations with the number of RCTs. It can be seen that RCTs of both *R. crenulata* and *R. wallichiana* all took place in China while the

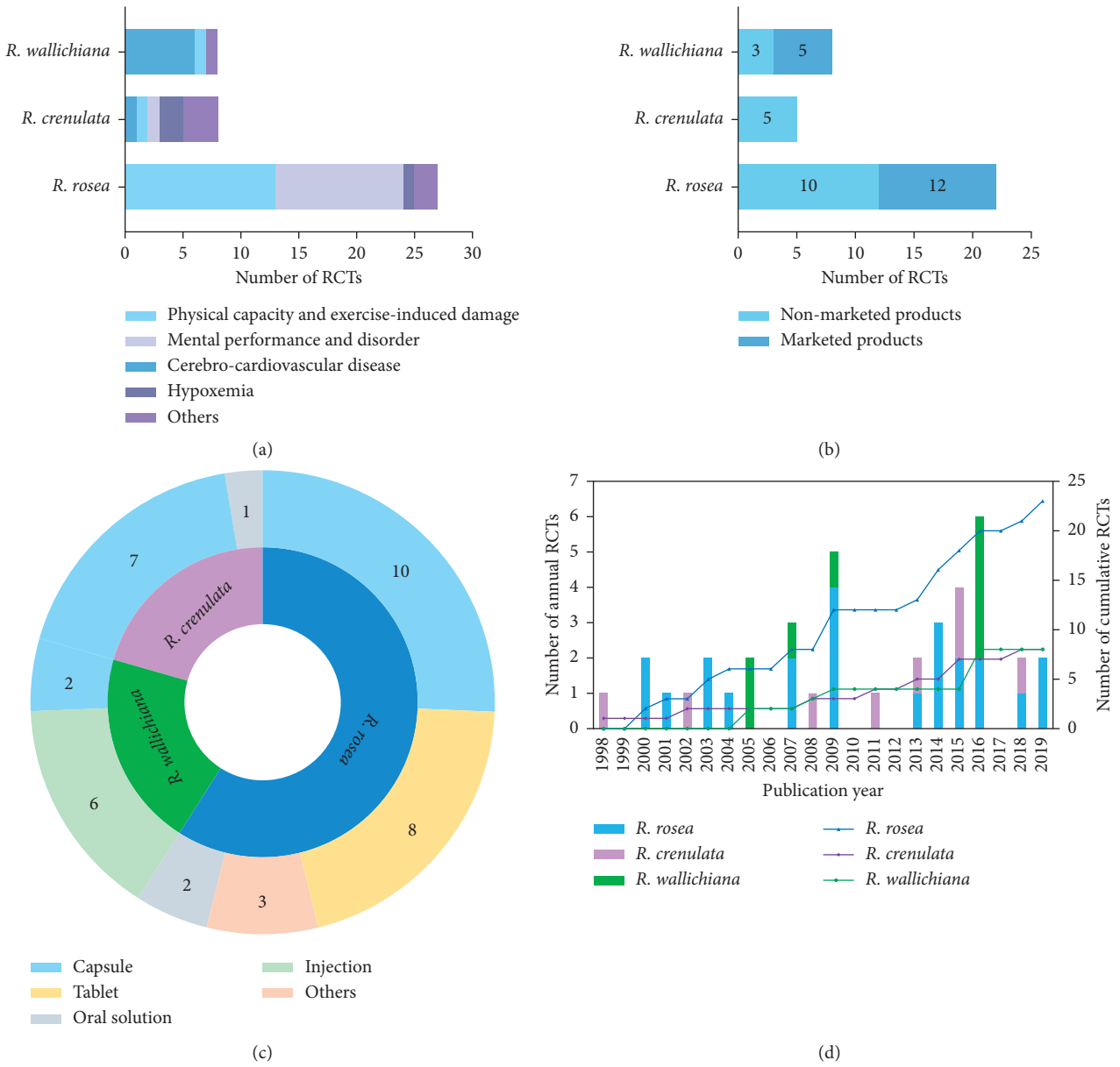
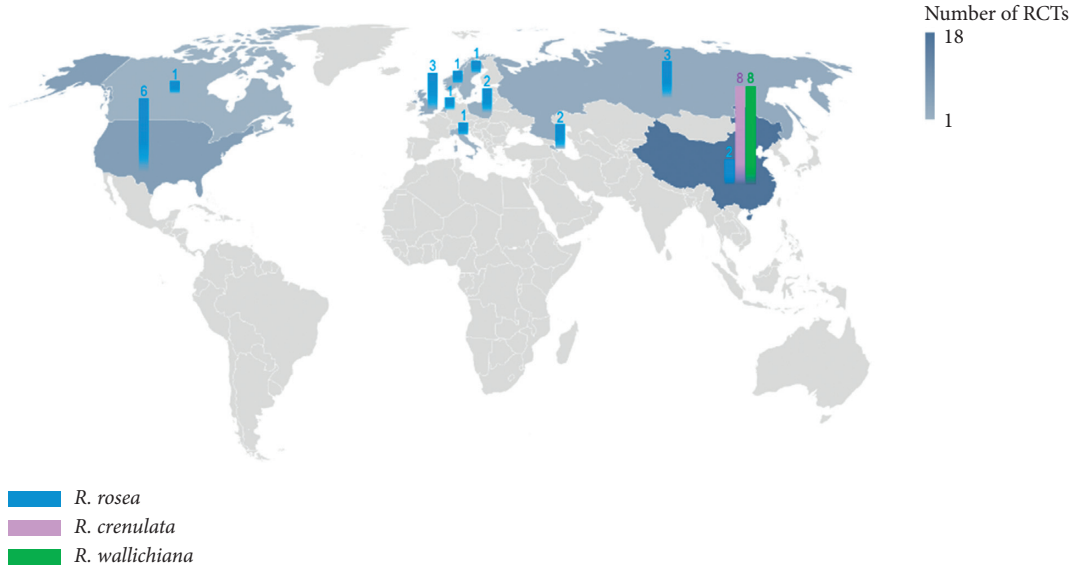


Figure 2: Continued.



(e)

Figure 2: Number of Rhodiola RCTs by focused functions, nonmarketed/marketed products, formulation, publication year, and locations of RCTs. (a) The number of RCTs by focused functions. (b) The number of RCTs by the use of nonmarketed/marketed products as an intervention in trials. (c) The number of RCTs by the formulation of intervention products in trials. (d) The annual/cumulative number of RCTs by the publication year. The blue column represents the annual number of RCTs of *R. rosea*. The purple column represents the annual number of RCTs of *R. crenulata*. The green column represents the annual number of RCTs of *R. wallichiana*. The blue curve represents the cumulative number of RCTs of *R. rosea*. The purple curve represents the cumulative number of RCTs of *R. crenulata*. The green curve represents the cumulative number of RCTs of *R. wallichiana*. (e) The number of RCTs by the locations of the trials.

locations of RCTs of *R. rosea* are across Europe, Asia, and North America, including Russia ($n = 3$), UK ($n = 3$), Poland ($n = 2$), Sweden ($n = 1$), Norway ($n = 1$), Italy ($n = 1$), Netherlands ($n = 1$), Armenia ($n = 2$), China ($n = 2$), USA ($n = 6$), and Canada ($n = 1$). It is obvious that the USA is the country where RCTs of *R. rosea* took place most. In addition, locations of RCTs of *R. rosea* also clustered in Europe with a number of more than a half.

3.3. CONSORT Evaluation. In Figure 3, the given scores of each study for each item in accordance with the compliance with the CONSORT statement are presented in the form of a heat map, with the studies sorted by the publication year. Apparently, none of the included studies met all the CONSORT statement criteria. The overall CONSORT compliance of the RCTs of the three *Rhodiola* species is poor as many of the items display a great proportion of light color area which means that the items were inadequately reported. For example, regarding item 1a "Identification as a randomized trial in the title", only 4 of the 23 RCTs of *R. rosea*, 3 RCTs of *R. crenulata*, and 1 RCTs of *R. wallichiana* reported it. For another item 7a "How sample size was determined", only 5 RCTs of *R. rosea*, 1 RCTs of *R. crenulata*, and none of the RCTs of *R. wallichiana* reported it. In the case of item 14a "Dates defining the periods of recruitment and follow-up", only 2 RCTs of *R. rosea*, 2 RCTs of *R. crenulata*, and none of the RCTs of *R. wallichiana* reported it.

The score of each RCT of each species and the average score of each species are presented in Figure 4. The scores of the 39 RCTs range from 0.03 to 0.88, and the overall mean scores for RCTs of *R. rosea*, *R. crenulata*, and *R. wallichiana* are 0.33, 0.25, and 0.17, respectively. In addition, the standard deviation (SD) of scores of *R. rosea*, *R. crenulata*, and *R. wallichiana* is 0.2, 0.25, and 0.07, respectively, which means that the homogeneity among reporting quality of *R. rosea* and *R. crenulata* is much smaller than that of *R. wallichiana*.

Although the overall scores of RCTs of the three *Rhodiola* species do not differ from each other much, their scores vary from item to item (Figure 5). Firstly, RCTs of these three species are all in poor compliance with the CONSORT statement regarding the items including title and abstract, sample size, randomization, participant flow, recruitment, outcomes and estimation, ancillary analyses, generalizability, registration, protocol, and funding. Moreover, RCTs of *R. crenulata* are also in very poor compliance with the items of background and objective, outcomes, blinding, statistical methods, baseline data, and limitations, while RCTs of *R. wallichiana* are also of poor reporting quality regarding the items of background and objective, trial design, outcomes, blinding, baseline data, limitations, and interpretation.

All of the above-highlighted items are with scores below 0.3. On the other hand, there are still several items with relatively good compliance and with scores above 0.7. RCTs of *R. rosea* are in good compliance with items including background and objectives and interpretation while for RCTs of *R. crenulata*, they are in good compliance with the item of numbers analyzed and harms. As for RCTs of *R.*

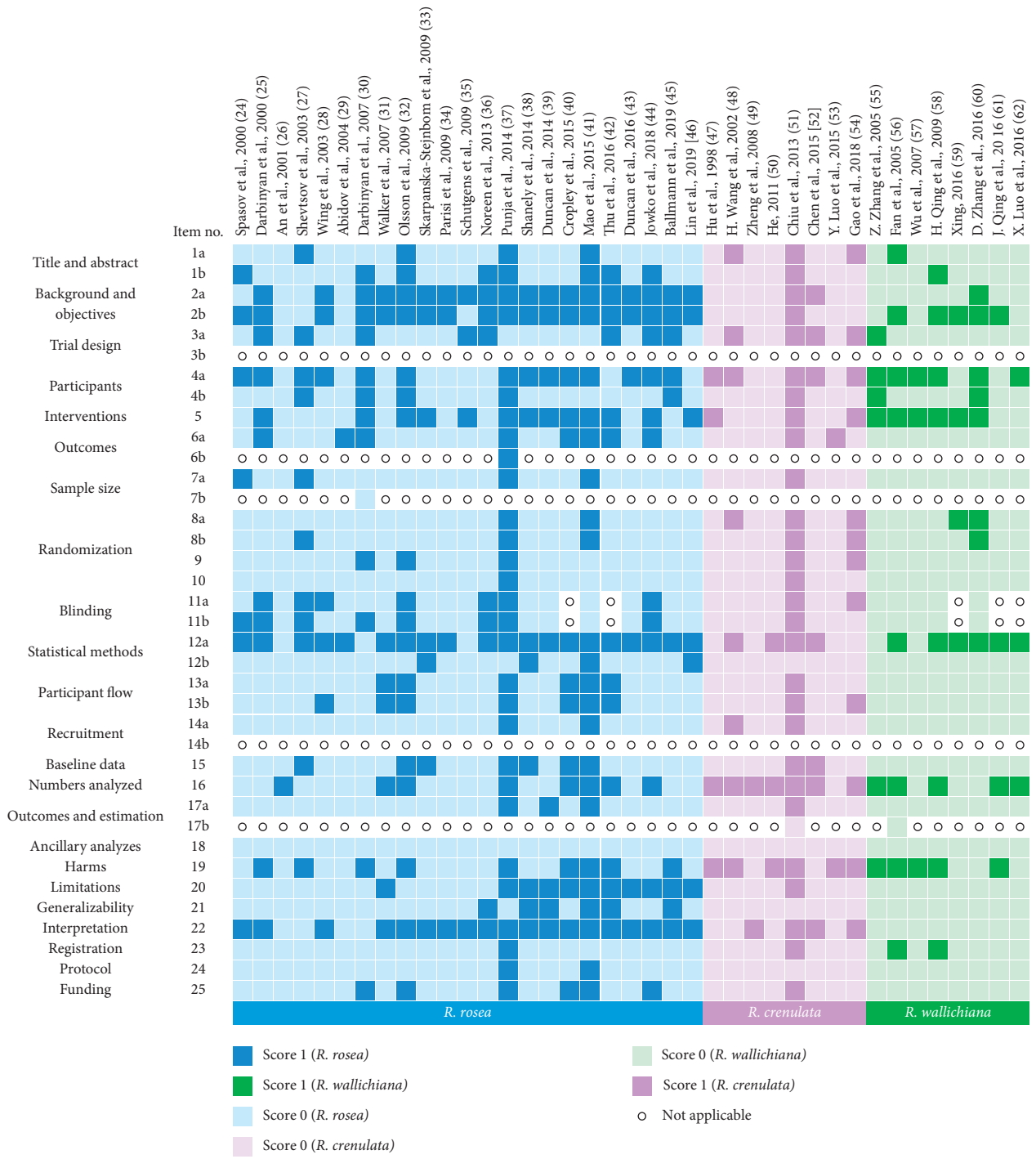


Figure 3: Scoring based on the compliance with the CONSORT statement of each RCT of *R. rosea*, *R. crenulata*, and *R. wallichiana* on subdivided items (Title and abstract: 1a, 1b; Background and objectives: 2a, 2b; Trial design: 3a, 3b; Participants: 4a, 4b; Interventions: 5; Outcomes: 6a, 6b; Sample size: 7a, 7b; Randomization: 8a, 8b (Sequence generation), 9 (Allocation concealment mechanism), 10 (Implementation); Blinding: 11a, 11b; Statistical methods: 12a, 12b; Participant flow: 13a, 13b; Recruitment: 14a, 14b; Baseline data: 15; Numbers analyzed: 16; Outcomes and estimation: 17a, 17b; Ancillary analyses: 18; Harms: 19; Limitations: 20; Generalizability: 21; Interpretation: 22; Registration: 23; Protocol: 24; Funding: 25) [24–62].

wallichiana, they are in good compliance with the items of interventions.

In order to explore the impact of publication year on the CONSORT adherence, especially after 2010 when the CONSORT statement was updated, the CONSORT scores of the RCTs of each *Rhodiola* species were classified in years and are presented in Figure 6, as well as the annual average score. As the results of RCTs of *R. rosea* show, among the years before 2010, the average scores of 2001 and 2004 are particularly low, which are 0.03 and 0.06, respectively, while

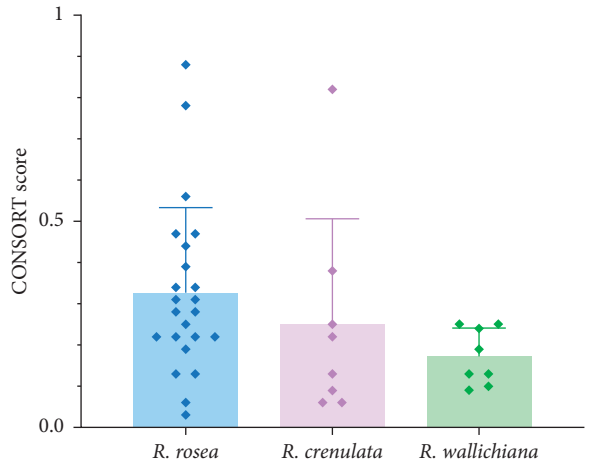


Figure 4: The CONSORT score of each RCT of *R. rosea*, *R. crenulata*, and *R. wallichiana* with the mean score and SD of each species.

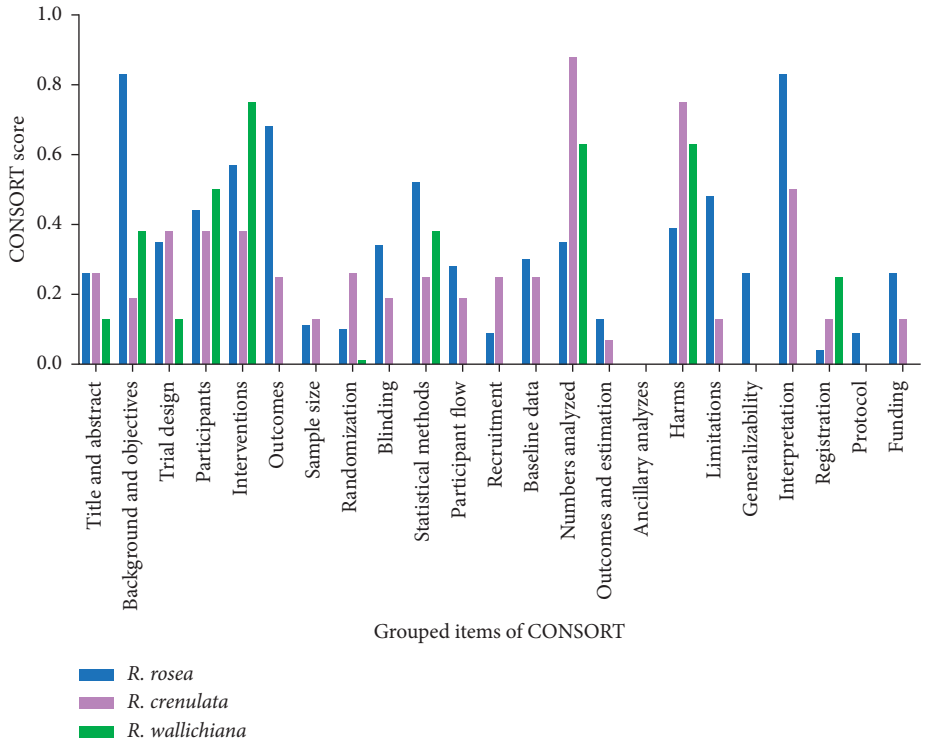


Figure 5: The overall CONSORT score of RCTs of *R. rosea*, *R. crenulata*, and *R. wallichiana* on grouped items of CONSORT.

the remaining four years (2000, 2003, 2007, and 2009) are around 0.4. As for the years after 2010, the average scores for 2014 and 2015 are 0.49 and 0.63, respectively, making them the two highest-scoring years among all. However, the scores of the remaining years do not improve much compared with those of the years before 2010. For the RCTs of *R. crenulata*, the average score of each year before 2010 is below 0.3. Among the years after 2010, the average score of 2013 reaches 0.82, but the scores of the other three years are all low, which are below 0.4. With respect to the RCTs of *R. wallichiana*, after 2010, a total of 4 studies were published all in 2016. However, the annual average score only reaches 0.15 which is a bit different from those of the years before 2010. As for the specific items, back to Figure 3, it is obviously that for RCTs of *R. rosea*, the compliance with CONSORT on limitations and generalizability has been significantly improved since 2013. To be specific, on the item of limitations, 10 of the 11 RCTs published after 2010 were all rated with score 1 while only 1 RCT published before 2010 was rated with score 1. As for the item of generalizability, 6 of the 11 RCTs published after 2010 were rated with score 1 while none of the RCTs published before 2010 were rated with score 1. However, on other items of RCTs of *R. rosea*, as well as the items of RCTs of *R. crenulata* and *R. wallichiana*, the

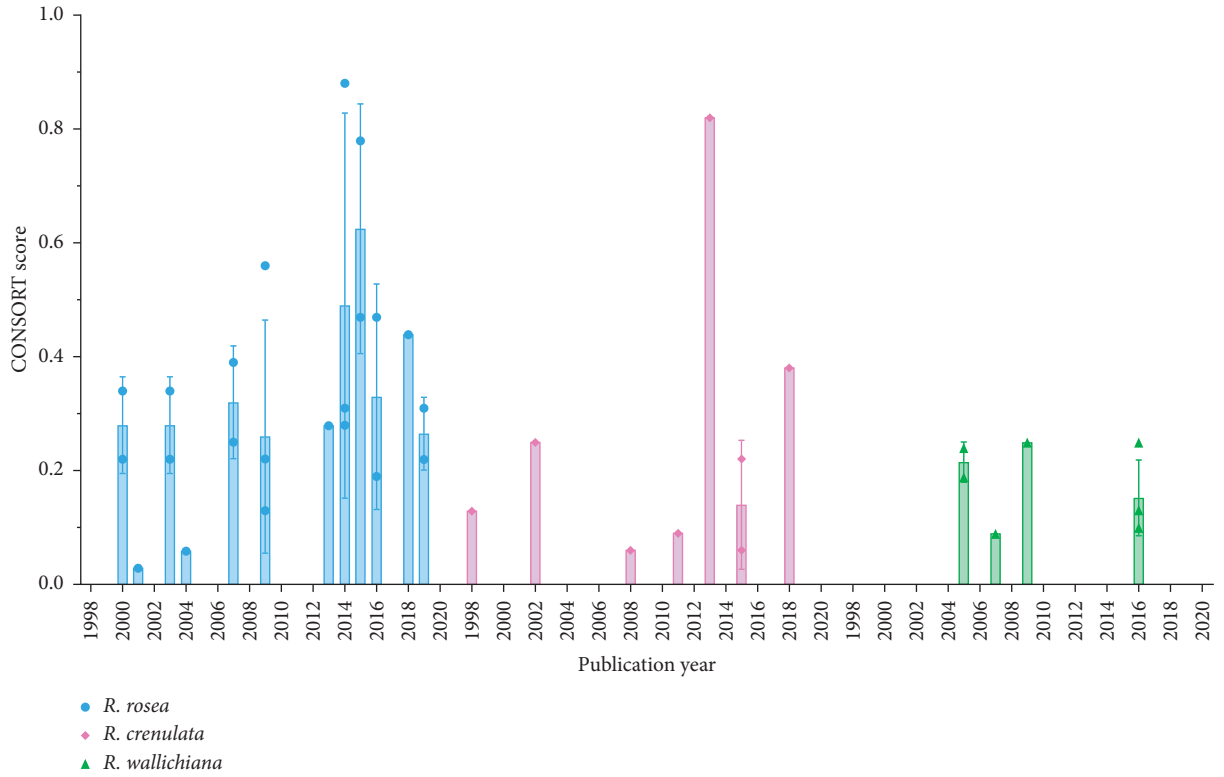


Figure 6: The CONSORT score of each RCT of *R. rosea*, *R. crenulata*, and *R. wallichiana* by publication year (mean ± SD).

compliance with CONSORT on them was not improved over time.

In addition to the publication year, the sample size and trial length were also investigated to examine if they have correlations with the CONSORT score (Figures 7(a) and 7(b)). Due to the missed information about the trial length of one RCT of *R. rosea*, there were only 22 RCTs of *R. rosea* included in Pearson's *r* analysis. Unexpectedly, the results of Pearson's *r* indicated a nonsignificant and weak correlation between the CONSORT score and the sample size of RCTs of *R. rosea*, *R. crenulata*, or *R. wallichiana* (*R. rosea*: $r = 0.265$, $p = 0.222$; *R. crenulata*: $r = 0$ .088, $p = 0.836$; *R. wallichiana*: $r = 0.49$, $p = 0.218$). Likewise, between the CONSORT score and the trial length of RCTs of *R. rosea*, *R. crenulata*, or *R. wallichiana*, a nonsignificant and weak correlation was also found (*R. rosea*: $r = 0.34$, $p = 0.122$; *R. crenulata*: $r = 0.6$, $p = 0.116$; *R. wallichiana*: $r = -0.333$, $p = 0.42$). In Figures 7(a) and 7(b), the correlations between the CONSORT score and the sample size/trial length are graphically presented.

3.4. Risk of Bias Assessment. As seen from Figure 8 in which the judgment of risk of bias regarding the six domains of the included studies is presented, the majority of the included studies of the three *Rhodiola* species were judged to have an unclear risk of bias in most domains due to the insufficient reporting. Among the studies of *R. rosea* and *R. wallichiana*, none of them was assessed to have a low risk of bias in all the six domains while one study of *R. crenulata* was found to reach a low risk of bias in all the domains. In Figure 9, the judgements of risk of bias were further summarized by percentage. There are studies of *R. rosea* which have a high risk of bias in domains of performance bias, detection bias, attrition bias, or reporting bias with percentages not exceeding 9%. For the other risks of bias, approximately 17% of studies of *R. rosea* were judged to have high risk of bias due to the industry sponsorship issue [38] or the lack of sample size calculation along with small sample size and nonsignificant results [28, 31, 46]. For both studies of *R. crenulata* and *R. wallichiana*, no high risk of bias was found during the assessment. For the assessment in studies of *R. wallichiana*, the percentages of high risk of bias in selection bias, performance bias, and detection bias are 38%, 38%, and 50%, respectively, which are relatively high compared with the other two species. At last, among the studies of *R. crenulata*, no high risk of bias was found.

4. Discussion

The current study identified 23 RCTs of *R. rosea*, 8 RCTs of *R. crenulata*, and 8 RCTs of *R. wallichiana* for the evaluation of their reporting quality based on the CONSORT 2010 statement and the assessment of their methodological quality (risk of bias) using the Cochrane Risk of Bias tool. Prior to the CONSORT evaluation and risk of bias assessment, the study characteristics including nonmarketed/marketed products, formulation, focused functions, publication year, and locations of RCTs were investigated to give an overview of the status of current RCTs of *Rhodiola*. The results of the CONSORT evaluation showed that the

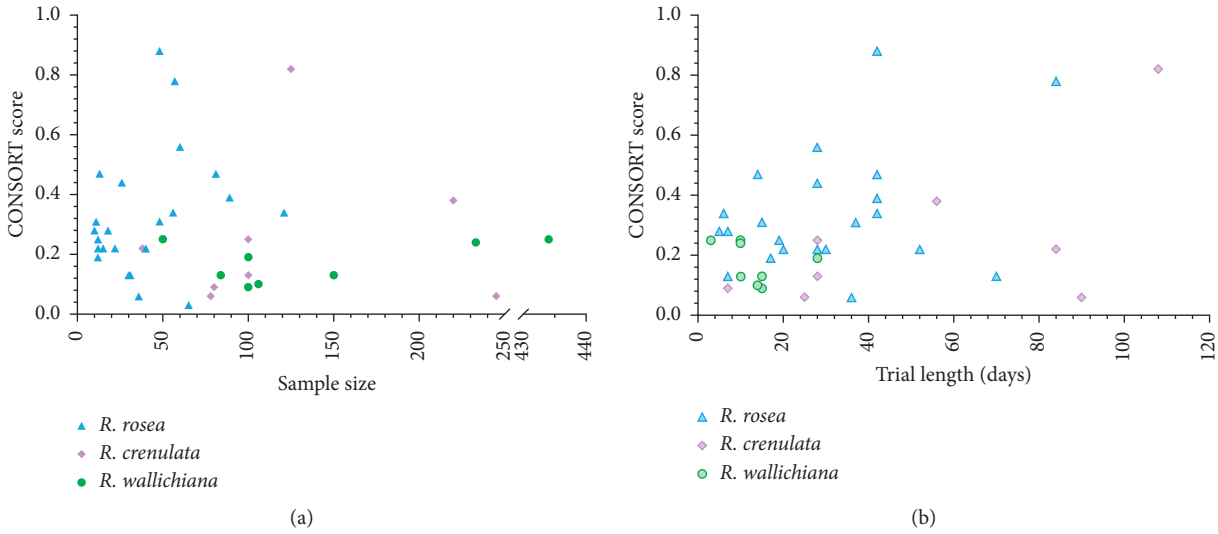


Figure 7: The correlation between the CONSORT score and sample size/trial length. (a) The correlation between the CONSORT score and sample size of RCTs of *R. rosea*, *R. crenulata*, and *R. wallichiana*. (b) The correlation between the CONSORT score and trial length of RCTs of *R. rosea*, *R. crenulata*, and *R. wallichiana*.

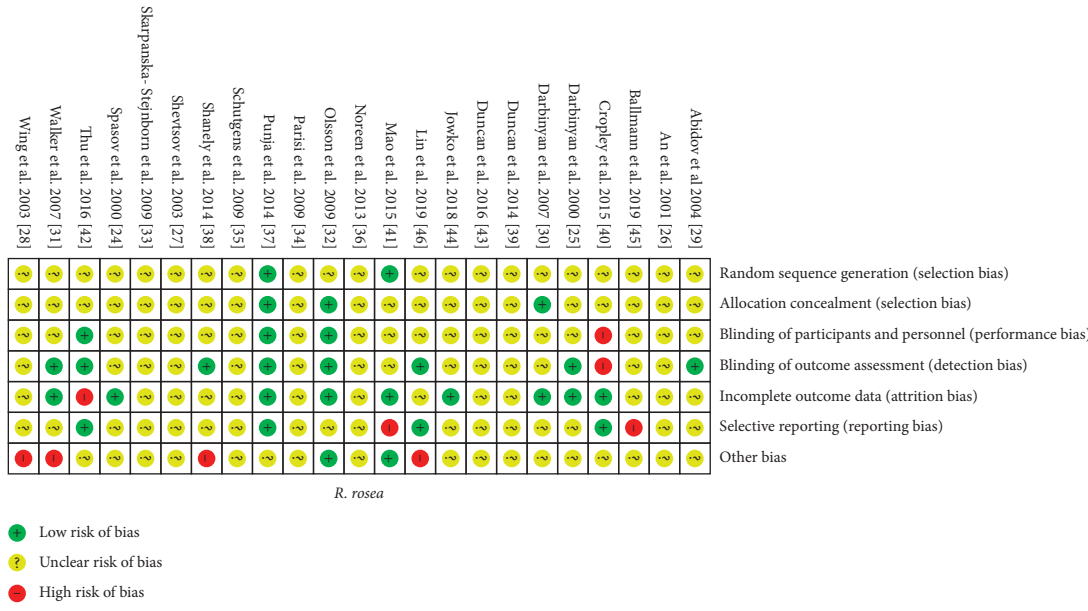


Figure 8: Continued.

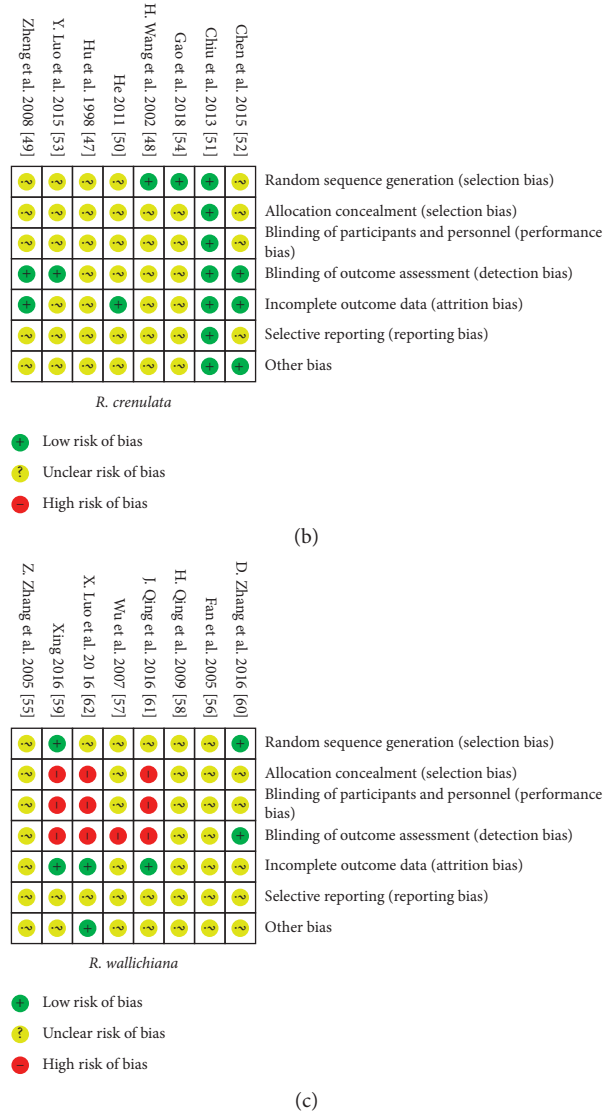


Figure 8: Risk of bias summary for the individual included studies. (a) Risk of bias summary for the individual RCTs of *R. rosea*. (b) Risk of bias summary for the individual RCTs of *R. crenulata*. (c) Risk of bias summary for the individual RCTs of *R. wallichiana*.

reporting quality of the included studies was generally poor. Furthermore, most of the included studies were judged to have an unclear risk of bias in most domains due to the limited reporting information in the assessment of Cochrane Risk of Bias.

The distribution of the locations of RCTs of *R. rosea*, *R. crenulata*, and *R. wallichiana* is consistent with the distribution of respective *Rhodiola* resources [9], implying that the *Rhodiola* researchers still focus on utilizing the local resource currently. In western countries, *R. rosea* is the most widely used species [63]. Products of *R. rosea* have already been on the market, and most of them are sold as food supplements [64]. In China, a wide variety of *Rhodiola* species are used. *R. crenulata* is the official species listed in the Chinese Pharmacopoeia (version of 2020), while some other species are also commonly used, such as *R. wallichiana*, *R. angusta*, and *R. sachalinensis* [65, 66]. Specifically, *R. wallichiana* is the only species that has been developed to injection for treating stable angina pectoris associated with coronary heart disease [67]. In light of the diverse functions of *Rhodiola*, more and more products have been developed. However, a high-value product, as a drug or functional food, needs support from sufficient clinical trial data. In our study, there are 39 RCTs of *Rhodiola* included, and the number of RCTs of *R. rosea* ($n = 23$) is almost three times of the number of RCTs of *R. crenulata* ($n = 8$) and *R. wallichiana* ($n = 8$), respectively, which means that more RCTs of *R. crenulata* and *R. wallichiana* are needed.

In addition to sufficient clinical trial data, the quality of clinical trial data is more important for the support of high-value products. However, the overall quality of the current RCTs of *Rhodiola* is poor. Taking the item "Title and abstract" as an example, it seems very easy to be adherent to; however, the CONSORT scores of the RCTs of the three species are all below 0.3 which means poor compliance with this item. Only a total of 8 RCTs were identified as the

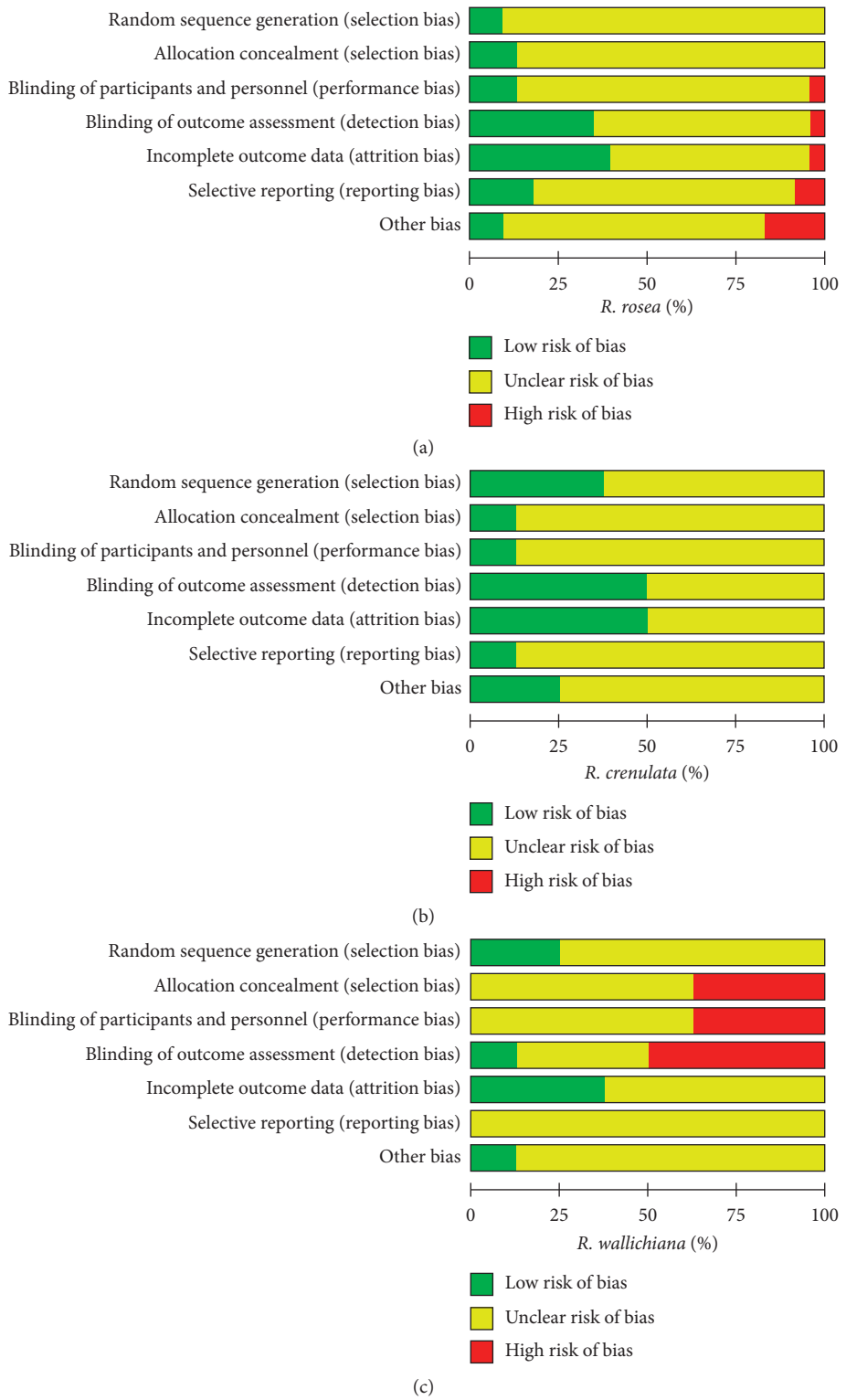


Figure 9: Percentage of risk of bias of the included studies. (a) Percentage of risk of bias of RCTs of *R. rosea*. (b) Percentage of risk of bias of RCTs of *R. crenulata*. (c) Percentage of risk of bias of RCTs of *R. wallichiana*.

randomized trials in the title. For the 26 RCTs which did not fully report item 1b "Structured summary of trial design, methods, results, and conclusions", most of them lacked the information about the trial design. Actually, the title and abstract are the important parts of a study. In a conventional literature screening method when conducting a systematic review for RCTs, a reviewer usually decides whether to include a study for further screening based on the examination of the title and abstract [68]. Those studies that have identified randomization in the title and have structured abstracts with sufficient information will help reviewers quickly screen the literature in the initial screening. Otherwise, there is a possibility that the studies will be excluded by the reviewers. Furthermore, an abstract with insufficient information or inappropriate presentation might sometimes mislead those decision-makers who cannot get access to the full-text reports and make a wrong healthcare decision [69]. Therefore, the title and abstract should be paid sufficient attention to by the researchers when they write the trial report.

In the method section, sample size calculation is another item with poor CONSORT compliance in the present study. In total, only 6 (15%) articles (5 RCTs of *R. rosea*, 1 RCT of *R. crenulata*, and 0 RCTs of *R. wallichiana*) reported how the sample size was determined. In line with our study, several other studies also found that the calculation of sample size was seldom reported [13, 70, 71]. A study also pointed out that researchers, reviewers, and editors do not attach much importance to the sample size calculation [72]. However, the sample size calculation is actually of importance. In our study, three RCTs reporting nonsignificant results were judged to have a high risk of bias just because they recruited a small sample of participants and did not report the sample size calculation. A too small sample size often fails to detect statistically significant relation or difference, which is also well known as Type II error [73]. Consequently, a null trial that uses a small sample size and does not specify the sample size calculation will raise the reviewers' concern about the validity of its results. However, an overlarge sample size could magnify the detection of differences, highlighting statistical differences which are not clinically relevant. Besides, it would also cause waste of budget and could involve ethical problems. Hence, how the sample size was calculated should be reported so that the reviewers can examine whether the sample size of the study is sufficient and appropriate.

Randomization and blinding are crucial parts of the methodology in an RCT report. Detailed information of randomization and blinding being reported can reduce the bias and thus improve the validity of the study [74]. However, the RCTs of *Rhodiola* seem to have a low quality of these two important items. A study conducted by Ishaque et al. [20] evaluated the safety and efficacy of *R. rosea* for mental and physical fatigue by systematically reviewing the clinical trials of *R. rosea*. The results showed that the majority of the clinical trials unclearly reported the method of randomization and allocation concealment, and almost half of the included studies had an unclear or high risk of bias of blinding. Likewise, a meta-analysis of *R. wallichiana* preparation in the treatment for unstable angina pectoris also indicated the insufficient reports of the randomization method among the included RCTs [65]. Consistent with the results of the above two studies, in this study, the compliance with randomization and blinding of the included RCTs is also poor. The item randomization in the CONSORT statement is composed of 4 items with respect to sequence generation, allocation concealment mechanism, and implementation. Among all the RCTs included, only 2 (1 RCT of *R. rosea* and 1 RCT of *R. crenulata*) fully complied with all 4 items of randomization. 29 RCTs even did not report any of the 4 items at all. As for blinding, likewise, the majority of the included RCTs of the three *Rhodiola* species did not adequately report the blinding information, and the risk of bias regarding blinding of participants and personnel and blinding of outcome assessment is even high in 38% and 50% of RCTs of *R. wallichiana*, respectively. The high risk of bias was attributed to the incomplete blinding or lack of blinding. To improve the validity of RCTs of *Rhodiola*, researchers should give enough attention to the improvement of randomization and blinding.

In clinical trials, the report of adverse events is essential since safety is the basic requirement of a drug. Nevertheless, in the current study, nearly half of the included RCTs (15 RCTs of *R. rosea*, 1 RCT of *R. crenulata*, and 3 RCTs of *R. wallichiana*) did not mention if there were adverse events. As can be seen from the above results, the compliance with harms of RCTs of *R. rosea* is poorer than that of the RCTs of the other two species. This might attribute to the fact that most of the RCTs of *R. rosea* examined the effect on improving mental or physical function, rather than treating a certain disease. Researchers may, therefore, not take the adverse events seriously enough. In contrast to the RCTs of *R. rosea*, RCTs of *R. crenulata* and *R. wallichiana* focused more on treating diseases, such as cerebro-cardiovascular disease and COPD. Therefore, researchers were more cautious in observing and reporting any adverse events, which resulted in a higher proportion of reporting harms. However, no matter whether the RCT investigates a disease or not, the reporting of adverse events should be paid enough attention to, especially for herbal drugs, since there are many kinds of ingredients in herb and some of them are even not acquainted by scientists. Taking *Rhodiola* as an example, there are more than 100 ingredients reported [9]. This is different from western medicine in which ingredients are relatively few and the effects are easier to be predicted. In addition, among the RCTs of *R. wallichiana*, 6 used *R. wallichiana* injections as interventions. Disappointingly, 3 of them did not mention any detail of the harms. The safety of herbal injections has become a public concern since the Yuxingcao injection caused a series of severe adverse events and was subsequently suspended in 2006 [75]. Herbal injections are considered to have a higher risk of adverse events than any other formulation of herbal drugs and conventional injections [76]. In view of the above, adverse effects should be paid great attention to when conducting relevant clinical trials.

The overall qualities of RCTs of the three *Rhodiola* species range from 0.17 to 0.32, which means that they do

not differ from each other too much. Nevertheless, on some items such as background and objectives, blinding, outcomes, and interpretation, differences among their qualities are significant. Overall, CONSORT grouped items of RCTs of *R. rosea* and *R. crenulata* were nearly all reported, indicating that RCT reports of *R. rosea* and *R. crenulata* are with a relatively complete structure based on the CONSORT. As for RCT reports of *R. wallichiana*, more than half of the grouped items were not reported at all. Authors should give adequate attention to each item on the CONSORT checklist. An RCT report with a complete structure of the CONSORT and adequate reporting of each item can provide the reviewers with sufficient information to evaluate the effectiveness of the RCT.

The reporting quality of RCTs is believed to be affected by some factors, such as sample size. A large and appropriate sample size is considered to be associated with better quality of RCT reporting [77]. In a previous study conducted by Kodounis et al. [14], a significant association was found between sample size and the quality of reporting in a univariate analysis. It is not hard to explain and understand this association. As the sample size increases, the expenditure and manpower invested in RCTs will also increase, which results in an aspiration of researchers to produce a good quality RCT report. Therefore, from this perspective, the increase in sample size is believed to improve the quality of reporting. With the same logic, we believe that the increase in trial length can also improve the quality of reporting. Thus, we investigated the correlation between the CONSORT score and the sample size, as well as the trial length, respectively. However, the results showed weak correlations, either for sample size or for trial length. Compared with Kodounis's study [14], our limited sample size of the included RCTs may be responsible for the result of the weak correlation. Additionally, the publication year is possibly a confounding factor of this negative result. The quality of reporting is supposed to improve over time, especially after 2010 when the CONSORT statement was updated for a more complete version. Among the included RCTs in our study, however, the CONSORT scores of most RCTs published after 2010 are actually similar to those of the RCTs published before 2010. Several RCTs published after 2010 are even in poorer compliance compared with some RCTs published before 2010 based on the CONSORT score. But three RCTs with high scores which are above 0.8 were not published after 2010. In addition, on specific items, the compliance with only two items of RCTs of *R. rosea*, such as limitations and generalizability, has been improved significantly after the CONSORT statement was updated in 2010. Obviously, the CONSORT 2010 statement has a limited influence on the quality of reporting among RCTs of *Rhodiola*.

To improve compliance with CONSORT, researchers should be trained with CONSORT before they conduct the clinical trial and take the CONSORT seriously when they write the report. In addition to the effort the researchers should make, journals can also make contributions. A previous study has revealed that the adoption of CONSORT by journals is related to the improvement of the quality of RCT reports [78]. Specifically, the poor CONSORT compliance is sometimes attributed to the restriction of journal format and word count, such as the part of title and abstract [79]. To resolve this problem, in addition to condensing the language on the basis of keeping the complete information by authors, journals are suggested to adjust the format and word count in response to the CONSORT, and the submitted manuscript should have high CONSORT compliance.

Only when the transparency of RCT reports is guaranteed can the risk of bias of the report be assessed properly. In our study, due to the inadequate reporting of the included RCTs, the risk of bias of most of them in most domains was assessed to be unclear, indicating the unclear methodological quality. Meanwhile, some RCTs of *R. rosea* and *R. wallichiana* were found to have a high risk of bias regarding the allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, funding, and sample size calculation, which lower the validity of those studies. For the RCTs of *R. crenulata*, although no high risk of bias was found among them, the validity of their results should be taken with a grain of salt due to a large proportion of the unclear risk of bias. Overall, the results of the included RCTs of *Rhodiola* which were evaluated to have an unclear or high risk of bias should be interpreted with caution.

In this study, there are also some limitations. Firstly, we planned to cover the RCTs of all the *Rhodiola* species, but eventually, there are only three species included in the study, and one species was excluded due to limited trials. The RCTs of other *Rhodiola* species may be missed due to the limited included publication language. In journals of other publication languages, there may be more eligible RCTs of the current included three species. Secondly, only peer-reviewed and full-text journal articles were included. Those reports such as conference abstracts are usually not full-text available while the CONSORT statement and Cochrane Risk of Bias are only suitable for the quality evaluation for full-text trial reports. In addition, the articles that have not been peer-viewed will raise certain concerns about the validity. In view of these concerns, we only included peer-reviewed and full-text journal articles, and the quality of those articles without peer review and full text remains unknown. Thirdly, the sample size in our study is relatively small, especially for RCTs of *R. crenulata* and *R. wallichiana*. In the future, when *Rhodiola* is investigated further, more RCTs would be included in the quality assessment for further investigation. Finally, we only included RCTs of *Rhodiola* single treatment. The quality of RCTs with *Rhodiola* combination treatment remains unknown. Therefore, the result of this study only represents the quality of RCTs with *Rhodiola* single treatment published in English and Chinese.

5. Conclusion

According to the reporting quality evaluation in this study, there is inadequate reporting among the included RCTs of *R. rosea*, *R. crenulata*, and *R. wallichiana*. Furthermore, in the assessment of the risk of bias, most of the included studies were found to have an unclear risk of bias, which raises the

concern about their validity. Therefore, *Rhodiola* researchers should use these clinical pieces of evidence with caution, and more RCTs with high reporting quality and good methodological quality are needed. In order to achieve the high quality of RCTs of *Rhodiola*, researchers are suggested to rigorously comply with the CONSORT statement when designing the trial and writing the report. We believed that with the improvement of the quality of RCTs, the development of *Rhodiola* products will attract more attention and be of higher value.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

X. L. and W. C. performed the literature search and selection, data extraction, quality evaluation, and data analysis. S. W., X. L., and W. C. discussed the result interpretation. X. L. drafted the manuscript. Y.W., S. W., H. H., Y. X., and Z.L. revised the manuscript. All authors reviewed and approved the final manuscript. Xiuzhu Li1 and Weijie Chen have contributed equally to this work.

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Review Article

Potential Acupoint Prescriptions and Outcome Reporting for Acupuncture in Atopic Eczema: A Scoping Review

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Background. Acupuncture is considered a complementary therapy for atopic eczema. The aim of this scoping review is to identify, examine, and summarize the potential acupoint prescriptions and outcome reporting regarding the clinical trials of acupuncture for eczema. *Methods*. We searched different databases from inception to September 30, 2020. The data were screened and extracted to identify the potential acupuncture prescription and examine the variation in outcome reporting, outcome measurement instruments (OMIs), and measurement time points in clinical trials of acupuncture. *Results*. A total of 116 clinical studies were included. The acupoint combination of LI11 and SP10 was used frequently. The core acupoint association networks were acupoints LI11, SP10, ST36, SP6, and LI4. For clinical trials of acupuncture, a total of 6 outcome distinct domains were identified in the 32 outcome measurements. The most frequently reported outcome was the eczema area, which was reported 97 times (83.6%, 97/116). Immune system outcomes were assessed in 15 outcome measurements, which totally reported 37 times. Adverse events were reported 51 times. TCM syndrome, which could reflect the characteristics of TCM, was reported 4 times. 29 outcomes (90.6%, 29/32) were provided definitions or OMIs. Among these outcomes, the outcome measurement times ranged from 0 to 34. *Conclusions*. This scoping review provides potential knowledge that should be considered as priority in future research of acupuncture for eczema.

1. Introduction

Atopic eczema (AE) is a chronic inflammatory skin disease, clinically characterized by exacerbations and remissions of eczematous skin with inflammation, pruritus and excoriations, scaling, dry skin, and susceptibility for cutaneous bacterial and mycotic infections [1]. In the past 30 years, lifetime prevalence has shown a worldwide increase, which plateaus at 10–20% in developed countries and continues to increase in China and many developing countries but lower [2].

The concurrent use of terms such as "eczema," "atopic eczema," "atopic dermatitis," "atopic eczema/dermatitis syndrome," and "neurodermatitis" has led to confusion and inconsistency in their application. In China, AE is also called as chronic eczema (CE) [3]. Therefore, the nomenclature review committee of the World Allergy Organization has proposed the use of "eczema" as a unifying term, and this term is also used throughout the text of this review [4].

Acupuncture was frequently applied in cases of allergy [5]. In recent years, it has been increasingly utilized as an adjuvant therapy to conventional treatment of eczema [6]. A large number of clinical trials related to acupuncture for eczema have been published in various journals [7]. However, evidence of acupuncture for eczema keeps unclear. The treatment prescriptions are various, and outcome reporting showing heterogeneity makes it impossible to merge data or compare efficacy of different interventions in a systematic review.

The aim of this scoping review is to identify potential treatment prescriptions and compare the outcome reporting for acupuncture clinical trials with the existing core outcome domains developed by the Harmonizing Outcome Measures for Eczema (HOME) consensus, which may help acupuncturists establish potential prioritized treatment prescriptions and choose appropriate outcomes in their future research.

2. Methods

2.1. Search Strategy. We conducted a systematic electronic search from Medline, PubMed, Cochrane Library, CBM, CNKI, Wanfang Database, and VIP Database from their inception to September 30, 2020. The search terms included acupuncture, acupoints, electroacupuncture, needle warming therapy, autologous whole blood injection, autohemotherapy, acupoint injection, catgut implantation, filiform steel needle, fire needle, plum-blossom needle, atopic dermatitis, chronic eczema, and atopic eczema. Language was restricted to Chinese and English. The search strategy is shown in the supplementary materials (available here).

2.2. Inclusion Criteria and Exclusion Criteria. The inclusion criteria are as follows:

① Population: patients diagnosed with either AD, AE, or CE were included as participants in this study [2, 8, 9]. There were no restrictions on age, sex, race, or region.

② Intervention: the included studies examined the use of acutherapy (acupuncture therapy). The studies could use multimodal interventions, but acutherapy had to be included.

③ Comparison: comparison of the intervention group with the control group in addition to treatment methods other than acutherapy, such as drugs or other forms of traditional Chinese medicine.

④ Outcomes: no restriction.

⑤ Study types: all kinds of clinical studies including randomized clinical trials, observational studies, and case series were included in this scoping review.

The exclusion criteria are as follows:

Comments, animal experiments, and narrative reviews were excluded.

2.3. Literature Identification. We imported the results from different databases into NoteExpress 3.2.0 [10] and then deleted duplicate studies. Two reviewers (Zeng Y. J. and Zhang J. L.) conducted data identification independently. They reviewed the abstracts and full texts and extracted relevant information from the included studies. If the studies involved in comparison of therapeutic effects of different acupoint prescriptions, then the most effective acupoint prescription according to the criteria of therapeutic effect was extracted. Any discrepancies were resolved by consensus.

2.4. Data Extraction. The following information was extracted pertaining to the reference title, first author's name, publication year, study location, study design, intervention and comparisons, duration, results, and outcome measurements. These data were charted in a custom-made data extraction file and double checked by ZZ.

2.5. Data Analysis. To overcome some weaknesses of previous studies and to determine whether acupuncture requires a different outcome set that will reflect particular effects of acupuncture on eczema symptom management, the outcomes reporting was analyzed by description analysis. Two researchers (ZZ and DZ) merged the overlapping outcomes according to the definition of core outcome domains recommended by HOME consensus including signs, symptoms, quality of life, and long-term control and grouped individual outcomes into the appropriate outcome domain independently. In addition, immune system outcomes and adverse events were added to outcome domains. These outcome sets are often preferred in what to consider as evidence relative to efficacy and safety in clinical trials of acupuncture on eczema.

Treatment prescriptions were analyzed by SPSS Modeler 18.0 [11]. We first analyzed the frequency of acupoints and meridians with text mining. Then, we determine common acupoint combinations, the support degree of different acupoint combinations, and the core acupoint association network of acupuncture for eczema by association rule mining. We obtain the core acupoints using hierarchical cluster analysis. We also analyzed the co-occurrence matrix of the top 18 acupoints using Heml 1.0.3.7 [12]. The results are illustrated by a heat map.

3. Results

3.1. Study Identification. A total of 1332 records were identified from the literature search, and a total of 116 eligible studies were included. The flowchart of the review is shown in Figure 1.

3.2. Characteristics of the Inclusive Studies. The characteristics of the inclusive studies are shown in Supplementary Table 1. The studies included were published between 1998 and 2020. Most of the studies (81%, 94/116) were published in 2010 and after. The majority of the studies were conducted in China ($n = 111$). The rest of the studies originated from Germany, United States, and Korea. Most of the studies used a randomized controlled trial design with two or three arms ($n = 91$). Nine studies used a control design with simple randomized assignment of participants to the experimental and control groups. 14 studies used a single group design such as a longitudinal prospective design. The duration of treatment sessions ranged from 7 d to 140 d throughout the studies, while two studies did not report duration. The

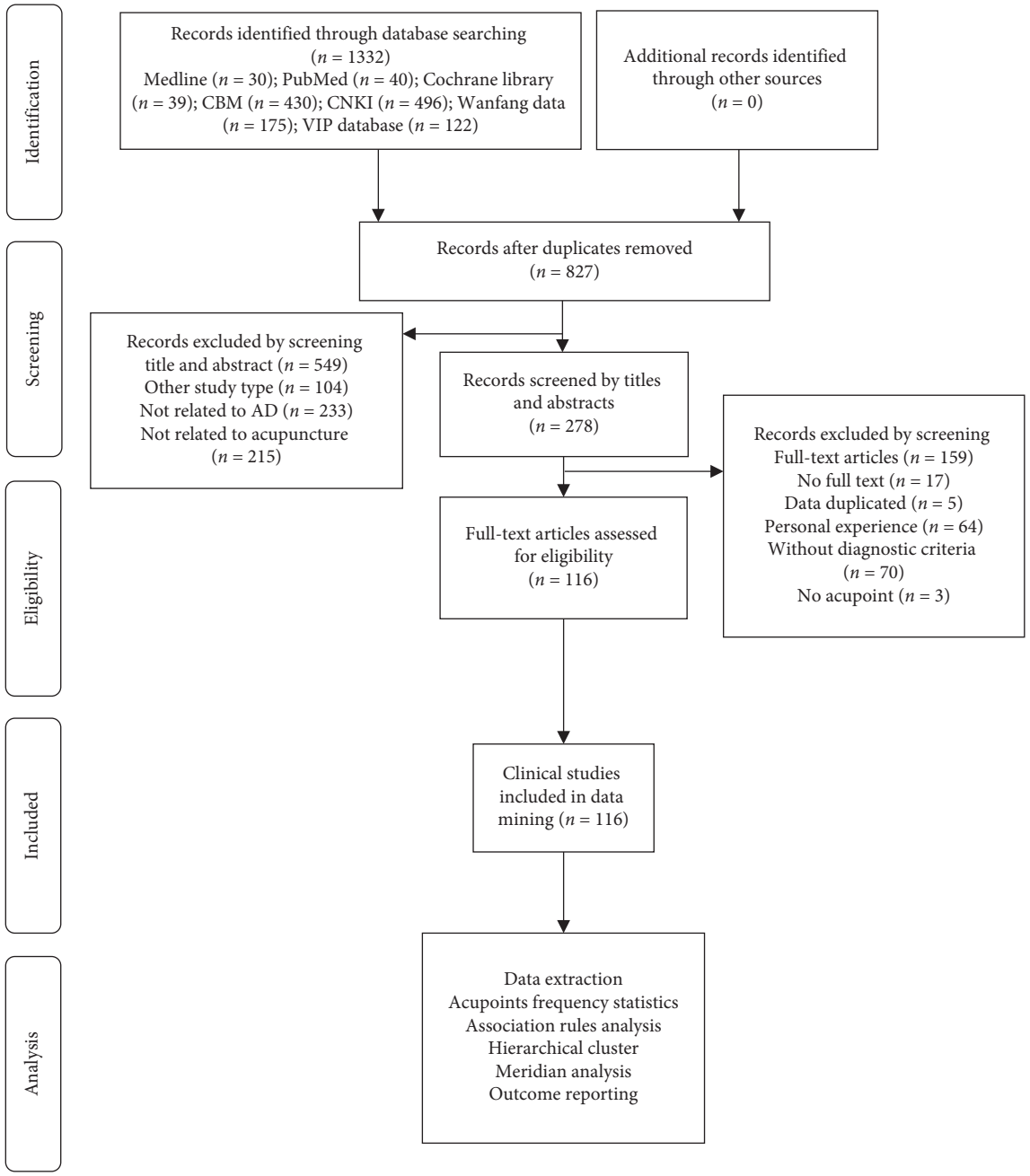

Figure 1: Flow chart of the research process.

sample sizes of all the studies range from 10 to 323, with an average of 82 participants. Intervention procedures varied among the 116 studies. Most of the studies (44%) included investigated manual acupuncture. Only two studies discussed electroacupuncture. Other studies involved the assessment of acupoint therapies such as autologous whole blood injection ($n = 21$), acupoint injection ($n = 18$), catgut implantation ($n = 7$), fire needle ($n = 24$), and plum-blossom needle ($n = 7$). Non-RCT studies discussed treatments such as acupuncture, autologous whole blood injection, acupoint injection, catgut implantation, fire needle, and plum-blossom needle ($n = 23$). All of the included studies demonstrated statistically significant ($P < 0.05$) improvements in at least one targeted eczema outcome.

3.3. Results of Data Mining

3.3.1. Frequency Statistics of Acupoints and Meridians. Acupoint frequency was analyzed based on the acquired 116 acupoint prescriptions, which involved 73 acupoints. The

Table 1: Common acupoint group of acupuncture for eczema.

No.	Acupoints	Frequency
1	LI11, SP10	58∗
2	LI11, ST36	48
3	SP10, ST36	40∗
4	LI11, SP10, ST36	37
5	SP6, LI11	31
6	SP6, SP10	29
7	LI11, SP6, SP10	28
8	SP6, ST36	27
9	LI11, SP6, ST36	25
10	LI4, LI11	24
11	ST36, SP6, SP10	23
12	LI4, SP10	20
13	LI4, SP10, LI11	20
14	LI4, ST36	18
15	LI4, ST36, LI11	17

∗The frequency of two-acupoint combinations includes that of the two acupoints in three-acupoint combinations.

top 15 acupoints used frequently were LI11, SP10, ST36, Ashi, SP6, LI4, SP9, BL20, BL13, BL17, GV14, CV4, ST25, PC6, and BL18, in turn. There were 5 acupoints with frequency ≥30, which were Ashi, LI11, SP10, ST36, and SP6. A total of 12 meridians, two extra meridians, and one Ashi point were used in acupuncture for eczema. There are 14 meridians recorded in the 116 acupoint prescription. The top 5 meridians included the Spleen Meridian of Foot-Taiyin (SP), Large Intestine Meridian of Hand-Yangming (LI), Stomach Meridian of Foot-Yangming (ST), Bladder Meridian of Foot-Taiyang (BL), and conception vessel (CV). The most frequently used meridian was SP, which was used 127 times and involved 5 acupoints. Detailed information can be checked in Figure 2.

3.3.2. Common Acupoint Combinations. A total of 15 common acupoint combinations were frequently used over 17 times. There were four acupoint combinations with frequency ≥35: LI11 and SP10, LI11 and ST36, SP10 and ST36, and LI11, SP10, and ST36. The most frequently used acupoint combination was LI11 and SP10, with 58 times. LI11 and ST36 was used 48 times, SP10 and ST36 was used 40 times, and LI11, SP10, and ST36 was used 37 times. Detailed information can be checked in Table 1. The co-occurrence matrix of the 18 acupoints is presented in Figure 3, which were consistent with the common acupoint combinations.

3.3.3. Association Rules of Acupoints. There were 13 acupoint combinations with confidence levels ranging more than 80% and support degree more than 20%, of which SP6, SP10⟶LI11 was listed as the association rules of 96.55%, indicating that when SP6, SP10 was selected, the probability of selecting LI11 was 96.55%. Also, the highest support degree was SP10⟶LI11, which was 54.31%. The association rules for acupoints are described in Table 2. The core acupoint association network of acupuncture for eczema is listed in Figure 4. The degree of support from strong to weak was acupoint LI11 combined with SP10, ST36 with LI11, SP6 with LI11, SP6 with SP10, and LI4 with LI11.

3.4. Outcomes Reporting in Clinical Trials of Acupuncture for Eczema. For included clinical trials, a total of 6 outcome distinct domains were identified in the 32 outcome measurements (Table 3). Seven (21.9%, 7/32) outcomes were reported only once. The most frequently reported outcome was the eczema area, which was reported 97 times (83.6%, 97/116). Immune system outcomes were assessed in 15 outcome measurements, which totally reported 37 times. Adverse events were reported 51 times. TCM syndrome, which could reflect the characteristics of TCM, was reported 4 times.

29 outcomes (90.6%, 29/32) were provided definitions or OMIs. 17 (53.1%, 17/32) outcomes were provided one OMI or definition, 4 (12.5%, 4/32) outcomes were provided two OMIs or definitions, and 8 (25.0%, 8/32) outcomes were provided more than two OMIs or definitions. In addition, among these outcomes, the outcome measurement times ranged from 0 to 34, and the median time was 6.5. Itch and eczema area had more measurement times than other outcomes did.

4. Discussion

The meridians, acupoint combinations, and core acupoints identified from the studies included in this scoping review broadly cover the characteristics of acupuncture prescriptions that could be offered in eczema treatment providing antipruritus and anti-inflammatory effect. Acutherapy offers an area for further clinical practice and research for treating eczema.

From the previous systematic review, we found that the majority of clinical trials of acupuncture for eczema are in low methodological quality and quality of evidence [13–15], so it is difficult to draw a definite conclusion. In addition, the researchers and clinicians may use different acupoints, so it is impossible for some clinical trials to include in systematic review when there is heterogeneity in prescription. From the results of data mining, the core acupoints may provide potential prescription to researchers.

This scoping review suggests that specific outcome measures for future clinical trials of acupuncture are clinician-reported signs, patient-reported symptoms, health-related quality of life and long-term control, TCM syndrome, immune system outcomes, and adverse events, which are different form the HOME. In addition, researchers choose different outcome measurement instruments, so some clinical trials will be excluded from systematic reviews, which produce waste and lower the value of research. For acupuncture clinical trials, following the HOME core outcome set for eczema may help improve the consistency of outcomes and outcome measurement instruments.

The theory of TCM is totally different from that of western medicine. When clinicians use acupuncture, the

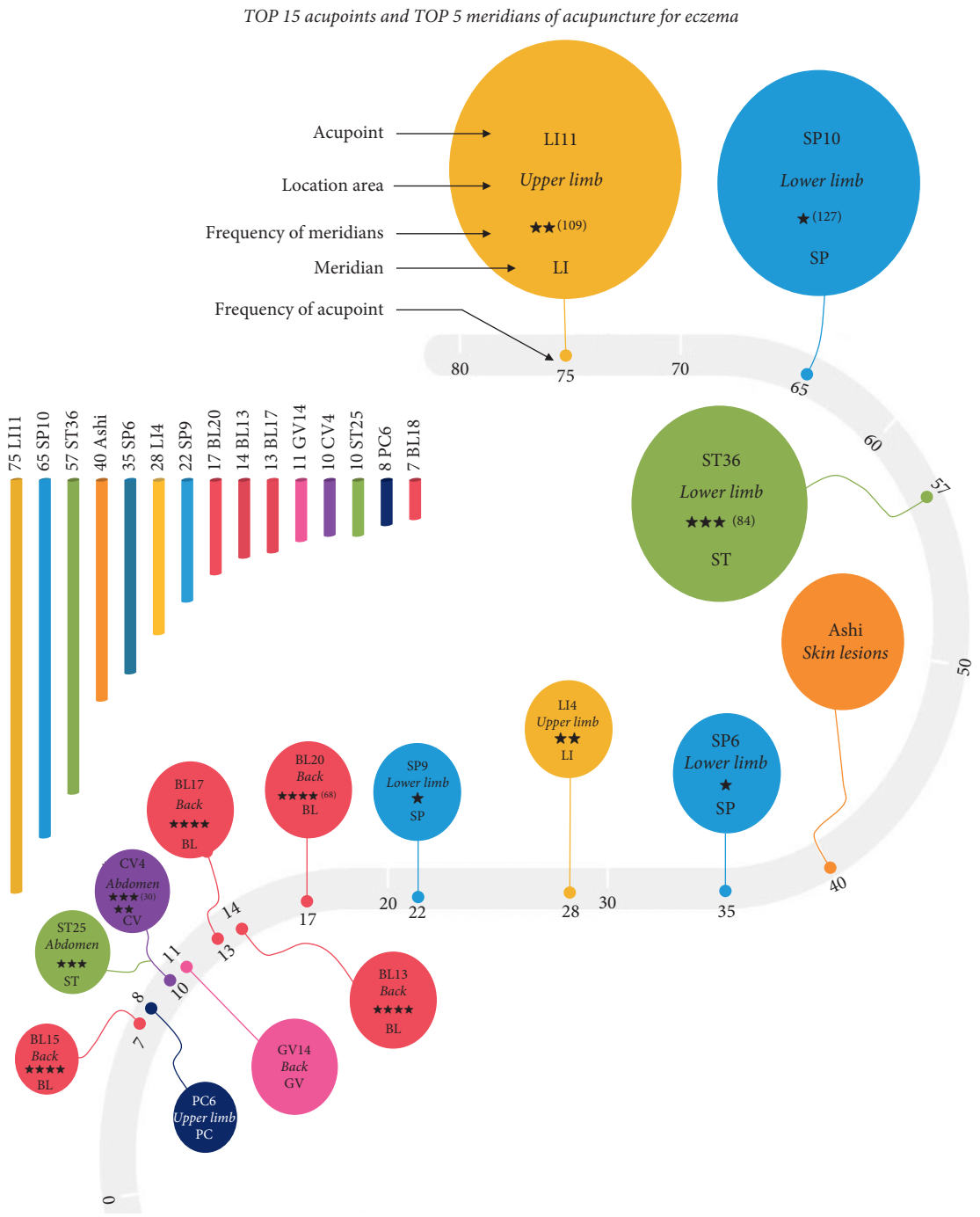

Figure 2: The top 15 acupoints and top 5 meridians used in acupuncture for eczema.

TCM syndromes are important factors that should be considered in the process of treatment and efficacy evaluation. Therefore, in the core outcome set for eczema, TCM syndromes should be considered. In addition, the HOME is developed by stakeholders from developed countries, and the perspectives of professionals and patients from low- and middle-income countries are missed. Therefore, the core outcome set for eczema should be achieved consensus in researchers, clinicians, and patients in China.

The current research had several strengths. To our knowledge, this was the first study that used bioinformatics methods and searched Chinese and English databases to assess which acupoints have been used to treat eczema. First, previous research was limited in that it could only "qualitatively" interpret the characteristics of acupoint prescriptions used in eczema treatment. We overcame this limitation by extracting significant "quantitative" characteristics using the association rule mining. Second, we explored core acupoints used in eczema treatment. Moreover, we evaluated

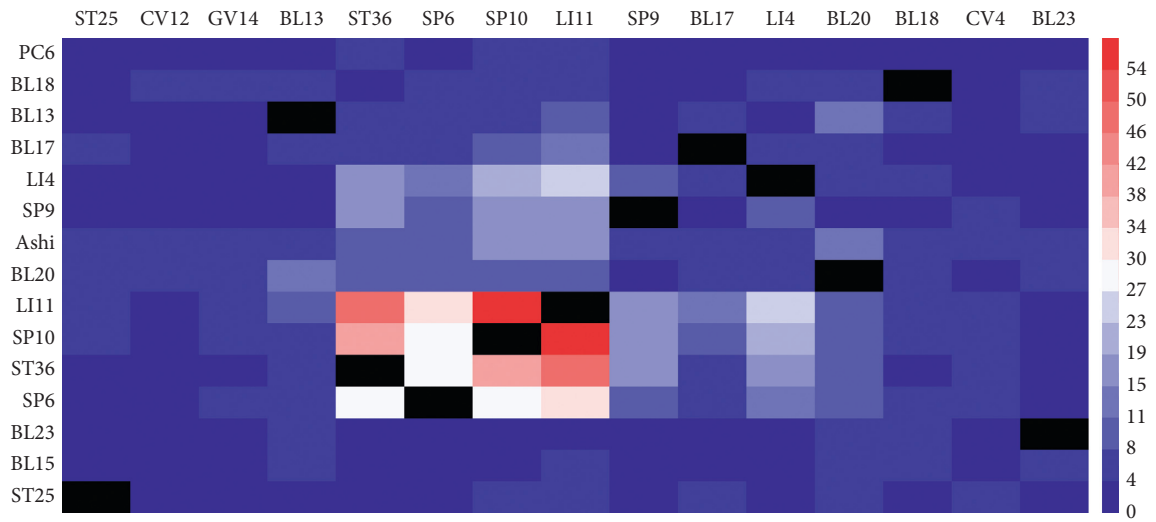

Figure 3: Co-occurrence matrix of acupoints.

Table 2: Association rules of acupoints.

Number	Acupoints	Confidence level (%)	Support degree (%)
1	SP6, SP10⟶LI11	96.55	25.00
2	SP6⟶LI11	93.94	28.45
3	SP6, ST36⟶LI11	92.59	23.28
4	ST36, SP10⟶LI11	92.50	34.48
5	SP10⟶LI11	92.06	54.31
6	SP6, LI11⟶SP10	90.32	26.72
7	SP6⟶SP10	87.88	28.45
8	ST36⟶LI11	87.27	47.41
9	LI4⟶LI11	85.71	24.14
10	SP6, ST36⟶SP10	85.19	23.28
11	LI4, LI11⟶SP10	83.33	20.69
12	SP6⟶ST36	81.82	28.45
13	SP6, LI11⟶ST36	80.65	26.72

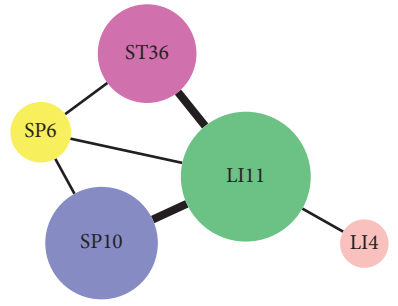

Figure 4: Core acupoint association network of acupuncture for eczema. The size of the circle represents the frequency, and the width of the line indicates the support degree.

the quality of outcome reporting, OMIs/definitions, and outcome measurement times.

5. Limitations

Our research had several limitations. The present study was based on the frequency of prescription used in the clinical practice and literature. For this reason, we could not evaluate new candidate acupoints emerging from recent experimental studies. Relatedly, we did not evaluate the clinical effectiveness of each prescription and adjunct points prescribed based on syndrome differentiation in our study. Finally, the acupoint combinations and core acupoints of acupuncture for eczema were rarely confirmed by large-scale multicenter clinical trials

Table 3: The outcomes reporting in clinical trials of acupuncture for eczema.

Domains/outcomes	Outcomes reporting (n)	OMIs/definitions	Measurement time point (*n*)
Clinical signs			
Erythema	65	SCORAD, EASI, Likert scale, Chinese self-made questionnaire	30
Oozing/crust	21	SCORAD, EASI, Likert scale, POME	18
Induration/edema/papulation	64	SCORAD, EASI, Likert scale, Chinese self-made questionnaire	31
Excoriation	59	SCORAD, EASI, Likert scale, POME, Chinese self-made questionnaire	29
Lichenification	60	SCORAD, EASI, Chinese self-made questionnaire	28
Dryness	9	SCORAD, POME	13
Lesion area	97	SCORAD, EASI, Likert scale, Chinese self-made questionnaire	32
Skin temperature	2	Infrared radiation thermometer	3
Symptoms			
Itch	91	VAS, EIQ, POME, Chinese self-made questionnaire	34
Bleeding	1	POME	3
Cracks	1	Chinese self-made questionnaire	2
Sleep loss	10	VAS, ISI, PSQI, SRSS, Likert scale	13
TCM symptoms	4	Chinese self-made questionnaire	8
Heath-related quality of life			
Quality of life	20	DLQI, EPQOLS	11
Long-term control of flares			
Recurrence rate	20	—	28
Immune system outcomes			
BAT	1	FLOW-CAST basophil activation test	3
BASO	1	Blood routine examination	2
EOS	3	Automated hematology analyzer, blood routine examination	5
CD3+	2	Flow cytometry	3
CD4+	4	Flow cytometry	11
CD8+	4	Flow cytometry	11
IFN-γ	3	ELISA	4
TNF-α	2	ELISA	3
IL-2	3	ELISA	4
IL-4	5	ELISA	5
IL-5	3	ELISA	4
IL-12	1	ELISA	2
IL-18	1	ELISA	2
IgE	5	Immunoturbidimetry assay, ELISA	11
CRP	1	ELISA	3
Others			
Adherence/compliance	20	—	0
Adverse events/side effects	51	—	20

BAT: basophil activation test; EOS: eosinophil count; BASO: basophil; CRP: C-reactive protein; EASI: Eczema Area and Severity Index; POEM: Patient-Oriented Eczema Measure; EIQ: Eppendorf Itch Questionnaire; VAS: Visual Analogue Scale; SCORAD: Scoring Atopic Dermatitis Index; DLQI: Dermatology Life Quality Index; EPQOLS: quality of life scale for chronic eczema; ISI: Insomnia Severity Index; PSQI: Pittsburgh Sleep Quality Index; SRSS: Sleep Self-Rating Scale.

or animal experiments. Further clinical/experimental studies are needed to assess whether the results derived from our research have real meaning for applications.

6. Conclusions

The preference of core acupoints is LI11, SP10, ST36, SP6, and LI4 in acupuncture clinical studies on eczema. In the future, the researchers could focus on them to prove the efficacy of potential core acupoints. However, researchers should use the core outcome set for eczema to improve the consistency of outcomes and outcome measurement instruments so that the clinical trials will be not exclude from systematic reviews because of the heterogeneity of outcome reporting and outcome measurement instruments.

Abbreviations

AE: Atopic eczema
AD: Atopic dermatitis

AMED: Japan Agency for Medical Research and Development
ARM: Association rule mining
BASO: Basophil
BAT: Basophil activation test
BL: Bladder Meridian of Foot-Taiyang
CBM: Chinese biomedical medicine
CCT: Clinical control trial
CE: Chronic eczema
CMCC: Chinese medical current content
CNKI: China National Knowledge Infrastructure
CRP: C-reactive protein
DLQI: Dermatology Life Quality Index
EA: Electroacupuncture
EASI: Eczema Area and Severity Index
EIQ: Eppendorf Itch Questionnaire
EOS: Eosinophil count
EPQOLS: Quality of life scale for chronic eczema
ERK: Extracellular regulated protein kinases
GB: Gallbladder Meridian of Foot-Shaoyang
GV: Governor vessel
IFN: Interferon
Ig: Immunoglobulin
IL: Interleukin
ISI: Insomnia Severity Index
LI: Large Intestine Meridian of Hand-Yangming
LR: Liver Meridian of Foot-Jueyin
POEM: Patient-oriented eczema measure
PSQI: Pittsburgh Sleep Quality Index
RCT: Randomized control trial
SCORAD: Scoring Atopic Sermatitis Index
SP: Spleen Meridian of Foot-Taiyin
SRSS: Sleep Self-Rating Scale
ST: Stomach Meridian of Foot-Yangming
TNF: Tissue necrosis factor
VAS: Visual Analogue Scale.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

ZZ and ML drafted the manuscript. YZ, JZ, and YL extracted data from articles. ZZ and YZ contributed to the data extracting and assessment. DZ, RQ, and HS revised the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

The detailed search strategy and characteristics of the inclusive studies are shown in Supplementary materials. . (*Supplementary Materials*)

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Review Article

Methodological Considerations in N-of-1 Trials of Traditional Chinese Medicine

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More and more scholars choose N-of-1 trials for TCM clinical research. However, the quality of the experimental designs was uneven. Accumulating more than eight years of experience in exploring the N-of-1 trials of TCM, the authors and their team searched the related literature in main Chinese and English databases, referenced to relevant Chinese and international guidelines. The design, implementation, and data analysis of N-of-1 trials of TCM are still in in-depth exploration and practice. "Carryover effect" may affect the design and quality of the trials. Individualized treatment should be guided by the classic theories of TCM. It is expected to formulate reasonable observation periods and pairs and closely integrate individual and group statistical analysis.

1. Introduction

In the context of making decisions about an individual patient's care, N-of-1 trials have been considered to be among the most relevant and rigorous study designs for assessing treatment efficacy; they are listed as "level 1" evidence in the Oxford Centre for Evidence-Based Medicine 2011 levels of evidence [1]. As with crossover trials, N-of-1 trials eliminate confounding by covariates since each patient serves as his or her own control. The use of multiple crossovers within well designed N-of-1 trials increases confidence in the reliability of the results [2]. With the establishment and publication of CONSORT extension for reporting N-of-1 trials (CENT) in 2015, and the formulation of CENT for TCM in 2019 [3], N-of-1 trials are attracting more and more attention from scholars in China and around the world, and its application scope is also expanding.

We have cooperated with scholars from the Department of Clinical Epidemiology and Biostatistics, McMaster University (pioneer of N-of-1 trials), to explore the methodology of N-of-1 trials of TCM since 2012. This method is welcomed by patients because it embodies individual thinking and is close to the clinical practice of TCM. However, there are some problems in the current N-of-1 trials methodology of traditional Chinese medicine. We searched the published literature of N-of-1 trials of traditional Chinese medicine in major Chinese and international databases and want to summarize the rules and find out the corresponding countermeasures.

2. Methods

2.1. Eligibility Criteria. We included studies that fulfilled all of the following criteria: (1) journal articles published in English or Chinese, (2) the trial design included at least 2 trial cycles (pairs), and (3) studies or protocols of N-of-1 trails related to TCM.

2.2. Exclusion Criteria. Studies or protocols that met any of the following criteria were excluded: (1) meeting abstract, review, letter, commentary, editorial, book, or pamphlet, (2) studies that did not meet the design of N-of-1 trails, and (3) duplicate publication.

2.3. Search Strategy. We retrieved N-of-1 trials related to TCM published in English or Chinese in journals indexed by PubMed, Web of Science, the Cochrane Library, CNKI (China National Knowledge Infrastructure), China Biology Medicine (CBM), WANFANG MED DATA, and China Science and Technology Journal Database (VIP) and used "Single-patient trials," "N-of-1," "single case," "individual patient and randomized," "Single-Case Experimental Design (SCED)," "TCM," or "CM" as the search terms. The searching was from the inception of the databases to October 2020. We came up with two main retrieval methods to improve the breadth and accuracy of retrieval.

Retrieval Method 1: Chinese database was retrieved by "N-of-1 trails" OR "single-case," and then literatures related to TCM were manually screened out from the results. Due to the small number of N-of-1 trials literatures, most of which are published in the field of traditional Chinese medicine and have good relevance to traditional Chinese medicine, this method is indeed feasible.

Retrieval Method 2: retrieve English database by "Traditional Chinese Medicine AND N-of-1." In the English database, N-of-1 trials literatures have been published in many fields and are not highly relevant to the field of traditional Chinese medicine. This method is adopted in the English database, and a single word (such as "N-of-1") is used to search to improve the detection rate of literatures and avoid omission.

2.4. Study Selection and Data Abstraction. Teams of two reviewers, working in duplicate, independently screened titles and abstracts of all citations identified in our search. We obtained the full text of all articles that either reviewer deemed as potentially eligible. We assessed the articles according to CENT 2015 and CENT for TCM [2, 3], combined with 8-year experience of our team in the exploration of N-of-1 trials for TCM. We resolved disagreement through discussion and, when unsuccessful, with the help of a third author.

2.5. Data Analysis. We summarized the categorical variables with numbers and percentages for all analyses.

3. Results

3.1. Screening Process of the Research. Among 141 identified citations, 53 articles were retrieved for full-text screening. We included 22 research articles related to the N-of-1 trails of TCM. Of the 22 clinical reports, 2 of the trials were designed for only one cycle (pair) and could not be strictly classified as the N-of-1 trials, and 1 trial is indeed multiple baseline design (MBD), so all of the 3 trials were removed. Finally, a total of 19 articles proved eligible (Figure 1).

3.2. General Characteristics of Included Studies or Protocols. The first report of N-of-1 trials of TCM was published in 2010 [4], and 14 articles (73.68%) were published in recent 5 years. Among the 19 included papers, a total of 11 diseases were involved. Two papers did not report specific disease (the research object was TCM syndrome), and the TCM dosage forms were mainly decoction and capsule. The number of cases in each study was at least 1 and at most 50. Most of the results showed that N-of-1 trials of TCM are feasible and reflect the advantages of individualized treatment.

3.3. The Reason and Purpose of Carrying Out N-of-1 Trials in TCM Research. The reason and purpose of carrying out N-of-1 trials in TCM research are as follows: (1) most of the authors believe that N-of-1 trials can give full play to the characteristics of TCM individualized treatment (treatment based on syndrome differentiation) and embody the patient-centered trial design, so it is suitable for TCM clinical efficacy evaluation; (2) to evaluate the efficacy of TCM syndromes [5]; (3) to study the dose-effect relationship of Bezoar antihypertensive capsules and explore the individualized diagnosis, treatment, and evaluation of TCM [4]; (4) by comparing the efficacy of TCM syndrome differentiation with fixed prescription, to evaluate the feasibility of the N-of-1 trials of TCM and explore the individualized treatment evaluation method suitable for TCM research [6–9]; (5) to provide evidence for guiding doctors and patients to rationally administer Chinese patent medicine [10]; (6) some scholars think that N-of-1 trials need smaller sample size, easy to carry out with flexible design, so it can be a good complement to large randomized clinical studies [11]; (7) trying to solve the controversy of TCM clinic practice [6].

3.4. The Design of N-of-1 Trials of TCM

3.4.1. Run-In Period. A run-in period occurs before a trial begins and is typically used to initiate trial medications (for example, preliminary observation on the prescription, dosage, and effect of TCM), determine tolerability, assess potential compliance with study regimens, identify adverse effects in a timely manner, or allow for washout of medication effects a participant was taking before formal enrolment in the trial [2, 12]. As traditional Chinese medicine is a kind of the mixture of herbs, its half-life period is difficult to determine biochemically. Professor Gordon Guyatt suggested that preliminary trials should be conducted to determine the washout periods along with the clinical experience of the researchers. Taking the changes of the patients' self-rated symptom scores as the main outcome, preliminary trials can obtain the onset time after administration and the efficacy maintenance time after drug withdrawal, so as to determine the observation period and washout period [8, 13]. This approach has been adopted by several authors [6–9, 14, 15].

Run-in period is particularly important for N-of-1 trials of TCM. However, only 4 of the 19 literatures contain preliminary trials, accounting for 21.05% of the included literatures. Therefore, the setting of run-in period has not received enough attention in N-of-1 trials of TCM.

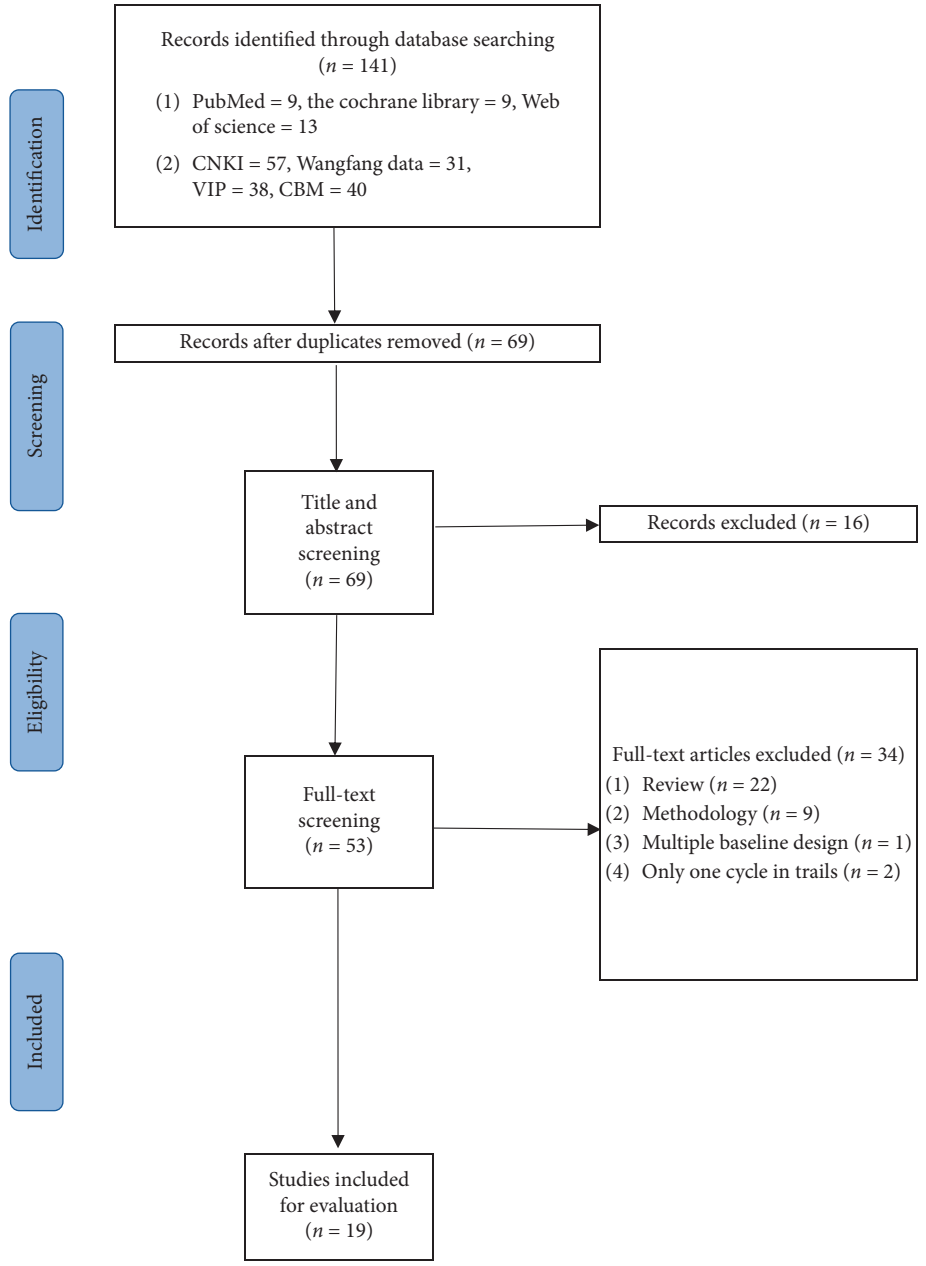

Figure 1: Search flowchart. CNKI, China National Knowledge Infrastructure; VIP, China Science and Technology Journal Database; CBM, China Biology Medicine.

3.4.2. Washout Period. A washout period may occur between treatments to allow the effects of one treatment to wear off before proceeding with the next (that is, to reduce carryover effect) [16]. The length of washout period is generally determined by consulting the half-life of the medication. Since it is difficult to determine the process of TCM metabolism and half-life period, relatively reasonable washout period is defined mainly by the following two methods:

(1) Conducting preliminary trial and combining the clinical experience of the researchers to determine a relatively reasonable washout period [8, 13] (see Section 3.4.1 of this article).

(2) To determine the length of washout period by known half-life of the main active components of TCM or TCM compound. For example, Li et al. determined the one-week washout period by the known half-life of the active components of Semen Cuscutae and astragalus membranaceus [17].

There are some ethical problems in setting washout period when patients cannot receive active treatment. If the time is too long, it will be difficult to be accepted by the subject. There were two solutions: (1) not stopping the medication between the two periods, while the outcomes were measured in the end of each period, and the time (days or weeks) before the day(s) of outcome measure was

supposed to be the washout period [7, 13]. (2) Chen et al. proposed the N-of-1 trials without washout period and their corresponding mixed effect models. The detection of carryover effect with the help of these mixed effect models is helpful to correct the influence of individual factors and period effects and compare the effects of the two kinds of interventions [16, 18].

Zi et al. [6] demonstrated the trial design of stopping medication for one week between the two treatments of TCM, which was originally aimed at extending the washout period of the former medication. Unexpectedly, it was welcomed and affirmed by most subjects, as their symptoms did not rebound during the week of stopping medication, and it was comfortable for the stomach.

3.4.3. Length of the Observation Period and Pair (Cycle). There were 10 trials involving three pairs [6, 8, 9, 14, 19–24] (the partial list is shown in Table 1). The maximum length of each period was 8 weeks [11, 20], most were 4 weeks (taking medicine for 3 weeks and stopping medication for 1 week) [4, 6, 8–10, 19, 21, 23]; 1 study was 3 weeks (taking medicine for 2 weeks and stopping medication for 1 week) [14]; 2 trials had 2 pairs (each pair contained two periods of 14 days each) [15, 25]. Some of the trials had long periods with the length of one pair up to 18 weeks [17]. Long trial time may increase the interference of unpredictable factors and decrease the subject's compliance. Most researchers selected four weeks as the length of the (observation) period.

3.4.4. Randomization and Blinding. Randomization and blinding are important principles of N-of-1 trials. Randomization is mainly carried out on several pairs of cross-sectional trials of the same N-of-1 trial and has been implemented in almost all the N-of-1 trials of TCM. However, three N-of-1 trials failed to be blinded [22–24]. Even though some literatures reported the application of blinding, the reports of implementation method were not complete and transparent [21, 23, 25]. The forms of TCM include decoction, granule, and capsule. Due to the unique perception, taste, and smell of traditional Chinese medicine, it is extremely difficult to find a control drug that is exactly the same as the test drug [3, 26]. It is especially difficult for decoction or granule to make its control (simulation agent). In the N-of-1 trials where the same patient alternates between trial and control drugs, requirements for the biofidelity of the simulated (control) drug are higher than in a parallel randomized controlled trial. Haiyin Huang et al. reported in their study that the two traditional Chinese medicine decoctions may be similar in appearance and size, but there may still be subtle differences in taste and smell. To overcome the interference of this difference, they told the participants that the test and control decoctions may be effective regardless of taste and smell. Even if there are differences, most participants did not know which formula they were assigned to because they did not show a preference for a certain decoction [6, 7].

3.5. Choice of Treatment and Control

3.5.1. Individualized Treatment. On the basis of disease differentiation, TCM researchers can carry out highly individualized treatment based on syndrome differentiation according to patients' different TCM syndromes. A total of 8 literatures [6–9, 13, 21, 22, 24] set the treatment based on syndrome differentiation as the treatment scheme in the trials. It not only met the requirements of N-of-1 trials but also retained the characteristics of TCM treatment based on syndrome differentiation. Zhang et al. [21] studied the therapeutic effect of TCM on postoperative patients with hypertensive cerebral hemorrhage according to the different syndromes of the four patients. For example, one case was diagnosed as hyperactivity of Liver Yang Syndrome and was given Tianma Gouteng decoction. In another case, purgatory phlegm decoction was used for phlegm dampness Mengshen syndrome, and later it was changed into Shenfu decoction. Placebo was used as the control for all of the four cases. Huang et al. [7] stipulated in the TCM medication program that the treatment based on syndrome differentiation can not only be performed individually but also be adjusted according to the patient's condition and syndrome changes during the whole process of N-of-1 trial while the control drug is always fixed. It conforms to the principle of "applying proper therapeutic measure in line with season, local conditions, and individuality" in TCM. In a series of N-of-1 trials, the different formulations may have different therapeutic effects, which presents new challenges for data integration analysis.

3.5.2. Fixed Prescription for the Treatment of the Same TCM Syndromes. According to the purpose and requirements of the study, this format was adopted in three clinical reports: Huang [13] included subjects clinically diagnosed as kidney Yin deficiency syndrome and observed the efficacy of Wang et al.'s decoction in the treatment of kidney Yin deficiency syndrome, which can be regarded as a type of TCM syndrome differentiation and treatment. Hui Wang compared the efficacy of two different doses of Bezoar antihypertensive capsules in patients with the same syndrome type (hyperactivity of liver fire type) [4]. Liu compared the effect of Liuwei Dihuang capsule and placebo on TCM syndrome in patients with the same syndrome type (liver-kidney Yin deficiency syndrome) [15].

3.5.3. Selection of the Control. The selection of appropriate control based on the research direction is crucial for N-of-1 trials. At present, there are mainly the following kinds of controls used in the N-of-1 trials of TCM:

(1) Placebo Control. Based on the analysis of the included literatures, it was found that few scholars chose placebo as the comparison of decoctions, which may be related to the simulation difficulty of TCM decoction. Sun et al. [20] selected the placebo with the taste, smell, and appearance most similar to ginkgo drop pills as the control. Liu et al. [19] used lactose, edible pigments, and bitters and added 10%

Table 1: Characteristics of some representative N-of-1 trials of TCM.

Author	Reasons for conducting N-of-1 trials	*n*	Run-in period	Treatment	Control	Pairs	Observation period	Randomization and blinding	Primary outcome measures	Statistical methods	Conclusion
Yuhong et al. 2013 [10]	Providing evidence for rational use of LDD.	50	No	LDD	Placebo	3	4 weeks	Yes	Likert scale, individual completion rate, response rate, and posttrial decision-making	Individuals: the self-designed criteria; population: meta-analysis	Nonresponders ceased the LDD. The positive significance is to avoid the unreasonable drugs use.
Huang et al. 2018 [7]	Exploring the establishment of clinical efficacy evaluation methods in line with the characteristics of individualized diagnosis and treatment of TCM.	17	Yes	Individualized herbal decoction	Standard decoction for stable bronchiectasis	3	4 weeks	Yes	7-point Likert scale	Individuals: paired *t* test; population: mixed effects mode	Optimizing the combined analysis of individual and group data, the improvement of statistical models may make contribution in establishing a method of evaluating clinical efficacy in line with the characteristics of TCM.
Wang et al. 2010 [4]	To study the dose-effect relationship of Bezoar antihypertensive capsules and explore the individualized diagnosis, treatment, and evaluation of TCM.	11	No	High-dose Bezoar antihypertensive capsules	Low-dose Bezoar antihypertensive capsules	3	4 weeks	Yes	Blood pressure, TCM symptom score	Paired *t* test, meta-analysis	Bezoar antihypertensive capsule can be used for mild to moderate hypertension particularly for lowering systolic blood pressure.
Chen et al. 2020 [11]	N-of-1 trials can provide more flexible clinical trial design for TCM and require a smaller sample size.	10	No	Modified SJZD with mesalazine placebo	Mesalazine with SJZD placebo	3	8 weeks	Yes	Visual analogue scale (VAS) of symptom score	Mixed effects mode	This article is a protocol and there is no conclusion.
Liu et al. 2018 [15]	To evaluate the efficacy of TCM syndromes by N-of-1 trials.	24	Yes	Liuwei Dihuang capsule	Placebo	2	2 weeks	Yes	5-point Likert scale	*t* test, ANOVA for repeated measurement, and meta-analysis	N-of-1 trials can be used to evaluate the therapeutic effects of TCM syndromes.

Table 1: Continued.

Author	Reasons for conducting N-of-1 trials	*n*	Run-in period	Treatment	Control	Pairs	Observation period	Randomization and blinding	Primary outcome measures	Statistical methods	Conclusion
Zhang et al. 2012 [21]	N-of-1 trials enable scientific evaluation of the individualized diagnosis and treatment of TCM.	4	No	Individualized herbal decoction and basic treatment	Basic treatment and Chinese medicinal decoction placebo	3	4 weeks	Yes	TCM symptom score, serum IL-6	*t* test	It is feasible to apply N-of-1 trials in clinical research of TCM.
Wang et al. 2016 [9]	To evaluate the feasibility of N-of-1 trails of TCM.	1	Yes	Individualized herbal decoction	Standard decoction for stable bronchiectasis	3	4 weeks	Yes	7-point Likert scale	Paired *t* test	N-of-1 trails reflected the advantage of TCM individualized treatment in this patient, providing with the highest rank of evidence for the patient.
Yu et al. 2012 [24]	Individualized treatment of TCM is the unique advantage.	3	No	Individualized herbal decoction	The basic treatment	3	4 weeks	Randomization but no blinding	TCM symptom score, SCr, and Ccr	*t* test	N-of-1 trails for the clinical studies of TCM are useful and feasible.

Abbreviations: TCM, traditional Chinese medicine; LDD, Liuwei Dihuang decoction; ANOVA, analysis of variance; SJZD, Sijunzi Decoction; SCr, serum creatinine; Ccr, creatinine clearance rate.

experimental medicine as the placebo control for the granules of treatment based on syndrome differentiation. Liu et al. [15] selected the black kerneled rice and prepared *Rehmannia* root (10 : 1 ratio) as the control of Liuwei Dihuang capsule. At present, it is difficult for TCM placebo to simulate the taste and smell perfectly. Some scholars have reduced the difficulty of TCM placebo simulation through the choice of dosage form. For example, Yuhong [10] chose the soft capsule from among the Liuwei Dihuang honey bolus, water pill, liquid, soft capsule, and other dosage forms in order to make it easier for the placebo to imitate the experimental drug.

(2) Related TCM Prescription as the Control. It is mainly used for the control of TCM decoction. Xue et al. [8] selected *Bronchiectasis Stabilization Decoction* (fixed formula) as the comparison of treatment based on syndrome differentiation. Zi et al. [6] selected the individualized decoction removed of heat-clearing TCM as the control of the individualized decoction in the N-of-1 trials on the treatment of bronchiectasis and studied the role of heat-clearing TCM in the stable period of bronchiectasis. Li et al. [17] used the prescription removed of the main effective drugs (astragalus membranous, semen Cuscutae) as the control.

(3) Conventional Basic (Western Medicine) Treatment. As a control, conventional basic treatment is mostly used to compare the efficacies of TCM treatment and Western medicine treatment in the same situation, or the efficacies of a single treatment regimen and a combination of treatment regimen. Yu et al. [24] chose the conventional basic treatment as the control of the prescription based on syndrome differentiation without blinding. Jiao et al. [22] used telmisartan as the control in the N-of-1 trials to observe the treatment of IgA diabetic nephropathy with Modified Huangqi Chifeng Decoction combined with telmisartan.

Some combined application schemes of TCM and Western medicine adopted double-blind and double-simulation form, indicating that the degree of blinding in some N-of-1 trials for TCM has reached a relatively high level [14, 21].

3.6. Outcome Measures

3.6.1. Selection of Outcome Measures. The characteristics of the N-of-1 trials are to fully respect the choice of the patient, to determine the main problems to be solved jointly by the doctor and the patient. Almost all of the included reports took the clinical symptom score that the patient is most concerned with as an important outcome. Some studies used objective signs, such as blood pressure at a given time [4] and 24-hour sputum volume [6–9, 13]. Some studies used laboratory indicators, such as serum IL-6 [21], 24h urinary protein quantification [22], blood routine [14], serum creatinine (SCr), and creatinine clearance rate (Ccr) [24].

Safety outcomes include regular observation of adverse events related to the trials, measurement of blood and urine routine, liver and kidney function, and electrocardiogram, if necessary, terminating the experiment and unblinding. Good outcomes should be easy to repeat measurement, sensitive, and generally recognized as effective.

3.6.2. Minimal Clinically Important Difference (MCID). The most direct outcomes that best reflect the needs of patients are the clinical symptoms which trouble patients (such as cough, wheezing, loss of appetite, and insomnia) and the quality of life scale. The 7-point Likert scale recommended by Professor Guyatt, in which the patients used a diary form to self-evaluate the severity of the symptoms which they were concerned about (usually 4 to 8 symptoms) on a daily basis, was more intuitive when supplemented by visual analogue scales (VAS). Its significant advantage is that its reliability and its MCID have been fully proofed before its application. In the 7-point Likert scale, an average difference of 0.5 was defined as the minimum clinically important difference (MCID) [27, 28]. Three included literatures have been found to have adopted the 7-point Likert scale as an important outcome [6–8]. Two articles used either a 6-point Likert scale or a 5-point Likert scale [10, 15]. Some authors used some widely accepted scales [20, 25]. Some authors adopted TCM syndrome score [9, 19, 22]. The results of these scales or scores may be statistically significant but not clinically significant; therefore, in addition to reporting statistical differences, it is also necessary to explain whether the score or scale has demonstrated MCID and whether the difference has reached MCID. Besides, MCID is essential for converting quantitative outcomes into binary outcomes such as "effective/response rate."

3.6.3. Negative and Positive Results. Because N-of-1 trials have the scientific design of randomization and blinding, which reduces the selection bias, and the small sample size increases the probability of false negatives, the probability of negative results will inevitably increase. Among the results of the 19 TCM N-of-1 trials retrieved in this study, 3 were negative, or even though, there is a statistical difference ($P < 0.05$), but not clinically significant [4, 6, 10]. It should be recognized that a negative result from a high-quality N-of-1 trial is no less important than a positive result. For example, Yuhong et al. evaluated the clinical efficacy of Liuwei Dihuang soft capsule in the treatment of 50 patients with kidney Yin deficiency syndrome by a series of N-of-1 trials. The meta-analysis results showed that Liuwei Dihuang soft capsule and placebo had no difference in the efficacy of improving kidney Yin deficiency syndrome ($P > 0.05$). After the trials, the drug was discontinued in the nonresponders, and its positive significance was to avoid the abuse of patent Chinese medicines [10]. When interpreting negative results, we must first realize that negative results are as valuable as positive results and then make judgments based on the doctor's clinical experience.

3.7. Selection of Statistical Methods. In the early years, N-of-1 trials were mainly the self-control of individual cases, and

the statistics of repeated crossover data in multiple rounds (pairs). With the development of the N-of-1 trials methodology, it was found that a series of N-of-1 trials can summarize the overall treatment effect of a group and obtain relevant treatment information for individual participants [29, 30]. The ideal statistics of a series of N-of-1 trials should be the combination of individual statistics and group statistics: to extend the application of this method from a single case to the combination of individuals and groups. At the same time, it also makes it possible for clinical trials of TCM to treat chronic diseases to generally adopt a series of N-of-1 trials in the future.

CENT 2015 encourages the use of confidence intervals which is often possible for the analysis of important clinical differences [2]. However, the Chinese literatures for N-of-1 trials did not pay enough attention to this, and only a part of the literatures published confidence interval.

3.7.1. Statistics of Individual Data. The most attractive aspect of N-of-1 trials is that, by the comparison of two treatments for the individual patient, a hypothetical or potentially more effective prescription or treatment can be screened out. By repeating N-of-1 trials on the same patient, we can continually improve the effectiveness of the prescription or treatment for each individual. This is the ultimate goal of the research on individualized treatment (syndrome differentiation) by TCM [7]. However, statistical analysis of individual cases was not performed in 9 included articles [9, 14, 17, 19–21, 23–25], accounting for 47.36% of the included literature, which is a pity for the evaluation of individualized treatment of TCM. If the data were normally distributed, paired t test was preferred for single case statistical analysis. Paired Wilcoxon signed rank tests were conducted to analyze the data which were not normally distributed. Although paired t test was used in many literatures [6, 7, 11, 22], some N-of-1 studies also used t test (its statistical power is lower than paired t test) [25]. Since the same subject receives multiple treatments and measurements at different times, it is called repeated measurement data and has autocorrelation interference to the results. Guyatt's strategy was to average the data in a certain period of time (for example, averaging the data of 7 days as the average value of the week) and then perform statistical analysis [7, 27]. The data published in some literatures did not clearly indicate the time and frequency of measurements, which needs to be improved [21, 22].

3.7.2. Population Statistics for a Series of N-of-1 Trials. Commonly used statistical methods include meta-analysis, hierarchical Bayesian statistical method, or mixed effects model analysis [31]. However, hierarchical Bayesian statistical method has not been used in the N-of-1 trials for TCM. Among them, meta-analysis [4, 10, 15] was used in three articles, t test or paired Wilcoxon signed rank tests in 5 articles [8, 13, 22, 24, 26], and mixed effects model in 3 articles [6, 7, 11].

3.7.3. The "Carryover Effect" of TCM. CENT2015 requires the description of the statistical methods used to account for carryover effect, period effects, and intrasubject correlation. Only 3 articles have given some explanation on this requirement [6, 11, 15]. There are two articles mentioning that the "carryover effects" of traditional Chinese medicine may affect the results. Yuhong [10] speculated that a limitation of their study was washout period which has not been fully considered, which resulted in "carryover effects" of Liuwei Dihuang decoction (LDD) interfering with the differences between LDD and placebo. Huang et al. [7] discussed that "the nature of traditional Chinese medicine might not meet with a certain requirement of the classic N-of-1 trials perfectly: the treatment should have a rapid onset and stop acting soon after it is discontinued." They found that "the gap between the mean symptom scores of the individualized decoction and control decoction had a diminishing trend from first to third pairs in a series (14 cases) of N-of-1 trials (Figure 2), suggesting the possibility of "carryover effects.""

3.8. Follow-Up after the Trial. Only a few literatures have mentioned whether the treatment plan of the subject has changed, or whether there were long-term adverse reactions after the completion of the trials [10]. It is worthy of attention in future research.

3.9. Main Findings. Since the publication of the first N-of-1 trials of TCM [4], the practitioners of TCM and integrated TCM and Western medicine have made valuable explorations on the feasibility and rules of N-of-1 trials of TCM over the past 10 years. Hui Wang et al. proved that the high dose of Bezoar antihypertensive capsules could reduce the systolic blood pressure with the syndrome type of hyperactivity of liver fire. N-of-1 trials can be used as a management tool for clinical medical practice and preliminary exploratory research on new drugs [4]. Huang et al. [13] proposed a method to establish a relatively reasonable washout period through the run-in period of N-of-1 trials for TCM. Yuhong et al. [10] used the results of a series of N-of-1 trials to make the nonresponders stop the drug after the trials. The positive significance is to avoid the abuse of Liuwei Dihuang capsule. Liu et al. [5] found that the N-of-1 trials could be used to evaluate the efficacy of the treatment based on syndrome differentiation to improve TCM syndromes, and the results were reliable. Chen et al. [16] proposed a design of N-of-1 trials without washout period and its corresponding mixed effect model, which could be used to detect the carryover effect of TCM. Finally, the publication of CENT for TCM in 2019 [3] showed that the national TCM community have attached great importance to this individualized clinical trial method.

4. Discussion

In summary, N-of-1 trials of TCM have made great progress in the past decade. However, we still face many difficulties, such as certain difficulties in the implementation of blind methods, sometimes the selection of outcome measures was

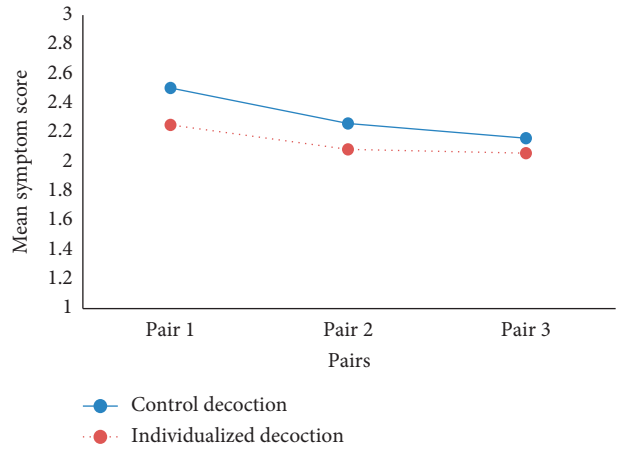

Figure 2: The gap between the mean symptom scores of the individualized decoction and control decoction had a diminishing trend from first to third pairs in a series (14 cases) of N-of-1 trials, suggesting the possibility of "carryover effects." Excerpted from "Huang Haiyin, Yang Peilan, and Wang Jie et al.'s Investigation into the Individualized Treatment of Traditional Chinese Medicine through a Series of N-of-1 Trials. Evid Based Complement Alternat Med. 2018; 2018 : 5813767. doi: 10.1155/2018/5813767."

unsatisfactory, and the statistical methods were not standardized, etc. There are other problems that manifested in the following aspects.

4.1. Existing Problems

4.1.1. The Contradiction between Treating Symptoms and Curing Root Causes. TCM treatment emphasizes that "the cure must be based on the etiology," rather than simply eliminating symptoms. This was the case with one of the core principles of naturopathic medicine: "Treat the Cause (tolle causam)" [32]. Although N-of-1 trials embody a high degree of individualization and are close to the clinical practice of TCM, they are most suitable for treating symptoms, especially chronic symptoms, rather than treating the root cause.

4.1.2. The Interference of "Carryover Effects". N-of-1 trials generally require that the studied drugs have the characteristics of fast onset, short half-life, and fast disappearance after stopping use [2]. The components of TCM are complex and the onset and expiration time (half-life) are relatively long, so it might not meet these requirements. "Carryover effects" may be an unfavorable factor affecting the design and quality of N-of-1 trials of TCM [7, 10, 16].

4.1.3. The Influence of Seasons and Time Rhythms. In addition to individualized treatment, traditional Chinese medicine believes that people's physiological and pathological changes will inevitably be affected by seasons, climate, and time rhythms. Therefore, the occurrence of diseases and the principles of health preservation and treatment are different in different seasons. In the treatment of chronic diseases, the patient's TCM syndrome type may also change. Therefore, in the N-of-1 trials of TCM, individualized prescriptions should also change according to the situation. Although Huang et al. [7] stipulated in the TCM medication program that the treatment based on syndrome differentiation can not only be performed individually but also be adjusted according to the patient's condition and syndrome changes during the entire process of the N-of-1 trial, how to overcome the influence of seasons and time rhythms in the N-of-1 trials of TCM is still challenging.

4.2. Development Trends. In recent years, hierarchical Bayesian statistical method has become one of the main statistical methods for a series of N-of-1 trials with remarkable characteristics [2, 29, 30, 33]. Some scholars discussed in detail the advantages of Bayesian method over general statistical methods, mainly including the following: (1) both individual and aggregate analyses can be simultaneously and coherently undertaken. (2) It is easy to introduce confounding variables [34–36], such as the gene type or different TCM syndrome types of different patients, the severity of bronchiectasis, sputum culture (whether *Pseudomonas aeruginosa* is positive), and the potential carryover effect of TCM. Regarding the "carryover effect," it is envisaged to adopt the mixed effect model proposed by Chen et al., which was supposed to detect the "carryover effect" [16, 18]. (3) A prior information can be introduced. Prior information, sample information, and posterior information are the three important types of information of the Bayesian model. The prior information comes from previous research. The sample information comes from the existing data, and the Bayesian model uses the sample information to update the prior information to obtain more reliable posterior information. In the hierarchical Bayesian model, previous data and new experimental data belong to a continuous data chain. When new experimental data is generated, the data chain is updated to produce more accurate posterior information [33]. Though having not been used in N-of-1 trials of TCM, hierarchical Bayesian statistical method is worthy of studying and promoting and is expected to improve the reliability and sensitivity of N-of-1 trials of TCM.

5. Conclusion

In short, the design, implementation, and data analysis of N-of-1 trials of TCM are still in in-depth exploration and practice. "Carryover effect" may affect the design and quality of N-of-1 trials of TCM. Individualized treatment should be guided by the classic theories of TCM. It is expected to formulate reasonable observation periods and pairs and closely integrate individual and group statistical analysis.

Disclosure

Haiyin Huang and Jiaqi An are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

H. Huang and J. An contributed equally to this work.

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Review Article

Outcome Reporting Variability in Trials of Chinese Medicine for Hyperlipidemia: A Systematic Review for Developing a Core Outcome Set

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Introduction. Hyperlipidemia is an underlying process behind cardiovascular disease. Chinese medicine (CM) may be effective in treating hyperlipidemia, but there is a lack of studies with high methodological quality. A major reason for this is heterogeneity in outcome reporting. Therefore, this study explores the degree of outcome reporting variation in CM trials for hyperlipidemia. It then generates a list of potentially important outcomes for developing a core outcome set (COS). *Methods*. A systematic review of literature focusing on studies of CM for hyperlipidemia was conducted. Outcomes were listed verbatim and grouped into 8 domains. Outcome frequency and definition uniformity were analyzed. *Results*. 3,702 studies and 452 individual outcomes were identified. These outcomes were reported 27,328 times, of which 1.6% were reported as primary outcomes, and 13.3% were defined. The most frequent outcome was total triglyceride, represented in 86.7% of the studies, followed by total cholesterol (86.0%), total effective rate (75.1%), high-density lipoprotein cholesterol (73.2%), and low-density lipoprotein cholesterol (60.5%). However, 43.6% of outcomes were reported only once. The largest outcome domain was "pathological or pathophysiological outcomes," which included 67.0% of outcomes. Of the "response rate related outcomes" domain, total effective rate was the most frequently reported outcome ($n = 2{,}780$), and 95.3% of the studies gave a clear definition. However, these definitions were often contradictory. Only 10 papers reported cardiovascular events, 3 of which referred to them as primary outcomes. Moreover, ten patient-reported outcomes were reported in the retrieved literature 19 times in total. The majority of the outcomes did not report measurement instruments (MIs) (269/453, 59.4%). MIs of the surrogate outcomes were reported more frequently. *Conclusion*. Outcome reporting in CM trials for hyperlipidemia is inconsistent and ill-defined, creating barriers to data synthesis and comparison. Thus, we propose and are developing a COS for CM trials for hyperlipidemia.

1. Introduction

Hyperlipidemia is a key underlying process for cardiovascular diseases [1]. It includes hypercholesterolemia, hypertriglyceridemia, and mixed hyperlipidemia [2]. The prevalence of hyperlipidemia among Chinese adults is 34.0%, and it is higher among males than females (41.9% and 32.5%, respectively) [3]. In the United States, an estimated 12.9% of adults have elevated total cholesterol (TC), and 6.2% have undiagnosed hypercholesterolemia [4]. Although hyperlipidemia is prevalent and has received attention from the medical community, it is often neglected by patients. Because clinical hyperlipidemia symptoms are not obvious, patients unfamiliar with it rarely receive serum lipid examinations until their cardiovascular system has already been damaged and symptoms have presented [5]. The awareness, treatment, and control rate of hyperlipidemia in China are low [6, 7], especially in men, aged <45, and especially in rural and Western areas [8]. Many studies have found that Chinese medicine (CM) provides relief to patients with hyperlipidemia [9–11]. One CM treatment, Xuezhikang, has been recommended by the Joint Committee for Developing Chinese Guidelines on Prevention and Treatment of Dyslipidemia in Adults [2].

While trials of the effectiveness of CM for hyperlipidemia are becoming more common, there remains a lack of studies with high methodological quality. Outcome measure is one of the key issues [12]. Several systematic reviews have reported heterogeneity in outcome reporting in many clinical trials [9–11, 13]. Therefore, it is difficult to synthesize clinical trial results with different outcomes. Developing a core outcome set would improve the quality of outcome reporting.

A core outcome set (COS) represents the minimum that should be measured and reported in all clinical trials for a specific condition to facilitate the comparison and combination of trials while researchers continue to explore other outcomes [14]. The Core Outcome Measures in Effectiveness Trials (COMET) initiative collates and stimulates relevant resources, both applied and methodological, to facilitate the exchange of ideas and information and to foster methodological research in this area [14]. The authors have registered COS development for hyperlipidemia with the COMET initiative [15]. Systematic review is a feasible and efficient approach to identifying and aggregating an inclusive list of outcomes being reported by researchers in a given area [16]. It is also recommended by the COMET initiative [17].

This systematic review is the first stage of developing a core outcome set for hyperlipidemia. It explores the degree of heterogeneity of outcome reporting in CM trials for hyperlipidemia and generates a list of potentially important outcomes which will be scored in a Delphi survey.

2. Methods

2.1. Protocol and Study Registration. This systematic review is a component of the development of a core outcome set for hyperlipidemia. It was registered on the COMET initiative website (registration number: 983) [15]. The protocol for the development of a COS for hyperlipidemia has recently been published [18]. We performed this systematic review in compliance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [19].

2.2. Eligibility Criteria

2.2.1. Type of Studies. Case reports, case-control studies, cohort studies, randomized controlled trials, or systematic reviews, published in either English or Chinese, were included. Studies published only as conference abstracts for which papers could not be accessed, despite sending requests to their authors, were excluded. This was done because conference abstracts are not representative of comprehensive outcome lists, due to their length limitations.

2.2.2. Types of Participants. Patients with hyperlipidemia were included, including high TC, high total triglyceride (TG), mixed high TC and high TG, high low-density lipoprotein cholesterol (LDL-C), and lowered high-density lipoprotein cholesterol (HDL-C), regardless of sex, age, or race. If a study focused on secondary hyperlipidemia, as opposed to other serious diseases, the full text was read to determine whether or not to include it.

2.2.3. Types of Interventions. Patients treated by CM alone or CM in combination with conventional medicine were included

2.2.4. Types of Outcome Measures. All studies reporting hyperlipidemia outcomes were included

2.3. Literature Search. Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, Embase, Wanfang Database, China National Knowledge Infrastructure (CNKI), and Chinese Biomedical Database (CBM) were searched, from inception to November 2020. The search strategy included three parts: hyperlipidemia disease, CM treatment, and study type. Boolean operators were used to combine the three parts. Truncations and wildcards were employed to optimize the search. The search strategy is available in Supplementary Materials S1.

2.4. Study Selection. Two independent reviewers (GL and RH) initially assessed eligibility by reading titles and abstracts. Any discrepancies were resolved either through discussion after critical review of the full text or by consulting a third author (XC). Duplicate studies were excluded. Studies that did not report hyperlipidemia outcomes were excluded as well.

2.5. Data Collection. Two reviewers (GL and RH) independently extracted the data by reading the full texts. Characteristics of each study were extracted, including title, publishing journal, author(s), year of publication, country,

authors' affiliation(s), funding, study type, treatment duration, patient source, and diagnosis criteria. The latter included blood lipids, CM syndrome pattern, type of hyperlipidemia, complications, follow-up duration, number of patients who withdrew, intervention details, names of outcomes and whether they had been specified as primary or secondary outcomes, definition of outcomes, time-point and method of outcome measurement, and adverse events.

To ensure the reliability of the analytical details, constant evaluation was conducted between the two reviewers before data extraction. Any disagreements were resolved by discussion and by consulting a third researcher (XC). Data then were extracted using EpiData 3.1 (EpiData Association, Denmark). In cases when the authors had given us missing data by e-mail or telephone, incomplete data were filled in.

To present and analyze the results, outcomes were grouped into eight domains by two researchers (GL and RH). The results were reviewed by another researcher and discussed by a research team. These included mortality related outcomes, pathological or pathophysiological outcomes, response rate related outcomes, cardiovascular events, symptoms or function related outcomes, adverse events or safety related outcomes, patient-reported outcomes, and resource utilization related outcomes.

2.6. Statistical Description and Analysis. Data analysis was performed with SPSS 18.0 (IBM SPSS Inc., Armonk, New York, USA). Definitions with the same outcome were extracted and compared. The number of outcomes in each domain and the number of measurement methods were calculated. The frequency of categorical variables was presented, as well as their means and standard deviations, or medians and interquartile ranges for quantitative data.

3. Results

3.1. Characteristics of Included Studies. The search strategy found 63,783 articles. Finally, 3,702 (3,614 in Chinese and 88 in English) were included after removing duplicates and other ineligible articles (Figure 1).

There were four main types of studies: (1) reviews articles ($n = 62$, 1.7%) including 57 systematic reviews; (2) experimental studies ($n = 2{,}872$, 77.6%) including randomized controlled trials (RCTs, $n = 2{,}394$, 64.7%), nonrandomized controlled trials ($n = 289$, 7.8%), and quasirandomized controlled trials ($n = 189$, 5.1%); (3) quasiexperimental studies ($n = 743$, 20.1%); and (4) observational studies ($n = 25$, 0.7%).

The included articles were all published between 1974 and 2020, and 87.0% had been published after 2000. Most of the studies included outpatients and patients with complications. Only 191 (5.4%) studies had funding support. Most of the studies (99.1%) were conducted in China. 1,178 (31.8%) of the studies only included primary hyperlipidemia, and 1,333 (36.0%) included patients with complications.

3.2. CM Syndrome Analysis. There were 933 studies reporting CM syndrome patterns, of which 357 included multiple CM patterns. In total, 315 verbatim CM patterns were reported, which were described 1,742 times. After processing and reclassification, 129 CM patterns were identified. The most frequent pattern was *phlegm dampness* (phlegm turbidity) which was reported in 365 studies. It was followed by the *phlegm dampness and blood stasis* pattern ($n = 277$), *qi stagnation and blood stasis* pattern ($n = 160$), *liver-kidney yin deficiency* pattern ($n = 159$), *spleen-kidney yang deficiency* pattern ($n = 114$), and *blood stasis* pattern ($n = 108$). There were 62 syndromes reported only once.

3.3. Results on Outcomes. A total of 452 individual recorded outcomes were identified and reported, from which there were 27,328 times (instances). These outcomes were categorized into 5 themes (death, physiological/clinical, adverse events, life impact, and resource use) and 8 domains (mortality related outcomes, pathological or pathophysiological outcomes, response rate related outcomes, cardiovascular events, symptom or function related outcomes, adverse events or safety related outcomes, patient-reported outcomes (PROs), and resource utilization related outcomes). Of note, "physiological/clinical" outcomes were mostly reported by researchers. Details of the five themes and eight domains including their definitions as well as the included outcomes are listed in Table 1.

The ten most frequently reported outcomes among the 3,702 included studies are listed in Table 2. The most frequent outcome was TG, representing 86.7% of the studies ($n = 3{,}211$), followed by TC in 86.0% of the studies ($n = 3{,}185$), total effective rate in 75.1% of the studies ($n = 2{,}780$), HDL-C in 73.2% of the studies ($n = 2{,}709$), and LDL-C in 60.5% of the studies ($n = 2{,}204$). Most of the studies neither used them as primary outcomes nor gave clear definitions (Table 2). Note that 197 (43.6%) outcomes were reported only once, and 157 (34.7%) were reported between 2 and 10 times (Supplementary Materials S2).

3.3.1. Mortality Related Outcomes. Mortality related outcomes were reported infrequently, and none reported a detailed definition. There were 2 outcomes relating to morality, all-cause mortality and death, in only 4 studies. One of these studies specified death as the primary outcome, and the other did not.

3.3.2. Pathological or Pathophysiological Outcomes. There were 303 different pathological or pathophysiological outcomes, which were subdivided into 30 subcategories (Table 3). These outcomes were reported 17,036 times, 62.3% of all reported outcomes. Thirty-seven outcomes pertained to blood lipid, the four most common of which were TG, TC, HDL-C, and LDL-C. These four outcomes were reported 11,345 times, accounting for 41.5% of all reported outcomes. Of these, only 310 (2.7%) were reported as primary outcomes, and 259 (2.3%) were defined.

3.3.3. Response Rate Related Outcomes. Twenty-seven individual outcomes measured disease recurrence or progression. Total effective rate was the most frequently

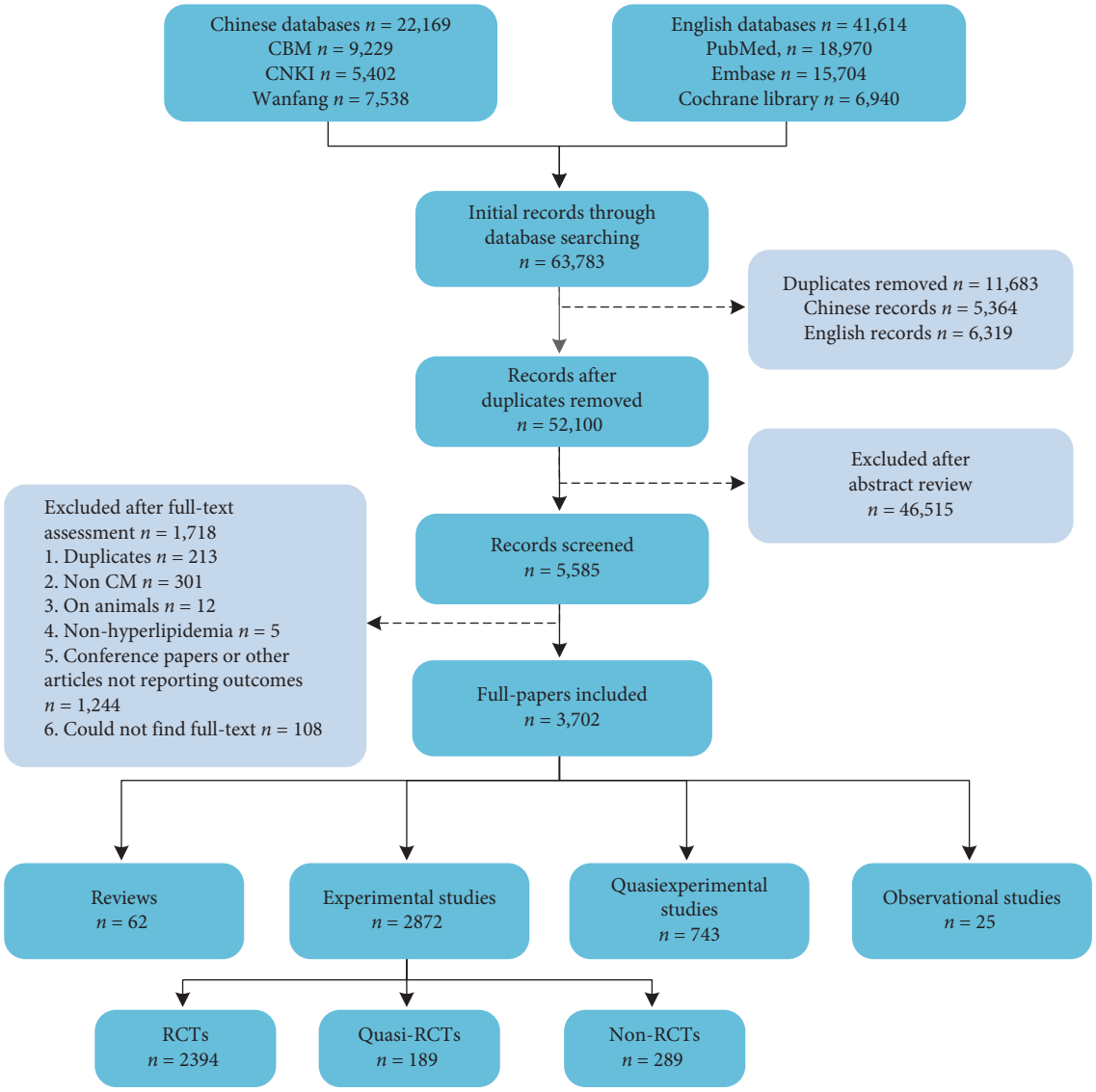

Figure 1: PRISMA diagram of studies searched and included in the systematic review.

reported outcome ($n = 2{,}780$), although only 18 studies referred to it as a primary outcome. Nevertheless, 2,648 (95.3%) studies defined it. However, based on a categorical assessment (e.g., "markedly improved," "improved," "slightly better," or "no effect"), the definition of total effective rate varied across studies. For example, some studies defined total effective rate as the sum of the "markedly improved" rate and the "improved" rate, while others stated total effective rate as the sum of the "clinical control" (blood lipid returning to normal after intervention) rate, "markedly improved" rate, and "improved" rate. Furthermore, the standard of "improved" also varied. For example, some studies specified that LDL-C that had declined by at least 20% had improved, while others defined improvement as LDL-C below 3.640 mmol/L after treatment.

3.3.4. Cardiovascular Events. 10 studies reported cardiovascular events, and 3 of them referred to it as a primary outcome.

3.3.5. Symptom or Function Related Outcomes. This section encompassed 6 subcategories: (1) clinical symptoms (non-CM), (2) CM symptoms or patterns, (3) endothelial function, (4) erectile function, (5) balanced capacity test, and (6) others, with 55 different outcomes. CM syndrome efficacy, CM syndrome score, and clinical signs and symptoms were the three most commonly reported outcomes, reported in 395 (10.7%), 333 (9.0%), and 295 (8.0%) of the studies, with an 85.3%, 42.3%, and 16.6% definition percentage, respectively. Based on reading their definitions, it was discovered that most of these three

Table 1: Outcome domains, definitions, number of individual outcome measurements in each domain, and frequency of outcomes reported of each domain.

Theme	Outcome domain	Definition of outcome domain	No. of outcomes	Frequency of outcomes reported
Total			452	27,328
I. Death	1. Mortality related outcomes	Outcomes related to short- and long-term survival/death rates and cause of death	2	4
II. Physiological/clinical	2. Pathological or pathophysiological outcomes	Outcomes related to the reporting of blood, or biochemical measures, within standard clinical practice or research	303	17,036
	3. Response rate related outcomes	Measures of disease recurrence or disease progression	23	2,835
	4. Cardiovascular events	Death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, unstable angina requiring hospitalization, coronary revascularization procedures, peripheral revascularization procedures, heart failure requiring hospitalization, stent thrombosis, and transient ischemic attack [1]	1	10
	5. Symptom or function related outcomes	Signs and symptoms of disease reported by clinicians	55	1,358
III. Adverse events	6. Adverse event or safety related outcomes	Forms of short-term and long-term complications	56	6,063
IV. Life impact	7. Patient-reported outcomes	Outcomes reported by patients themselves	10	19
V. Resource use	8. Resource utilization related outcomes	Economic outcomes related to healthcare	2	3

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Table 2: The ten most frequently reported outcomes among the 3,702 included studies.

Outcome name	Domain	No. of studies reported/total (%)	No. of studies reported as primary outcome/total (%)	No. of studies reported as secondary outcome/total (%)	No. of studies reported with definition/total (%)
TG	II. Pathological or pathophysiological	**3211**/3702 (86.7%)	**94**/3211 (2.9%)	12/3211 (0.4%)	**73**/3211 (2.3%)
TC	II. Pathological or pathophysiological	**3185**/3702 (86.0%)	**74**/3185 (2.3%)	**14**/3185 (0.4%)	**72**/3185 (2.3%)
Total effective rate	V. Response rate	**2780**/3702 (75.1%)	**18**/2780 (0.6%)	**6**/2780 (0.2%)	**2648**/2780 (95.3%)
HDL-C	II. Pathological or pathophysiological	**2709**/3702 (73.2%)	**63**/2709 (2.3%)	**17**/2709 (0.6%)	**63**/2709 (2.3%)
LDL-C	II. Pathological or pathophysiological	**2240**/3702 (60.5%)	**79**/2240 (3.5%)	**9**/2240 (0.4%)	**51**/2240 (2.3%)
Liver function	III. Adverse event or safety	**1199**/3702 (32.4%)	**1**/1199 (0.1%)	**6**/1199 (0.5%)	**10**/1199 (0.8%)
Renal function	III. Adverse event or safety	**1046**/3702 (28.3%)	**1**/1046 (0.1%)	**6**/1046 (0.6%)	**10**/1046 (1.0%)
Blood routine examination	III. Adverse event or safety	**958**/3702 (25.9%)	**1**/958 (0.1%)	**5**/958 (0.5%)	**9**/958 (0.9%)
Urine routine examination	III. Adverse event or safety	**873**/3702 (23.6%)	**1**/873 (0.1%)	**5**/873 (0.6%)	**7**/873 (0.8%)
ECG	II. Pathological or pathophysiological	565/3702 (15.3%)	0/565 (0.0%)	3/565 (0.5%)	7/565 (1.2%)

TG: total triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ECG: electrocardiogram.

Table 3: Subcategories of pathological or pathophysiological outcomes and frequency of outcomes reported in each subcategory.

No.	Outcome subcategories	No. of individual outcomes in each subcategory	Frequency of outcomes reported from this subcategory
Total		303	17,036
1	Blood lipid related outcomes	37	11,744
2	Blood glucose related outcomes	7	498
3	Steroid related outcomes	6	6
4	Protein related outcomes	43	845
5	Other lipid related outcomes	4	8
6	Carotid atherosclerotic plaque related outcomes	7	31
7	Heart function related outcomes	5	288
8	Hemorheology related outcomes	24	1,136
9	Cellular immune function related outcomes	5	8
10	Fluidity of cell membrane related outcomes	2	2
11	Inflammation related outcomes	7	118
12	Blood pressure related outcomes	2	363
13	Blood platelet function related outcomes	9	68
14	Blood coagulation function related outcomes	14	78
15	Enzymatic activity measurement outcomes	14	75
16	Hormone related outcomes	7	11
17	Insulin related outcomes	5	15
18	Imageological examination related outcomes	18	80
19	ECG related outcomes	2	622
20	Electrolyte related outcomes	8	99
21	Prostaglandin related outcomes	2	15
22	Stable no. of metabolites	1	1
23	Obesity related outcomes	7	15
24	Anemia related outcomes	4	4
25	Microcirculation related outcomes	10	12
26	Thrombotic test in vitro related outcomes	4	9
27	Blood gas analysis related outcomes	2	10
28	General items related outcomes	15	773
29	Physical examination related outcomes	5	16
30	Others	27	86

outcomes were measured by scales. For example, the obstruction of phlegm-dampness pattern grading scale measured the CM score. Tongue manifestation and pulse condition, two important CM symptoms, were reported 54 and 50 times. In addition, nitric oxide (NO) and endothelin were the most commonly reported endothelial function outcomes, reported by 54 and 39 studies, respectively. There were only 10 outcomes reported for erectile function, balanced capacity test, and other functions. Examples included erection quality and static balance with closed eyes.

3.3.6. Adverse Events and Safety Related Outcomes. Three subcategories (adverse events, adverse reactions, and safety outcomes) were considered part of the adverse event or safety domain, with 56 outcomes in total. Adverse events were any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. On the other hand, all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions [20]. In this paper, adverse events related outcomes only included outcomes with literal meaning of adverse events, such as the number of adverse events and adverse events rates; adverse reactions related outcomes also only included outcomes with literal meaning of adverse reactions, such as the number of adverse reactions and adverse reaction rate. Other safety related outcomes except adverse events and adverse reactions were included in safety outcomes, such as laboratory abnormalities. Liver function, kidney function, routine blood, and urine and stool tests were the most frequently reported outcomes, reported in 1,199 (32.4%), 1,046 (28.3%), 958 (25.9%), 873 (23.6%), and 376 (10.2%) studies, respectively. However, these 5 most common outcomes were rarely defined (the definition incidence rate was below 1%). Other safety related outcomes included hematological or biochemical measures, such as glutamic-pyruvic transaminase (ALT). With regard to adverse events and adverse reaction related outcomes, the most commonly reported outcome was adverse events (reported in 85 studies). The other 4 outcomes (incidence of adverse reactions, adverse reactions, incidence of adverse events, and total incidence of adverse reactions) were reported by 21 papers.

3.3.7. Patient-Reported Outcomes. Ten outcomes were patient-reported. Seven studies reported on quality of life, of which two used SF-36, one used an instrument designed by the researcher, and two used other scales. The rest did not specify the scales used. Additional outcomes were related to activity, health, patient satisfaction, eating behaviors, and mental states or symptoms; all were reported in the individual studies.

3.3.8. Resource Utilization Related Outcomes. Two outcomes related to resource utilization were reported in 3 studies: cost and treatment implementation time.

3.4. Outcome Measurement Instrument Results. The measurement instruments (MIs) for most outcomes were not reported (269/453, 59.4%). MIs for surrogate outcomes were reported more frequently; for example, enzymatic analysis and immunoturbidimetry were used for TC measurement. For some of the subjective outcomes, such as quality of life and CM syndrome efficacy, MIs were also reported. For example, CM symptom score scale, CM syndrome rating scale, dyslipidemia syndrome evaluation scale, and *spleen-strengthening and lipid-regulating therapy* questionnaire were used to measure CM syndrome efficacy.

4. Discussion

In this systematic review, variation and inconsistencies in the definition and reporting of outcomes were identified. 452 individual outcomes were reported in the included studies, while there was no single outcome reported in all studies. Of those, 43.6% were reported only once, and 34.7% were reported between two and ten times. Most studies did not distinguish primary outcomes from secondary outcomes, nor did they provide explicit definitions of the outcomes measured. Some provided only classification outcomes without detailed outcome measures such as blood lipids, routine blood test, renal function, or carotid ultrasonography. This heterogeneity complicates comparison and synthesis across studies. Thus, the studies' usefulness in promoting clinical practice progress was limited.

A positive finding was that blood lipid outcomes, such as TG, TC, HDL-C, and LDL-C, were reported by most studies. A composite outcome called total effective rate was also extensively reported. However, its definitions varied, inhibiting comparison across studies.

However, cardiovascular events and patient-reported outcomes were rarely reported. One reason may have been that the intervention periods for most of the included studies were not long enough to observe the cardiovascular events. It is also possible that hyperlipidemia is commonplace and usually does not affect patient quality of life [5].

In CM theory, pattern (also called syndrome) is a diagnostic conclusion based on pathological changes in a disease, at a certain stage [21]. A pattern often contains several CM symptoms, such as tongue manifestation or pulse condition. It is important for CM physicians to measure pattern and CM symptom changes when treating patients. Several CM outcomes were reported in this review such as CM symptoms score (primary symptoms and secondary symptoms), CM syndrome efficacy (single or multiple items), tongue manifestation, and pulse condition. However, the definitions and measurement instruments varied. This presents an obstacle to high-quality systematic review. Furthermore, these outcomes were poorly reported—CM syndrome efficacy was the most frequently reported CM outcome, despite only 395 of 3,702 studies reporting it.

Previous work has found heterogeneity in outcome reporting in hyperlipidemia studies. A 2015 systematic review of 35 trials of Zhibituo (a Chinese patented drug) in hyperlipidemia treatment reported 30 different outcomes [13]. A 2011 systematic review of 22 RCTs on Chinese herbal medicines for hypercholesterolemia found 20 individual outcomes, but no trial reported outcomes pertaining to cardiovascular events [11]. The present review covered a wide range of studies. This included not only RCTs, but also observational studies. It identified an exhaustive list of outcomes reported in studies of CM for hyperlipidemia, and it is the first stage of developing a core outcome set for clinical trials of CM for hyperlipidemia.

This review has several limitations, and its results should be interpreted with caution. Firstly, only published studies were included. This may have caused bias. For example, only researchers' and clinicians' viewpoints on outcomes were referenced, while patients and other stakeholders were not considered. However, as the present study is the first stage of developing a core outcome set, we will include several patients and other stakeholders in a future Delphi survey to capture their comments. Secondly, as a literature systematic review, publication lag bias is almost inevitable, which is another limitation of this review. The review compiled the outcomes in the included studies to form the core outcome set experts questionnaire for Delphi survey. In the follow-up survey, we will set up an open item for experts to fill in supplementary suggestions. We believe that invited clinical experts, especially those familiar with hyperlipidemia, will provide their appropriate supplements based on the latest knowledge, such as various clinical practice guidelines. Thirdly, only studies in English and Chinese were included. Thus, outcomes reported in other languages could have been omitted. In consideration of the plethora of studies included, we believe that all important outcomes for hyperlipidemia have been identified.

4.1. Implication for Future Research. As the first stage in the process of developing a core outcome set for hyperlipidemia, this review has demonstrated the heterogeneity of outcomes reported in the current literature. It has also generated a list of potentially important outcomes for the next step in hyperlipidemia COS development. In the future, the identified outcome will first be evaluated by the study advisory group, and then COS candidate items will be developed and scored in a Delphi survey. Finally, a consensus meeting will be held with clinicians, patients, and other key stakeholders to finalize items and definitions. A COS for hyperlipidemia will also be developed.

5. Conclusion

This systematic review has highlighted the fact that outcome reporting for CM trials of hyperlipidemia has been inconsistent and ill-defined. The results of this review will generate a list of potentially important outcomes for the next step in hyperlipidemia COS development.

Abbreviations

COS: Core outcome set
COMET: Core outcome measures in effectiveness trials
CM: Chinese medicine
HDL-C: High-density lipoprotein cholesterol
LDL-C: Low-density lipoprotein cholesterol
TC: Total cholesterol
TG: Total triglyceride
PRISMA: The preferred reporting items for systematic reviews and meta-analyses statement.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

It has been approved by the Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine.

Disclosure

This systematic review is the first part of the development of a core outcome set of CM clinical trials for hyperlipidemia. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the article; or in the decision to publish the results.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

XC, GL, and ZW devised the study. GL drafted the manuscript. RH and GL performed data collection and extraction, and analysis. WC made an update search and performed data collection according to the update result. XC resolved conflicting opinions between researchers and supervised the work. ZW reviewed the results. XC and ZW confirmed the final version after reviewing the literature. All the authors read and approved the final version of the manuscript prior to submission.

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Supplementary Materials

S1: search strategy. S2: outcome details. (*Supplementary Materials*)

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Research Article

Gastrointestinal Motility and Gut Hormone Secretion in response to Shenhuang Plaster in a Postoperative Ileus Rat Model

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Postoperative ileus (POI), a gastrointestinal function disorder, is a complication that arises from surgery. Shenhuang plaster (SHP) application to the Shenque acupoint (CV8) to promote the recovery of gastrointestinal function has achieved definite curative effects in clinical settings; however, the underlying pharmacological mechanism remains unknown. In this study, we evaluated the effects of SHP using a Sprague Dawley rat POI model. Then, gastrointestinal transit in different rat groups was evaluated by the movement of fluorescein-labelled dextran. Ghrelin, obestatin, motilin (MTL), and vasoactive intestinal peptide (VIP) plasma concentrations were measured via a radioimmunoassay. The expression of the ghrelin and obestatin receptors (GHS-R1α and GPR39) in the intestinal muscularis of rats in different groups was comparatively identified via western blotting. The results indicated that SHP application improved gastrointestinal motility in POI model rats. SHP application significantly increased ghrelin concentration and the expression of its receptor and inhibited obestatin concentration and the expression of its receptor in blood. Further, ghrelin concentration and the capability of gastrointestinal transit were positively correlated. Simultaneously, SHP application also promoted the secretion of other gastrointestinal motility hormones, such as MTL and VIP. Hence, these results provide evidence that SHP can promote the recovery of gastrointestinal transmission in POI rat models through regulation of ghrelin and other intestinal hormones.

1. Introduction

Postoperative ileus (POI) is a complication arising from abdominal or even non-abdominal surgery, generally manifested by varying degrees of abdominal pain, bloating, nausea, vomiting, weakened or missing bowel sounds, and even anastomotic leakage and infection [1]. POI is often considered the main reason for prolonged hospitalization and increased hospitalization costs [2], but its pathogenesis remains unknown. The influence of ghrelin on gastrointestinal motility has been investigated, and results from previous studies have indicated that the expression of ghrelin and its receptor (GHS-R1a, growth hormone secretagogue receptor) are repressed in POI [3]. This suggested that ghrelin may be a key regulator in POI.

In recent times, few effective treatments and therapies have been clinically useful in treating POI, including gastrointestinal decompression, anti-inflammatory rehydration, and nutritional supplementation [4]. However, the application of traditional Chinese medicine (TCM) external therapy presents advantages, such as exact curative effect, safe and easy application, and good patient compliance. For example,

TCM plaster application on acupoints is a therapeutic method that has been practiced for over a thousand years [5, 6], and the effectiveness of acupuncture practices on gastrointestinal motility improvement has been well documented [7, 8].

POI is a kind of "intestinal knot." Based on TCM theory, it is the obstruction of "Qi" (energy) in the intestinal tract, or the dysfunction of "Qi" [9, 10]. The Shenhuang plaster (SHP) is a "Qi"-promoting herbal formula consisting of Renshen (*Ginseng Radix Et Rhizoma*), Raw Dahuang (*Rhei Radix Et Rhizoma*), Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*), Zhishi (*Aurantii Fructus Immaturus*), Houpo (*Magnoliae Officinalis Cortex*), Dingxiang (*Caryophylli Flos*), and Wuzhuyu (*Evodiae Fructus*) [11, 12]. The Shenque acupoint, CV8, is located in the "Ren" meridian, and it is the intersection point of the "Ren," "Du," and "Sanjiao" meridians, according to TCM literature [13]. Therefore, a Chinese doctor has suggested that treatment at the Shenque point will stimulate the "Qi" circulating throughout the body [14]. SHP has been applied clinically for years, and in previous studies we demonstrated that SHP can improve POI in different model animals [15]. We hypothesized that SHP can influence the expression of ghrelin as well as other intestinal hormones. Therefore, in this study, we elucidate our hypothesis using a POI rat model.

2. Materials and Methods

2.1. Animals. Sixty Sprague Dawley (SD) rats (male, 260–310 g body weight) were obtained from Slack Laboratory Animals Co., Ltd (certificate number, SCXK 2013-0016; Shanghai, China). All rats were housed under specific-pathogen-free (SPF) conditions at a constant temperature ($23 \pm 2^{\circ}$C) and humidity ($55 \pm 10\%$) with standard rodent chow, water ad libitum, and a 12-hour light/dark cycle. All animal experiments were performed according to the "Regulations for the Care and Use of Laboratory Animals in Zhejiang Chinese Medical University," published by the Zhejiang Chinese Medical University. The institutional animal care and use committee of Zhejiang Chinese Medical University approved the study protocol, with certificate number 11722.

2.2. Chemical and Biochemical Materials. Fluorescein isothiocyanate- (FITC-) labelled dextran (70 kDa) was purchased from Thermo Fisher Scientific (Massachusetts, USA). Ghrelin, obestatin, motilin (MTL), and vasoactive intestinal peptide (VIP) (rat) radioimmunoassay kits were purchased from Phoenix Pharmaceuticals (California, USA). G Protein-Coupled Receptor 39 (GPR39) antibodies were obtained from Abcam (Cambridgeshire, UK). GHS-R1a (F-16; goat anti-mouse) primary antibody and rabbit anti-alkaline phosphatase- (AP-) labelled secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA). Isoflurane was purchased from Lunan Pharmaceutical Co., Ltd. (Linyi, China). The 0.9% sodium chloride injection was provided by Zhejiang Sapais Pharmaceutical (Jiaxing, China), and PBS phosphoric acid buffer was provided by Zhongshan Bio. Co., Ltd. (Beijing, China).

2.3. Equipment. The NanoDrop 3300 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA), SDS-PAGE electrophoresis system (BIO-RAD, California, USA), inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan), Allegra x-15r large capacity centrifuge (Beckman Coulter, Inc., California, USA), and ultra-low temperature freezer (ChangHong MeiLing Co. Ltd., Hefei, China) were used in this study.

2.4. Preparation of Shenhuang Plaster. The SHP recipe is an herbal mixture of 300 g Renshen (*Ginseng Radix Et Rhizoma*), 300 g Raw Dahuang (*Rhei Radix Et Rhizoma*), 300 g Danshen (*Salviae Miltiorrhizae Radix Et Rhizoma*), 200 g Zhishi (*Aurantii Fructus Immaturus*), 250 g Houpo (*Magnoliae Officinalis Cortex*), 125 g Dingxiang (*Caryophylli Flos*), and 125 g Wuzhuyu (*Evodiae Fructus*). These herbs were purchased from Chinese Herbal Pieces Co., Ltd. of Zhejiang Chinese Medical University, and they were qualified according to the standards noted in "The Pharmacopoeia of the People's Republic of China, 2015." The ingredient extracts and SHP were prepared by the Traditional Chinese Medicine Preparation Institute of Zhejiang Chinese Medical University, as previously described [11].

2.5. Rat Surgery. The SD rat POI model preparation has been previously described [16, 17]. Briefly, the animals were fasted for 24 h before the experiment, with free access to water. The abdominal cavity of an isoflurane-anesthetized rat was opened with surgical scissors under sterile conditions. A gauze soaked with normal saline was placed on both sides of the incision, and two cotton applications with normal saline were used to roll and wipe from one site of the small intestine (near the stomach end) to the other site (near the cecum). Then, the operation was carried out several times from top to bottom in the same manner. Congestion and oedematous tissue were more obvious after scrubbing. Finally, the abdominal cavity and incision were sutured via a double-layered closure. The time period of small intestine manipulation was maintained within 10–15 min, during which the movement of intestinal contents was observed.

2.6. Animal Experimental Design and Treatment. SD rats were randomly separated into four groups, consisting of the saline treatment group (Ctrl), SHP treatment group (Ctrl + SHP), surgery/saline treatment group (POI), and surgery/SHP treatment group (POI + SHP). SHP treatment indicates that rats were administered SHP at the Shenque (CV8) point immediately after surgery. The SHP or saline plaster was changed twice a day during the experiment.

2.7. Capability of Gastrointestinal Transit Evaluation. The gastrointestinal transit evaluation method was described previously [11]. The rats were fasting for 24 h before each measured time point: 6 h, 12 h, 24 h, 48 h, and 72 h. Then, 200 μL of FITC-labelled dextran (6.25 mg/ml, 70 kDa) was injected into each rat through the gastric tube. After 30 min, the rats were sacrificed by anaesthesia, and the abdominal

cavity was opened. The total gastrointestinal tract was divided into 15 segments: the stomach (Sto), small intestine SI1–SI10 (SI; 10 segments), cecum (Ce), and colon Co1–Co3 (Co; 3 segments). Each segment of the intestinal lumen was washed with saline. The intestinal contents were discharged by centrifugation at 12000 rpm for 15 min. The supernatant was collected, and absorbance was measured with a photometer at 494 nm. The percentage absorbance per segment was calculated using equation (1). The geometric centre (GC), which indicated the distribution of fluorescein in the gastrointestinal tract, was calculated using equation (2).

$$\text{Percentage absorbance per segment} = \frac{\text{absorbance per segment}}{\text{total absorbance}} \times 100, \quad (1)$$

$$\text{GC} = \sum \frac{(\text{percentage of absorbance per segment} \times \text{number of segments})}{100}. \quad (2)$$

2.8. Determination of Plasma Ghrelin and Obestatin Concentrations. Rat blood was collected 6 h after surgery, and the ghrelin and obestatin concentrations (pg/ml) in plasma were determined using a standard curve obtained via gamma radioimmunoassay and the non-equilibrium method. At 6, 12, 24, 48, and 72 h each, 2 ml of blood was collected from the peritoneal vein, anticoagulated, and centrifuged at 3000 rpm and 4°C for 15 min. Then, the supernatant was aspirated into an Eppendorf tube and frozen at −20°C for testing. The "I" radioactivity in the precipitate was determined using the ghrelin and obestatin rat ultrasensitive RIA Kit according to manufacturer's instructions.

2.9. Quantification of MTL and VIP Hormones. At the end of the experiment (72 h), 4 ml of peritoneal venous blood was collected into a 15 ml sterile blue-cap blood tube and allowed to stand at room temperature (18–25°C) for 2 h. Subsequently, it was centrifuged at 1000 rpm for 45 min, and the supernatant (serum) was removed and stored at −80°C. The MTL and VIP concentrations were determined via gamma radioimmunoassay, as previously described.

2.10. Western Blot Analysis. The expression of ghrelin and obestatin receptors in the intestinal muscularis of the different rat groups was compared via western blotting. After 72 h, the SD rats were sacrificed, and the colon (2–3 cm) was washed with 4°C PBS solution. The muscle layer was immediately cut off using surgery scissors, homogenized, and sonicated with radioimmunoprecipitation assay (RIPA) lysis buffer at 4°C. After 20 min centrifugation at 13500 rpm at 4°C, the supernatant was pipetted into a clean 1.5 ml centrifugation tube, and the protein concentration was quantitatively evaluated via the Bradford method. The target proteins were separated using 8% SDS polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins in the gel were electro-transferred to the polyvinylidene fluoride (PVDF) membrane at 65 V for 2 h. The transferred PVDF membrane was blocked with blocking buffer containing 5% skim milk at 26°C for 1 h and hybridized with specific antibodies at 4°C overnight. After washing twice with a tween washing buffer (PBST) for 1 h, the hybridized PVDF membrane was incubated with AP-labelled secondary antibody for another 1 h. Before the addition of colour developer (NBT/BCTP) assay solutions, excess secondary antibodies were rinsed off thrice with PBST buffer.

2.11. Statistics. Data for each group were expressed as means ± standard deviation (SD). The statistical software SPSS 22.0 was used for analysis. The mean comparison between groups was analysed using one-way analysis of variance (ANOVA). When the variance was uniform, the *t*-test was used. When the variance was not uniform, the calibration *t*-test and linear correlation analysis were used. $P < 0.05$ was considered statistically significant.

3. Results

3.1. SHP Promotes Gastrointestinal Motility of POI Model Rats. The FITC-labelled dextran GC in the intestine, which can intuitively indicate the speed of gastrointestinal motility, was used in this study to determine gastrointestinal motility. The GC value of the Ctrl group maintained a dynamic balance at each detection time (Figure 1). Meanwhile, the GC of the Ctrl + SHP group was greater than that of the Ctrl group at each time, indicating motility improvement by SHP; however, this was not statistically significant ($P > 0.05$). The GCs of the two surgery groups were significantly lower than those of the Ctrl group at 48 h ($^{***}P < 0.001$). At 72 h, the GC of the POI + SHP group recovered, but it was not significantly different from that of the Ctrl group. Hence, the GC of the POI group was significantly lower than Ctrl GC at 48 h ($^{***}P < 0.001$). The recoveries were also obtained. In detail, the GC values of the POI group were 6.98 ± 0.42, 5.37 ± 0.41, 4.57 ± 0.66, 4.95 ± 0.55, and 5.76 ± 0.54 at 6, 12, 24, 48, and 72 h, respectively. This shows a decrease within 24 h and a slow recovery after 24 h. The GC of the POI + SHP group decreased within 12 h and then recovered swiftly and significantly ($\Delta\Delta P < 0.01$, $\Delta\Delta\Delta P < 0.001$) compared to the POI group. The GC values of each group are listed in Table 1. This result indicated that SHP stimulated gastrointestinal motility.

3.2. SHP Stimulated the Expression of Ghrelin in Rats. After surgery, the ghrelin concentration in blood changed dynamically in the different groups (Figure 2). Ghrelin

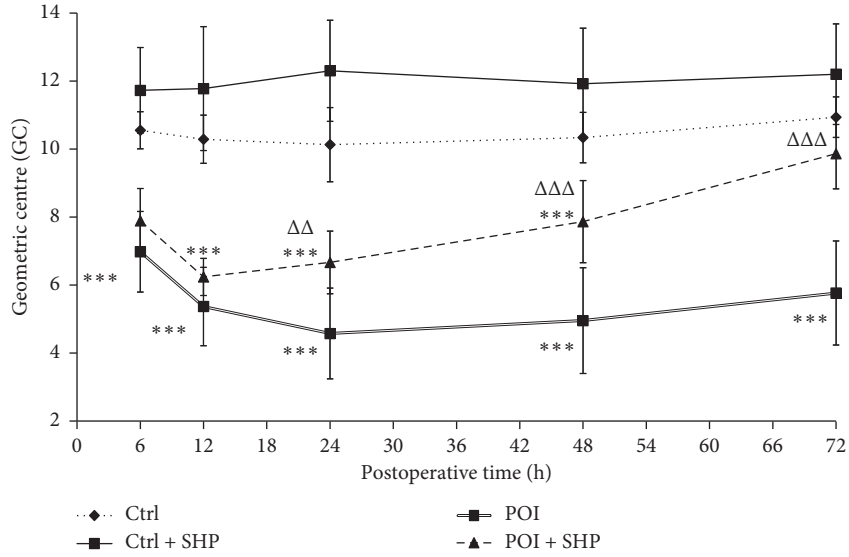

Figure 1: The dynamic change in the geometric centre of rats in different groups. The symbol *** indicates the statistical comparison with the Ctrl group ($P < 0.001$). The symbols △△ and △△△ indicate the statistical comparison with POI rats ($P < 0.01$ and $P < 0.001$, respectively). Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

Table 1: The GC values for each group.

Group	6 h	12 h	24 h	48 h	72 h
Ctrl	10.55 ± 1.68	10.29 ± 1.06	10.13 ± 1.09	10.34 ± 1.09	10.94 ± 1.04
Ctrl + SHP	11.73 ± 1.26	11.78 ± 1.82	12.30 ± 1.49	11.92 ± 1.63	12.20 ± 1.48
POI	6.98 ± 1.19	5.37 ± 1.15	4.57 ± 1.34	4.95 ± 1.56	5.76 ± 1.53
POI + SHP	7.89 ± 0.95	6.24 ± 0.55	6.66 ± 0.92	7.86 ± 1.21	9.86 ± 1.04

GC, geometric centre; Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

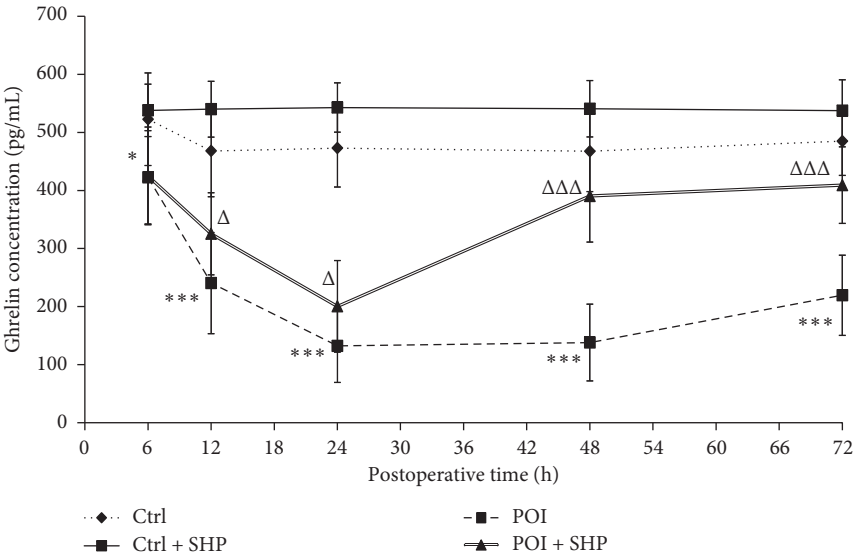

Figure 2: The concentration of ghrelin in different groups at different times. The symbols * and *** indicate the statistical comparison with the Ctrl group ($P < 0.05$ and $P < 0.001$, respectively). The symbols △ and △△△ indicate the statistical comparison with POI ($P < 0.05$ and $P < 0.001$, respectively). Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

concentrations in the Ctrl and Ctrl + SHP groups were maintained between 490 and 520 pg/ml from 12 to 72 h, respectively. However, no significant difference was observed between both groups ($P < 0.05$). Compared with the Ctrl group, the ghrelin concentration in the POI group decreased significantly, especially between 24 h (132.24 ± 22.20) and 48 h (138.06 ± 23.38). However, the ghrelin concentrations in rats in the POI + SHP group at 6,

12, 48, and 72 h were 425.03 ± 29.76, 325.28 ± 25.00, 390.43 ± 28.10 ($\Delta\Delta\Delta P < 0.001$, with the POI group), and 409.11 ± 23.29 pg/ml, respectively. The ghrelin concentration significantly recovered within 48 h. Hence, it remained lower than that of rats in the Ctrl group, but not significantly. Correlation analyses between ghrelin concentration and the GC of the gastrointestinal transit of rats in the POI + SHP group are shown in Figure 3, and the correlation coefficients for both physiological indices were 0.633, 0.836, 0.898, 0.935, 0.768, and 0.547 at 6, 12, 24, 48, and 72 h, respectively.

3.3. Dynamic Changes in Obestatin Concentrations among Different Groups. The obestatin concentrations in rats in different groups are listed in Table 2. No significant changes were observed in the two groups (Ctrl and Ctrl + SHP) of rats that underwent placebo surgery. Instead, the obestatin levels in rats in the POI group increased significantly at 12 h after surgery. Additionally, SHP administration attenuated obestatin expression in the POI group within the same period (12 h; Figure 4).

3.4. SHP Influenced the Expression of GHS-R1α and GPR39 in Smooth Muscle Cells of the Jejunum in Different Groups. GHS-R1α and GPR39 are the respective receptors for ghrelin and obestatin in intestinal smooth muscle cells. The effect of SHP on the expression of GHS-R1α and GPR39 was investigated via western blotting (Figure 5). Compared to the Ctrl group, GHS-R1α expression in the POI group was significantly repressed, and the effect of SHP treatment on GHS-R1α expression in the Ctrl + SHP and POI + SHP groups was evident. GPR39 expression was identical in the Ctrl and Ctrl + SHP groups but attenuated in the POI and POI + SHP groups.

3.5. Serum MTL and VIP Level of Rats Were Improved by SHP. To elucidate the effects of SHP on gastrointestinal motility, the transcription of MTL and VIP was semi-quantified using ELISA. Compared to the Ctrl group, the transcription of both gastrointestinal hormones in the Ctrl + SHP group increased, but not significantly (Figure 6). After surgery, the transcription of MTL and VIP was significantly diminished ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$), and SHP administration improved the effects of surgery ($\Delta P < 0.05$, $\Delta\Delta P < 0.01$).

4. Discussion

The pathogenesis of POI is complicated, and it is generally considered to be controlled by multiple factors, such as perioperative medication, gastrointestinal hormone changes, water-electrolyte disorders, and surgical trauma. One such major pathological change is small intestinal smooth muscle inflammation [1]. Currently, the aetiology and pathogenesis of POI remain unclear, and clinical treatment mainly relies on rapid rehabilitation concepts for comprehensive treatment, such as reducing surgical trauma, limiting fluid replacement, and early restoration of diet and activities, but the clinical effects of these therapies are not satisfactory [18]. The TCM treatment of POI is not unique, as POI is considered a syndrome that combines a deficiency and reality in TCM, which is classified as "intestinal obstruction." The pathogenesis of POI is mainly due to the suffocation of traumatic "Qi" and blood consumption. Moreover, a "Qi" deficiency impedes blood circulation [19, 20]. Therefore, improving "Qi" in the intestine could improve the symptoms of POI. Moreover, the prescription of SHP has complied with the TCM aetiology and pathogenesis theory of promoting the motility of "Qi." In the last decade, SHP application at the "Shenque" point has been clinically beneficial for POI and constipation treatment (unpublished clinical trial results), but the therapeutic mechanism remains unknown. SHP consists of seven herbs, which contain >100 chemical ingredients. Therefore, the mechanism of action is difficult to elucidate, due to its "multi-component, multi-target, and multi-pathway" properties. It has been reported that several active ingredients in TCMs have anti-inflammatory and gastrointestinal motility effects that are compatible with SHP [21–23]. Our previous studies showed that SHP enhanced gastric motility and could potentially serve as a novel therapeutic agent for chemotherapeutic constipation due to its anti-inflammatory potency [11].

POI is a common clinical complication following colon/rectal surgery [1]. Generally, small bowel peristalsis is restored 24 h after surgery, gastric peristalsis at 24 to 48 h, and colonic peristalsis between 48 and 72 h [24]. However, the recovery of gastrointestinal function in patients with POI is even longer. Serious complications, such as intestinal flora shift and multiple organ dysfunction syndrome, were reported in some cases [25]. In the current study, the GC was significantly larger in the POI group than in the Ctrl group at 72 h after surgery (Figure 1 and Table 1), indicating that this method was appropriate for POI rat model preparation.

In this study, the recovery of gastrointestinal transit (determined as GC) in the POI + SHP group was observed at 24 h. The recovery of ghrelin concentration in the serum was at 48 h (Figure 2), and the GC values were positively correlated with ghrelin concentrations in the POI + SHP group (Figure 3). On the contrary, the concentration of obestatin in the POI and POI + SHP groups increased after surgery. However, a significant difference was observed in the POI + SHP group (vs. POI group) 12 h after surgery (Figure 4). Additionally, the regulative activity of SHP on GHS-R1α [26] and GPR39 [27, 28] was identified in different groups via western blotting (Figure 5). The transcription of MTL and VIP in different groups was detected, and the influence of SHP on both hormones was elucidated (Figure 6).

Abnormal defecation is a pathological feature in many diseases and a major symptom in TCM diagnoses. Recent medical studies indicated that the enteric nervous system, smooth muscle, and interstitial cells are involved in gastrointestinal motility regulation [29, 30]. Some functional molecules have been discovered, such as protein hormones, ghrelin, obestatin [31], MTL [32], and VIP [33], which serve as key targets for the development and evaluation of novel therapies.

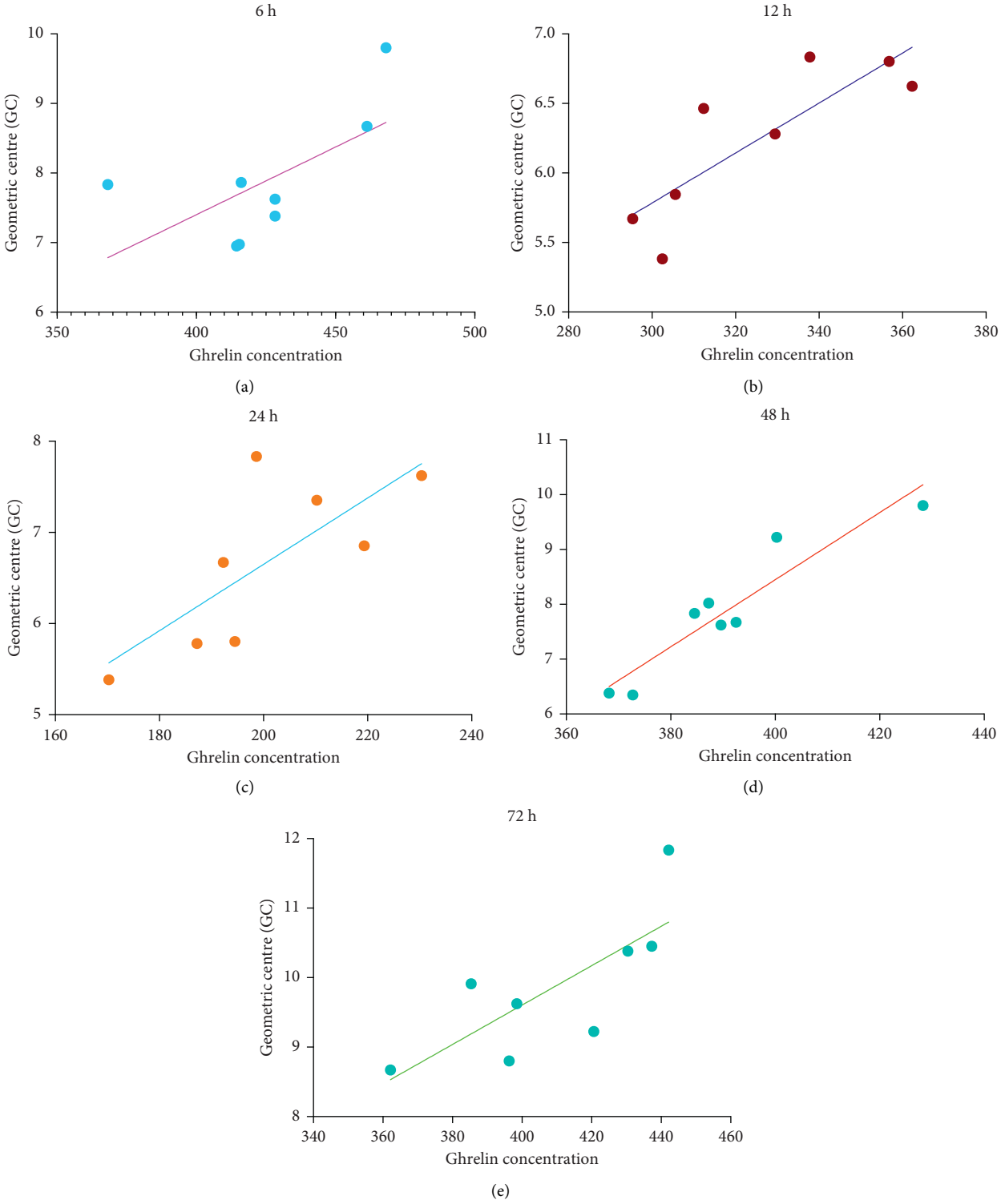

Figure 3: The correlation of changes between the ghrelin concentration and gastrointestinal transit centre of rats in the POI + SHP group. SHP, Shenhuang plaster; POI, postoperative ileus.

Ghrelin, an endogenous brain-gut peptide, first discovered by the Japanese scholar Kojima in 1999 in the stomach of rats, can directly promote gastrointestinal motility by binding to GHS-Rla [26, 34]. In this study, intestinal damage (in the POI group, but not in the Ctrl group) attenuated ghrelin expression from 6 h to 24 h after operation, in accordance with a previous study [35]. Then, the ghrelin concentration remained at a low level within 72 h. Compared to the POI group, the serum ghrelin level in the POI + SHP group increased significantly 24 h after surgery. However, there was no significant difference between the Ctrl and Ctrl + SHP groups (Figure 2), suggesting that SHP

Table 2: The obestatin concentration in each group at different time points.

Group	6 h	12 h	24 h	48 h	72 h
Ctrl	67.32 ± 8.16	67.09 ± 13.67	56.34 ± 14.37	55.84 ± 18.11	66.64 ± 16.68
Ctrl + SHP	54.59 ± 5.77	54.83 ± 12.46	55.04 ± 10.52	53.59 ± 11.51	57.67 ± 9.82
POI	78.97 ± 17.15	103.67 ± 21.27	106.32 ± 13.67	96.54 ± 16.55	91.98 ± 12.42
POI + SHP	73.06 ± 17.93	77.50 ± 13.96	77.03 ± 15.66	67.23 ± 12.10	70.54 ± 19.00

Note: the significant difference is noted in Figure 4. Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

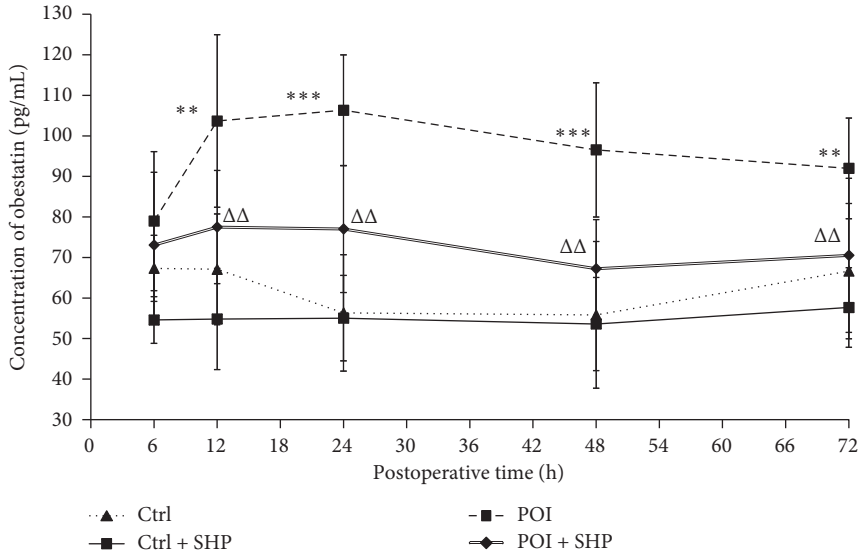

Figure 4: The concentration of obestatin in different groups at different times. The symbols ** and *** indicate the statistical comparison with the Ctrl group ($P < 0.01$and $P < 0.001$, respectively). The symbol △△indicates the statistical comparison with POI ($P < 0.01$). Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

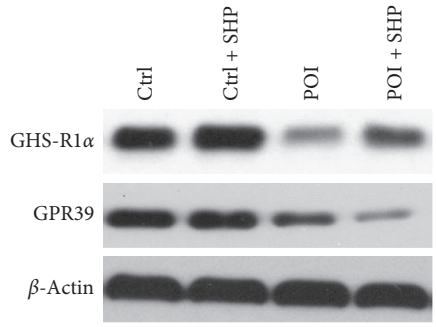

Figure 5: The expression of GHS-R1α and GPR39 in the colon muscularis of rats from different groups at 72 h after operation. Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

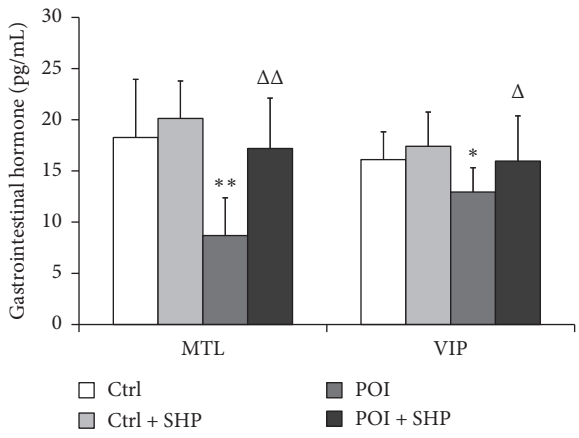

Figure 6: The transcriptional expression of MTL and VIP in rats from different groups at 72 h after operation. The symbols *, **, and *** indicate the statistical comparison with the Ctrl group, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. The symbols △ and △△ indicate the statistical comparison with POI, $P < 0.05$ and $P < 0.01$, respectively. MTL, motilin, MTL; VIP, vasoactive intestinal peptide; Ctrl, control; SHP, Shenhuang plaster; POI, postoperative ileus.

promoted gastrointestinal motility by improving ghrelin secretion in rats with damaged intestines, but not in healthy ones. A similar effect of SHP on GHS-Rla in the colon muscularis of rats from different groups was elucidated (Figure 5).

Obestatin, an active polypeptide in the blood circulation, manifests several biological functions, such as inhibition of thirst [36] and food intake [37, 38], improvement of memory retention [39], regulation of sleep quantity and quality [40], and gastrointestinal motility attenuation [31]. Obestatin, mainly secreted by the intestine, can control gastric emptying speed and reduce intestinal contraction [41], which means that obestatin inhibits intestinal motility. Our results indicated the inhibitory activity of SHP in POI-

induced obestatin (Figure 4) and GPR39 (Figure 5) overexpression. The results suggested that SHP application regulated ghrelin and obestatin expression simultaneously, possibly as a result of the similarity in ghrelin and obestatin gene codes [42].

MTL and VIP, which exist in several animals, are gastrointestinal motility-stimulating hormones. Hence, previous reports have noted an interaction between motilin and ghrelin in regulating GI motility [43]. Moreover, these two hormone peptides and their respective regulators share partial identities and structures [44], and this study showed that both hormones were influenced by surgery and SHP application (Figures 2 and 6). However, in humans, endogenous levels of ghrelin were not correlated with motilin levels [45, 46]. Therefore, we speculate that ghrelin and motilin have their own unique functions, apart from their similar activities in promoting gastrointestinal motility.

VIP, present in different organs, has been recognized as a neuropeptide [47], which can mediate several gastrointestinal functions, such as gastric acid and intestinal anion secretion, enzyme release from the pancreas, cellular motility, vasodilation, and intestinal contractility [48–50]. However, the function of its receptors, VPAC1 and VPAC2, in gastrointestinal motility stimulation remains unclear [33].

Meanwhile, the use of SHP as an external POI treatment avoids intravenous infusion and transgastric administration, which is especially beneficial for patients who need to limit fluid volume and undergo gastrointestinal surgery. SHP is safe to use and is also simple, inexpensive, and easy; however, further studies are required to evaluate this therapeutic approach.

5. Conclusions

SHP application at the "Shenque" acupoint is an effective and safe therapeutic method for POI amelioration. However, the pharmacological mechanism remains unknown because of its complicated ingredients and external application. In this study, we first demonstrated the gastrointestinal motility-promoting capability of SHP administered in a POI rat model. Following this, the influence of SHP application on the expression of ghrelin, obestatin, and their receptors in the colon muscularis was demonstrated. Further, improvements in SHP effects based on the concentration of the serum gastrointestinal hormones MTL and VIP were identified in POI rats, but elucidating the underlying mechanism of action requires further study.

Data Availability

All data used to support the findings of this study are included within the article, and these data can also be accessible on website https://fairsharing.org/collection/GastrointestinalMotilityandGutHormoneSecretioninResponsetoShenhuangPlasterinaRatModelofPostoperativeIleus.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Ms. Yanan Shi and Mr. Yingsong Zheng contributed equally as first authors, because they equally completed most of the experiments and data analysis. This study was designed and guided by Qiuhua Sun and Xiaohong Xu, and therefore they contributed equally as corresponding authors. The rat model and SHP application were finished by Ms. Jingming Xu. Dr. Ding was responsible for editing this submission. Qiyang Shou, Guiping Chen, and Ting Liu helped with animal anaesthesia treatment.

Acknowledgments

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Research Article

Development of a Computerized Adaptive Test for Quantifying Chinese Medicine Syndrome of Myasthenia Gravis on Basis of Multidimensional Item Response Theory

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Background. Making comprehensive management of myasthenia gravis (MG) is a challenge in clinical practice due to heterogeneity and multiple comorbidities among patients. *Aim*. To develop an end-to-end instrument for individualized assessment of MG in the perspective of Chinese medicine (TCM) with the application of multidisciplinary quantification approaches. *Methods*. A self-administrated questionnaire was developed integrating typical symptoms of MG and spleen-kidney deficiency syndrome on basis of the conceptual framework of TCM. With data collected in a multicenter cross-sectional study, confirmatory factor analysis together with multidimensional item response theory (MIRT) was used for evaluating the psychometric property of the questionnaire. A computerized adaptive test was developed based on the MIRT model, and scores of syndrome factors were calculated in simulation. A logistics regression model was also estimated for evaluating the consistency between the quantitative result and the clinical diagnosis of syndrome from clinical practitioners. *Result*. With 337 patients enrolled and assessed, the 14-item questionnaire was evaluated to be with adequate validity and reliability (Cronbach's alpha indices = 0.87, AIC = 195.827, BIC = 348.631, CFI = 0.921, RMR = 0.006, GFI = 0.954, RMSEA = 0.048, and $\chi 2/df = 1.782$). With adequate factor loadings of symptoms on related syndrome factor, the instrument was evaluated with preliminary interpretation and was suitable for evaluating patients with moderate severity of the spleen and kidney deficiency syndrome. *Conclusion*. Setting typical symptoms of MG together with systemic discomforts in a computerized adaptive test on the basis of MIRT, this study proposed an innovative research paradigm for quantifying individual condition in the perspective of TCM with application of interdisciplinary approaches.

1. Background

As an autoimmune neuromuscular disease, myasthenia gravis (MG) was reported to be mediated by autoantibodies targeting components of the neuromuscular junction [1]. Typical appearances of muscle weakness such as eyelids droops and fatigue result in reduction of daily activity and negtively affect the quality of life of patients. Restrictive respiratory failure caused by severe weakness of respiratory muscle could even lead to emergency known as MG crisis in 15% of MG patients [2]. The heterogeneity of clinical appearances of MG ranging from mild ocular deficits to severe widespread weakness posed a challenge for clinical assessment of MG patients [3]. Moreover, multiple comorbidities among patients with chronic disease were also commonly reported which made it difficult for the management of MG. Due to the disease heterogeneity, MG is increasingly acknowledged as a syndrome more than a single disease [4]. The goal of treatment of MG is to obtain remission and disease stability with the least symptoms and that was thought to be a challenge [5]. It is critical to introduce ideas and approaches from chronic disease management and develop instrument for comprehensive measurement and individualized monitoring of patients with MG [6].

Quantitative instruments were developed as assistive tools for individual assessment of MG patients [7]. For example, the Myasthenia Gravis Score [8] and the Myasthenia Gravis Composite [9] were developed and used for measuring the clinical outcome of MG by quantifying disease severity. The myasthenia gravis patient-reported outcome scale was developed for evaluating the quality of life of MG patients and supporting measurement of treatment effects in clinical trials about MG [10, 11]. These instruments served as practical tools for the management of MG offering quantitative scores as references for clinical diagnosis and decision of treatment. However, shortcomings of these scales are also obvious among which the lengthy setting of scalesalways resulted in reduction of compliance of patients during assessment. Moreover, the interpretability of the traditional assessment strategy was impaired since much information was lost while accumulating the scores with compensatory logic. The "one size fits all" approaches were reported to be without relevance. And individualized diagnosis and treatment approaches are required to match the heterogeneity of MG patients.

Traditional Chinese medicine (TCM) practitioners pursue individualized diagnosis and therapy by summarizing symptoms and signs of patients within the conceptual framework of syndrome differentiation. Falling within the scope of Flaccidity Syndrome in TCM theory, MG was known to be caused by deficiency of spleen. As a complementary and alternative medical approach, TCM therapies with herbs and acupuncture were reported to help releasing severity of muscular fatigue and improving quality of life of MG patients [12–18]. Pharmacological effects and pathogenesis of MG were also explored with the application of statistical and machine learning methods in the perspective of TCM [19, 20]. However, controversy remained about the abstract theory and empirical practice of TCM. Innovative research strategies should be established for measuring the efficacy of TCM therapy and further strengthening the interpretability of TCM theory.

With the purpose of quantifying abstract concepts in TCM theory, many innovative research paradigms were proposed with application of interdisciplinary methods including structural equation modeling (SEM) and multidimensional item response theory (MIRT) [21, 22]. In these studies, mathematical models were estimated bridging the gap between observable symptoms or signs and syndromes which were regarded as the latent trait of patients.In this way, individual condition could be quantified within a interpretable conceptual framework. With application of computer science and information technology, the traditional form of assessment was also shifted into a more efficient mode and that further enabled individualized evaluation on basis of quantitative model [23–25].

Aiming at providing a flexible approach to support clinical management of MG patients in the perspective of TCM, this article proposed an innovative strategy for quantifying TCM syndrome of MG with development of a computerized adaptive test (CAT) on the basis of MIRT.

2. Method and Materials

2.1. Data Source. A multicenter cross-sectional study was carried out in China from Jun 2008 to Aug 2013. Diagnosis criteria of MG was set referring to guidance from the Handbook of Clinical Neurology [26]. Patients diagnosed as MG in age between 14 and 75 were recruited from three research institutions including the First Affiliated Hospital of Guangzhou University of Chinese Medicine, the Guangzhou Second People's Hospital, and Guangdong Province Hospital of Chinese Medicine after informed consent. Patients aged less than 14 or over 75 and those with malignant thymoma or serious comorbid diseases such as renal failure or psychiatric diseases were excluded. Pregnant or breast-feeding women with MG were also kept out in this study. All patients diagnosed with spleen deficiency syndrome or spleen-kidney deficiency syndrome were asked to fill the self-administrated questionnaire and those who did not complete the assessment were excluded for further analysis.

2.2. Questionnaire and Conceptual Framework of TCM Syndrome Assessment. Aiming at quantifying the severity of TCM syndrome about MG, the instrument was designed under the conceptual framework of TCM theory. The self-administrated questionnaire consisted of two parts. Firstly, an introduction about the purpose of the assessment together with fields of individual information such as name, gender, and age was formed at the top of the scale. Secondly, items describing typical symptoms or signs of MG served as the major part of the assessment for evaluating the syndrome severity of MG patients.

According to previous reports about the prevalence of syndromes about MG, deficiency of both spleen and kidney was known as major pathogenesis of MG in TCM theory [27, 28]. Therefore, the conceptual framework of the instrument was set limiting the scope of assessment over major syndromes including spleen deficiency syndrome and spleen-kidney deficiency syndrome. A set of symptoms or signs as clues for syndrome differentiation was listed and discussed and then transformed into items with dichotomous options. A group of 3 clinical experts was invited taking the responsibility of evaluating and validating the content description and option settings of the items so as to ensure the rationalization of the questionnaire considering both clinical fitness and cultural competence.

Conceptual framework of spleen-kidney deficiency syndrome was shown in Figure 1(a) and items consisted of the framework were listed in Table 1. There were 14 typical symptoms in the framework among which 9 items were drafted for quantifying spleen deficiency including weakness of limbs, fatigue, blepharoptosis, inappetence, dysphagia, salivation, loose stool borborygmus, and sweating with the other 5 items for quantifying kidney deficiency items including weakness of waist and knee, weakness of neck, dyslalia, shortness of breath, and blurred vision. The instrument was reviewed and approved by the chief of experts before promoting the assessment.

Figure 1: Conceptual framework of spleen-kidney deficiency syndrome of myasthenia gravis. (a) Multidimensional model of spleen-kidney deficiency syndrome. (b) Unidimensional model of spleen-kidney deficiency syndrome

Table 1: Items of questionnaire about spleen-kidney deficiency syndrome of myasthenia gravis.

Index	Content	Option 1	Option 2	Related factor
1	Weakness of limbs	Yes	No	Spleen deficiency
2	Fatigue	Yes	No	Spleen deficiency
3	Blepharoptosis	Yes	No	Spleen deficiency
4	Inappetence	Yes	No	Spleen deficiency
5	Dysphagia	Yes	No	Spleen deficiency
6	Salivation	Yes	No	Spleen deficiency
7	Loose stool	Yes	No	Spleen deficiency
8	Borborygmus	Yes	No	Spleen deficiency
9	Sweating	Yes	No	Spleen deficiency
10	Weakness of waist and knees	Yes	No	Kidney deficiency
11	Weakness of neck	Yes	No	Kidney deficiency
12	Dyslalia	Yes	No	Kidney deficiency
13	Short of breath	Yes	No	Kidney deficiency
14	Blurred vision	Yes	No	Kidney deficiency

During the assessment, demographic details of the examinees including gender, age, occupation, and education were recorded in the first section of the questionnaire. Patients were also asked to fill in the items following the introduction of the professional clinical practitioner. And the practitioner took the responsibility of explaining the content to reduce misunderstanding of the items. Initial opinion of syndrome differentiation was made by a trained practitioner after the assessment. And another practitioner with senior title was in charge of making confirmation of the diagnosis. Once there was a conflicting idea that occurred toward the clinical diagnosis of the syndrome, a third practitioner with senior title would be invited to make a discussion for the final decision.

2.3. Development of CAT on Basis of the MIRT Model. As all the responses of the items were collected from patients, the 2-parameter logistic model was used for estimating the psychometric parameters of items. And quasi-Monte Carlo Expectation-Maximum (EM) estimation was used as the estimation algorithm. As to the parameters setting of the estimation, a limitation was also set with the maximum number of EM cycles as 2000 and the standard error tolerance criteria for the computation of the information matrix as 0.001. On basis of the MIRT model, the CAT was created with package mirtCAT [29] in *R* 3.6.2 and the logic of assessment was designed as follows: (i) Starting item was randomly selected in range of major appearances of MG including blepharoptosis, fatigue, and weakness of limbs. (ii) The maximum determinant of the information matrix was set as adaptive criteria of the assessment for the latent trait scores calculation. (iii) Stopping criteria of CAT were set with the delta of latent trait scores as 0.05 and the minimum standard error of each dimension as 0.3. Furthermore, a web-based questionnaire was designed offering an interactive interface for the assessment. As far as compliance of patients was concerned, the CAT assessment was carried out in simulation with the response of the original assessment.

2.4. Statistical Analysis. Descriptive analysis about demographic characteristics was carried out in SPSS 22.0. To evaluate the validity of the scale, Cronbach's alpha indices were analyzed in SPSS 22.0. The psychometric property of the items was evaluated with the assistance of package mirt in *R* 3.6.2 [30]. Construct validity of the multidimensional and unidimensional model shown in Figures 1(a) and 1(b) were evaluated referring to indices estimated in Confirmatory Factor Analysis (CFA) including Root Mean Square Residual (RMR), Root Mean Square Error of Approximation RMSEA), Comparative Fit Indices (CFI), and Goodness of Fit Indice (GFI). Multidimensional discrimination index (MDISC) and multidimensional difficulty index (MDIFF) of each item were also evaluated indicating reliability of item setting. Item information surface of each item was plotted as intuitive visualization of the property of the items and the entire assessment. With a CAT developed in *R* 3.6.2, multidimensional traits of each patient were estimated in stimulation. Setting clinical diagnosis of syndrome as reference, Receiver Operation Curve (ROC) was estimated and the area under the curve (AUC) was calculated in *R* 3.6.2 for evaluating the accuracy of the model.

3. Result

3.1. Demographic Analysis. As shown in Table 2, a total number of 337 patients were finally enrolled in this study with 12 cases excluded out of the unfinished assessment. Male took a larger percentage than female and the elder was less than the young and mid-age patients. The mean age of the sample was 37.947 ± 16.358 and the patients in youth and middle age took up a major proportion as 58.46% and 29.67% of the sample. Ranking with the frequency of clinical appearances as shown in Table 3, typical symptoms including blepharoptosis, weakness in limbs, fatigue, and dysphagia were most frequently reported and that is consistent with previous reports [31]. It should be noticed that systemic symptoms such as inappetence and shortness of breath were also commonly reported and that could be important factors influencing the quality of life of MG patients.

As to the validity and reliability of the instrument, consistency of the response of items in the assessment was evaluated to be adequate with Cronbach's alpha indices as 0.87. Split-half validity was also calculated in an acceptable condition as 0.87. The goodness of fit about the conceptual framework as construct validity of the instrument was also evaluated to be adequate with AIC = 195.827, BIC = 348.631, CFI = 0.921, RMR = 0.006, GFI = 0.954, RMSEA = 0.048, and $\chi 2/\mathrm{df} = 1.782$. As comparison, the fitness indices of the unidimensional model were estimated with AIC = 286.537, BIC = 393.500, CFI = 0.762, RMR = 0.009, GFI = 0.908, RMSEA = 0.077, and $\chi 2/\mathrm{df} = 2.99$.

Psychometric parameters including MDISC, MDIFF, and standardized factor loading were estimated with MIRT and shown in Table 4. All items were evaluated with adequate discrimination for assessment with MDISC over 0.5. Information characteristics and standard error curves of the items were plotted and shown in Figure 2. The setting of all items was evaluated to be adequate as most information and least standard error could be achieved for those with a moderate score of latent traits. And the trace surfaces showed in Figure 3 indicated that items were with adequate setting to discriminate patients in different severity. Moreover, with both latent traits scores in range (−2, 2), most information and least standard error could be achieved as the humps of the information surface shown in Figure 4. Settings about items of the instrument were evaluated to be proper therefore ensuring the assessment with adequate validity and reliability.

Factor loadings of symptoms on their related latent factor were also evaluated to be consistent with the conceptual setting. For spleen deficiency syndrome factor, fatigue and digestive discomforts such as dysphagia took a loading value over 0.5 as shown in Table 4. As far as kidney deficiency was concerned, shortness of breath and dyslalia together with the weakness of waist and knees took the highest loadings as 0.856, 0.823, and 0.712. The information surface and standard error of the test shown in Figure 4 showed that most information would be achieved for examinees with severity of both dimensions in a moderate range in (−3, 3).

As psychometric parameters of the items were estimated, the CAT was developed for individualized assessment of TCM syndrome of MG. Latent traits of patients were estimated with a stimulated assessment with multidimensional scores in the range of (−6, 6). Correlation between clinical

Table 2: Demographics and characteristics of the 337 myasthenia gravis patients.

Variables	Total ($n = 337$)	Proportion (%)
Age		
Youth (14–44)	197	58.46
Mid-age (45–59)	100	29.67
Elder (60–75)	40	11.87
Gender		
Female	185	54.89
Male	152	45.11
Syndrome		
Spleen and kidney deficiency	68	20.18
Spleen deficiency	269	79.82

Table 3: Frequency of symptoms reported with the 337 myasthenia gravis patients.

Item	Frequency	Percentage (%)
Blepharoptosis	286	84.87
Weakness of limbs	226	67.06
Fatigue	173	51.34
Dysphagia	158	46.88
Blurred vision	110	32.64
Inappetence	102	30.27
Weakness of waist and knee	101	29.97
Dyslalia	100	29.67
Salivation	91	27.00
Shortness of breath	85	25.22
Weakness of neck	83	24.62
Loose stool	66	19.58
Sweating	47	13.95
Borborygmus	30	8.90

Table 4: Estimated properties of the spleen-kidney syndrome model of myasthenia gravis.

Items	MDISC	MDIFF	Factor loading	
			Spleen deficiency	Kidney deficiency
Weakness of limbs	1.587	−0.641	0.682	0
Fatigue	2.841	−0.018	0.858	0
Blepharoptosis	2.177	1.612	0.788	0
Inappetence	1.263	0.870	0.596	0
Dysphagia	2.423	2.420	0.818	0
Salivation	1.141	1.097	0.557	0
Loose stool	1.093	1.581	0.54	0
Borborygmus	1.287	2.262	0.603	0
Sweating	0.999	2.142	0.506	0
Weakness of waist and knees	1.725	0.743	0	0.712
Weakness of neck	0.841	1.522	0	0.443
Dyslalia	2.463	1.989	0	0.823
Shortness of breath	2.816	1.790	0	0.856
Blurred vision	0.828	1.673	0	0.437

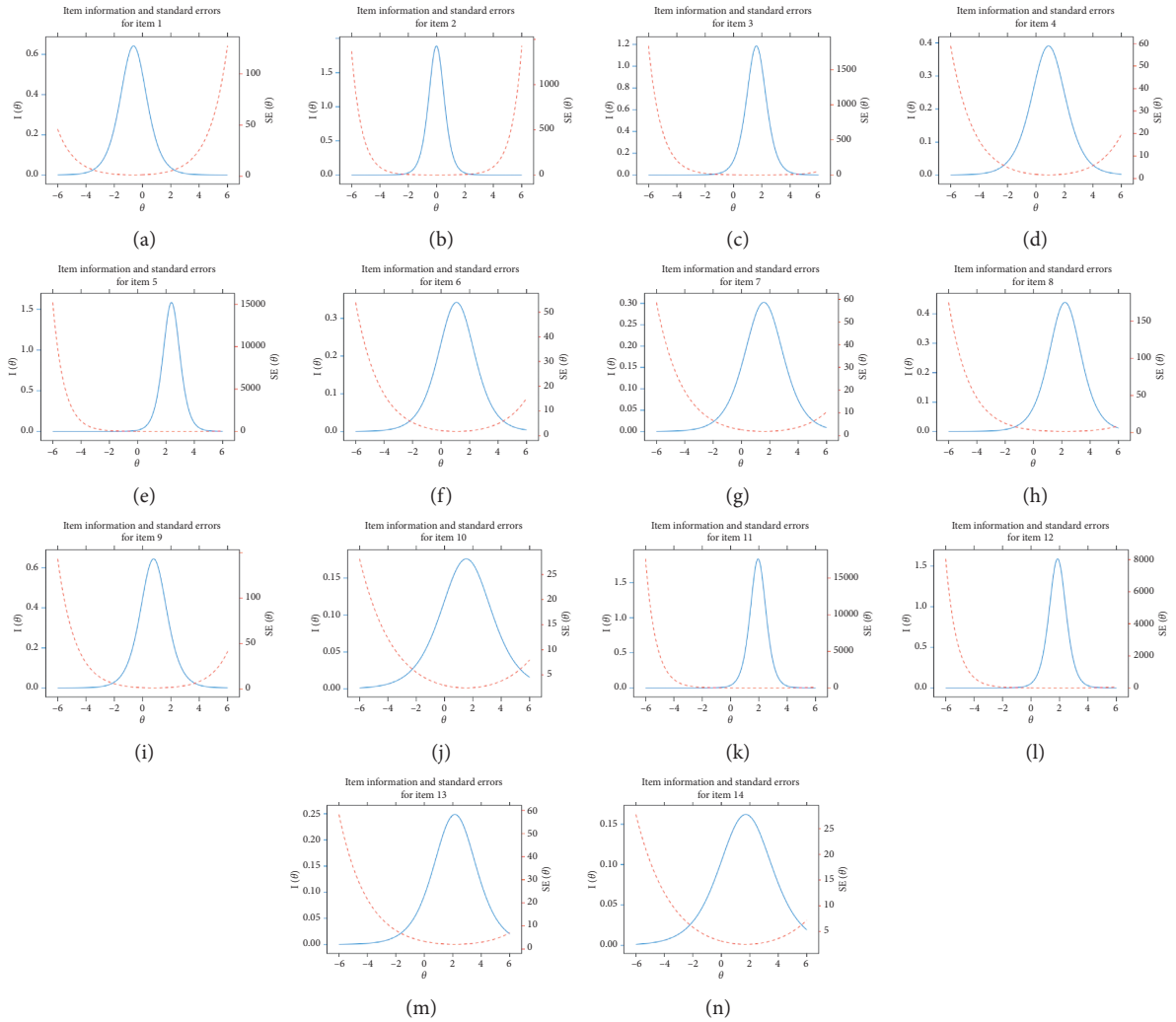

Figure 2: Item information and standard errors curves of the 14 items in the questionnaire.

diagnosis and latent trait scores was evaluated to be significant in logistics regression. Results of the regression analysis showed that the two latent traits were calculated to be statistically significant correlated with the clinical diagnosis of syndrome with the correlation coefficients as 2.088 ($p < 0.01$) and 6.593 ($p < 0.01$) as shown in Table 5.

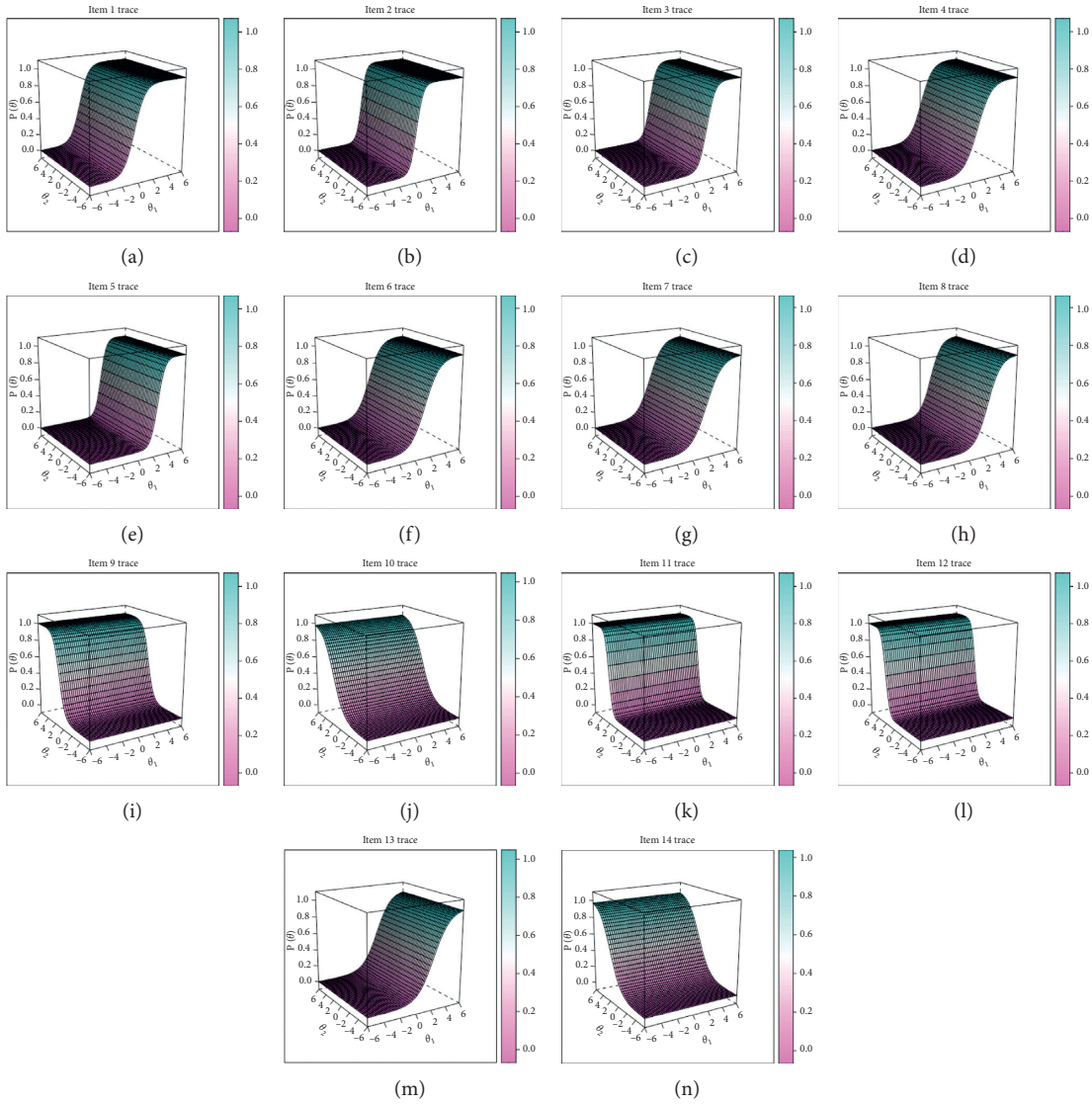

Figure 3: Trace surfaces of the 14 items in the questionnaire.

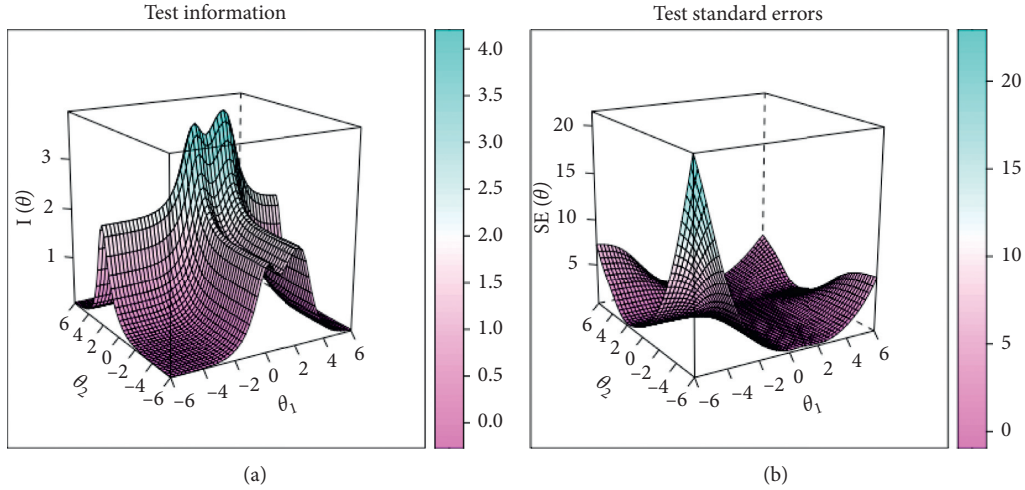

Figure 4: Information and standard error surfaces about the test with the 14-item questionnaire.

AUC was evaluated as 0.986 indicating that the predicted score was in adequate consistency with the clinical diagnosis of syndrome. Best performance with sensitivity as 0.926 and specificity as 0.974 could be achieved while setting the threshold of the model as −0.177 as shown in Figure 5.

4. Discussion

In this study, female preponderance was found with the male:female ratio evaluated as 1 : 1.21. And that is similar with studies reported in other regions of the world. [32, 33]. Although patients in different range of age took different corporation of the sample, no significant conclusion could be drawn because neither the onset time nor the duration information but only the attendance time was exactly recorded. While tracing back the development of MG, the diagnosis procedure of patients always lasted long and patients intended to seek treatment with TCM as an alternative approach.

Heterogeneity of MG patients in different gender, ages, duration of disease, and more importantly the comorbidity with different diseases made it a challenge to make comprehensive management of MG patients. As shown in Table 4, besides the most commonly reported symptoms, systemic appearances such as inappetence were calculated with frequency not lower than typical MG appearances. It should be noticed that these discomforts could be caused by multifactors including side effects of drugs. Therefore, it is a challenge to make a comprehensive interpretation of the clinical appearances of MG patients.

Standardized rules of diagnosis and treatment are important while individualized management is also essential to meet different requirements of patients. In the clinical practice of TCM, syndromes were concluded as summarization of systemic appearances in a conceptual framework. Accordingly, therapies were then designed to adjust the individual status with balance therefore achieving the goal for relieving the severity of all symptoms. Spleen-kidney deficiency syndrome was reported to be the major syndrome of MG patients in China. [16, 34]. In TCM theory, the kidney governs the bone and acts as the root of primordial Qi to dominate growth and development. And deficiency of primordial Qi directly influences growth, development, and muscular function. The transformation function of spleen provided nutrients for muscle and energy metabolism mainly relying on the transporting of Qi. Therefore, deficiency of spleen and kidney leads to failure in transporting food and nutrients that caused digestive disorders symptoms such as belching and loss of appetite. That further leads to disorder in nourishing muscle and makes muscles atrophied and become asthenic resulting in symptoms involving ocular, bulbar, respiratory and proximal limb muscles [28]. Following the conceptual framework of TCM, the model of assessment was conducted with two latent factors including spleen deficiency and kidney deficiency. Related symptoms were drafted as clues for differentiation of each dimension of syndrome factors. Setting typical symptoms of MG together with systemic discomforts in a uniformed baseline, the paradigm of the study was delighted by the idea of syndrome differentiation in TCM theory.

Table 5: Estimated parameters of logistics regression model with multidimensional scores from the computerized adaptive test.

Variable	Estimate	Standard error.	z-value	Pr (>|z|)
(Intercept)	−5.322	0.907	−5.866	0.000
Spleen deficiency	2.088	0.637	3.279	0.001
Kidney deficiency	6.593	1.145	5.760	0.000

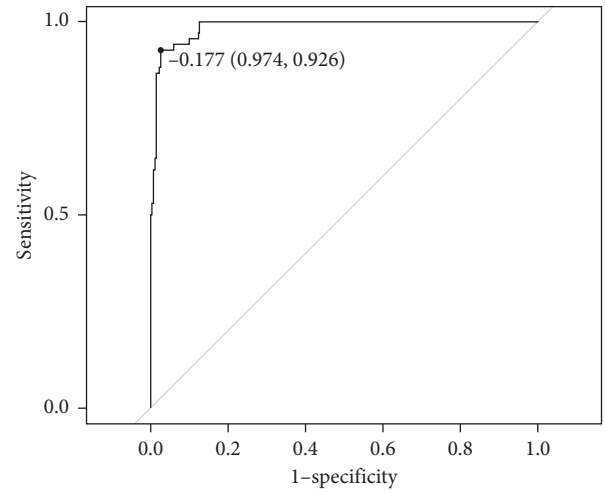

Figure 5: Receiver operating characteristic curve about the computerized adaptive test on basis of multidimensional model.

Interdisciplinary approaches also benefit us with the possibility to develop individualized approaches to assist the management of MG. We proposed an innovative end-to-end strategy with development of an individualized assessment for MG in this article not only meeting the requirement of chronic disease management but also out of the exploration about the modernized clinical practice of TCM. The quantitative syndrome differentiation model under the conceptual framework of disease-syndrome integration covered the most important clinical appearances for analyzing the major pathogenesis of MG and was evaluated with adequate consistency with the clinical diagnosis of syndrome. With psychometric property of the items estimated in MIRT, severity of spleen deficiency and kidney deficiency as latent traits of patients was quantified with different clinical appearances in combination. Setting all of the items in a standardized scoring procedure, the CAT on basis of MIRT model equipped designer with adaptive logic of assessment meeting the requirement of different situations. Uncompensated scoring algorithm also makes it more suitable for the individualized evaluation of patient with complex clinical appearances. The logistic regression model of spleen and kidney deficiency was also evaluated with adequate accuracy with AUC evaluated to be 0.925 referring to the clinical diagnosis of syndrome. And the regression model bridged the gap between the assessment and decision of syndrome differentiation in this way making the CAT an end-to-end instrument.

To our knowledge, this is the first study that proposed a multidisciplinary paradigm for quantifying TCM syndromes of MG with application of multidimensional latent traits analysis and computerized adaptive testing. However, there are several limitations in our research. Firstly, since the research region was limited in Guangdong province, there was much uncertainty about the representativity of the sample although the patients were enrolled from a multicenter study. Secondly, there could be information loss and bias introduced from the assessment due to the rough dichotomous responses recorded with the instrument. Design of the instrument should be modified by setting the items with graded options and extending the scope of assessment for other syndromes of MG besides spleen-kidney deficiency syndrome. Thirdly, research should be carried out for estimation and evaluation of the stability, rationality, and further extrapolation of the model with a representative sample before further application in clinical practice. Last but not the least, controversies still exist either about the complex concepts in TCM theory or the empirical strategy in its clinical practice for the lack of objective evidence as practical clues for diagnosis and clinical decision of treatment. Further research should be carried out focusing on the estimation of standardized criteria for syndrome diagnosis and therapy in place of the traditional empirical approach for the modernized practice of TCM.

5. Conclusion

The establishment of instruments with interdisciplinary approaches for quantification and management of chronic and rare diseases such as MG would benefit the patients with continuous monitoring of individual condition and further promoting efficiency of treatment and management of disease. Setting typical symptoms of MG together with systemic discomforts in a uniform quantification baseline in the perspective of TCM, this study provided an innovative research paradigm to assist individualized management of MG with application of multidisciplinary approaches including MIRT and CAT.

Abbreviations

TCM: Traditional Chinese medicine
MG: Myasthenia gravis
CFA: Confirmatory factor analysis
MIRT: Multidimensional item response theory
FGIDs: Functional gastrointestinal disorders
SEM: Structural equation modeling
CAT: Computer adaptive test
CFI: Comparative fit indices
GFI: Goodness of fit indices
RMSEA: Root mean square error of approximation
RMR: Root Mean Square Residual
AIC: The Akaike information criterion
χ^2: Chi-square
Df: Degree of freedom
MDISC: Multidimensional discrimination index
MDIFF: Multidimensional difficulty index
IIS: Item Information Surface
EM: Expectation-Maximum
ROC: Receiver operation curve
AUC: Area under the curve.

Data Availability

The data used to support the findings of this study are available from the corresponding author (e-mail: jlily0252@126.com) upon request.

Ethical Approval

This study was approved by the Clinical Research and Ethics Committee at the First Affiliated Hospital of the Guangzhou University of Chinese Medicine (No.2006BAI04A12).

Consent

All patients enrolled in this study signed informed consent.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Z.Y.H., L.J.L., and F.B.L. contributed toward the concept, data analysis, and manuscript writing and review; Z.Y.H., L.J.L., and F.B.L. were responsible for funding acquisition; Y.Y.Y., L.J.L., and F.B.L. contributed toward the patient management and data collection.

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Research Article

Using Surface Electromyography to Evaluate the Efficacy of Governor Vessel Electroacupuncture in Poststroke Lower Limb Spasticity: Study Protocol for a Randomized Controlled Parallel Trial

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Background. Lower limb spasticity is a common complication after stroke, which seriously affects the quality of life and rehabilitation of patients. There are different treatment methods for poststroke spasticity. It has been found in clinical practice that governor vessel electroacupuncture (GV-EA) can effectively relieve poststroke upper extremity spasticity, but the efficacy of treatment of lower extremity spasticity needs to be further verified. This study aims to design a randomized controlled trial to evaluate the efficacy of GV-EA in the treatment of poststroke lower limb spasticity. *Methods/Design*. This is a randomized, controlled trial. Patients ($N = 177$) will be randomized to receive routine therapeutic drug and rehabilitation treatment plus GV-EA (experimental group) or routine therapeutic drug and rehabilitation treatment plus EA (control group 1) or routine therapeutic drug and rehabilitation treatment (control group 2). All patients will receive 20 sessions of treatment for 4 weeks. The primary outcomes are the RMS value and the Modified Ashworth Scale. Secondary outcomes include the Fugl–Meyer Assessment for Lower Extremity (FMA-LE) and the Modified Barthel Index score. All outcome measures will be evaluated at the beginning and after the intervention (4 weeks). *Discussion*. This trial will observe the clinical effect of GV-EA on lower extremity spasticity after stroke, especially its influence on surface electromyography characteristics, and provide high-quality experimental evidence for the clinical application of GV-EA based on surface electromyography in the treatment of poststroke lower limb spasticity. *Trial Registration*. China Clinical Trials Registry No. ChiCTR1900027969. Registered on 7 December 2019.

1. Introduction

Due to the aging population, accelerated urbanization process, and residents' often-unhealthy lifestyles, there is an increased incidence of stroke [1]. According to statistics, 15 million people worldwide die each year from stroke [2]. Spasticity is one of the most common poststroke complications, and it is also an important cause of motor dysfunction. Among them, lower limb spasticity is manifested as muscle atrophy, pain, and joint contracture stiffness [3, 4]. Although lower limb spasticity is not life-threatening, it has become an important public health issue worldwide because of its high recurrence rate and disability, detrimental effects on functional recovery and quality of life, and high health care costs [5]. Epidemiological surveys have shown that the incidence of lower limb spasticity in Western countries after 6 months of stroke patients was about 42.6%, while as high as 65.7% in China [6, 7]. According to statistics, the medical expenses were 4 times higher for stroke patients with spasticity than those of patients without spasticity [8, 9]. Physical

therapy, pharmacological therapies, and botulinum toxin seem to be the most common choices for the treatment of lower limb spasticity. However, every conventional approach has its limitations. There is no uniform standard for the treatment parameters and stimulation intensity of physical therapy. Pharmacological therapies have potential side effects, such as drowsiness, nausea, and vomiting. Long-term injection of botulinum toxin may cause serious adverse reactions such as muscle weakness and dyspnea [10]. Therefore, there is a need for a safer, more effective, and less side effect treatment method for poststroke lower limb spasticity.

Acupuncture has been widely applied to treat poststroke spasticity; it is considered by the World Health Organization (WHO) as an alternative and complementary strategy for the treatment and improvement of stroke [11]. Besides, clinical trials and meta-analysis results have demonstrated that acupuncture can improve balance function, reduce spasticity, increase muscle strength, and improve quality of life [12]. It is also more and more popular with clinicians and patients for its unique advantages of significant efficacy, low price, and small side effects. However, the efficacy differences between different acupoints are uncertain. Few trials have involved the comparison of different acupuncture points to verify the efficacy differences between different acupuncture methods. Governor vessel (GV), also known as "the sea of the yang meridians," is one of the eight extra meridians. GV passes through the kidney, spinal cord, and brain, so it can treat various system diseases closely related to these parts [13]. Our previous study has demonstrated that GV-EA can significantly reduce the root mean square value of the biceps and triceps and the Modified Ashworth Scale score and increase the Fugl–Meyer Assessment for upper extremity motor function score and the Modified Barthel Index score, which can effectively improve the degree of upper limb spasticity, motor function, and ability of daily life [14]. At the same time, studies in modern medicine have shown that GV-EA effectively increases blood flow and partial pressure of oxygen in the brain, thereby accelerating the self-repair ability of the brain tissue. It also has a protective effect on neuronal damage after cerebral ischemia and promotes brain function reorganization [15].

The methods of clinical assessment of limb spasticity mainly include subjective evaluation methods and objective evaluation methods. Among them, the objective evaluation methods include neurophysiological measures and biomechanical measures. The methods mainly rely on certain instruments to determine the severity of spasticity through corresponding measurement indicators, which can make up for the lack of standardization and precision of subjective evaluation scales [16]. Therefore, we selected acupuncture points frequently used clinically and rehabilitation treatment as the control group to design the clinical trial to explore the efficacy of GV-EA in poststroke lower limb spasticity based on sEMG technology, which can provide a strong evidence-based medical basis for further popularizing the application of GV-EA in the treatment of lower limb spasticity in patients with stroke.

2. Methods and Analysis

2.1. Trial Design. This is a parallel-design and randomized controlled trial. The subjects will be recruited from the Rehabilitation Center of the First Affiliated Hospital of the Henan University of Chinese Medicine. We selected 177 patients who meet the predefined criteria and randomly divided them into 3 groups, with 59 patients in each group: experimental group (GV-EA), control group 1 (conventional EA), and control group 2 (rehabilitation treatment). All patients will receive 20 sessions of treatment that last for 4 weeks and will have sEMG measurements before and after treatment. The efficacy of GV-EA relative to conventional EA and rehabilitation treatment will be analyzed after data collection. The study flow chart is shown in Figure 1. An example template for the content of admission plans, interventions, and evaluations is shown in Table 1. This protocol is guided by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [17].

2.2. Randomization. All included participants will be randomly assigned to three groups at a ratio of 1:1:1: experimental group and control group 1 and control group 2 following the randomization principle, which employs block randomization to generate random-number sequence using SPSS software and an independent statistician who is not involved in treatment and outcome assessment will be responsible for processing the results. Meanwhile, it prepares predetermined sealed opaque envelopes and these envelopes containing the group information strips will be hidden until informed consent is obtained.

2.3. Blinding. This is a single-blinded study; because of the nature of acupuncture treatment, none of the acupuncturists involved in this trial can be blinded to the assignments. Patients will be told that they will receive one of the three effective interventions randomized after enrolment. Each patient will know the type of treatment that they accepted, but they will not know the other two types. The intervention of each patient will be performed in a personal space separated to refrain the communication between patients and will be asked to wear an eye patch when they receive treatment. Data managers and statisticians are not clear on the allocation of patient groups. Therapists, data managers, and statisticians are not allowed to communicate with others about the treatment of patients.

2.4. Recruitment. Posters, pictures, and videos related to the study will be produced to help participants understand the purpose and program of this study while explaining the advantages and disadvantages of the treatment and relevant safety measures to be taken during the trial. In addition, we will use websites and hospital-based WeChat ads for recruitment. According to the above-mentioned inclusion and exclusion criteria, we will make a preliminary judgment and screening of the possibility of inclusion for each participant

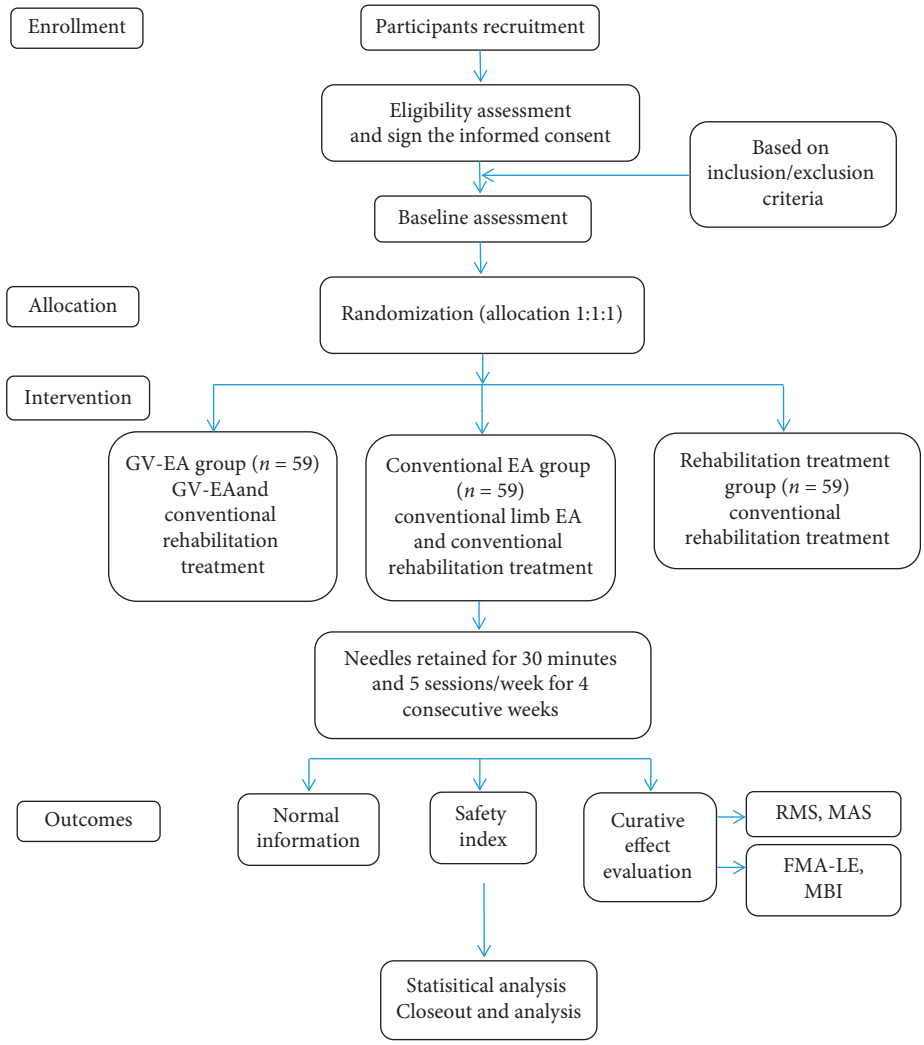

Figure 1: Flowchart of the trial. GV-EA: Governor Vessel electroacupuncture; EA: electroacupuncture; RMS: root mean square; MAS: the Modified Ashworth Scale; FMA-LE: the Fugl–Meyer Assessment for Lower Extremity; MBI: the Modified Barthel Index.

and finally determine whether the subject is included based on the results of the relevant examination.

2.5. Inclusion and Exclusion Criteria

2.5.1. Inclusion Criteria. Participants meeting the following criteria will be included:

(1) Patients who meet the diagnostic criteria of stroke by computed tomography (CT) or magnetic resonance imaging (MRI) (including ischemic and hemorrhagic stroke).

(2) Patients with the Modified Ashworth Scale of grade I and above, grade III and below.

(3) First onset, subacute stage of stroke (the onset time is 3 weeks to 6 months).

(4) Males or females with an age range between 20 and 70 years.

(5) Patients with stable vital signs, no cognitive impairment, and cooperate with treatment.

(6) Patients who voluntarily agree and sign the informed consent form.

2.5.2. Exclusion Criteria. Participants will be excluded if they meet any of the following criteria:

(1) Patients with other serious diseases that may affect treatment outcomes and life-threatening, such as cardiac disease, hepatic disease, pulmonary disease, renal disease, diabetes, osteoporosis, or bleeding tendency.

(2) Patients with systemic infection, or severely unstable.

(3) Patients who are pregnant, lactating, or preparing for pregnancy.

(4) Patients with severe cognition obstacles who cannot cooperate with the treatment.

Table 1: Qualification screening and informed consent will be completed before the assignment. After allocation, each patient will be treated within 4 weeks. The clinical outcome will be evaluated twice: after allocation and after treatment. Adverse events will be recorded on the case report form at any time during treatment.

Timepoint	Enrollment	Allocation	Treatment period			
	−1 week	0 weeks	1 week	2 weeks	3 weeks	4 weeks
Enrollment						
Eligibility screen	X					
Baseline	X					
Informed consent	X					
Medical history	X					
Merger disease	X					
Allocation		X				
Interventions						
Group A			X	X	X	X
Group B			X	X	X	X
Group C			X	X	X	X
Assessments						
RMS		X				X
MAS		X				X
FMA		X				X
MBI		X				X
Safety evaluation		X				X
Needle sensation			X	X	X	X
Adverse events			X	X	X	X

(5) Patients who are participating in other clinical trials.

2.6. Interventions. The trial is divided into 3 groups, namely, experimental group (GV-EA group), control group 1 (conventional EA group), and control group 2 (rehabilitation treatment group). These three groups of patients will receive different treatments 5 times a week for 4 weeks in separate compartments. Meanwhile, each patient will receive the same routine therapeutic drug and rehabilitation treatment. In addition to these two treatments, the experimental group also has electroacupuncture on governor vessel acupoints, and the control group 1 has electroacupuncture on conventional acupoints.

2.6.1. Routine Therapeutic Drug Treatment. All patients use blood pressure control, blood lipid adjustment, blood sugar control, and nutritional nerve drug as routine therapeutic drug treatment following the physicians' instruction.

2.6.2. Rehabilitation Treatment. According to the actual situation of all patients, we will follow the theory of neurodevelopment and provide each patient with the same rehabilitation treatment that will include the affected limb position, passive stretching exercises, muscle strength training, proprioceptive neuromuscular facilitation exercises, Bobath therapy exercises, and Brunnstrom therapy exercises.

2.6.3. Acupuncture Interventions. In China, stroke patients are familiar with acupuncture treatment. Thus, it is easy for patients to determine whether fake acupuncture is used. And because an important purpose of this trial is to make up for the lack of comparison of the efficacy differences between different acupuncture acupoints in the current experiment, placebo acupuncture was not selected. Therefore, we decided to perform clinical conventional electroacupuncture as the control group rather than sham acupuncture and placebo acupuncture.

The interventions will be performed by acupuncturists with more than 3 years of experience in rich clinical acupuncture practice. The acupuncture treatment group used sterilized stainless steel disposable acupuncture needles (0.3∗30 mm). After the needle is inserted to a certain depth of points, it will be lifted and thrust or twirled and rotated for Deqi sensation. After feeling of "Deqi"(the sensations including soreness, numbness, distention, or heaviness), it was connected to the electroacupuncture device (G6805-2A, Shanghai Huayi Medical Instrument Co., Ltd., China, dense waves 2–5 times/second, frequency 2.5 Hz), once a day, every 30 minutes, 5 times a week, for 4 weeks.

2.6.4. Experimental Group. In addition to the two routine treatments, acupuncture will also be performed at Dazhui (GV14), Shendao (GV11), Jinsuo (GV8), Mingmen (GV4), and Yaoyangguan (GV3) for patients in the experimental group. The specific operation method is as follows: generally take the prone position, punctured obliquely to a depth of 0.5–1.0 cm. After the needle is inserted, manipulations will be applied for the "Deqi" sensation. The locations are specifically presented in Table 2.

2.6.5. Control Group 1. The acupuncture will also be performed at the hemiplegic side Blood sea (SP10), Zusanli (ST36), Yinlingquan (SP9), Yanglingquan (GB34), and Sanyinjiao (SP6) for patients in control group 1. The selected

Table 2: Location of acupoints for treating poststroke lower limb spasticity.

	Acupoints location
Dazhui (GV14)	In the depression inferior to the spinous process of the seventh cervical vertebra (C7), on the posterior median line
Shendao (GV11)	In the depression inferior to the spinous process of the fifth thoracic vertebra (T5), on the posterior median line
Jinsuo (GV8)	In the depression inferior to the spinous process of the ninth thoracic vertebra (T9), on the posterior median line
Mingmen (GV4)	In the depression inferior to the spinous process of the second lumbar vertebra (L2), on the posterior median line
Yaoyangguan (GV3)	In the depression inferior to the spinous process of the fourth lumbar vertebra (L4), on the posterior median line

acupoints in this group were high-frequency acupoints based on data mining, the clinical experience of famous acupuncturists of the past, and theoretical research of Chinese medicine for acupuncture treatment of poststroke spasticity [18]. Specific operation: generally take the supine position, perpendicular insertion 1.0–1.5 cm. After "Deqi", the electrode will be connected to the needle.

2.6.6. Control Group 2. Control group 2 only received the two routine treatments.

2.7. Sample Size. The purpose of this study is to evaluate the efficacy of GV-EA in the treatment of poststroke lower limb spasticity. Based on previous studies and clinical experience, the effectiveness rate of conventional EA and rehabilitation treatment is about 75% and 60%, and it is expected that electroacupuncture of GV acupoint will reach 90% [19]. Type 1 error is assumed at 0.05, type 2 error is assumed at 0.1, and a standard deviation is 10. PASS15.0 software (NCSS Statistical Software, Kaysville, UT, USA) has been used to determine the sample size, and the minimum sample size is 53 subjects for each group. Considering a dropout rate of 10%, a total of 177 subjects will be required with 1:1:1 allocation to each group (59 participants per group) for this study.

2.8. Outcome Assessments. The observation indexes of result measurement include three parts: baseline, safety indicators (general physical examination, routine blood tests, routine urine tests, routine stool tests, electrocardiograms, and liver and kidney function tests), and clinical efficacy observation indexes. All evaluations will be performed before and after treatment.

2.9. Primary Outcomes. The main result is surface electromyography (sEMG) measurement and the Modified Ashworth Scale (MAS).

2.10. Surface Electromyography (sEMG) Measurement. The sEMG, also known as dynamic electromyography, is a widely used technology in rehabilitation research and provides quantifiable information on the myoelectric output of a muscle [20]. It is a noninvasive inspection method for recording bioelectric signals during muscle activity through surface electrodes and is an essential test tool for studying the functional status of the neuromuscular system. At the same time, it provides a scientific basis for clinical treatment. Commonly used evaluation indicators for sEMG include frequency and time-domain indicators, among which time-domain indicators mainly include the root mean square (RMS). Among them, the RMS value reflects the change of the amplitude of the sEMG signal voltage in the time dimension and variation of relevant muscle strength [21]. Its change mainly reflects the number of motor units activated during muscle activity, the type of motor units participating in the activity, and the degree of synchronization. The sEMG can also distinguish between spasticity and contracture. When the joint resistance measured by passive stretching increases and the antagonistic myoelectric signal is not significantly different from the resting state, it is a contracture, and the electromyographic signal is significantly increased, which is spasticity.

Before and after the treatment, the sEMG signal system (BioNeuro Infiniti, Thought Technology Ltd., Canada) was used to collect the electromyographic signal values of the quadriceps, hamstrings, calf triceps, and tibialis anterior muscles of the hemiplegic lower limbs. With the patient in the sitting position, a 75% alcohol cotton swab will be used to clean the skin on the measured muscle surface to remove oil on the skin surface, reduce electrical resistance, and increase the electrical conductivity between the surface electrode and the skin. After cleaning, according to the anatomical position of the muscle and the direction of the muscle fiber, the disposable surface electrode sheets are, respectively, pasted on the muscle belly of the measured muscle. Select the program measurement mode, passively stretch the tested muscles, passively stretch 3 times, relax 3 times, 5 s each time, take RMS value as the detection index, and calculate cocontraction ratios (CCR); the specific calculation formula is

$$\text{CCR} = \text{antagonist muscle RMS}/(\text{active muscle RMS} + \text{antagonist muscle RMS})$$

3. The Modified Ashworth Scale (MAS) [22]

The MAS is the most widely used spasticity assessment and has the advantages of simplicity, time savings, and convenient operation, which includes 6 levels. To facilitate data recording and analysis and statistical analysis, grades 0, I, I+, II, III, and IV are converted into 0, 1, 2, 3, 4, and 5 points, respectively. The higher the MAS score is, the more serious the spasticity is considered as shown in Table 3.

3.1. Secondary Outcomes. The Fugl–Meyer Assessment for Lower Extremity (FMA-LE) and the Modified Barthel Index score (MBI) were the secondary observation outcome indicators.

Table 3: The MAS specific scoring rules.

	Modified Ashworth Scale
1	No increase in muscle tone
1	Slight increase in muscle tone, manifested by a catch or by minimal resistance at the end of the range of motion (ROM) when the affected part(s) is (are) moved in flexion or extension
1+	Slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM
2	More marked increase in muscle tone through most of the ROM, but affected part(s) easily moved
3	Considerable increase in muscle tone, passive movement difficult
4	Affected part(s) rigid in flexion or extension

4. The Fugl–Meyer Assessment for Lower Extremity (FMA-LE)

The FMA-LE is a widely recognized and valid clinical measure of poststroke motor impairment severity assessment. The lower extremity has a maximum score of 34 points divided into motor, balance, sensory, range of motion, and joint pain and a higher score indicates better lower limb movement function [23, 24]. The FMA-LE is the most common scale for evaluating lower limb motor function after stroke.

4.1. The Modified Barthel Index Score (MBI). The MBI is mainly used to assess the patient's daily living ability, including control urination, eating, grooming, toileting, transfer, walking, dressing, bathing, and going up and down stairs. The total score is 100 points. A higher score indicates the worst daily living ability. The MBI classification can be divided into without dependence (100 points), mild dependence (61~99 points), moderate dependence (41~60 points), severe dependence (1~40 points), and completely dependent (0 points) [25].

4.2. Safety Evaluation and Adverse Events. Any adverse events and how they are dealt with will be recorded throughout the treatment process. Adverse events related to acupuncture treatment included severe pain, fainting, bleeding, or other any discomfort. During the intervention period, the researchers will pay close attention to the patient's condition. If any discomfort occurs to the patient, the intervention will be stopped immediately and the patient's condition will be dealt with accordingly. Any medical occurrence detailed information will be reported in detail in case report forms (CRFs). The primary researchers will review all adverse events periodically, and the Ethics Committee will have access to the interim results.

4.3. Data Collection and Management. The data of all patients will be recorded on the CRFs, which include observation time points, outcome measures, adverse events, safety evaluations. Two independent researchers will input the data into the Excel spreadsheet, and each patient's personal privacy information will be protected. Data will be safely kept by the data researchers of our team and monitored by the Ethics Committee of the First Affiliated Hospital of the Henan University of Chinese Medicine each year as shown in Table 1.

4.4. Statistical Analysis. Statistical analysis will be performed using SPSS 25.0 software for statistical analysis. To measure baseline, a chi-square test or analysis of variance (ANOVA) is used to compare demographic data and other basic data at baseline. Continuous variables are expressed in terms of mean ± standard deviation when following a normal distribution. If not, the data will be shown by medians and interquartile ranges. Frequencies and percentages will be used to count the data. The paired t-test will be used to compare the differences between the groups before and after treatment, including the RMS value, FMA-LE, and MBI score. The comparison among the three groups before and after treatment will be analyzed by variance (ANOVA). For the MAS, the Kruskal–Wallis test will be used. The test level is set to 0.05, and $P < 0.05$ means the difference is statistically significant.

4.5. Quality Control. To reduce the potential bias and ensure the quality of this trial, all acupuncturists and assessors will be required to receive standard training prior to the beginning of the trial. The content of the training course is provided by the protocol, including recruitment, interventions, and assessment process. Whether the data is complete and accurate will be monitor by a qualified clinical trial expert from the Clinical Research Center of the First Affiliated Hospital of the Henan University of Chinese Medicine. All clinical trial experts are independent of the sponsor. In addition, a quality control team will be established to supervise whether the experimental procedure meets the standard guideline. Statistical analysis will be performed when the total number of samples collected reaches 89 cases. The primary investigator will obtain these interim results and will decide whether to continue the trial. We will stop the trial if the efficacy of the patients in the experimental group is much lower than the other two groups in the outcomes of the interim data [26].

5. Discussion

Lower limb spasticity will severely limit the patient's trunk antigravity activity function and daily and social life ability recovery and even induce severe complications such as pneumonia and deep vein thrombosis [27]. It is of great

significance to find effective treatment measures to reduce lower limb spasticity after stroke. This study is designed to evaluate the efficacy and safety of GV-EA in the treatment of poststroke lower limb spasticity.

5.1. Acupuncture Points Selection. The GV walks in the middle of the back and intersects with the three yang meridians of hand and foot and the Yang Link Vessel many times. Due to the connection between the GV and the yang meridians, it is also called "the sea of the yang meridians" [28]. Unblock Yang takes the regulation of Du as the first, so acupoints on the GV are selected. In this trial, the selected acupoints GV14, GV11, GV8, GV4, and GV3 are all the GV points. Among them, GV14 is the intersection of the GV and the three yang meridians of hand and foot, that is, "confluence of the yang". GV8 acupoints mainly treat convulsions, strong spine, limbs, and tendons and have a good effect on spastic hemiplegia caused by stroke [29, 30]. EA at the above-mentioned acupoints can effectively promote the circulation of yang qi in the GV to the whole body. Blood flows with qi, and qi and blood flow to the end of the limbs so that the muscles and veins of the limbs can be nourished by qi and blood, thereby relieving limb spasticity.

5.2. Outcome Measurements Selection: Surface Electromyography (sEMG). The sEMG evaluation is an objective method to obtain the electromyographic signal during muscle activity, which is achieved by placing surface electrodes on the muscle layer [31]. It can perform quantitative and qualitative analysis on the function of muscles and can study multiple muscles on the body at the same time. It has the advantages of noninvasiveness, safety and reliability, convenient operation, and objective quantification [32]. The evaluation of nerve and muscle function and rehabilitation effects of stroke patients has gained more and more attention. The muscle strength classification scale and muscle spasticity detection methods that are still in use until now are subject to great subjectivity; the detection results are difficult to accurately quantify and qualify so that subjective evaluation methods are questionable and limited in clinical applications [33]. And through the analysis of sEMG signal, it can reflect the level of motor unit recruitment and synchronization and can study the functional status and control mechanism of the nerve and muscle of stroke patients with hemiplegia to evaluate the rehabilitation effect and guide that rehabilitation process has become an important tool in the field of rehabilitation [34, 35].

One important limitation of our study is that as it is an acupuncture trial, it is impossible to execute double-blind procedures. To reduce this kind of bias, each group of patients will be treated in a different room, and each group of patients will be prohibited from talking. In addition, all the acupuncturists participating in this study will conduct unified training and strictly regulate the acupuncture operation to maximize the elimination bias. In addition to the required scales, sEMG is also used in the evaluation to reduce the interference of subjective factors. We expect that the results of this trial will provide evidence on the efficacy and safety of GV-EA in the treatment of poststroke lower limb spasticity. This will provide a practical and effective treatment method for the clinic, which has very important social significance.

Abbreviations

GV-EA: Governor vessel electroacupuncture
EA: Electroacupuncture
sEMG: Surface electromyography
SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials
CT: Computed tomography
MRI: Magnetic resonance imaging
PASS: Power analysis and sample size
SPSS: Statistical Package for the Social Sciences
RMS: Root mean square
MAS: Modified Ashworth Scale
FMA-LE: Fugl–Meyer Assessment for Lower Extremity
MBI: Modified Barthel Index
CRFs: Case report forms.

Data Availability

No data were used to support this study.

Ethical Approval

This study has been approved by the Ethics Committee of the First Affiliated Hospital of Henan University of Chinese Medicine (reference no. 2019HL-103-01).

Consent

Written informed consent will be obtained from all participants. All personal information about potential and enrolled participants will be confidential.

Disclosure

The study funders have no role in the study design, data collection and management, and manuscript writing publication.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

RQL proposed the concept for this trial and designed the study. JWL contributed to the conception, design, and manuscript writing. JJM, YYW, YCH, and WXH helped search the literature and assisted in the recruitment of patients. XDF, KQS, and SY participated in the revision and editing of this manuscript. All the authors approved the final version of the manuscript.

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Supplementary Materials

Additional file 1: Completed Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) 2013 Checklist: items addressed in this clinical trial protocol. Additional file 2: STRICTA 2010 checklist of information to include when reporting interventions in a clinical trial of acupuncture (expansion of Item 5 from CONSORT 2010 checklist). Additional file 3: Informed consent form. (*Supplementary Materials*)

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Research Article

Development of an Assessment Tool of Menstrual-Cycle-Related Signs and Symptoms Based on Thai Traditional Medicine Principles for Evaluation of Women's Health

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Background. Utilization of Thai traditional medicine (TTM) was considered in menstrual-cycle-related signs and symptoms (MCSs) to evaluate women's health. TTM clinicians diagnosed the MCSs by signs, symptoms, and associated factors of patients including a physical examination to find patterns of imbalance elements and the origin of the disorder to optimize treatment. Thus, the purpose of this study was to develop a new assessment tool, the menstrual-cycle-related signs and symptoms questionnaire (MCSQ) based on TTM principles for evaluation of women's menstrual health. *Methods*. The items and components of the MCSQ were adjusted by TTM expert consensus using the Delphi technique. The content validity of the MCSQ was quantified by the content validity index (CVI). MCSQ were examined by construct validity and internal consistency reliability using exploratory factor analysis (EFA) and Cronbach's α coefficient, respectively. *Results*: All 19 experts (100%) responded to the questionnaires in the three rounds of the Delphi technique. The MCSQ showed high content validity of individual items (I-CVI = 0.83–1.00) and high overall content validity of the questionnaire (S-CVI/AVE = 0.98). Overall, 429 of 432 participants completed the questionnaire (99.31%). After factor analysis, the final MCSQ was divided into two sections, which consisted of 49 items. The first had 23 items focusing on the MCSs. And, the second had 14 items of personal and medical data including 12 items of associated factors. Cronbach's α coefficient of the final MCSQ was 0.87, and that of each component was between 0.32 and 0.82. *Conclusions*. This study reports a new MCS questionnaire tool, which was developed from TTM knowledge to evaluate women's health. This questionnaire showed an acceptable level of validity and reliability. Thus, it is also expected to be useful in clinical practice and ongoing research on evaluation of women's health.

1. Introduction

In most women, the menstruation period brings a variety of uncomfortable symptoms and inconveniences affecting daily life. Menstrual-cycle-related signs and symptoms (MCSs) are a group of physical and/or emotional manifestations that occur before or during the menstrual period, mostly identified as either premenstrual syndrome (PMS) or dysmenorrhea. These MCSs are the most prevalent menstrual problems afflicting reproductive-age women. In reproductive-age women, the prevalence of MCSs during menstruation varies between 16% and 91% [1]. The first systematic review and meta-analysis of PMS reported that the pooled prevalence of PMS worldwide was 47.8 % [2]. Although dysmenorrhea and PMS are health problems that occur in a relatively short-time period and the symptoms can disappear without treatment, both symptoms often occur every menses cycle for many years [3, 4]. Severity of symptoms directly affects the quality of life and indirectly affects social and economic development, including impaired daily living activities, and often augmented social involvement. Some adolescents and women may require absence from school or work, and may have impaired work productivity [4, 5]. Moreover, women who reported PMS at baseline were at greater risk of menopausal symptoms and risk of postpartum depression development [6, 7], and women with a history of severe dysmenorrhea symptoms before pregnancy have a higher risk of developing psychological distress during pregnancy [8]. Menstruation is an important indicator of health and quality of life in women: the reproductive endocrine system is associated with reproductive health; it affects mental health, fertility, and leads to menopause [6, 9].

Thai traditional medicine (TTM) has long been used for the treatment of menstrual disorders. According to the TTM principles, menstruation is an important part of women's health care because menstruation can be a principal cause of illnesses or diseases in women [10]. There is a specific TTM textbook about menstrual disorders and women's diseases called "Mahaa-Chotarat." The MCSs in TTM principles are called "Lohit-Pokkathi-Thod (in Thai language)." It is characterized by symptoms and signs linked to the menstrual cycle occurring before and/or during the menstrual period and resolving before or during the onset of menstruation such as fever, low back pain, depression, irritability, characteristics of menstrual blood, and leucorrhea. This disorder is considered a dysfunction of the wind and fire elements that have an impact on water elements or effects on the blood system. Women have menstrual-cycle-related signs and symptoms; if they do not receive proper treatment, these symptoms can develop into more severe disease, such as skin disorders, mental problems, uterine abscess, or infertility and cancer [10].

The instruments and measurements are an integral component of routine patient assessment for practitioners, which ensures appropriate treatments are selected; therefore, a variety of tools for the assessment of MCSs were developed and widely used for screening or diagnostic tests such as Menstrual Distress Questionnaire (MDQ) [11], Menstrual Symptom Questionnaire (MSQ) [12], Premenstrual Symptoms Screening Tool (PSST) devised by Steiner, MacDougall and Brown 2003 [13, 14], and Calendar of Premenstrual Experiences (COPE) [15]. But, these tools focus on measuring the severity or impact of MCSs and were developed based on a conventional Western medicine context. Thai traditional medical clinicians evaluate and diagnose the MCSs by collecting signs, symptoms, and the associated behaviors of the patients. Interestingly, TTM practitioners also inspect via physical examination to diagnose for abnormalities, illness, or disease. A guided proper treatment utilizing TTM principles considers the signs and symptoms to be a reflection of the fundamental pathology of the internal systems and organs; therefore, assessing the signs and symptoms that occur leads to the fundamental pathology of the diseases. In addition, we also assess lifestyles, food and drink consumption, sleeping, mental health, and the environment to elucidate the causes of diseases, because these factors usually influence menstruation and the occurrence of MCSs according to TTM principles. Moreover, the TTM is an individualized personalized medicine approach that treats each patient on the basis of their own individual pathology. According to this foundational knowledge of TTM, we sought to determine whether it is possible to create such a tool through this study for assessment of the MCSs by applying TTM principles and knowledge.

Therefore, this study aimed to develop a menstrual-cycle-related signs and symptom questionnaire (MCSQ) based on TTM principles that can assess the associated factors, the severity of the MCSs, and identify the internal system or organ that is the cause of the MCSs. It is expected that this questionnaire and its guidelines will be clinically useful for women's self-care and prevention with precision TTM treatment.

2. Methods

The study employed a sequential mixed methods design consisting of two phases. The first phase was qualitative research conducted to establish the questionnaire from the literature review and interviewing TTM experts. Subsequently, the second phase was quantitative research for questionnaire development. The validity of the developed MCSQ was evaluated by using content validation and structural validation. The assessment of the reliability within each component of the questionnaire utilized internal consistency reliability. The pattern of this research is shown in Figure 1.

2.1. Investigation of Menstrual-Cycle-Related Signs and Symptoms Using TTM Principles. In this study, the theoretical framework about pathogenesis of MCSs was generated based on a review of the TTM literature and interviewing TTM experts–TTM clinicians who have experience in the treatment of menstrual disorders and scholars of TTM theory.

2.2. Developing the MCSQ Items through the Delphi Method. The Delphi technique is considered desirable to reach consensus in a field where incomplete knowledge is evident

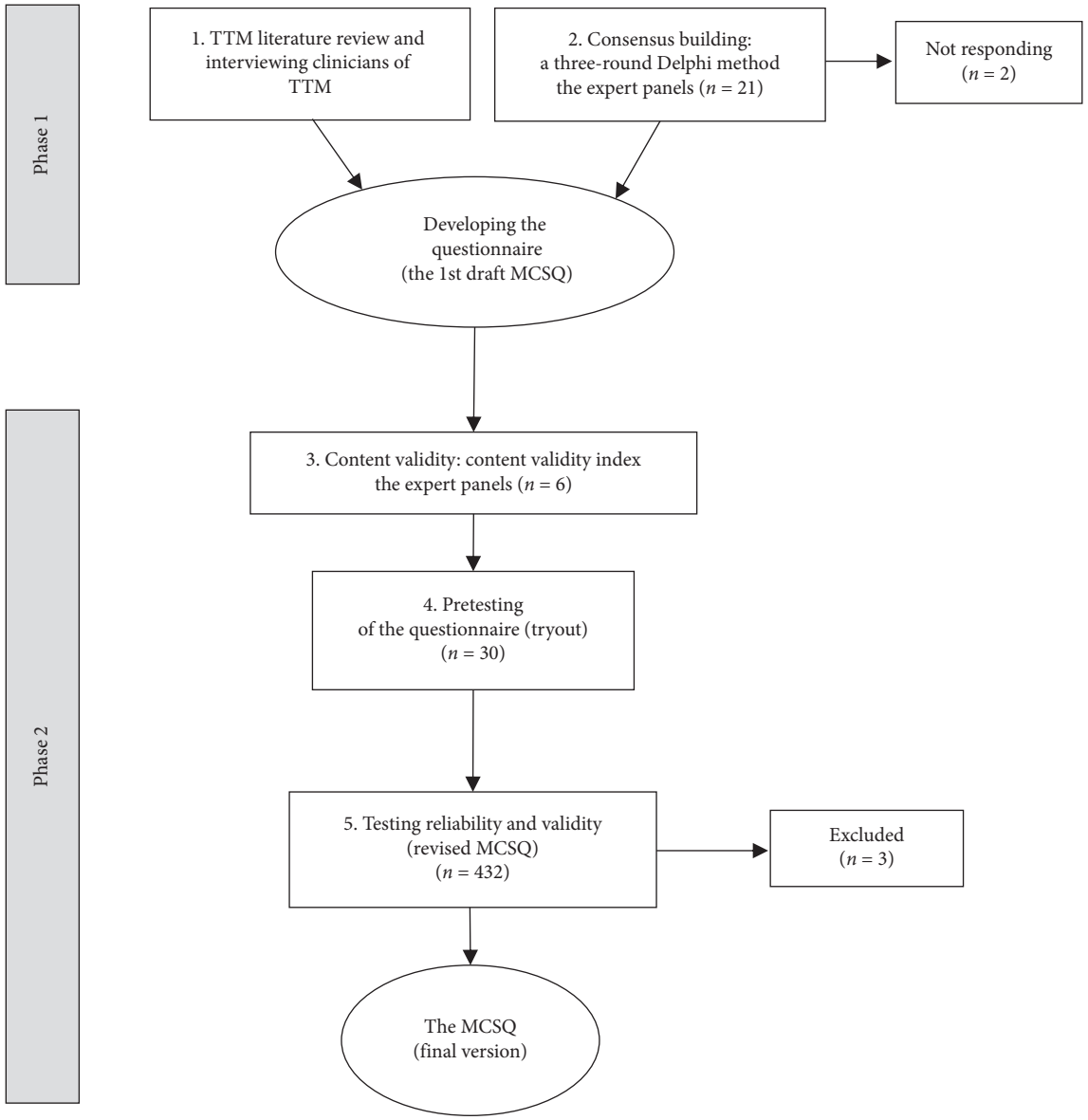

Figure 1: Phases and steps in developing the menstrual-cycle-related questionnaire (MCSQ).

[16]. It was used to develop the appropriate MCSQ items. The Delphi method consists of repeated questionnaire surveys from experts without face-to-face meetings. It requires at least two or three rounds of the questionnaire survey if the first round is an open-ended questionnaire [17]. This study used a three-round Delphi method to establish a consensus on the essential content of assessing the cause and severity of menstrual-cycle-related symptoms based on TTM principles. The open-ended questionnaire was explored in the 1st round, and the structured questionnaire was sent by mail in 2nd and 3rd rounds.

2.2.1. Selection of the Expert Panel and Sample Size. The number of experts in the Delphi technique were calculated by Thomas T. Macmillan found that more than 17 experts or equal resulted in a minimal error value (error level 0.02) [18]. Thus, the sample size was chosen for this study to be 17–21 participants. Purposive sampling was used for the selection of the TTM experts. The experts in the Delphi technique were selected from the list of expert committees of the Thai Traditional Medical Council with clinical experience in treatment for menstrual-cycle-related symptoms or menstrual disorders with TTM theoretical knowledge greater than 10 years.

2.2.2. The Delphi Questionnaire. The first round of the Delphi Technique received an open-ended questionnaire, which was modified from a review of literature in TTM theory and interviewing TTM experts. This open-ended questionnaire was also used for interviewing 19 experts. After that, this questionnaire was modified into a structural questionnaire and was used in the 2nd round of Delphi questions. This 2nd round questionnaire would be sent back to the 19 experts to determine the importance level of items

by a 5-point Likert rating scale; 1-strongly unimportant; 2-unimportant; 3-neutral; 4- important, and 5-strongly important. However, this structural questionnaire in the 2nd round was confirmed by the same 19 experts in the 3rd round. The time duration between 2nd and 3rd rounds was 2 weeks.

2.2.3. The Delphi Analysis. In the 1st round, the open-ended questionnaire, a list of all contents was analyzed using content analysis. In the 2nd round, the importance level of each item of the draft questionnaire was evaluated and showed an outcome as median and interquartile range (IQR) [The IQR is the value of data distribution]. In the 3rd round (final round), the experts received a questionnaire which showed the median score and IQR of each item from the 2nd round. The experts were given an opportunity to revise their rating or retain the previous scores for confirmation of answers. The new median score and IQR of items in 3nd round were calculated to assess the level of consensus per question [19]. The items with the median score greater than or equal to 3.5 (high level of importance) and IQR less than or equal to 1.5 would be accepted to be important items that reached the consensus threshold [20]. These accepted items were used to establish the first draft MCSQ.

2.3. Evaluation of Validity and Reliability of the MCSQ

2.3.1. Content Validation. The content validity index (CVI) of each item was evaluated by 6 experts who had experience in Thai traditional medicine for more than 10 years and were licensed to practice Thai traditional medicine. The expert panel provided a score of each item based on its relevance using a 4-point ordinal scale, with score 1 meaning "not relevant," score 2 "somewhat relevant," score 3 "quite relevant," and score 4 "highly relevant." At the end of each item was a clinical notation for the expert's comments. Relevance evaluation of each item under the pattern of TTM principles for menstrual-cycle-related signs and symptoms were calculated with the item-content validity index (I-CVI) and the scale-content validity index (S-CVI). The I-CVI of each item equal or greater than 0.83 was selected.

Subsequently, the revised MCSQ was used in a preliminary trial of 30 reproductive women (age 18–45 years with a clinically normal menstrual cycle) for evaluation on content, language, and time to complete the survey. Then, the questionnaires were revised and edited for clarity. Internal consistency was measured by Cronbach's alpha.

Through the results of the revised questionnaire, it was concluded that 63 items from 75 items were suitable. This questionnaire was divided into 2 sections: Section 1: Menstrual-cycle-related signs and symptoms (MCSs section) had 27 items; this section represented the signs and symptoms related to the menstrual cycle and the importance of identification of the internal organs and system that are the likely cause of the MCSs. Section 2: Associated factors (AF section); this section emphasizes personal information, behaviors, and other factors that affect the occurrence of MCSs. The AF section was divided into two parts. Part 1: Multiple-choice questions (14 items) that inquired about personal data, medical history, and gynecologic history, and Part 2: Rating scale questions (22 items) that queried the frequency of associated factors which affect the occurrence of menstrual-cycle-related signs and symptoms.

All items were rated on a 5-point rating scale as follows. For the question items related characteristics of menstrual blood, leucorrhea, and associated factors, the scale ranged from 0 "never" to 4 "always" to assess the frequency of the event. The scale of the question items indicated menstrual-cycle-related symptoms ranged from 0 "none" to 4 "extremely severe" to assess the severity of signs and symptoms.

2.3.2. The Field-Testing of the Revised Questionnaire for Evaluation Validity and Reliability. Because the purported afflicted population is large and the true proportion is not known, the sample size of this study was calculated by Cochran's sample size formula. Assuming the maximum variability, which is equal to 50% ($p = 0.5$) and taking 95% confidence level with ±5% precision, the calculation for required sample size was set as follows: $p = 0.5$ and hence $q = 1–0.5 = 0.5$; $e = 0.05$; $z = 1.96$:

$$\begin{aligned} n_0 &= \frac{z^2\, pq}{e^2} \\ &= \frac{(1.96)^2\,(0.5)\,(0.5)}{(0.05)^2} \\ &= 384.16 \\ &= 385, \end{aligned} \tag{1}$$

where n_0 is the sample size, z is found in statistical tables that contain the area under the normal curve, e.g., $z = 1.96$ for 95% level of confidence, e is the desired level of precision, p is the estimated proportion of an attribute that is present in the population, and q is a margin of error or it was calculated from $1 - p$.

The sample size of the field testing was expanded by 10% of the power calculated sample size in anticipation of incomplete surveys with deficient answers or missing questionnaires. The questionnaires were equally distributed to 6 study sites (the sample size per study site: 428/6 = 71.3 = 72); therefore, the sample size of this study was 432. Comrey and Lee (1992) have described sample sizes of 300 as "good" and 500 as "very good" for factor analysis [21]. According to this guideline, the sample of 432 participants was deemed acceptable for exploratory factor analysis.

This study utilized purposive sampling from 6 regions with one province representing each region, namely, Pathum Thani (Central region), Prachin Buri (Eastern region), Phetchaburi (Western region), Loei (Northeastern region), Phayao (Northern region), and Songkhla (Southern region).

The revised MCSQs were distributed to 432 Thai women of reproductive age, 18–45 years, who were receiving

services at the Thai Traditional Medicine department in hospitals or Thai traditional medical clinics. The inclusion criteria were: (1) female aged 18–45 years, (2) women having a menstrual cycle between 21 and 35 days, and (3) women that understand and communicate in the Thai language.

Exclusion criteria were: (1) amenorrhea (missing more than 2 menstrual cycles before participating in the study), (2) pregnancy or breast feeding within one year before participating in the study, (3) abdominal surgery within one year before participating in the study, (4) childbirth within one year before participating in the study, (5) history of abortion within one year before participating in the study, and (6) taking medication to treat a mental health disorder. The survey was conducted from December 2019 to February 2020.

2.4. Statistical Analysis. The data analysis was divided into two phases concomitant with the process of questionnaire development. The qualitative research in the first phase used content analysis. The second phase as quantitative research used the descriptive statistics including frequency or percentage, range, mean, and standard deviation (SD). The MCSQ (final version) score in each section and component were summarized as a percentage, mean, and SD.

The content validity of the questionnaire in quantitative research was assessed using the item-level content validity index (I-CVI) and the scale-level content validity index (S-CVI). The I-CVI is calculated as the number of experts providing a rating of 3 or 4 for each item divided by the total number of experts. The acceptable standard for the I-CVI should be more than 0.78 (considered "excellent") and the S-CVI should be more than or equal to 0.9 [22].

Construct validity was evaluated using exploratory factor analysis (EFA). In this study, the MCSQ was split into two sections, and each section was evaluated for its construct validity separately. EFA was used to evaluate the validity of the component of the items mainly due to the TTM principal component aspects. The underlying structure of the revised MCSQ items was evaluated by principal component exploratory factor analysis using varimax rotation with Kaiser normalization. The criterion applied to retain the component was an eigenvalue ≥1.0 for that component. The factor loading for each item to meet this condition was set at 0.40 or more [23, 24].

The internal consistency or the reliability of the rating scale of the items and each component of the final MCSQ were measured by calculating Cronbach's alpha coefficient and corrected item-total correlation. The acceptable values of Cronbach's alpha were above 0.70 [25, 26] and the corrected item-total correlation was greater than or equal 0.20 [27].

2.5. Ethical Considerations. The expert panel and participants were approached by the researchers and invited to complete the questionnaire voluntarily through informed consent. They were informed about the objectives of the study, and that their personal information would be protected and would not be revealed to others. This study received approval from the Human Research Ethics Committee of Thammasat University No.1 (Faculty of Medicine) (MTU-EC-ES-1-162/61).

3. Results

3.1. Phase 1: Developing the Menstrual-Cycle-Related Symptoms Questionnaire (MCSQ)

3.1.1. The Menstrual-Cycle-Related Signs and Symptoms Using TTM Principles. According to the official Thai traditional medicine textbook named "Paet-SaatSong-Kror" (in Thai language), the human body consists of four elements: Earth element (all organs), water element (all liquid in the body such as blood, saliva, urine, etc.), wind element (the circulation of blood, the signal transmission of the nervous system, the movements of the body, and the transportation of gases in the respiratory system), and the fire element (such as the metabolic system, endocrine system, chemical mediators). All functions of the organ and liquid system are controlled by the wind and fire elements [28]. Vedcha-SukSaa textbook (in Thai language) states that disorders or diseases are caused by the impaired function of elements. The causes of disease are age, environment (hot and cool weather), behaviors (eating, sleeping, excreting, bodily movements), tastes and types of food and drinks, and emotion. All of these factors affect the balance elements in the body [29].

Thai traditional medical scripture regarding women's diseases and menstruation called "Mahaa-Chotarat (in Thai language)" states that the irregularity of blood and menstruation are the principal causes of illnesses or diseases in women. The fire and wind elements are the basis regulators for all functions of organs (physical and mental processes) including the smooth flow of blood throughout the body. Blood is the water element that circulates and nourishes various organs within the body. Accordingly, if the function of the fire and wind elements is impaired, the blood circulation will be interrupted affecting various emotional and physical symptoms. Menstruation is directly related to the blood or water element, the wind element, and the fire element. Before menstruation, the body has more power of the fire, wind, and water (blood) elements than usual. The increasing power of these elements affects the function of organs inside the body. According to TTM principles, MCSs are the group of physical and/or emotional symptoms that occur before and/or during the menstrual period and disappear before or after the onset of menstruation. Most MCSs are caused by the following organ malfunctions: muscle and liver, joint and bone, gallbladder (in the TTM principles, it is also responsible for controlling heat), skin, heart, and intestine and mesentery (in TTM principles organs work together). Moreover, there will be irregular characteristics of menstrual blood and leucorrhea (color, texture, and smell) that indicate that the blood and uterus have a disorder. These MCSs are caused by an impaired function of the fire and wind elements affecting the malfunctions of blood and related organs. Accordingly, TTM clinicians use these signs and symptoms to examine and

find the organ or system inside the body, which is the origin of the disorder.

According to TTM principles and the TTM clinician tenet, the MCSs are influenced by several external and internal factors, as shown in Table 1.

These factors exert an effect on the function of the fire and wind elements, and blood, leading to an increase in the fire element and/or the wind element causing the poor circulation of blood and giving rise to menstrual-cycle-related symptoms and signs. The mechanism of MCSs according to the principle of TTM is shown in Figure 2.

Consequently, this study utilized the described theoretical framework to guide the development of the questionnaire for assessing the MCSs. The two important issues of this questionnaire were the MCSs and associated factors that can reflect the origin of the disorder.

3.1.2. Questionnaire Development via Delphi Method. Nineteen out of the 21 experts agreed to participate in the Delphi study and all of them completed the three rounds of the Delphi questionnaire; the flow of the participation is shown in Figure 1. The expert panel was selected from different TTM operational levels comprising 12 TTM clinicians from clinics or hospitals and 7 experts from the university. The characteristics of TTM experts are given in Table 2.

The expert panel was asked about the appropriate domains and items associated with assessing MCSs based on the TTM principle. There was a consensus on the important items including physical and/or emotional symptoms that occur before or during menstruation, the characteristics of menstrual blood and leucorrhea, and the factors affecting MCSs were determined to be the appropriate clinical essentials and items for this assessment.

The final round contained a total of 75 items, each item having a median ≥ 3.50 (high level of importance) and IQR ≤ 1.50. The summarized detail of the questionnaire is shown in Table 3. In section 1: MCSs section, because characteristics of menstrual blood and leucorrhoea have similar content in some items, experts recommended including items with consistent content to be one item to reduce the number of items and redundancy, resulting in 9 remaining items of characteristics of menstrual blood and leucorrhoea. The expert panel suggested that these items should use the frequency score to assess the severity of menstrual blood and leucorrhoea (if a woman always has irregular menstrual blood or leucorrhea, indicating the blood was terrible). In Section 2: Associated factors section, due to the associated factors being so detailed and involving a lengthy number of items, experts recommended including items with consistent content to be one item to reduce the number of items and redundancy, resulting in the 35 remaining items of associated factors. Thus, the first draft MCSQ included 61 items in two sections: the menstrual-cycle-related signs and symptoms section (the MCSs section) had 26 items and the associated factors section (the AF section) had 35 items. Each section incorporated a number of subcategories according to the theoretical framework (the content above) and the expert consensus.

The MCSs section subcategories focus on the important signs and symptoms that reflect the problematic internal system or organ, including the following five dimensions: (1) the signs reflect the problem of the blood in the uterus (characteristics of menstrual blood and leucorrhea), (2) the symptoms reflect the problem of the musculoskeletal system, (3) the symptoms reflect the problem of the intestine, (4) the symptoms reflect the problem of the gallbladder (it is the controller of the heating system inside the body), and (5) the symptoms reflect the problem of heart (mind aspect).

The AF section subcategories addressed aspects of the causes that affect the occurrence of MCSs, including the following seven dimensions: (1) personal data, (2) medical history, (3) Ob-Gynecologic history, (4) the types of food and drink, (5) The behaviors and health problems, (6) the environment (hot and cool), and (7) the emotion and feeling. The details of items of MCSQ in the 3rd round of the Delphi method are shown in Supplementary Table 1.

3.2. Evaluation of Validity and Reliability of the MCSQ

3.2.1. Content Validity of the MCSQ. After finishing the Delphi method, the content validity was performed by five experts to evaluate the content validity of the first draft MCSQ. All items (61 items) of this questionnaire showed excellent content validity (I-CVI = 0.83 and 1.00). The average scale content validity (S-CVI/AVE) was 0.98, above the cut-off of 0.90. There were 2 additional items as recommended by experts. The items were later adjusted or changed for clarity according to experts' suggestions. Thus, the revised MCSQ included 63 items in two sections. The items with CVI of the revised MCSQ are shown in Supplementary Table 2. The pretesting of the revised MCSQ showed Cronbach's α coefficient was 0.85 overall, indicating good internal consistency and appropriate item homogeneity.

3.2.2. The Evaluation of MCSQ by Construct Validity. The questionnaires were distributed to 432 reproductive-age Thai women. Two women were excluded and one woman did not complete the questionnaire; therefore, there were 429 participants or useful questionnaires (response rate of 99.31%). The mean age of the participants was 26.00 ± 7.27 years (aged 18–45 years). The majority of participants were single (81.4%), 15.8% of the participants were married, and the remaining 2.9% was either separated or widowed. The participants reported menarche at 11.00 ± 4.20 years old. The general information of participants is shown in Table 4.

Eight items of the revised draft MCSQ were removed before analyses via EFA because 2 items of menstrual-cycle-related symptoms had high skewness and kurtosis values (2 and 4, respectively), showing nonnormal distribution of the data, and six items of associated factors had low internal consistency (the items were negative or less than 0.2). The revised MCSQ consisted of 55 items in two sections: the MCSs section had 25 items and the AF section had 30 items.

Table 1: The external and internal factors affecting MCSs according to TTM knowledge.

Factors affecting MCSs according to TTM knowledge [10, 29]	Example of the factors
Internal factors	
Personal data	Age, major elements in the human body, obesity
Medical history	Underlying illness or health problems (migraine, asthma, atopic dermatitis, insomnia, constipation, etc.), accident, abdominal surgery
Ob-gynecologic history	Pregnancy and childbirth history, postpartum care, abortion, curettage, contraceptive use Gynecological disease, age at menarche, first time menstrual-cycle-related symptoms began, family history (grandmother/mother/sister/twin)
Emotion	Angry, irritability, anxiety, stress, sad, depressed
Behaviors	Skipping meals, being in the same position for a long time, working excessively, sleep deprivation, often sleeps late
External factors	
Types of food	Uncooked food or raw food, strong-flavored food, preserved food, fried food, oily food
Types of drink	Energy drinks, carbonated drinks, iced drinks, frappe, coconut juice, alcoholic drinks, caffeinated drinks
Environment	Contact with chemicals, hot weather, cold weather, climate change immediately

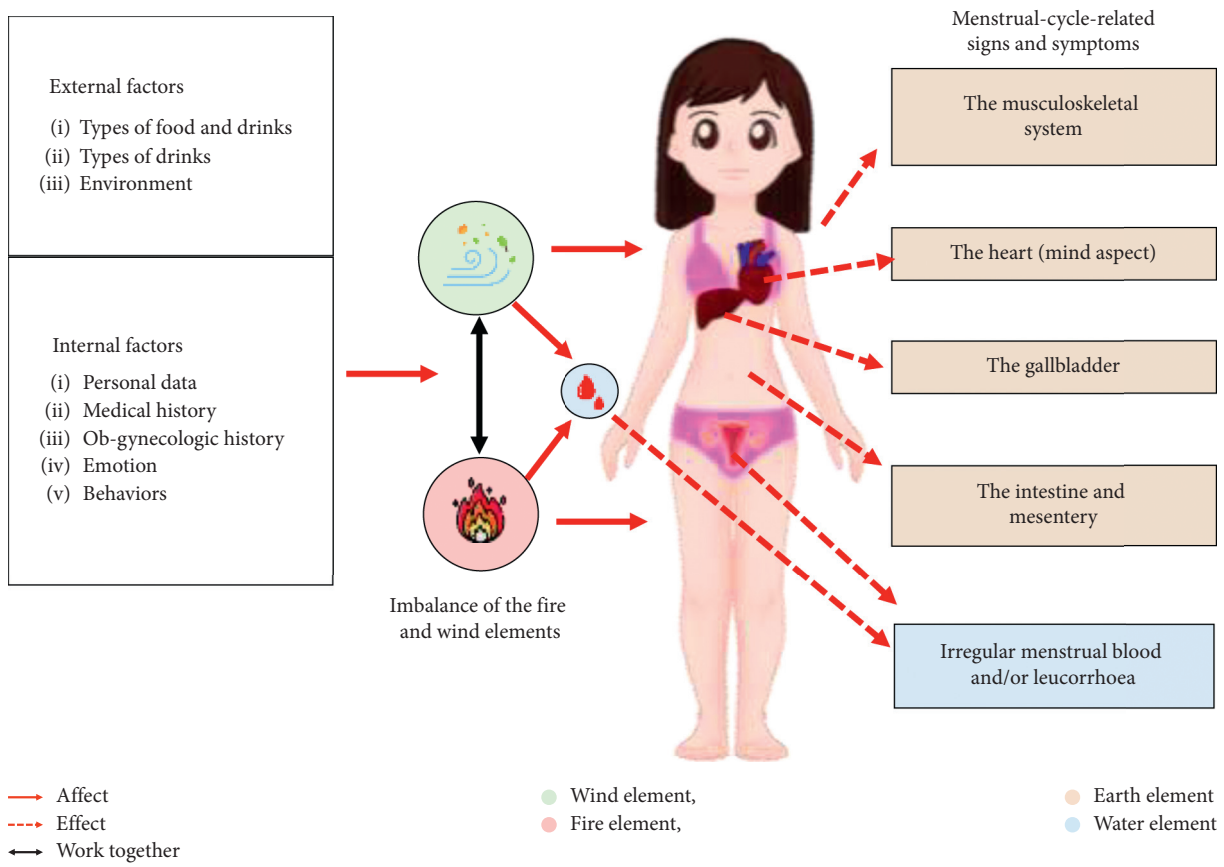

Figure 2: TTM theoretical concept of menstrual-cycle-related signs and symptoms.

In the AF section, only 16 items of the AF in the rating scale part were evaluated using EFA.

This study performed principal component analysis (PCA) to explore the factor structure of the MCSQ. The factor analysis was conducted in each section of the questionnaire. *The MCSs section* (section 1), the value of the Kaiser–Meyer–Olkin (KMO) was 0.89 and Bartlett's test of sphericity ($\chi^2 = 3342.770$, df = 300, p value ≤ 0.001) indicated that the data had adequacy and was appropriate for the analysis model. The AF section (section 2), the data also met the Kaiser–Meyer–Olkin's sample adequacy criteria (0.75, minimum acceptable level 0.60), as well as those for Bartlett's test of sphericity ($\chi^2 = 1154.917$, df = 120, p value ≤ 0.001) for the appropriateness of using the analysis model. Thus, it was

Table 2: Characteristic of the TTM expert ($N = 19$).

Characteristics	N (%)
Gender	
Male	7 (37%)
Female	12 (63%)
Clinical experience	
5–10 years	11 (58%)
11–20 years	5 (26%)
>20 years	3 (16%)
Current position	
TTM clinician	
Operational level	6 (32%)
Experienced level	3 (16%)
Senior level	3 (16%)
Head of Department of Thai Traditional Medicine in university	2 (11%)
University professor	5 (26%)

Table 3: Summary of the third-round questionnaire (Delphi method).

Section 1: Menstrual-cycle-related signs and symptoms (total 32 items)	
Subsection 1.1 Characteristics of menstrual blood and leucorrhoea (15 items)	The signs reflect the problem of the blood in the uterus
3 items: 4.00 > median ≤ 4.50, IQR ≤ 1.50	The irregular menstrual blood flow (3 items)
12 items: median > 4.50, IQR ≤ 1.50	The irregular color of menstrual blood (3 items)
	The irregular texture of menstrual blood (2 items)
	The irregular smell of menstrual blood (2 items)
	Menstrual blood clots (2 items)
	The irregular characteristics of leucorrhea (3 items)
Subsection 1.2 Menstrual cycle-related symptoms (17 items)	The symptoms reflect the problem of the musculoskeletal system (7 items)
7 items: 3.50 > median ≤ 4.00, IQR ≤ 1.50	The symptoms reflect the problem of the intestine and mesentery (4 items)
9 items: 4.00 > median ≤ 4.50, IQR ≤ 1.50	The symptoms reflect the problem of the gallbladder (3 items)
1 item: median > 4.50, IQR ≤ 1.50	The symptoms reflect the problem of heart (mind aspect) (3 items)
Section 2. Associated factors (total 43 items)	
Internal factors (28 items)	Personal data (3 items)
4 items: 3.50 > median ≤ 4.00, IQR ≤ 1.50	Medical history (3 items)
15 items: 4.00 > median ≤ 4.50, IQR ≤ 1.50	Ob-gynecologic history (8 items)
9 item: median > 4.50, IQR ≤ 1.50	The behaviors and health problems (11 items)
	The emotion and feeling (3 items)
External factors (15 items)	
4 items: 3.50 > median ≤ 4.00, IQR ≤ 1.50	The types of food and drink (11 items)
11 items: 4.00 > median ≤ 4.50, IQR ≤ 1.50	The environment (hot and cool) (4 items)

acceptable to adopt a factor analysis to test the construct validity of this questionnaire.

The results of EFA, with PCA as the extraction method, and Varimax, with Kaiser normalization as the rotation method, revealed that the MCSs section consists of 5 components that can explain 51.27% of the total variance, and the AF section consisted of 5 components that can explain 54.21 % of the total variance. Mean and SD of each item, each component; factor loading; commonalities; eigenvalues; and variances of each section are presented in Tables 5 and 6. Section 1: MCSs section, as shown in Table 5, Component 1 was "the problem of blood in the uterus," Component 2 was "the musculoskeletal system," Component 3 was "the heart (mind aspect)," Component 4 was "the digestive system (intestine and mesentery)," and Component 5 was "the irregular menstrual blood."

Section 2: AF section, as shown in Table 6, Component 1 was "the emotion and feeling," Component 2 was "the type of drinks," Component 3 was "the type of food," Component 4 was "the environment," and Component 5 was "the behavior and health problem." The intercorrelation of subscale of both sections is shown in Supplementary Tables 3 and 4.

There were five items that belong to the inappropriate component according to the TTM theoretical conceptual construct and they were removed before calculating Cronbach's α coefficient of each component. Cronbach's α coefficient for each section was 0.87 (the MCSs section) and

Table 4: General information of participants ($n = 429$).

Characteristic	n (%)
Mean age ± SD (years)	26.00 ± 7.27
Mean age of menarche (SD) (years)	11.00 ± 4.20
Marital status	
Single	349 (81.4%)
Married	68 (15.8%)
Widowed/divorced/separated	12 (2.9%)
Education	
Lower than bachelor degree	53 (12.4%)
Bachelor degree	345 (80.4%)
Graduate degree	31 (7.2%)
Menstrual disorder in family	
Yes	57 (13.3%)
No	252 (58.7%)
Unknown	120 (28.0%)
Contraceptive use	
Never used	322 (75.0%)
Used to, but no longer using	75 (17.5%)
Used to and still in use today	32 (7.5%)

0.69 (the AF section). Cronbach's α coefficient of subscale of both sections are given in Tables 5 and 6.

Finally, the MCSQ consisted of 49 items divided into two sections, 35 items in ten components which were rating-scale questions (MCSs section 23 items in five components and AF section 12 items in five components), and 14 multiple-choice questions (AF section). The complete list of items and their components in the final MCSQ are given in Table 7.

3.2.3. The MCSQ Score of Participants. Accordingly, the MCSQ is the screening tool to assess the severity of MCSs, which indicates the problematic organ or system inside the body, and assesses the causes of the disorder. The total score of the MCSs section was the sum of the scores for the 23 items (score range 0–92). The 25th percentile was defined as the lowest score group. The 75th percentile was defined as the highest score group. The mean score of MCSs section was 25.78 ± 12.01, indicating that the participants had the severity of menstrual-cycle-related signs and symptoms at a mild level according to this study's criteria. This study showed that all participants had at least one organ or system which had a problem related to the menstrual cycle. About 99.07% of the participants had menstrual-cycle-related symptoms caused by the musculoskeletal system. The total score of the AF section was the sum of the scores for the 12 items (score range 0–48). The mean score of associated factors section was 21.40 ± 6.24 (the score at a fair level according to this study's criteria). The MCSQ scores of each section and subscale are given in Table 8, and the score interpretation is given in Supplementary Table 5.

In addition, five components of the AF section were tested by the extreme groups of MCSs section using the unpaired Student's t-test. The AF score comparisons between the lowest group and highest group are given in Table 9. The total score in the component "Emotion and feeling," "Types of food," "Environment," "Behavior and health problem," and the total score of AF section had statistically significant differences between the lowest and highest score groups. This study revealed that the total score of the MCSs section has a moderate correlation with the total score of the AF section ($r = 0.41$, p value ≤ 0.001).

4. Discussion

The purpose of this study was to develop and evaluate the initial validity and reliability of the menstrual-cycle-related symptoms questionnaire (MCSQ) based on TTM principles. The MCSQ is a questionnaire for assessing the severity of MCSs and associated factors in reproductive-age women. Moreover, this tool can indicate the problematic organ or system inside the body. Because TTM knowledge regarding assessing the menstrual-cycle-related signs and symptoms is unclear, this study attempted to develop a questionnaire using mixed-method research. This is the first study to develop a MCSQ based on TTM principles for reproductive-age Thai women. The results are supportive of initial evidence of its reliability and validation in the healthy reproductive-age Thai women. This study used items from the interviews and the Delphi method in which the participants were TTM clinicians and experts; information was obtained directly from experienced TTM clinicians and experts, providing a perspective of the clinical experience.

4.1. Thai Traditional-Medicines-Related MCSs. According to TTM principles and tenets, MCSs are the results of dysfunctions or imbalance of the fire and wind elements, and the impairment of blood that affects the function of organs inside the body [10]. The results of the Delphi study revealed that almost all MCSs are mentioned in TTM scriptures, and the expert panel discussed that these MCSs were sufficient as these were common symptoms that should be treated (breast tenderness and abdominal cramps were added because they are common symptoms in the present day). "Fever before or during menstruation" is an important menstrual-cycle-related symptom based on TTM theory; it is associated with the severity of menstrual problems. Moreover, this symptom indicates the dysfunction of the fire element, which can be compared with changes in hormonal levels of estrogen and progesterone that cause imbalances in the body and cause the body to have less immunity to diseases [30]. In principles of both Buddhism and TTM, the heart is a living embodiment of mind, and mind is one of nature that knows emotion. This is the reason why the symptoms of the heart system described in TTM scripture are further related to emotion and psyche than the symptoms of heart disease [31]. According to the knowledge of TTM clinicians, all of the menstrual-cycle-related symptoms often are related to the behaviors or activity of each person—if a woman sits for a long time during a day or works hard, it causes muscle strain and leads to the occurrence of musculoskeletal symptoms during the menstrual period, or if a woman often has a negative emotion (depression, stress, irritability), it shows that the elements in the heart have impairment, which leads to the occurrence of negative emotions during the menstrual

Table 5: Mean, SD, factor loading communalities, eigenvalues, variances, and Cronbach's α coefficient for the menstrual-cycle-related signs and symptoms section.

Menstrual-cycle-related signs and symptoms	Score		Communalities					Components
	Mean	SD	1	2	3	4	5	
The problem of blood in the uterus								
1. Vaginal discharge is an abnormal color with a bad smell	0.52	0.93	0.709					0.534
2. A lot of vaginal discharge and vaginal discharge is eggy, clear, stretchy, or thick white	1.66	1.25	0.660					0.483
3. Before the menstrual period, have vaginal discharge and vaginal itching	0.71	0.96	0.654					0.559
4. Menstrual blood smells stronger than usual or smells like rotten meat	0.55	0.82	0.643					0.528
5. Thick texture menstrual blood	0.99	1.10	0.616					0.480
6. Menstrual blood looks like egg whites	0.90	1.05	0.537					0.375
7. A lot of menstrual blood clots are released every day or are of large size (greater than or equal to 2 centimeters).	0.93	1.01	0.471					0.369
8. Pubic symphysis pain or burning feel in the vagina∗	0.51	0.81	0.419					0.359
The musculoskeletal system								
9. Breast pain	1.68	0.99		0.722				0.557
10. Abdominal cramps	1.85	1.13		0.704				0.622
11. Muscle pain/lower back pain	1.89	1.00		0.695				0.603
12. Hot and cold flushes or fever	0.87	0.88		0.562				0.508
13. Fatigue	1.42	1.06		0.551				0.573
14. Joint pain/bone pain	0.94	1.10		0.419				0.446
The heart (mind aspect)								
15. Anxiety and/or tension	1.05	1.05			0.840			0.783
16. Depression and/or crying	1.28	1.13			0.808			0.769
17. Irritability and/or anger	1.77	1.02			0.591			0.620
18. Insomnia or waking up with a fright	0.77	0.99			0.499			0.438
The digestive system (intestine and mesentery)								
19. Colic in the abdomen or flanks	0.44	0.72				0.669		0.537
20. Loose/watery stools five or six times a day	0.65	0.87				0.626		0.449
21. Abdominal bloating	0.98	1.04				0.601		0.504
Irregular menstrual blood (the impairment of blood)								
22. Light or heavy menstrual bleeding throughout the cycle (using less than 2 pads per day or more than 4 pads per day).	1.26	1.05					0.612	0.458
23. Many colors of menstrual blood in one period	1.72	1.34					0.605	0.456
24. Headache/dizziness∗	1.10	1.06					0.532	0.532
25. Menstrual blood color is pale red, bright red, orange, dark red, dark brown, or black.	0.98	1.02					0.429	0.273
Eigenvalue			3.18	3.05	2.73	1.99	1.83	
% of variance explained			12.71	12.20	10.93	7.98	7.45	
% of variance explained overall					51.27			
Reliability								
Cronbach's α coefficient			0.77	0.80	0.82	0.58	0.45	

∗It was the item that belongs to the wrong component according to the theoretical concept and was removed before calculating Cronbach's α coefficient of each component.

period. Furthermore, the associated factors were the indicators necessary for TTM pattern assessment on menstrual problems. Associated factors consist of the types of food and drink, taste and flavor of food and drink, behavior, health problems, emotion, and environment. All of them can influence menstruation, e.g., strong-flavored foods, ice-beverages, hard work, hotness, coldness, bad emotion, and high stress. These were generally referred to as causes of diseases according to TTM principles [32]. These associated factors will affect the menstrual cycle if they occur regularly.

4.2. MCSQ Development, Validity, and Reliability. This study performed EFA to explore the latent structure of the MCSQ. The factor analysis was conducted separately in each questionnaire's section because each section assesses a different dimension—the signs and symptoms, and the causes of the disorder. Two items of MCSs that were removed because they had high skewness and kurtosis values were "Rash/urticaria/bruise or a burning feeling on the skin" and "Nausea and/or vomiting." The researcher hypothesized that both of these items were not the common symptoms in this study's participants. Construct validity was used to evaluate whether or not the questionnaire corresponded to the theoretical design upon which the questionnaire was based [23]. Results of the analysis showed that the structure of this tool was rather in accordance with the theoretical framework (Figure 2). Some symptoms may appear in different

Table 6: Mean, SD, factor loading communalities, eigenvalues, variances, and Cronbach's α coefficient for the associated factors section.

Associated factors	Score		Components					Communalities
	Mean	SD	1	2	3	4	5	
Emotion and feeling								
1. You are irritable or angry	1.72	1.01	0.848					0.749
2. You are bored, depressed, or in despair	1.42	1.06	0.789					0.662
3. You feel anxious or worried	1.98	1.11	0.789					0.722
Types of drinks								
4. You like to drink ice-beverages or frappe	2.77	1.05		0.778				0.632
5. You like to drink caffeine beverages (e.g., chocolate, tea, carbonated beverage, energy drinks, coffee)	2.09	1.30		0.763				0.634
6. You like to eat fried food, oily food, bakery products, e.g., streaky pork, bacon, cheese, bread, cakes∗	2.25	0.93		0.661				0.515
Types of food								
7. You eat preserved food and/or uncooked food, e.g., fruit preserves, sashimi, and medium to raw meat	1.49	0.93			0.742			0.572
8. You like to eat strong-flavored foods, e.g., extremely spicy, extremely sour	2.32	1.12			0.679			0.632
9. You like to drink alcoholic drinks∗	0.78	0.93			0.578			0.394
Environment (hot and cold)								
10. You sit for a long time during the day (more than 30 minutes/time)∗	2.14	1.17				0.676		0.518
11. In one day, you must enter and exit the area with temperature differences (hot and cold)	2.17	1.21				0.563		0.383
12. You work or stay in a bad environment for a long time a day (in the area too hot or cold/exposed to or inhaling chemicals)	1.20	1.14				0.509		0.372
Behavior and health problem								
13. You have sleep problems, e.g., insomnia, waking up with a fright in the middle of the night	1.54	1.06					0.600	0.603
14. You travel to cities or places with a different climate from your current place of residence∗	0.76	0.80					0.522	0.468
15. You work hard (using a lot of energy or muscle power)	1.27	0.95					0.494	0.451
16. You have constipation	1.45	1.10					0.425	0.364
Eigenvalue			2.39	1.84	1.59	1.55	1.31	
% of variance explained			14.97	11.49	9.92	9.67	8.17	
% of variance explained overall					54.21			
Reliability								
Cronbach's α coefficient			0.81	0.62	0.53	0.38	0.32	

∗It was the item that belongs to the wrong component according to the theoretical concept and was removed before calculating Cronbach's α coefficient of each component.

components, hot and cold flushes/fever, fatigue, and waking up with fright/insomnia as examples, which are the symptoms that reflect a problem of the gallbladder according to the TTM scripture, but it can occur in the musculoskeletal system and the heart too. Accordingly, it might be the reason why "the problem of the gallbladder" component did not happen [10, 33]. Nevertheless, some components and item of each section in the MCSQ were not satisfactory including the following:

(1) The MCSs section: the questions which asked about the "problem of the blood in the uterus" and "irregular menstrual blood" were combined into one answer because each question involved overlap in the irregular characteristics of menstrual blood and leucorrhea.

(2) The AF section: the "types of food" component and the "types of drink" component should be combined into one component because both components have similar underlying concepts, i.e., the taste of food and drink affects the balance of the fire and wind elements, and blood.

(3) From the PCA result, the "headache or dizziness" item and "pubic symphysis pain or burning feeling in the vagina" item should belong to the musculoskeletal system component rather than "irregular menstrual blood" component, or "characteristics of menstrual blood and leucorrhea" component. And, four items of the associated factors, including "eating fried food, oily food, bakery products" item, "drinking alcoholic drinks" item, "sitting for a long time during a day" item, and "travel to cities or places with a different climate from your current place of residence" item, belong to the wrong component. Considering this contradiction, these items were

Table 7: The complete list of items and their components in the final MCSQ.

Section 1. Menstrual-cycle-related signs and symptoms (rating scale)
1.1 The problem of blood in the uterus
1. You have thick texture menstrual blood.
2. Your menstrual blood looks like egg whites.
3. Your menstrual blood smells stronger than usual or smells like rotten meat.
4. You have a lot of menstrual blood clots which are released every day, or large-size clots (greater than or equal to 2 centimeters).
5. You have a lot of vaginal discharge that you need to put on a sanitary pad and your vaginal discharge is eggy, clear, stretchy, or thick white.
6. Your vaginal discharge is an abnormal color with a bad smell (abnormal color: yellow, green, brown or blood).
7. Before the menstrual period, you have vaginal discharge and vaginal itching.
1.2 Irregular menstrual blood
8. You have light or heavy menstrual bleeding throughout the cycle (using less than 2 pads per day or more than 4 pads per day).
9. Your menstrual blood color is pale red, bright red, orange, dark red, dark brown, or black.
10. In one period, you have many colors of menstrual blood.
1.3. The musculoskeletal system
11. Hot and cold flushes or fever
12. Muscle pain/lower back pain
13. Joint pain/bone pain
14. Fatigue
15. Breast pain/tender breasts
16. Abdominal cramps
1.4. The digestive system (intestine and mesentery)
17. Loose/watery stools five or six times a day
18. Colic in the abdomen or flanks
19. Abdominal bloating/abdominal discomfort
1.5 The heart (mind aspect)
20. Waking up with a fright/insomnia
21. Irritability and/or anger
22. Depression and/or crying
23. Anxiety and/or tension
Section 2. Associated factors (rating scale)
2.1. Emotion and feeling
24. You feel anxious or worried.
25. You are irritable or angry.
26. You are bored, depressed, or in despair.
Section 2. Associated factors (rating scale)
2.2. Types of drink
27. You like to drink ice-beverages or frappe.
28. You like to drink caffeine beverages (e.g., chocolate, tea, carbonated beverage, energy drinks, coffee).
2.3. Types of food
29. You like to eat strong-flavored foods, e.g., extremely spicy, extremely sour.
30. You eat preserved food and/or uncooked food, e.g., fruit preserves, sashimi, and medium to raw meat.
2.4. Environment
31. You work or stay in a bad environment for a long time a day (in the area too hot or cold/exposed to or inhaling chemicals).
32. In one day, you must enter and exit the area with temperature differences (hot and cold).
2.5. Behaviors and health problems
33. You work hard (using a lot of energy or muscle power).
34. You have sleep problems, e.g., insomnia, waking up with a fright in the middle of the night.
35. You have constipation.
Section 2. Associated factors (multiple choice)
2.6. Personal data
36. How old are you?
37. Date and time of birth
38. Weight and height
2.7. Medical history
39. What is your health problem or underlying disease?
40. Have you ever had an accident that injured your lower back or lower abdomen?
41. Have you ever had abdominal surgery?

Table 7: Continued.

Section 1. Menstrual-cycle-related signs and symptoms (rating scale)
2.8. Ob-gynecologic history
42. How old were you when your first period start?
43. Have you ever given birth to a child?
44. What postpartum care did you have?
45. Have you ever miscarried?
46. Have you ever had a curettage?
47. Have you ever used hormonal birth control?
48. Has your grandmother or mother had a menstrual disorder history?
49. When did your menstrual symptoms first occur?

Table 8: The final MCSQ scores of each section and subscale of participants ($n = 429$).

Section	No. of items	Total score	Mean ± SD (min, max)	Percentage score	Level ∗ number (percentage)			
					None	Mild	Moderate	Severe
1. Menstrual-cycle-related signs and symptoms section	23	92	25.78 ± 12.01 (3.72)	28.02	0	294 (68.5)	134 (31.2)	1 (0.2)
1.1. The problem of blood in uterus	7	28	6.24 ± 4.62 (0.22)	22.29	31 (7.2)	309 (72.0)	85 (19.8)	4 (0.9)
1.2. Irregular menstrual blood	3	12	3.96 ± 2.37 (0.11)	33.00	36 (8.4)	218 (50.8)	164 (38.2)	11 (0.6)
1.3. The musculoskeletal system	6	24	8.65 ± 4.37 (0.23)	36.04	4 (0.9)	221 (51.5)	180 (42.0)	24 (5.6)
1.4. The digestive system (intestine and mesentery)	3	12	2.07 ± 1.97 (0.10)	17.00	116 (27.0)	262 (61.1)	46 (10.7)	5 (1.2)
1.5. The heart (mind aspect)	4	16	4.86 ± 3.37 (0.16)	30.38	35 (8.2)	230 (53.6)	145 (33.8)	19 (4.4)
					Very good	Good	Fair	Bad
2. Associated factors section	12	48	21.40 ± 6.24 (3.40)	44.58	0	97 (22.6)	317 (73.9)	15 (3.5)
2.1. Emotion and feeling	3	12	5.13 ± 2.71 (0.12)	42.75	18 (4.2)	168 (39.2)	194 (45.2)	49 (11.4)
2.2. Types of drinks	2	8	3.81 ± 1.69 (0.8)	47.63	9 (2.1)	92 (21.4)	257 (59.9)	71 (16.6)
2.3. Types of food	2	8	4.85 ± 2.01 (0.8)	60.63	3 (0.7)	56 (13.1)	199 (46.4)	171 (39.9)
2.4. Environment	2	8	3.37 ± 1.84 (0.8)	42.13	31 (7.2)	110 (25.6)	239 (55.7)	49 (11.4)
2.5. Behaviors and health problems	3	12	4.25 ± 2.03 (0.12)	35.42	10 (2.3)	240 (55.9)	170 (39.6)	9 (2.1)

∗According to the criteria for interpreting results of each section that was set by the researcher.

Table 9: The associated score comparison between the lowest and highest groups.

Subscale	Lowest group		Highest group		t	p value
	n	Mean (SD)	n	Mean (SD)		
Emotion and feeling	117	3.73 (2.36)	113	6.54 (2.70)	−8.43	<0.001
Types of food	117	3.47 (1.55)	113	3.92 (1.82)	−2.02	<0.05
Types of drink	117	4.48 (1.88)	113	5.00 (2.11)	−2.01	NS
Environment	117	3.02 (1.82)	113	3.68 (1.83)	−2.75	<0.05
Behavior and health problem	117	3.55 (2.02)	113	5.39 (1.97)	−7.00	<0.001
Total score of associated factors	117	18.24 (5.98)	113	24.54 (5.93)	−8.02	<0.001

SD = standard deviation, NS = not significant (p value ≥ 0.05).

removed from the questionnaire. However, the removed items in this study should be further modified and could be used for further reflective development in the next study.

The internal consistency was analyzed for reliability within each component. Cronbach's α coefficient of two components in the MCSs section ("the digestive system" component and "irregular menstrual blood" component),

and four components in the AF section (except emotion and feeling component) was relatively low (0.32 to 0.62). This would suggest that the items of each component did not correlate very well overall. The lack of correlation may also be due to each question involving observable symptoms or characteristics, difficulty in answering the question with clarity, or insufficient number of items for each component to determine a clear relationship.

The MCSQ score norm could not be used because our population was not representative of the Thai women in general. Results of the MCSQ score were just a preliminary analysis for determining the score of these participants. This questionnaire needs more complete validation to determine a normal standardized score of the questionnaire. The AF score comparisons between the lowest group and the highest group were evaluated differently between groups. The result showed significant statistical differences in four components, and the lowest group and the highest group had total AF scores with significant statistical differences. The "type of drinks" was, however, not statistically different between groups. The researcher hypothesized that most participants have a mild to moderate level of severity, resulting in the score of this component not being different between groups and the score of some components being only slightly different. Thus, further study of this hypothesis needs to be tested and proven in women with and without the MCSs.

5. Limitations

This study has some limitations as the Delphi technique is a new method for TTM research. The Delphi method is a questionnaire development technique based on purposive sampling; it has the potential risk for bias in expert selection and representativeness [34]. However, the study was designed to set distinct criteria for expert selection to ensure some degree of homogeneity among the experts.

Due to practical limitations, content validity was tested only on a small group of experts, that is, using the recommended lowest number of experts (6 persons) [22], and only one round of expert reviews was performed.

The limitations of the MCSQ in the reliability and validity paragraph test include: (1) using a retrospective self-reported measure where there is a risk of incorrect recall and inflated or deflated answers to reflect the severity of symptoms and frequency of behavior that were associated factors. Participants could possibly forget the exact severity level of the symptom or frequency of the behavior, when required to recall after a long period (six months). (2) The MCSQ consisted of many items and participants had to spend a long time to complete it (about 20 minutes), which could make them bored and potentially not paying close enough attention to their responses. (3) Due to the fact that the MCSQ was designed for reproductive-age women who want to assess their menstruation, the survey was initially conducted with healthy participants who had normal menstruation. Hence, the samples in this study may not be a good representation of the overall population of Thai women, and its specificity to a population who had menstrual problems is unknown and must be tested. Lastly, because this questionnaire is multidimensional and designed according to the TTM theoretical framework, to complete validation, this questionnaire should be assessed for the confirmatory factor analysis (CFA) to test-retest reliability. Moreover, the norm and scoring system should be determined. Further studies should recruit various groups of Thai reproductive-age women, e.g., women who have menstrual problems, to ensure its generalization, and clinical research should be applied and evaluated.

6. Conclusions

The MCSQ is the first menstrual questionnaire that was developed using the TTM principles as the framework. This questionnaire was developed for reproductive-age women. The MCSQ is a self-assessment questionnaire consisting of 49 items divided into two sections, MCSs and associated factors. The questionnaire included 35 items in ten components which were rating-scale questions and 14 multiple-choice questions. The MCSQ shows moderate to high content validity of individual items (I-CVI ranges: 0.83–1.00) and high content validity of the overall questionnaire (S-CVI/AVE = 0.98). The construct validity testing showed the structure of this tool rather was in accordance with the principle of TTM. The internal consistency of each component was moderate to good (alpha value range: 0.32 and 0.82) and this could be further optimized. As a result, this tool needs more extensive validation and rigorous verification before it is used in future research and clinical practices. However, the developed MCSQ will be a useful guideline for clinical treatment and encourages women's personalized self-care for either the reduction of the severity of the MCSs to having normal menstruation and/or the increasing of the effectiveness of personalized therapeutic treatment.

Abbreviations

AF:	Associated factors
CFA:	Confirmatory factor analysis
COPE:	Calendar of premenstrual experiences
CVI:	Content validity index
EFA:	Exploratory factor analysis
I-CVI:	Item-content validity index
MCSQ:	Menstrual-cycle-related symptoms questionnaire
MCSs:	Menstrual-cycle-related signs and symptoms
MDQ:	Menstrual distress questionnaire
MSQ:	Menstrual symptom questionnaire
PSST:	Premenstrual symptoms screening tool
S-CVI:	Scale-content validity index
S-CVI/AVE:	Average scale content validity
TTM:	Thai traditional medicine.

Data Availability

The data came from the questionnaire survey results which can be made available upon request.

Ethical Approval

This study received approval from the Human Research Ethics Committee of Thammasat University, No.1 (Faculty of Medicine) (MTU-EC-ES-1-162/61). The research method adhered to the tenets of informed consent and the Declaration of Helsinki. The expert panel and participants were informed about the objectives of the study and their personal information was protected and would not be revealed to others.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

KS who is a Ph.D. student together with AI designed and conducted the study. AI is the supervisor of KS. AI is Director of the Center of Excellence in Applied Thai Traditional Medicine, the organization that provided funding for this study, and AI is the corresponding author of this work. PS is co-supervisor of KS; she gave advice to and supervised KS on the design and statistical analysis of this study. PW provided advice on research methodology and statistics. WP assisted KS with calculation of data; AI, PS, BO, and NMD were involved in writing, analysis, and revision and approval of the manuscript.

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Supplementary Materials

Table 1 shows median, interquartile range, and percentage of expert agree of each item in the third-round questionnaire (Delphi method). Table 2 shows the content validity index of the first draft MCSQ. Table 3 shows an intercorrelation of the subscale of the menstrual-cycle-related signs and symptoms section (Pearson's r). Table 4 shows an intercorrelation of the subscale of the associated factors section (Pearson's r). Table 5 shows the interpretation of total score and the score of each domain in the final MCSQ. (*Supplementary Materials*)

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Research Article

Evaluation of the Effectiveness of Cinnamon Oil Soft Capsule in Patients with Functional Dyspepsia: A Randomized Double-Blind Placebo-Controlled Clinical Trial

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Background. Different effects of cinnamon and its oil in traditional medicine in the treatment of diseases, including gastrointestinal diseases, were reported. The aim of this study is to evaluate the efficacy and safety of cinnamon oil (*Cinnamomum zeylanicum*) in patients with functional dyspepsia in a double-blind, randomized placebo-controlled trial. *Methods*. Soft gelatin capsule was made using the rotary die process, and the final capsule was standardized based on its cinnamaldehyde amount and analyzed by high-performance liquid chromatography (HPLC) method. Sixty-four patients with symptomatic functional dyspepsia were randomized to receive cinnamon oil soft capsule ($n = 29$) or sesame oil soft capsule as placebo ($n = 35$) for 6 weeks. The primary efficacy variable was the sum score of the patient's gastrointestinal symptom (five-point scale). Secondary variables were the scores of each dyspeptic symptom including severity of vomiting, sickness, nausea, bloating, abdominal cramps, early satiety, acidic eructation/heartburn, loss of appetite, retrosternal discomfort, and epigastric pain/upper abdominal pain, as well as any reported adverse events. *Results*. The results showed that, after 6 weeks of treatment, the cinnamon oil and placebo groups significantly decreased the total dyspepsia score compared to the baseline at the endpoint ($P < 0.001$). However, there was no significant difference between the cinnamon oil and placebo groups in terms of the baseline and endpoint values of the outcome variables ($P = 0.317$ and $P = 0.174$, respectively). Two patients in the cinnamon oil group complained of rashes, and three patients in the placebo group complained of nausea. *Conclusion*. This study showed significant improvements in gastrointestinal symptom score in both treatment and placebo groups. However, there was no significant difference between the cinnamon oil and sesame oil groups in terms of the baseline and endpoint values of the outcome variables. This study was registered as https://clinicaltrials.gov/ct2/show/IRCT20170802035460N2, 29 December 2017, in the Iranian Registry of Clinical Trials with https://www.IRCT.ir.

1. Background

Functional dyspepsia (FD), a common gastroduodenal disorder, is defined by individual symptoms. Symptoms of functional dyspepsia such as postprandial fullness, early satiety, epigastric burning, or epigastric pain are often related to meal, but not to the abdominal pain, and affect one in five people in the community [1–3]. Effective therapies for functional dyspepsia are limited although acid secretion inhibitors, H2 blockers, proton pump inhibitors,

prokinetics, *H. pylori* eradication treatment, antidepressants, psychotherapy, and mirtazapine may provide some symptom relief in clinical practice [1–4]. As there is no satisfactory medication for the treatment of FD, and the control of symptoms is a more realistic end point, more effective therapies should be presented as the main goal of treatment to achieve fewer adverse effects than conventional medications [2, 4]. Traditional medicines in treating functional gastrointestinal disorders are valuable sources for new drug discovery [2].

Commercial cinnamon is the inner bark of the *Cinnamomum zeylanicum* tree belonging to the Lauraceae family. Cinnamon, a fragrant spice plant, is a native of Sri Lanka and is commonly known in the trade as Ceylon cinnamon or Sri Lankan cinnamon [5, 6]. Besides food applications, the *Cinnamomum zeylanicum* has also been used for some health benefits including antimicrobial, antioxidant, anticholesterolemic, antiviral, antidiabetic, antitumor, analgesic, and antigastric ulcer effects [5, 7]. Due to the different effects of cinnamon and its oil in traditional medicine in the treatment of diseases, including gastrointestinal diseases, and since there have been no studies on its beneficial effects on dyspepsia, placebo-controlled trials are clearly needed to substantiate the efficacy of cinnamon oil. For this reason, we aimed to evaluate the efficacy of cinnamon oil (*Cinnamomum zeylanicum*) in the treatment of patients with functional dyspepsia with respect to the intensity of dyspeptic symptoms [8, 9].

2. Methods

2.1. Participants. A double-blind, randomized placebo-controlled clinical trial was conducted between April 2018 and May 2019 at an outpatient special clinic of Kermanshah University of Medical Sciences. A total of 64 patients with symptomatic functional dyspepsia were included in the study, and the allocation ratio was almost 1 : 1. This study was carried out in accordance with the Declaration of Helsinki and the research Ethics Committee of the Kermanshah University of Medical Sciences. Our study adheres to CONSORT guidelines, and the protocol was approved by the Ethics Committee of the Kermanshah University of Medical Sciences, Iran. All subjects were made aware of the content of the study, and written informed consent was obtained from each patient. For inclusion in the study, participants were required to be older than 18 years and younger than 80 years and their diseases are confirmed by complete medical evaluation, as well as patients who sign the testimonials and cooperate during the study and patients who have not been treated with cinnamon oil soft capsule during the last month. Exclusion criteria included the following: patients with inflammatory bowel disease, pure gastro-esophageal reflux, peptic ulcer disease, or irritable bowel syndrome; patients with a history of gastrointestinal system surgery; and pregnant and breast-feeding women.

2.2. Preparation of the Materials

2.2.1. Cinnamon Oil. The dried barks of cinnamon were procured from local herbal market (Isfahan, Iran, 2016) and identified by the botanist of Herbarium Center at Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (*Cinnamomum zeylanicum* Nees, Voucher No. PMP-908).

To prepare the cinnamon oil, according to the instruction of traditional manuscript (Qarabadin-e-kabir: a well-known Persian pharmacopeia), 500 g of plant's bark coarse powder was soaked 40 days in 3.26 L sesame oil (Golkaran Co., Kashan, Iran) in a glass closed vessel and exposed to the sun during extraction. Traditional cinnamon oil was obtained by filtration of supernatant and kept in dark containers.

2.2.2. Cinnamon Oil Soft Capsules. Soft gelatin capsule was made using the rotary die process (Figure 1). In this formulation, traditional cinnamon oil was inserted into soft shell consist of gelatin, water, plasticizers, and preservative. The final capsule was standardized based on its cinnamaldehyde amount (55–75% of cinnamaldehyde as the significant pharmacological component of cinnamon essential oil) and analyzed by high-performance liquid chromatography (HPLC) method.

2.2.3. HPLC Analysis of Cinnamon Oil. The cinnamaldehyde content of cinnamon oil was determined using a Shimadzu 10AD HPLC system (Kyoto, Japan) equipped with a column oven. Data integration was performed using Shimadzu Class-VP software. Separation was achieved using a Waters μBondpak C18 column (4.6 mm × 250 mm) and the column temperature was maintained at 30°C. The mobile phase comprising methanol (A) and 0.05% phosphoric acid (B) at a flow rate of 1.0 ml/min was used to elute the target components with a gradient program (0–5 min, 5%A; 5–10 min, 5%A to 35%A; 10–15 min, 35%A to 60%A; 15–18 min, 60%A to 80%A; 18–22 min, 80%A; 22–25 min, 80%A to 5%A, followed by 5 min equilibration between injections). The sample injection volume was 10 μl and detection wavelength was set at 280 nm. The quantitation was by reference to a standard curve of cinnamaldehyde in sesame oil. Cinnamaldehyde was extracted from standards and samples using methanol in water by solvent extraction.

2.3. Study Design, Assessments, and Treatment. After obtaining informed consent, eligible patients after colonoscopy were assessed at baseline for demographic characteristics (gender, age, height, weight, duration of functional dyspepsia, marital status, smoking, coffee drinker, tea drinker, regular meals, diet, stress, and previous drug-taking). They were then randomized to receive either cinnamon oil soft capsule ($n = 29$) or sesame oil soft capsule as placebo ($n = 35$). Sesame oil was purchased from Golkaran Agro-Industry Co., Kashan, Iran, obtained from Iranian white sesame seeds. Chemical composition analysis of this sesame oil showed that it contains 75 to 80% of liquid fatty acids (oleic and linoleic acids), 15% of solid fatty acids (palmitic, stearic, and arachidic acids), and 1% of lecithin. In this way, one soft capsule was used orally three times a day for a period of 6 weeks. For randomization procedure, we

Figure 1: Cinnamon oil soft capsules.

used a random number table. Odd number was allocated to an intervention group and even number was allocated to the placebo group. The capsule box of both groups looked identical, so in addition to the physicians and researchers, the patients were also blinded to the drug allocation. Figure 2 shows a flow chart of the trial procedure.

To calculate the sample size, we first conducted a pilot study. Six patients who had functional dyspepsia were recruited and randomly assigned in two groups (placebo and cinnamon oil) for 6 weeks. The percent of bloating in two groups was 0% and 34% in cinnamon oil and placebo group. Using equation (1) ($\alpha = 0.05$, $\beta = 0.1$) and taking into account 50% probability of falling in each group, the estimated minimum sample size was 32 and the total sample size was 64.

$$n = \frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2 \left[P_1\left(1 - P_1\right) + P_2\left(1 - P_2\right)\right]}{\left(P_1 - P_2\right)^2} \cong 21. \quad (1)$$

2.4. Statistical Methods. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 16). Primary characteristics (age, height, weight, gender, marital status, duration of having functional dyspepsia, the status of being smoker, coffee drinker, and tea drinker and having regular meals, diet, stress, and previous drug-taking) and outcomes (gastrointestinal symptom score (GIS), as well as number of participants with any observed or reported adverse reaction) were compared between the patients in the cinnamon oil soft capsule and those in the placebo groups using the chi-square and Mann–Whitney tests, respectively. A Wilcoxon signed rank test was used for statistical comparison of values obtained before and after the intervention. All statistical tests were 2-sided, with the significance level set at 0.05.

3. Results

The HPLC analysis displayed that there was 2 ± 0.09 mg/g cinnamaldehyde in each cinnamon capsule. A total of 64 patients with symptomatic functional dyspepsia completed the clinical trial, 29 patients in treatment group and 35 patients in placebo group. The average age of patients was 39.7 years, at least 19 and at most 64 years. The patients in the treatment group included 10 males and 19 females. Also, the patients in the placebo group included 11 males and 24 females. The characteristics of the patients in both groups are listed in Table 1. There were no significant differences in the basic characteristics of the 2 groups ($P > 0.05$).

Patients were evaluated prior to and following 6 weeks in terms of the severity of vomiting, sickness, nausea, bloating, abdominal cramps, early satiety, acidic eructation/heartburn, loss of appetite, retrosternal discomfort, and epigastric pain/upper abdominal pain, as well as number of participants with any observed or reported adverse reaction. Symptom severity was assessed by a valid 5-point Likert scale: none (0), slight (1), moderate (2), severe (3), and very severe (4). The gastrointestinal symptom score (GIS) is used for the outcome measurement as a sum score, with its highest value of 40 points representing the most severe symptom intensity [10]. Based on the results, the GIS sum score showed nearly equal baseline values, with values of 20.72 and 21.94 for cinnamon oil and placebo groups, respectively (Table 2). In both groups, the GIS showed an improvement during 6 weeks (in the cinnamon oil group by 4.52 units, and in the placebo group by 7.19 units). The cinnamon oil and placebo groups significantly decreased the total dyspepsia score compared to the baseline at the endpoint ($P < 0.001$). However, there was no significant difference between the cinnamon oil and placebo groups in terms of the baseline and endpoint values of the outcome variables ($P = 0.317$ and $P = 0.174$, respectively) (Table 2). Moreover, the major symptoms analyzed in comparison of both groups are shown in Table 3. In all cases, the dyspepsia symptom score decrease compared to baseline was numerically greater in the placebo group than in the cinnamon oil group. As can be seen, the nausea score was decreased from baseline in the cinnamon oil group significantly compared to the placebo at the endpoint ($P = 0.012$). However, the decreases of other dyspepsia symptoms scores from baseline in the cinnamon oil group were not significant compared to the placebo at the endpoint ($P > 0.05$) (Table 3).

Two patients in the cinnamon oil group complained of rashes, and three patients in the placebo group complained of nausea. No patient reported any other adverse events during the follow-up period in both groups.

4. Discussion

In this randomized double-blind placebo-controlled clinical trial, the efficacy and safety of cinnamon oil that has been claimed to be effective for treatment of patients with functional dyspepsia were assessed. To our knowledge, this study is the first evaluating cinnamon oil effects in functional dyspepsia. According to the potential pharmaceutical agent proven in several studies, it is possible to say that administration of this plant to the diet possibly attenuates the symptoms of gastrointestinal diseases.

Cinnamon, in addition to being a combination of antioxidants, anti-inflammatory, antimicrobes, antidiabetic, anticancer, hypoglycemic, and cardiovascular-reducing agents, has been reported to have beneficial effects on neurological disorders, including Parkinson's disease and Alzheimer's disease [11].

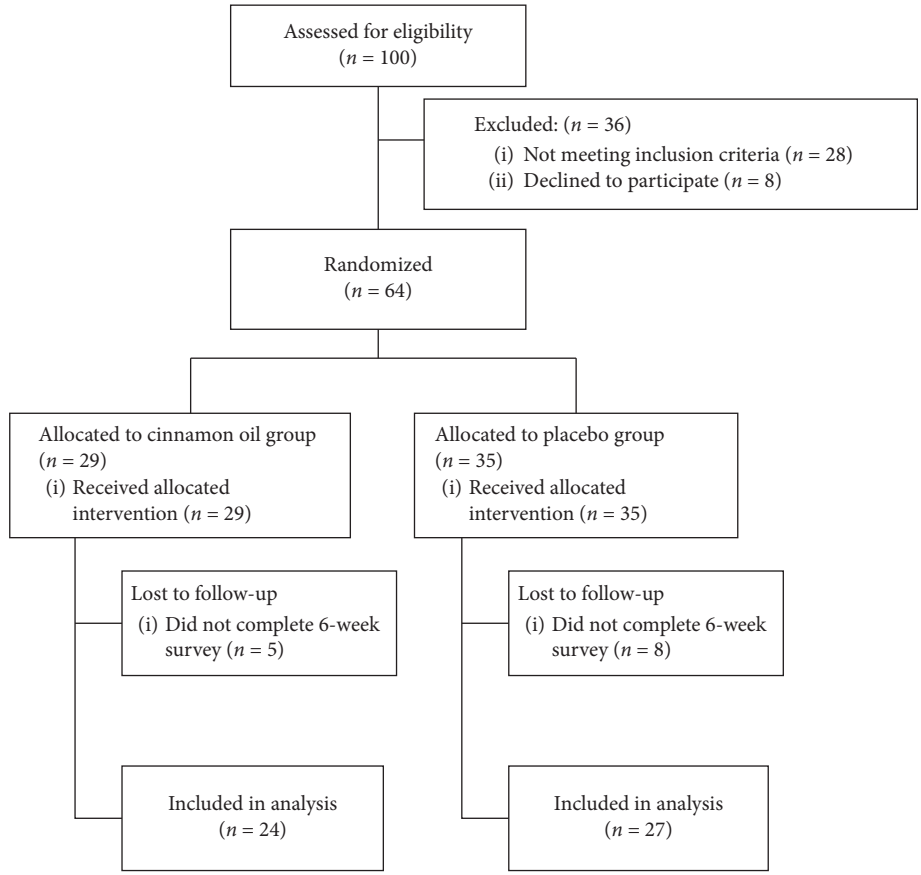

Figure 2: Flow chart of the trial.

The protective effect of cinnamon ethanolic extract against carbon tetrachloride-induced liver injury in rats was investigated in 2012. Administration of ethanolic extract of cinnamon at different concentrations for 28 days can act as a potent hepatoprotective agent in poisoned rats, leading to a marked increase in the levels of catalase and superoxide dismutase enzymes and a decrease in the alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase [12]. Sahu et al. conducted a study to evaluate the skeletal muscle relaxant activity of aqueous extract of *Cinnamomum zeylanicum* compared to metocarbamol as a standard drug in white mice. Cinnamon aqueous extract showed better muscle relaxant effect than standard drug (metocarbamol, 60 mg/kg). This aqueous extract also showed fewer side effects compared to metocarbamol, which had less adverse effects attributed to its antioxidant properties [13]. Im et al. showed that polyphenolic content affects the antidiabetic activity and safety of cinnamon extract. Extracts that had increased 45 and 75% gallic acid equivalents of polyphenol content when administered to diabetic rat (200 mg per kg for 30 days) showed higher hypoglycemic and hypolipidemic effects than standard aqueous extract containing 15% gallic acid equivalents [14]. The results of Bharti et al.'s study showed that the antioxidant activity of cinnamon essential oil was higher than aqueous and alcoholic extracts. On the other hand, collagenase inhibitory activity in aqueous and alcoholic extracts was 25% and 30%, respectively, whereas in cinnamon essential oils it was maximum, 35%. Also, the antibacterial activity of cinnamon essential oil against all tested bacteria was significantly higher than the different cinnamon extracts [15].

Clinical studies on the efficacy of cinnamon oil have been mainly performed on blood pressure, blood glucose, lipid levels, and glycosylated hemoglobin levels, in patients with type 2 diabetes mellitus. The results showed that cinnamon oil is characterized by antidiabetic effects [16–19]. Ulrica von Arnim et al. performed a multicenter placebo-controlled double-blind study with 315 individuals to investigate the efficacy of herbal drug STW5 for patient with functional dyspepsia. Patients received 3×20 drops/day of STW5 or placebo, and a significant improvement was reported after 8 weeks by GIS score [20]. Clinical study of *Mentha pulegium* extract in fifty male and female patients was conducted to reduce symptoms of functional dyspepsia. 330 mg of extract was administered 3 times a day for 2 months in participants. They found that *Mentha pulegium* extract can significantly decrease total dyspepsia score, and some symptoms, including upper abdominal dull ache, bloating, belching, and stomach pain, compared to the placebo. Moreover, no significant change was observed in other symptom scores in the extract group [21].

Table 1: Baseline characteristics at inclusion.

Characteristics	Treatment group *n* (%)	Placebo group *n* (%)	*P* value
Mean age (years)	41.9	37.63	0.2**
Height (cm)	168.12	167.23	0.692**
Weight (kg)	72.21	71.83	0.905**
Duration of functional dyspepsia (months)	64.13	145	0.067***
Gender			
Male	10 (34.5)	11 (31.4)	0.796
Female	19 (65.5)	24 (68.6)	
Marital status			
Single	10 (35.7)	9 (27.3)	0.478
Married	18 (64.3)	24 (72.7)	
Smoking			
No	26 (92.9)	33 (100)	0.207*
Yes	2 (7.1)	0 (0)	
Coffee drinker			
No	26 (89.7)	32 (100)	0.102*
Yes	3 (10.3)	0 (0)	
Tea drinker			
No	3 (10.3)	2 (5.9)	0.654*
Yes	26 (89.7)	32 (94.1)	
Regular meals			
No	10 (35.7)	5 (15.2)	0.063
Yes	18 (64.3)	28 (84.8)	
Diet			
No	27 (96.4)	31 (93.9)	1*
Yes	1 (3.6)	2 (6.1)	
Stress			
No	5 (17.2)	30 (90.9)	0.456*
Yes	24 (82.8)	3 (9.1)	
Previous drug-taking			
No	6 (20.7)	6 (17.6)	0.759
Yes	23 (79.3)	28 (82.4)	

Table 2: The GIS index in the cinnamon oil and placebo groups before and after 6 weeks of intervention. The data are expressed as mean.

	Cinnamon oil	Placebo	Between-groups *P* value
Baseline score	20.72	21.94	0.317
After 6 weeks	16.2	14.75	0.174
Within-group *P* value	<0.001	<0.001	

Table 3: The dyspepsia symptom score decrease compared to baseline in the cinnamon oil and placebo groups. The data are expressed as mean.

	Cinnamon oil	Placebo	Between-groups *P* value
Vomiting	0.0000	0.0000	1
Sickness	0.1667	0.3077	0.733
Nausea	0.0000	0.4231	0.012
Bloating	1.0400	1.3929	0.191
Abdominal cramps	0 .7200	0.8462	0.804
Early satiety	0.1250	0.5000	0.059
Acidic eructation/heartburn	0.1905	0.5000	0.214
Loss of appetite	0.0833	0.4167	0.252
Retrosternal discomfort	0.1250	0.2963	0.481
Epigastric pain/upper abdominal pain	0.4348	0.6154	0.69

5. Conclusion

This study showed significant improvements in gastrointestinal symptom score in both treatment and placebo groups. The cinnamon oil and placebo groups significantly decreased the total dyspepsia score compared to the baseline at the endpoint ($P < 0.001$). However, there was no significant difference between the cinnamon oil and placebo groups in terms of the baseline and endpoint values of the outcome variables ($P = 0.317$ and $P = 0.174$, respectively). Of course, for the condition, functional dyspepsia is subjective symptomatic condition which is heavily affected by placebo.

To our knowledge, this study is the first evaluating cinnamon oil effects in functional dyspepsia. However, the sample size was small and the experimental design was too simple without further analysis, which affects the reliability of this study. In addition, the short duration of patient follow-up was an important limitation of this study. Further studies are required to verify the efficacy and safety of the formula for symptom management on a larger scale, for a longer duration, and with different dosages.

Abbreviations

FD: Functional dyspepsia
HPLC: High-performance liquid chromatography
GIS: Gastrointestinal Symptom Score.

Data Availability

The numerical data used to support the findings of this study are included within the article, and the rough data are available from the corresponding author upon request.

Ethical Approval

This study was approved by the Ethics Committee of the Kermanshah University of Medical Sciences, Iran, and was registered in 29 December 2017 with https://www.IRCT.ir (number: IRCT20170802035460N2).

Consent

All subjects were made aware of the content of the study, and written informed consent was obtained from each patient.

Disclosure

Titles of proposal were accepted in the research committee and ethical committee of KUMS http://research.kums.ac.ir/webdocument/load.action?webdocument_code=1000&masterCode=3008912.

Conflicts of Interest

The authors declare no conflicts of interest.

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Review Article

The Use of Multiple Primary Outcomes in Randomized Controlled Trials of Chinese Herbal Medicine

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Background. Multiple primary outcomes are commonly used in randomized controlled trials (RCTs) of Chinese herbal medicine (CHM). Analysis and interpretation of the results of CHM RCTs with many outcomes are not clear. No previous studies have systematically assessed the use of multiple primary outcomes in this area. This study aimed to assess the reporting of multiple primary outcomes and the statistical methods used to adjust multiplicity in RCTs of CHM. *Methods*. Search for RCTs of CHM published in English between January 2010 and December 2019 in MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL) was undertaken. We randomly selected 20% of the included RCTs as the analyzing sample of this study. The number of multiple primary outcomes, the methods used to adjust the multiplicity in statistical analysis and sample size estimate, and the trial information were collected. For RCTs that adopted multiple primary outcomes without the multiplicity adjustment, we used Bonferroni correction to adjust. *Results*. 227 CHM RCTs were included in our study. 92 (40.5%) failed to report what their primary outcome was. Of 135 (59.5%) RCTs that reported primary outcome, 93 (68.9%) reported one and 42 (31.1%) reported more than one primary outcome (range 2–5). Of 42 RCTs that reported multiple primary outcomes, only 5 adjusted for multiple outcomes. If multiplicity had been accounted for using Bonferroni correction, 10 (37.0%) RCTs that reported a significant result had demonstrated a nonsignificant result, giving the adjusted P value. Only one of the 42 RCTs calculated sample size based on multiple primary outcomes. Adopting multiple primary outcomes showed a slow growth trend with the publication year. The proportion of primary outcome reported explicitly in RCTs was different in terms of the nationality of the first author ($P = 0.004$), in which mainland China has the lowest proportion (55.8%). The highest percentage of the studies with primary outcome reporting explicitation was mental and behavioural disorders (83.3%), and the most frequently adopting multiple primary outcomes were studies on the disease of the nervous system (66.7%). The percentage of reporting primary outcome explicitly was associated with sample size ($P < 0.001$); for the percentage of RCTs adopting multiple primary outcomes, there was no statistically significant difference ($P = 0.739$). *Conclusions*. Multiple primary outcomes are prevalent in CHM RCTs. However, appropriate methods are not usually taken in most of the analyses to safeguard the inferences against multiplicity. Sample size estimation based on multiple primary outcomes is still lacking. These issues complicate the interpretability of trial results and can lead to spurious conclusions. Guidelines to improve analyzing and reporting for multiple primary outcomes in CHM RCTs are warranted.

1. Introduction

Chinese herbal medicine (CHM) alone, or in combination with Western medicine (WM), has been widely used for patients with different diseases in mainland China [1–4]. Since the first randomized controlled trial (RCT) of CHM was published in 1982 [5], RCTs have been widely used to assess the clinical efficacy of CHM [6]. Although ICH Harmonised Tripartite Guideline Statistical Principles Clinical Trials E9 (ICH E9) recommends RCT designed with a single primary outcome [7], the effect of interventions is always multidimensional. A single outcome is insufficient to describe all the effects of an intervention on a complex disease in RCTs. However, multiple health outcomes may need to be investigated to assess all the relevant aspects of the disease. These multiple health outcomes are often correlated, especially for this efficacy on both physical and psychological outcomes. Then, multiple primary outcomes are often incorporated in RCTs due to interest in characterizing how a treatment influences a range of responses [8]. CHM, namely, Chinese herbal formulas, are composed of ingredients chosen to function in combination with each other and are particularly reflective of this practice. In WM, medications are usually prescribed individually for a specific effect. In Chinese herbal formulas, each herb has a different role to help the human body achieve harmony [9]. Therefore, reporting more than one primary outcome in CHM trials may be appropriate because a single measure may not sufficiently characterize the effect of a Chinese herbal formula on a broad set of domains [10, 11]. Multidimensional primary outcomes, which can incorporate the laboratory test, traditional Chinese medicine- (TCM-) diagnosed information (e.g., tongue coat, pulse, face color, and mind), and clinician-concerned and patient-reported outcomes have been proposed [12, 13]. When there is a lack of clear consensus on the most important clinical outcome, combined with the need to examine clinical effectiveness on related outcomes spanning disparate domains, encourage the use of multiple primary outcomes [14].

Normally, researchers often specify an outcome to serve as the primary one, with some other outcomes listed as secondary to adhere to the statistical design principle. While it is common to collect and report multiple primary measures in practice, the appropriate and efficient analysis for multiple primary outcomes is not fully established [14–16]. Choosing an appropriate method for dealing with multiple primary outcomes is important because clinical interpretations can be difficult for those multiple conflicting results.

There are mainly four kinds of approaches accounting for multiple outcomes that have been proposed, assessed, and reviewed [15]. The most common method for analyzing multiple primary outcomes is separate testing of each individual outcome, sometimes with but most often without adjustment for multiple testing [16, 17]. In terms of statistical principle, this method increases the probability of making at least one false significant result, and this could lead to an erroneous conclusion [18]. The second method is controlling the Type I error for multiplicity and the most common technique observed was the Bonferroni adjustment [19]. The third approach involves combining the multiple outcomes into a single (composite) outcome and performing a single test [20]. The fourth method uses global testing using simultaneous (joint) tests [21].

Furthermore, the sample size estimation is an important part of designing RCT. The number of primary outcomes and the correlations among them should be considered when estimating the sample size, which, if optimal, could help to ensure that the trial is efficient, ethical, and cost-effective. For trials with a single primary outcome, the sample size estimation is often univocal. While for trials with multiple primary outcomes, these outcomes and the correlations among them should be prioritized before the sample size estimation [22, 23].

We assessed the prevalence of reporting and adopting multiple primary outcomes in RCTs of CHM. CHM RCTs were chosen because they have a profound social and economic cost and are the focus of a number of prevention and intervention trials. The use of multiple primary outcomes in CHM RCTs is particularly common because efficacy mechanism complexity is multifaceted. Clinicians may be interested in the impact of a CHM on different aspects. For RCTs that reported multiple primary outcomes but without the multiplicity adjustment, we used Bonferroni correction to adjust.

2. Materials and Methods

We conducted the current study, which focused on CHM trials published in English databases from 2010 to 2019. Given the large number of the published studies, we randomly selected 20% of them. We aimed to describe the following: (1) the prevalence of RCTs reported primary outcome, (2) the prevalence of RCTs that adopted multiple primary outcomes, (3) the percentage of multiple adjustment for the multiple primary outcomes in the process of statistical analysis and sample size estimation, and (4) factors distributed in primary outcome reporting explicitation and adopting multiple primary outcomes.

We present the following article in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist.

2.1. Search Strategy. MEDLINE, EMBASE, and CENTRAL (Cochrane Central Register of Controlled Trials) were searched by JH, and only RCTs published in English between 2010 and 2019 were selected. Medline was used to obtain articles that matched "clinical trials" and included the keywords "chinese herbal medicine" or "traditional Chinese medicine". The detailed MEDLINE search strategy is available in Supplementary Materials.

2.2. Eligibility Criteria. RCTs published in English language were selected if they were parallel, crossover, factorial and N-of-1 trials, and studying oral CHM alone or in combination with other interventions, with different preparation forms (e.g., oral liquid, tablet, capsule, pill, granule, and decoction). There is no limitation on diseases. All the

following were excluded: (1) phase I or pharmacokinetics trials, (2) for healthy subjects, (3) self-described preliminary or pilot studies, (4) follow-up or secondary analysis of the original data, and (5) protocols or conference paper.

In addition, RCTs were excluded if the studies focused on nontraditional Chinese herbs; plant extract product is also excluded because it is approved as a nonherbal product by China's Food and Drug Administration (FDA) and it belongs to the same category as WM, which is out of the rules of TCM.

2.3. Selection of Studies. Firstly, we imported 39,116 related records into the reference manager software and built a database. Secondly, we used the random sampling method used in other studies [16, 24, 25] to select target samples for analysis. SAS for Windows (version 9.4; Order Number: 9C1XJD) was used to generate a 20% random sampling number table and 7,824 records were selected. We numbered and sorted the selected records. Thirdly, four reviewers (YXH, XJW, RZ, and CYW), divided into two groups (in pairs), individually and independently screened the titles and abstracts of the selected studies to determine those potentially met the inclusion criteria and 475 related trials were found. Finally, we then obtained the full text of these trials and independently reviewed to find the exact trials that met the inclusion criteria; 227 trials were picked out. Any inconsistency during this process was resolved by discussion with a third party (JH and XL).

2.4. Data Extraction. For each RCT, the results in the abstract and the methods used for sample size estimation and statistical analysis were examined. The numbers of primary outcomes, secondary outcomes, and methods (if any) used to account for multiple primary outcomes were extracted. An outcome was identified as primary if it was explicitly stated in the abstract, methods, results, or tables or if it was clearly implied in the aims of the RCT. We also considered the outcome as primary outcome if it had been explicitly referenced in the sample size estimation. Other outcomes were extracted as secondary outcomes. Side effects and adverse events were not extracted. In addition, publication details (e.g., year, authors, and journal), participants, disease (coded by the International Classification of Disease revision 10 (ICD-10)), interventions, sample size, and sample size estimation were also extracted.

2.5. Data Analysis. Firstly, we performed a descriptive statistical analysis for all the extracted information of the included RCTs. For RCTs that reported multiple primary outcomes but without the multiplicity adjustment, we used Bonferroni correction to adjust, which is based on the probability of obtaining a false positive. It is a method where the significance level is divided by the number of primary outcomes and then compares each single outcome's P value with the adjusted level of a/K rather than a, where K is the total number of primary outcomes.

Factors distributed in primary outcome reporting explicitation and adopting multiple primary outcomes, including countries and sample size, were examined by chi-square test. A P value of 0.05 was used to assess statistical significance. Analyses were performed using SAS for Windows (version 9.4; Order Number: 9C1XJD).

3. Results

3.1. Screening of Included Studies. We identified and selected 227 RCTs of CHM that met the inclusion criteria. Details of the study screening process can be seen in Figure 1.

3.2. Basic Characteristics of Included RCTs. Of the 227 CHM RCTs, 197 (86.8%) were conducted from mainland China, and 193 (85.0%) were designed with two arms, 28 (12.3%) with three arms, and 6 (2.6%) with four arms. The sample size ranged from 12 to 3,143 participants (median: 115, quartile range [IQR] 72–228). Table 1 summarizes the characteristics of these trials.

3.3. Primary Outcomes and Adjustment. The median number of outcomes was 4 (IQR 3 to 6, range 1–14) in 227 CHM RCTs (Figure 2). Of the 227 RCTs, 92 (40.5%) did not clearly specify any primary or secondary outcome, 93 (68.9%) explicitly reported a single primary outcome, 42 (31.1%) reported multiple primary outcomes (in which 24 RCTs had 2 outcomes, 12 had 3 outcomes, 5 had 4 outcomes, and 1 had 5 outcomes).

Of the 42 RCTs with multiple primary outcomes, only 5 (11.9%) had adjusted for multiple primary outcomes, in which three of them used Bonferroni correction and two used Benjamini–Hochberg adjustment. Of the remaining 37 RCTs, ten of them reported "$P < 0.05$" in the full text instead of the actual P value. Then, we used Bonferroni's adjustment to account for the multiplicity in the other 27 RCTs with P value. Of the 27 RCTs, ten (37.0%) that reported an effective intervention would have drawn different conclusions giving the adjusted P value.

3.4. Sample Size Estimation. Sixteen (38.1%) of the 42 trials that reported multiple primary outcomes did not report the process of estimating sample size. Twenty-five of the trials reported sample size estimation based on one outcome. Only one RCT reported sample size estimation that involved more than one primary outcome [26]. This study adopted 3 primary outcomes, 3 sample sizes of these outcomes were estimated with a total significance level of 5% according to Bonferroni correction of the P value ($P < 0.017$), and then the largest value was selected for the final sample size.

3.5. Viewing the Results by Publication Year and Countries. In general, the percentage of primary outcome reported explicitly was increasing by year between 2010 and 2019, from 22.2% in 2010 to 92.0% in 2019. Adopting multiple primary outcomes showed a slow growth trend with the publication year (Figure 3). The proportion of primary

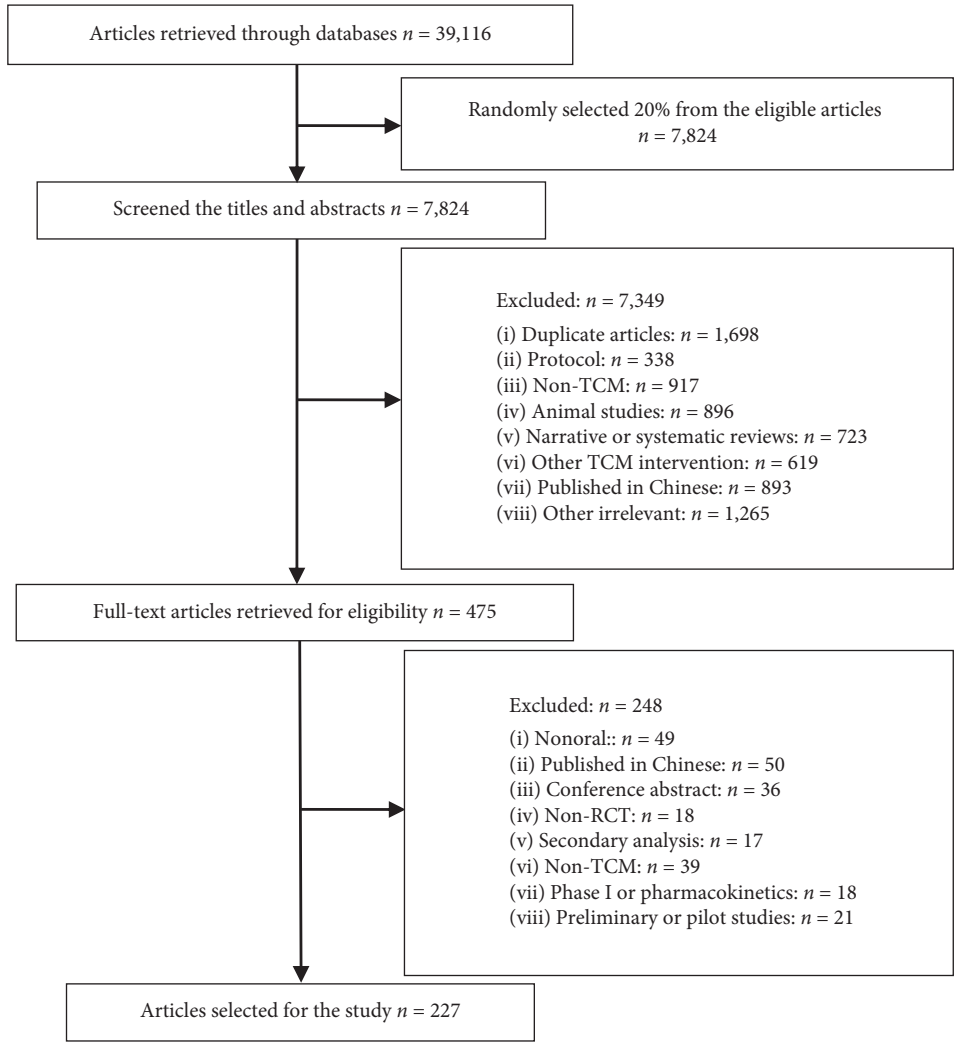

Figure 1: Flow chart of study selection.

Table 1: Summary of the characteristics of included RCTs.

Variable		Number of RCTs ($n = 227$)	Variable		Number of RCTs ($n = 227$)
Year	2010	18 (7.9%)	*Country*	Mainland China	197 (86.8%)
	2011	20 (8.8%)		Hong Kong	11 (4.8%)
	2012	22 (9.7%)		Taiwan	8 (3.5%)
	2013	31 (13.6%)		Singapore	2 (0.9%)
	2014	19 (8.4%)		America	2 (0.9%)
	2015	28 (12.3%)		Australia	2 (0.9%)
	2016	18 (7.9%)		Brazil	1 (0.4%)
	2017	21 (9.2%)		India	1 (0.4%)
	2018	25 (11.0%)		Iran	1 (0.4%)
	2019	25 (11.0%)		Netherlands	1 (0.4%)
Arms per trial	2	193 (85.0%)	*Sample size*	Korea	1 (0.4%)
	3	28 (12.3%)		Range	12–3,143
	6	6 (2.6%)		Median (IQR)	115 (72–228)

outcome reported explicitly in RCTs was different in terms of the nationality of the first author ($P = 0.004$; see Table 2), in which mainland China has the lowest proportion (55.8%).

3.6. Viewing the Results by Disease Area. According to ICD-10 classification, the highest prevalence of the included RCTs focused on circulatory disease ($n = 36$), followed by the genitourinary system ($n = 28$) and digestive system ($n = 27$).

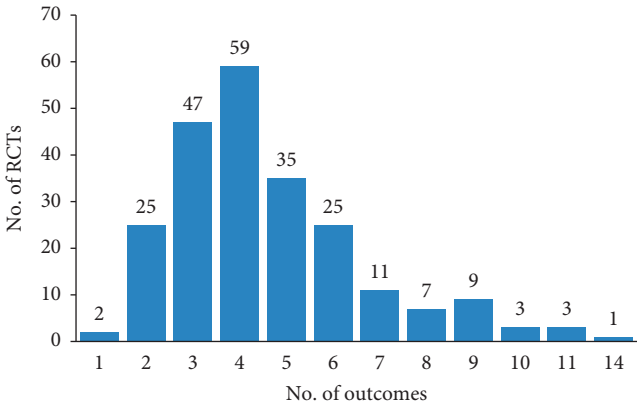

Figure 2: Number of reported outcomes in 227 included RCTs.

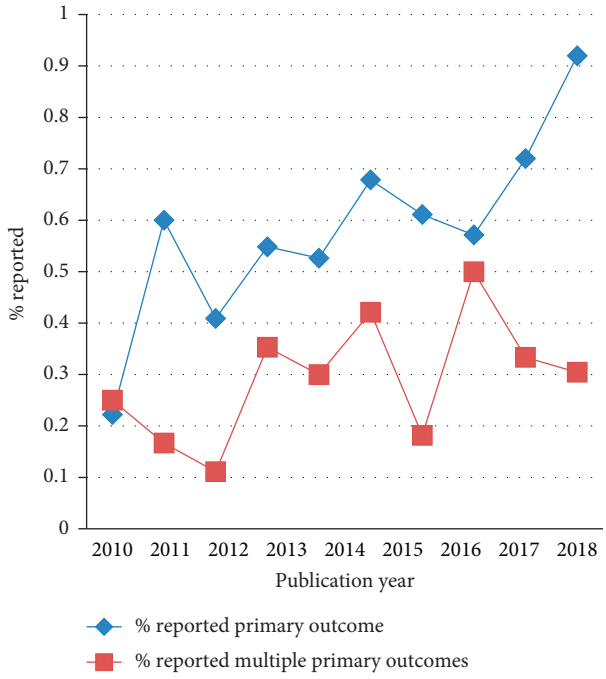

Figure 3: Percentage of RCTs that reported primary outcome and primary multiple outcomes by publication year.

The highest percentage of the studies with primary outcome reporting explicitation was mental and behavioural disorders (83.3%), followed by diseases of the respiratory system (80.9%); diseases of the skin and subcutaneous tissue (75.0%); and symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified (75.0%).

The most frequently adopting multiple primary outcomes were studies on the disease of the nervous system (66.7%), followed by mental and behavioural disorders (60.0%) and certain infectious and parasitic diseases (50.0%; see Table 3).

3.7. Viewing the Results by the Sample Size. Based on the quartiles, the sample size could be divided into three levels of small (sample size <72), medium (72 to 227), and large (≥228). The percentage of reporting primary outcome explicitly was associated with sample size ($P < 0.001$). For the percentage of RCTs adopting multiple primary outcomes, there was no statistically significant difference ($P = 0.739$; see Table 4).

4. Discussion

We randomly selected 227 RCTs published in English between 2010 and 2019 and analyzed the consistency in the reporting and analysis of multiple primary outcomes in CHM. Among the representative, 40.5% did not clearly specify any primary outcome. This suggested the reporting of primary outcome explicitly is relatively low in trials of CHM. Failure to reporting primary outcomes may lead to selective outcome reporting [27]. The International Standards for Clinical Trials Registries established by the World Health Organization has stated that both the primary and secondary outcomes should be defined and prespecified [28]. CONSORT statement also claimed that primary outcomes should be clearly and explicitly stated in all peer-reviewed published RCTs [29]. Our study demonstrated that the specification and explicitation of the primary outcome in clinical trials of CHM need to be improved. Inexplicit primary outcome reporting also has been reported in some previous studies in pediatrics, depression, neurology, and psychiatry research areas [14–17]. The percentage of reporting primary outcome in our study was generally lower than these studies, although the percentage had an upward trend with publication year.

In our study, nearly one-third (31.1%) of included RCTs adopted multiple primary outcomes, while only 5 of these 42 RCTs adjusted for the multiplicity. For the statistical analysis, separate testing of each individual primary outcome, without adjustment for multiple testing, was the most commonly used method to deal with the multiplicity in currently published CHM trials.

A familiar drawback of this approach is the probability of obtaining statistically significant results due to the chance may increase [14, 18]. Practically, what we were concerned about is that it can be falsely concluded that a treatment has significant benefits when the results are actually due to chance, rather than to treatment efficacy (Type I error). When multiple outcomes are analyzed without any adjustments, the Type I error would increase. In our study, for the trials that did not account for multiplicity, we used Bonferroni correction and found that 10 (37.0%) that reported an effective intervention would lead to false positive conclusions. That implied the control of Type I error rate for the multiple primary outcomes is critical.

There are a variety of statistical adjustment methods that can be used to control the Type I error for multiplicity [30]. In particular, the P value-based approaches are the most commonly used. These approaches can be classified into two types: single-step and multistep procedures. The Bonferroni method is a single-step procedure that is usually recommended because of its simplicity and broad applicability [19], even though it was considered to be conservative when the outcomes are positively correlated [31]. Holm procedure is a multistep, step-down procedure [32], while the

Table 2: The primary outcome reporting percentage with the first author's country.

Country of first author	No. of RCTs	No. of RCTs that reported primary outcome	No. of RCTs that adopted multiple primary outcomes
Mainland China	197	100 (55.8%)	37 (33.6%)
Hong Kong	11	10 (90.9%)	1 (10.0%)
Taiwan	8	6 (75.0%)	2 (33.3%)
Other countries	11	9 (81.8%)	2 (22.2%)
Total	227	135 (59.5%)	42 (31.1%)
P value		0.004	0.239

Table 3: Disease classification (ICD-10) of RCTs reporting primary outcomes and multiple primary outcomes.

Disease classification (ICD-10)	No. of RCTs	No. of RCTs that reported primary outcome	No. of RCTs that adopted multiple primary outcomes
Certain infectious and parasitic diseases	8	2 (25.0%)	1 (50.0%)
Neoplasms	19	9 (47.4%)	1 (11.1%)
Endocrine, nutritional, and metabolic diseases	22	14 (63.6%)	5 (35.7%)
Mental and behavioural disorders	12	10 (83.3%)	6 (60.0%)
Diseases of the nervous system	14	6 (42.9%)	4 (66.7%)
Diseases of the circulatory system	36	22 (61.1%)	6 (27.3%)
Diseases of the respiratory system	21	17 (80.9%)	5 (29.4%)
Diseases of the digestive system	27	12 (44.4%)	3 (25.0%)
Diseases of the skin and subcutaneous tissue	8	6 (75.0%)	1 (16.7%)
Diseases of the musculoskeletal system and connective tissue	15	10 (66.7%)	3 (30.0%)
Diseases of the genitourinary system	28	16 (57.1%)	5 (31.2%)
Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified	8	6 (75.0%)	1 (16.7%)
Others	9	5 (55.5%)	1 (20.0%)
Total	227	135 (59.5%)	42 (31.1%)

Table 4: The primary outcome reporting percentage with sample size.

Sample size	No. of RCTs	No. of RCTs that reported primary outcome	No. of RCTs that adopted multiple primary outcomes
Small	52	24 (46.1%)	9 (37.5%)
Medium	118	59 (50.0%)	17 (28.8%)
Large	57	52 (91.2%)	16 (30.8%)
Total	227	135 (59.5%)	42 (31.1%)
P value		0.000	0.739

Hochberg procedure is step-up [33], which are useful for outcomes with any degree of correlation.

Some other statistical analysis methods can also be used to multiple primary endpoints without the need to adjust *P* values. A comprehensive evaluation method can combine the multiple outcomes into a single (composite) outcome, using a variety of pooling rules or scoring algorithms, such as taking a simple average of the outcomes or using conjunctive or compensatory rules, and then test treatment difference on this composite outcome [20]. The global statistical test can provide a univariate test statistic to describe overall benefit and respect the correlated nature of the multiple outcomes instead of multiple statistical tests [21]; this approach is useful to test a treatment's global benefit based on multiple outcomes [34].

Since the holism perspective of TCM, as well as the multidimensional of the reported outcomes (patient-reported, laboratory test, clinician-rated and TCM syndrome outcomes, etc.), it is not practical to identify a single most important outcome as the primary outcome to summarize the effect of CHM [35]. Our previous study also had proposed an efficacy evaluation system with multiple primary outcomes, which is based on the holism benefit of TCM, integrated the primary outcome by three domains: western medicine-specific outcome, TCM syndrome outcome, and quality of life [12].

Determine the sample size that guarantees the prespecified power is an important task in the design phase of clinical trials, and the sample size estimation should be based on the primary outcomes. When a single primary outcome is used, the estimate of sample size has been well studied [36] while when estimating the sample size for trials with multiple primary outcomes, these outcomes and the correlations among them should be considered [15, 22, 23].

For the included studies that adopted multiple primary outcomes, only one RCT estimated sample size based on multiple primary outcomes. Others just used one primary outcome to estimate sample size, while this maybe causes insufficient power to find statistically significant results. The simple and most commonly used adjustment method is using a multiplicity-adjusted significance level within the estimate, estimating for all the primary outcomes, and then selecting the largest sample [37].

In order to help improve practice in this area, we suggest that all CHM RCTs report the following:

(i) The authors should clearly specify a single primary outcome of the trial or multiple primary outcomes along with a strategy to account for multiplicity

(ii) The authors should consider the use of more principled methods to minimize the chance of spurious results due to multiplicity by accounting for multiple primary outcomes

(iii) The authors should report the sample size estimation and use all primary outcomes with a multiplicity-adjusted significance level in the estimation for multiple primary outcomes RCTs

(iv) The authors should specify a limited number of secondary outcomes, along with a justification for their inclusion

(v) The authors should adopt the CONSORT guidelines, the current ICH guidelines, and other related standards or act, which could help improve the timely dissemination and appropriate interpretation of results from clinical trials

5. Strengths and Limitations

To our knowledge, this is the first study to present an overview of multiple primary outcomes adopting and adjustment in CHM RCTs. We chose to assess a random 20% sample as we believe this represents a comprehensive and feasible sample. We focused on studies published in English because those RCTs are believed as having higher methodological quality and more rigorous publication standards than those published in Chinese [38, 39]. Hence, if a significant problem exists in this group, then our findings will likely underestimate the extent of the problem in all CMH RCTs.

This study also has some other limitations. The included studies compromised both confirmatory and exploratory clinical trials, whereas, for explanatory trials, the major objective of which is to frame future research or explore new hypotheses, the multiplicity adjustment consideration is less important. As a comparative effect design, rigorous multiplicity adjustment and Type I error control in exploratory trials may lead to difficulty in achieving the major objectives. Thus, the finding in our study may be potentially exaggerated. Therefore, additional research on a wider scope and specific types of design is needed to furtherly assess the multiplicity adjustment in CHM.

6. Conclusions

From the selected sample of randomized controlled trials on Chinese herbal medicine, this study demonstrated that the primary outcome reporting was generally inexplicit. Multiple primary outcomes were commonly adopted while the multiplicity adjustment was rarely addressed. An appropriate statistical method for analysis and sample size calculation to safeguard the inferences against multiplicity should be used.

Data Availability

The data used to support the study are available from Professor Jing Hu (hujingebm@163.com).

Disclosure

The funders had no role in study design, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Supplementary Materials

PRISMA 2009 checklist. PRISMA 2009 flow diagram. MEDLINE (OVID) search strategy. (*Supplementary Materials*)

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Research Article

A Novel Evaluation System of Psoriasis Curative Effect Based on Bayesian Maximum Entropy Weight Self-Learning and Extended Set Pair Analysis

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Background. Psoriasis is a complex skin disease and difficult to evaluate, and this study aimed to provide an objective and systematic approach for evaluating the efficacy of psoriasis. *Methods*. We sought to construct a Bayesian network from sixteen indicators in four aspects of psoriasis (skin lesion conditions, laboratory indexes, quality of life, and accompanying symptoms) and obtained weights of each index by combining the analytic hierarchy process with maximum entropy self-learning. Furthermore, we adopted stability analysis to calculate the minimum sample size of the system. The extended set pair analysis was utilized to evaluate the efficacy based on improved weights, which overcomes the limitation of set pair analysis (unable to evaluate the efficacy with uncertain grades and thresholds). *Results*. A total of 100 psoriasis vulgaris patients were included to evaluate the curative effect by the system. We obtained the weights of each index and the Euclidean distance for efficacy evaluation of 100 patients. The sensitivity analysis proved that the results had no significant change with the variation of single patient's indexes, which indicated that our results were stable to assess the effectiveness. *Conclusions*. We provided an available method of comprehensive effective evaluation of various indicators of psoriasis and based on both subjective and objective weights.

1. Introduction

Psoriasis is a complex, chronic, immune-mediated inflammatory skin disorder that affects approximately 125 million people worldwide [1]. Moreover, complications are associated with increased exacerbations in subjects with psoriasis, including diabetes, metabolic syndrome, and chronic obstructive pulmonary disease [2–4]. As a refractory systemic disease, psoriasis has a great impact on human health, and some can even be life threatening [5, 6]. Due to the complexity and severity, it is currently difficult to evaluate the efficacy of psoriasis [7]. Although varied evaluation tools and models have been practiced to evaluate the efficacy of diseases, there are still some shortcomings including the inability to integrate multiple indicators to construct an evaluation system and the lack of impartiality in evaluating the weights of various indicators [8–10].

In the Bayesian network, a directed acyclic graph is constructed to intuitively reflect the potential relationship between factors, and a conditional probability distribution table is used to reflect the strength of association [11]. A Bayesian network can reflect the multifactor relationship of psoriasis, so we adopted it to evaluate the efficacy. Meanwhile, the importance of each factor affecting the evaluation of the curative effect is dissimilar, so a reasonable weight needs to be provided [12]. The Analytic Hierarchy Process (AHP) is based on expert experience and was relatively prejudiced [13]. Interestingly, maximum entropy can

identify the probability distribution that is most consistent with the cost function and makes the fewest assumptions [14]. To obtain the comparatively actual weight, we combined AHP with maximum entropy to maximize the entropy of the evaluation network through self-learning [15]. On the basis of the evidence mentioned above, we evaluated the efficacy quantitatively through extended set pair analysis (ESPA) based on multifactorial network and reasonable weights [16].

In the current study, we aimed to develop a comprehensive efficacy evaluation system of psoriasis vulgaris, based on Bayesian maximum entropy self-learning and ESPA. A total of 100 patients were included by this system for efficacy evaluation. It is expected to provide a new approach for curative effectiveness evaluation of psoriasis and other complex diseases.

2. Methods

2.1. Patients. Adult patients (between 18 and 65 years of age) were eligible to participate if they satisfied the condition of both Western and traditional Chinese medicine (TCM) diagnosis standards for psoriasis vulgaris. The trial was performed in accordance with our previous study [17]. The study was approved by the Ethics Committee of the Yueyang Hospital of Integrated Traditional Chinese and Western Medicine (approval no. 2019-028). All participants provided written informed consent before entering the study.

2.2. Case Study Design. Eligible patients received oral TCM herbal medication tailored to the participant's disease progression. Medication was administered twice every day during the intervention phase. After 8 weeks of treatments, clinical efficacy was assessed, and blood samples were collected for all eligible patients. The measurement of laboratory indexes was detected in accordance with our previous study [18], which is described in the supplementary materials.

2.3. Bayesian Network Construction. A Bayesian network consists of a directed acyclic graph (DAG) and a series of conditional probability tables (CPTs). The nodes represent random variables, and edges represent the conditional dependences among nodes in a DAG [19]. In our study, the nodes were the indexes related to psoriasis. The conditional probability of each node was obtained according to the relationship between the indexes. An overview of the study flow is shown in Figure 1.

2.4. Calculation of the Initial Weight (IW) by the AHP. We invited experts to score according to the significance of the indexes (Supplementary Table S1). Then, the IWs of indexes were calculated by the AHP according to the following steps [20]. The scores were regarded by the expert, and the IWs were back in calculation if they failed to pass the consistency test.

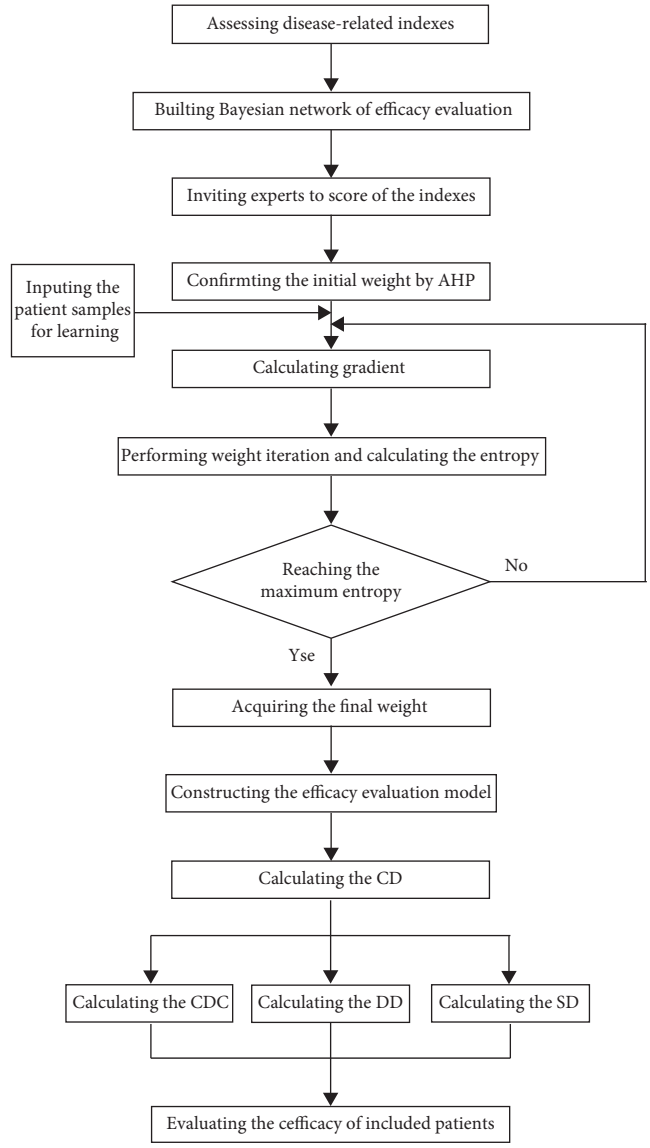

Figure 1: Schematic of the process.

2.5. Calculation of Maximum Entropy and the Final Weight (FW) by Self-Learning. In the process of self-learning, the maximum entropy was taken as the output condition by the gradient descent [21]. The IWs were input into the system to self-learn. Finally, the FW of the indexes was calculated according to the Bayesian network.

2.6. Efficacy Evaluation by ESPA. The efficacy of patients was evaluated by ESPA as described in the previous studies [16]. For the data of included patients in the index k, the greatest and smallest value of the data represented the upper threshold u_k and lower threshold v_k, respectively. The assuming arbitrary value x_{kl} belongs to $[v_k, u_k]$. In this study, if the smaller values mean a better level of the effect, the connection degree (CD) of the patient l with respect to the index k could be calculated by equation (1). Conversely, if the greater values of data mean a lower level of efficacy, then the CD could be calculated by equation (2) [22].

$$\mu_{kl} = \frac{x_{kl}(x_{kl} - v_k)}{u_k(u_k - v_k)} + \frac{2(u_k - x_{kl})(x_{kl} - v_k)}{u_k(u_k - v_k)}\, i + \frac{(u_k - x_{kl})(u_k + v_k - x_{kl})}{u_k(u_k - v_k)}\, j, \tag{1}$$

$$\mu_{kl} = \frac{(u_k - x_{kl})(u_k + v_k - x_{kl})}{u_k(u_k - v_k)} + \frac{2(u_k - x_{kl})(x_{kl} - v_k)}{u_k(u_k - v_k)}\, i + \frac{x_{kl}(x_{kl} - v_k)}{u_k(u_k - v_k)}\, j, \tag{2}$$

where μ_{kl} denotes CD of the patient l with respect to the index k, i indicates the uncertainty coefficient of discrepancy and its value range is $[-1, 1]$, and j connotes the contradictory coefficient with the value defined as -1.

After that, the similarity degree (SD) was proposed to reflect a couple of patients' similarities, and the range of SD was $[0, 1]$. Then, the ideal patient denoted a patient with the optimal response and was availed to make a comparison with the patients who are evaluated for the efficacy. As set pair analysis (SPA) defines [23], the CD of an ideal patient was calculated by the following equation:

$$u^* = 1 + 0 \cdot i + 0 \cdot j, \tag{3}$$

where u^* denotes the CD of an ideal patient.

Moreover, the Euclidean distance (ED) was set to evaluate the SD of each patients, and the specific calculation method was mentioned in Yan's study [16]. The comprehensive evaluation was confirmed, and the weights were also considered in the calculation of the ED [3]. The formula is given as follows:

$$d(\mu_l, u^*) = \left\{ \sum_{k=1}^{m} \left[\omega_k \left(1 - S(\mu_{kl}, u^*)\right) \right] \right\}^{(1/2)}, \tag{4}$$

where $d(\mu_l, u^*)$ denotes the ED between the assessed patient l and the ideal patient, μ_{kl} connotes the CD of the assessed patient l in index k, $S(\mu_{kl}, u^*)$ represents the SD of the CD for patient l and the ideal patient, and index k is the FW of index k.

2.7. Stability and Sensitivity Analysis. The stability analysis was practiced to test the rationality of the sample size. When the sample size changed, the corresponding entropy and self-learning times were determined by the Bayesian maximum entropy self-learning model. The sample size was set from 5 to 100, and the interval was 5. This process was repeated 20 times.

As inputs of some indexes are uncertain, the results were affected by the uncertainties. Consequently, a sensitivity analysis was performed to check the consistency. Each patient was set as the variable sample in turn, and data in each index were set as the variable inputs. Assuming an error of ±10% in the inputs determined, that is to say, the range of input values was between 90% and 110% of the reference values [24]. Herein, the interval of input values was set as 1%. Then, ED with different input values were made by ESPA.

3. Results

3.1. Results of the Index Weights. A total of 100 psoriasis vulgaris patients were included from outpatients of 20 clinical centers. The characteristics of the patients and 16 indicators are shown in Table 1. The details are provided in Supplementary Table S2.

To assess the efficacy of psoriasis vulgaris, we sought to construct the Bayesian network with four layers (Figure 2). The top layer was efficacy evaluation, that is, the target value of the network. The intermediate layers corresponded to the attribute values for the upper layer, and the target values for the next layer. The second layer consisted of four aspects of psoriasis vulgaris, including skin lesion conditions, laboratory indexes, quality of life, and accompanying symptoms. The third layer contained psoriasis area and severity index (PASI), body surface area (BSA) [25], squamous cell carcinoma antigen (SCC-Ag), tumor necrosis factor-α (TNF-α), interleukin (IL) and complement levels [26], Dermatology Life Quality Index (DLQI), Self-Rating Anxiety Scale (SAS) and Self-Rating Depression Scale (SDS) [27], the scales of Xerostomia Questionnaire (XQ), Cleveland Clinic Score (CCS), and Pittsburgh Sleep Quality Index (PSQI) [28–31]. Of these, IL comprised of IL-10, IL-17, IL-22, and IL-23, and the complement involved complement 3 (C3) and complement 4 (C4) [26]. Each node of the bottom layer was the attribute value of assessment, which was as well the outcome measure of this study.

Each abovementioned index contributed variously to the efficacy evaluation. Therefore, we confirmed the IW by the AHP according to expert score (Supplementary Table S3). Then, we performed consistency checks (Supplementary Table S4). The results passed the consistency check, so the weights were available for the efficacy evaluation, whereas the AHP required repeated artificial modification of the judgment matrix, and the evaluation of each index with different experts tended to be various [13]. Therefore, AHP was combined with the Bayesian network and self-learning to obtain more unbiased and accurate results. The Bayesian maximum entropy self-learning weights were obtained when the entropy values were the maximum. Furthermore, the FW was calculated according to the hierarchical relationships of the Bayesian network (Table 2).

To further demonstrate the availability of our method, we compared the weights from two methods (AHP and AHP with maximum entropy self-learning). The results revealed that the weight orderings of recognized indexes of psoriasis (PASI and BSA) were same, which confirmed our method was reliable to an extent. Interestingly, the weight orderings of complement (C3 and C4), quality of life (DLQI, SAS, and SDS), and accompanying symptoms (PSQI, XQ, and CCS) ascended after maximum entropy self-learning, which was consistent with the latest research that psoriasis is a systemic disease [1–4, 7]. Unexpectedly, the weights of IL-17, IL-22,

Table 1: The characteristics of the patients and the indexes.

Characteristics	n or means	Percentages or stds	Minimums	Maximums
Male	59	59%	—	—
Ages, yrs	45.04	10.65	21	65
PASI	7.91	2.96	1.30	17.60
BSA (%)	9.57	2.84	3.00	17.00
SCC-Ag (ng/mL)	20.94	28.78	1.17	143.69
TNF-α (pg/mL)	9.38	4.49	3.70	41.70
C3 (g/L)	0.91	0.20	1.71	0.48
C4 (g/L)	0.47	2.38	0.13	24.00
IL-23 (pg/mL)	912.43	210.94	509.99	1446.99
IL-22 (pg/mL)	5.73	3.96	2.62	27.93
IL-17 (pg/mL)	5.07	1.77	1.93	9.11
IL-10 (pg/mL)	4.77	0.86	2.90	7.90
DLQI	5.58	3.18	0	15.00
SAS	36.75	9.13	25.00	68.75
SDS	32.44	10.10	25.00	80.00
XQ	16.20	9.31	0	43.00
CCS	9.74	4.97	0	21.00
PSQI	9.28	5.67	0	21.00

Stds, standard deviations; PASI, psoriasis area and severity index; BSA, body surface area; SCC-Ag, squamous cell carcinoma antigen; TNF-α, tumor necrosis factor-α; C3, complement 3; C4, complement 4; IL, interleukin; DLQI, Dermatology Life Quality Index; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale; XQ, Xerostomia Questionnaire; CCS, Cleveland Clinic Score; PSQI, Pittsburgh Sleep Quality Index.

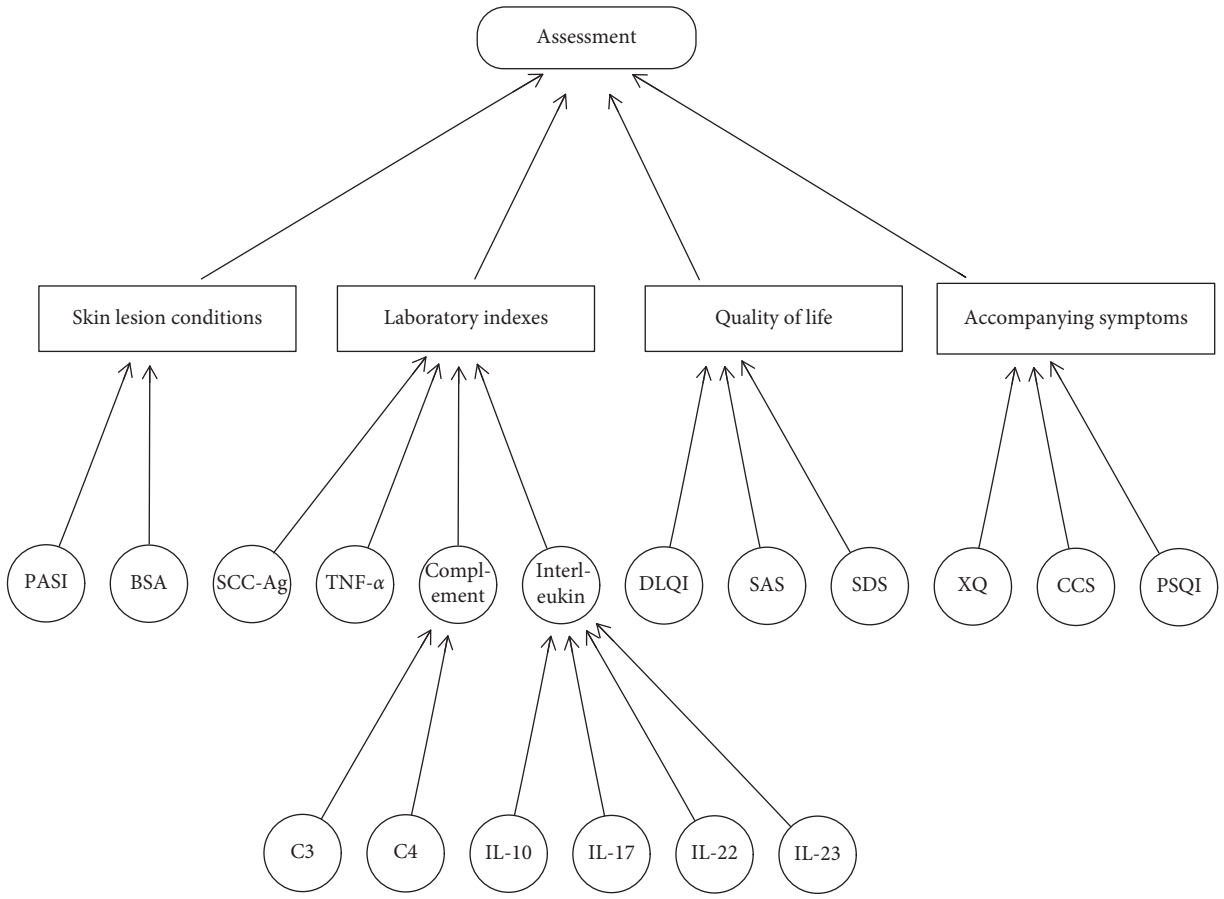

Figure 2: The Bayesian network of efficacy evaluation of psoriasis vulgaris.

and IL-23 were decreased that were correlated to the interaction between the interleukin family, or the specificity of interleukins in psoriasis is actually not high.

3.2. Stability Analysis. Considering the influence of sample size, stability analysis was carried out. The weights and entropy gradually altered and finally tended to be stable in

Table 2: Comparison of the weight by the AHP and FW.

Indexes			Weights by AHP	FW
Skin lesion conditions	PASI		0.3126	0.1362
	BSA		0.1839	0.1182
	SCC-Ag		0.0392	0.0544
	TNF-α		0.0682	0.0545
Laboratory indexes	Complement	C3	0.0119	0.0385
		C4	0.0059	0.0385
	Interleukin	IL-10	0.0134	0.0171
		IL-17	0.0483	0.0171
		IL-22	0.0409	0.0171
		IL-23	0.0380	0.0171
Quality of life	DLQI		0.0290	0.0845
	SAS		0.0209	0.0846
	SDS		0.0330	0.0844
Accompanying symptoms	XQ		0.0593	0.0912
	CCS		0.0274	0.0421
	PSQI		0.0681	0.1047

AHP, analytic hierarchy process; FW, final weight.

the course of self-learning (Figures 3 and 4(a)). It indicated the results are relatively stable when the sample size is 100. On this basis, we explored the relationship among sample size, weights, self-learning times, and entropy values. When sample size input was increased, the entropy altered. In addition, the error bars of the entropy and weights were shorter gradually, indicating that the larger the sample size is, the more stable the entropy and weights are (Figures 4(b) and 5). Besides, the self-learning times showed a linear increase ($r = 0.987$) with the growth of the sample size (Figure 4(c)), revealing that the computing cost was enhanced with increasing sample size. Next, we showed the relations between the three. The vertical error bars and horizontal error bars varied with the numbers of self-learning increasing. With the increase of sample size, the entropy was more stable, and the numbers of self-learning increased, which reminded the three-way interaction (Figure 4(d)). Above, the stability analysis demonstrated that entropy increases from starting levels and becomes gradually stable with the growth of sample size and self-learning times. Approximately 50 patients were needed to make the model stable.

3.3. Results of Efficacy Evaluation. We included 100 patients for efficacy evaluation based on the results of stability analysis.

Table 3 shows means and standard deviations (Stds) of all patients with respect to each index (complete results are in the Supplementary Table S5). The SD reflected the similarities between the patient and the ideal patient. When the value of SD was closer to 1, it indicated that a single index of the patient being tested was more similar to that of an ideal patient.

Aggregating SD of each index to obtain ED: the ED from small to large corresponded to the efficacy from superior to inferior (Table 4). The results showed that the patients with preferable curative effect were L1 (0.3196), L18 (0.3713), L17 (0.3722), L37 (0.3906), and L21 (0.4126), whereas those with unfavorable effect were L100 (0.7904), L90 (0.7749), L61 (0.7656), L82 (0.7469), and L99 (0.7400). Interestingly, ESPA was feasible for efficacy evaluation, overcoming the major limitation of uncertain grades and thresholds.

3.4. Sensitivity Analysis. As the inputs of some indexes of efficacy evaluation are uncertain, efficacy evaluation results were affected by their uncertainties. Thus, a sensitivity analysis was performed to check the consistency of the obtained efficacy evaluation ranking. If the change of a patient's data affected the thresholds of the indexes, the ED of other patients changed accordingly. Sensitivity analysis was performed on 100 patients, where 20 patients are randomly shown in Figure 6. The other patients' ED had no noticeable changes when a patient's indicators were changed. This result illustrated that the ESPA efficacy evaluation model is relatively stable.

4. Discussion

Psoriasis is an immune-mediated chronic inflammatory skin disease with a high incidence. Till now, the question of how to comprehensively evaluate the treatment for psoriasis is a major clinical issue [32]. In this study, we first constructed a novel evaluation system of psoriasis by adopting Bayesian maximum entropy self-learning and ESPA.

The most obvious finding to emerge from the analysis was that PASI and BSA, as the most common evaluation tools of psoriasis, were the core indicators with the highest weights either before or after self-learning, which is consistent with expert knowledge and clinical experience. Guidelines of European Association of Dermatology and venereal Diseases recommended that PASI score and BSA should be the first choice of an objective indicator [33]. Even though the United States guidelines indicated that PASI score is cumbersome, it is still employed as a criterion for assessing the severity of psoriasis patients [34]. One previous study showed that compared with Patients Global

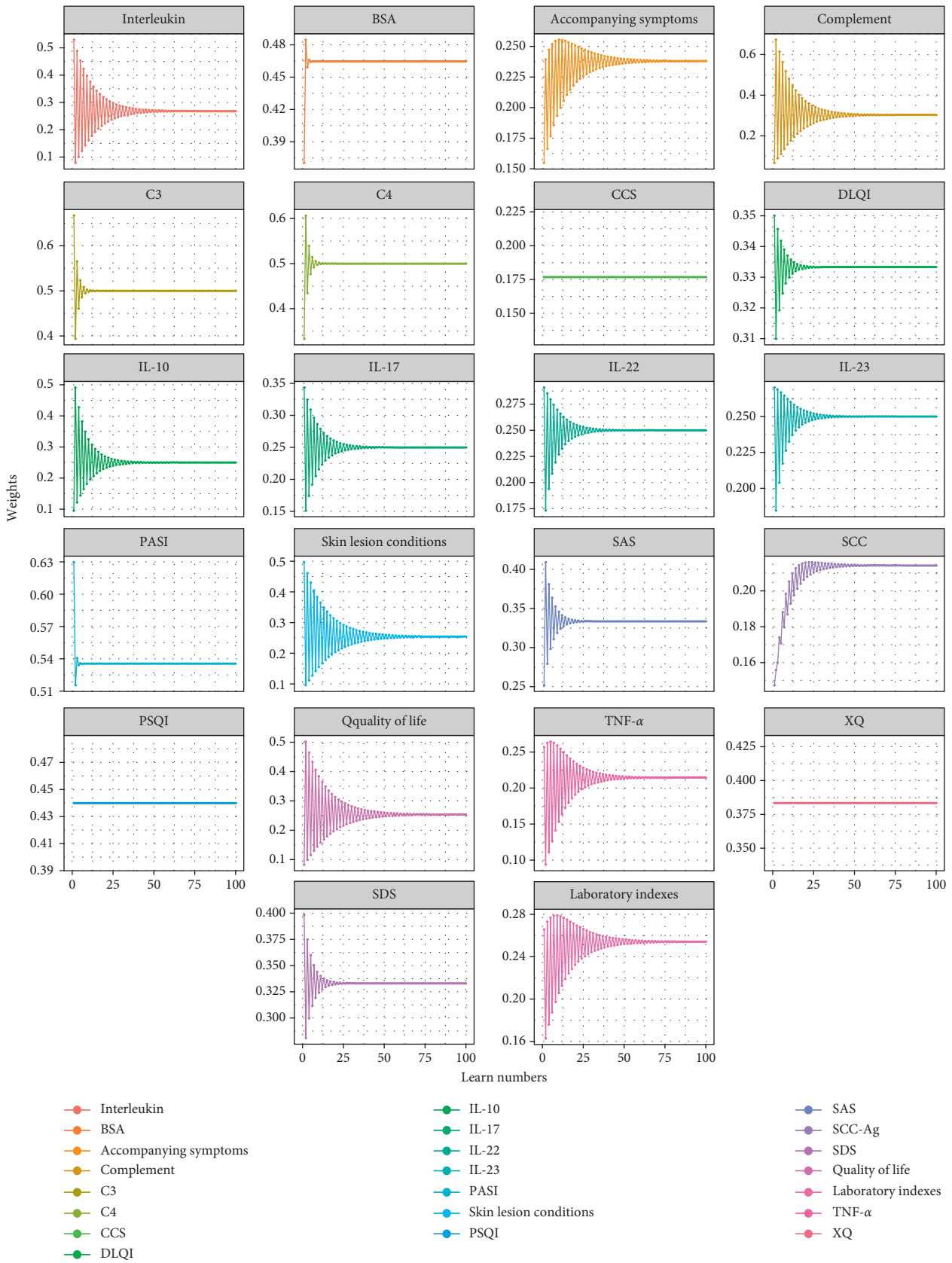

Figure 3: The weights of each index altered in self-learning progress.

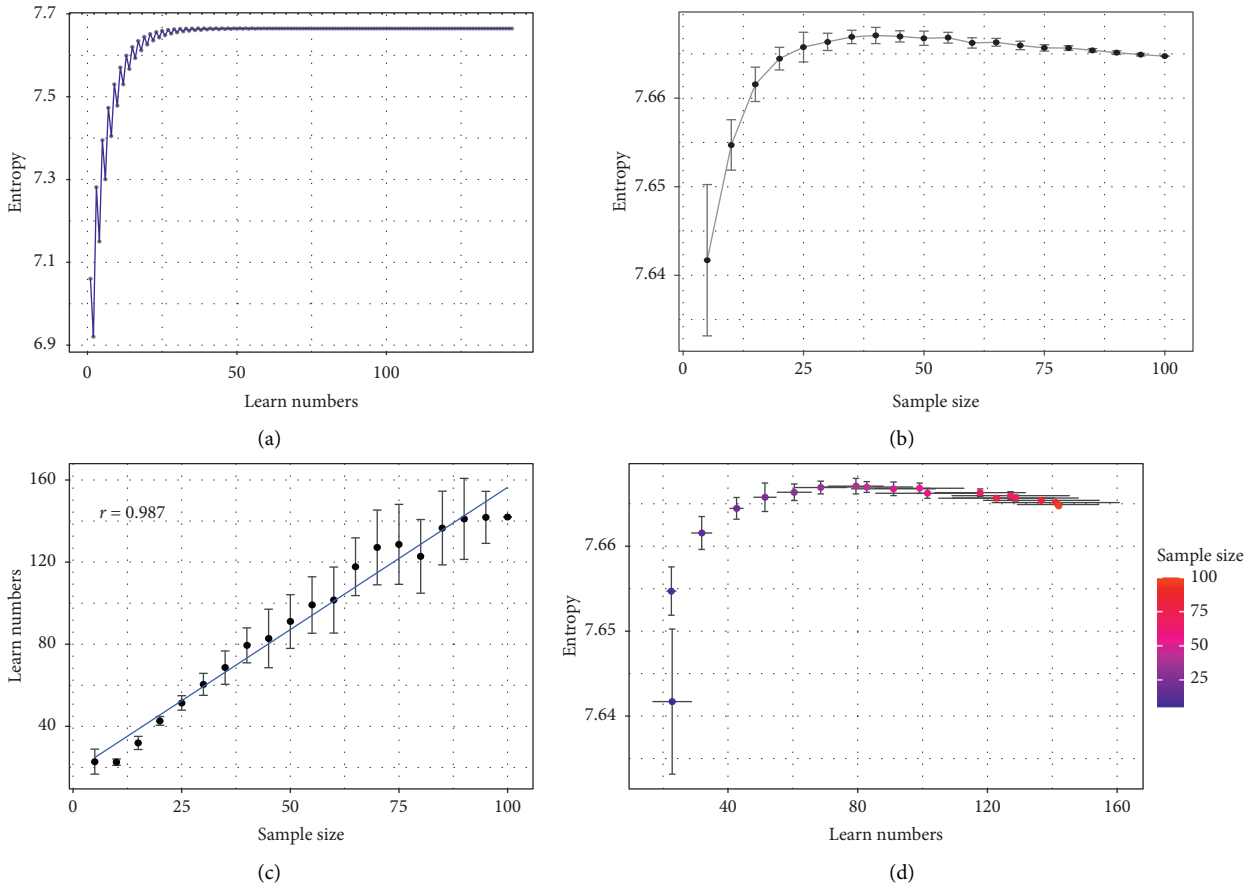

FIGURE 4: The stability and sensitivity analysis results of the evaluation system of the psoriasis curative effect. (a) The entropy was incremental and tended to be stable in increasing with numbers of self-learning when n is 100. (b) The maximum entropy varied when the sample size was from 5 to 100. (c) The self-learning times showed a linear increase ($r = 0.987$) with the growth of the sample size. (d) The relationship among entropy, self-learning times, and sample size.

Assessment, BSA and PASI have higher weights, which is consistent with our result [35].

One interesting finding was that the weights of C3 and C4 increased significantly in the self-learning system. Previous studies showed that the levels of C3 and C4 in patients with psoriasis were significantly higher, and C3 could be considered as a reliable marker of cardiac metabolic risk in psoriasis [36, 37]. Animal experiments have demonstrated that psoriasiform dermatitis was significantly alleviated and a marked reduction in C3 has been observed throughout when the S100A9 gene was deleted, in an imiquimod-induced mouse psoriasis model [38]. These studies indicate that there is a correlation between psoriatic lesions and C3 level, and the complement factor should be monitored as a considerable indicator of evaluation in various therapeutic interventions. Combining the AHP and maximum entropy criterion, we acquired more subjective and accurate results than the AHP on the weights of C3 and C4. However, C3 and C4 have not been integrated into the existing system, and the current study will fill this gap.

Besides, accompanying symptoms and quality of life received higher weights after self-learning. Previous research suggested that DLQI should be recognized as a major indicator and has even assisted in deciding patient-specific treatment strategies [27, 39]. In addition, the current study showed that the weight of SAS is higher than that of the AHP. One study has revealed that the psoriasis patients are more prone to anxiety than normal people, even though there is no obvious correlation between anxiety and the severity of psoriasis lesion, suggesting that doctors should not ignore the anxiety level of patients with mild psoriasis [40].

What is curious about this result is that the weight of interleukin was significantly lower than that by the AHP, and there were few differences among IL-10, IL-17, IL-22, and IL-23. In contrast, these indicators were considered as essential indicators in one of our earlier studies [26]. On the one hand, the reason may be related to the interactions among these indicators, and the evidence of the complex interplay has been proven. Previous studies reported that IL-23 stimulates the process of IL-17 secretion by Th17 cells, while the secretion of IL-22 needs to be encouraged by Th17 [41]. Moreover, enhancing IL-22 could lead to inhibit the production of IL-17 or stimulate the production of IL-10 [42], indicating that there may be a mechanism of interaction among IL-17, IL-23, and IL-22, which needs to be further unraveled. Another possible explanation for this is that it likely relates to specificity of interleukin. The

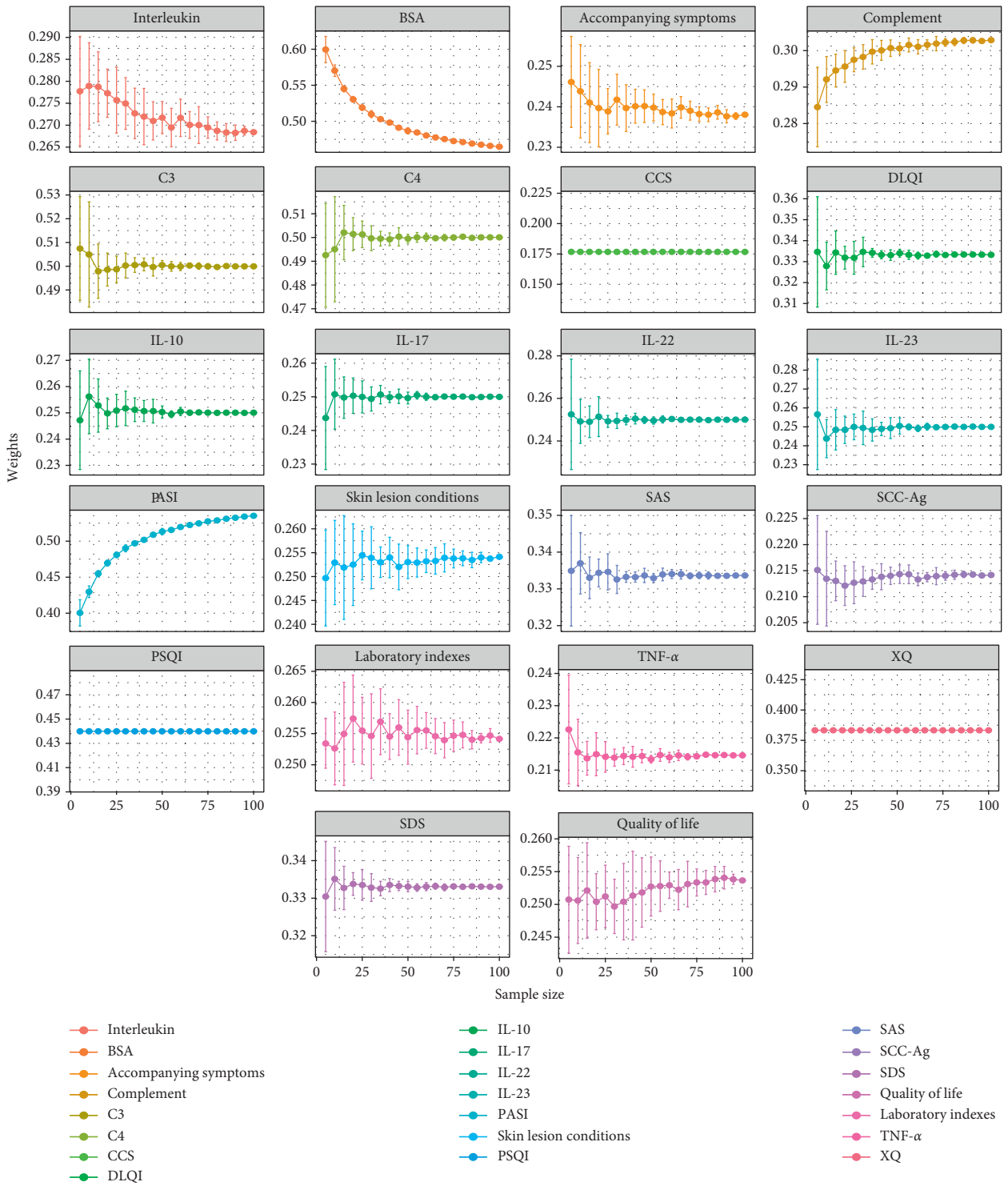

Figure 5: The weight altered when the sample size was from 5 to 100.

Table 3: The characteristics of SD of each index.

Indexes	PASI	BSA	SCC	TNF	C3	C4	IL-23	IL-22	IL-17	IL-10	DLQI	SAS	SDS	XQ	CCS	PSQI
Means	0.5512	0.5085	0.8359	0.8104	0.6274	0.9859	0.5832	0.8511	0.5620	0.4328	0.5792	0.7254	0.8555	0.5761	0.3095	0.5377
Stds	0.1497	0.1503	0.1904	0.1106	0.1336	0.0742	0.1753	0.1517	0.1971	0.1258	0.1929	0.1770	0.1738	0.2003	0.1007	0.2085

SD, similarity degree.

Table 4: The ED of 100 patients.

Serial number	Patients	ED
1	L1	0.3196
2	L18	0.3713
3	L17	0.3722
4	L37	0.3906
5	L21	0.4126
6	L6	0.4328
7	L16	0.4449
8	L14	0.4507
9	L32	0.4563
10	L3	0.4591
11	L10	0.4791
12	L29	0.4813
13	L35	0.4843
14	L26	0.4851
15	L5	0.4872
16	L15	0.4939
17	L38	0.4966
18	L8	0.4974
19	L19	0.5016
20	L62	0.5059
21	L51	0.5070
22	L28	0.5123
23	L31	0.5132
24	L46	0.5152
25	L2	0.5168
26	L57	0.5237
27	L27	0.5249
28	L7	0.5250
29	L50	0.5287
30	L11	0.5290
31	L53	0.5293
32	L23	0.5318
33	L9	0.5348
34	L59	0.5354
35	L25	0.5363
36	L40	0.5411
37	L78	0.5411
38	L41	0.5423
39	L4	0.5452
40	L73	0.5468
41	L64	0.5581
42	L52	0.5611
43	L36	0.5616
44	L42	0.5642
45	L75	0.5765
46	L76	0.5837
47	L44	0.5852
48	L92	0.5905
49	L45	0.5915
50	L33	0.5918
51	L34	0.5921
52	L63	0.5951
53	L22	0.5971
54	L58	0.6017
55	L56	0.6055
56	L72	0.6070
57	L83	0.6080
58	L66	0.6082
59	L71	0.6091
60	L97	0.6136
61	L20	0.6148
62	L77	0.6192
63	L55	0.6212
64	L49	0.6218
65	L86	0.6225
66	L30	0.6231
67	L70	0.6242
68	L95	0.6297
69	L87	0.6385
70	L47	0.6454
71	L13	0.6520
72	L74	0.6609
73	L80	0.6618
74	L88	0.6664
75	L65	0.6691
76	L94	0.6696
77	L91	0.6725
78	L96	0.6753
79	L39	0.6772
80	L81	0.6785
81	L69	0.6801
82	L79	0.6807
83	L68	0.6823
84	L24	0.6829
85	L67	0.6846
86	L54	0.6895
87	L89	0.6953
88	L12	0.7016
89	L48	0.7036
90	L60	0.7108
91	L43	0.7213
92	L85	0.7264
93	L84	0.7330
94	L94	0.6696
95	L95	0.6297
96	L96	0.6753
97	L97	0.6136
98	L98	0.7360
99	L99	0.7400
100	L100	0.7904

ED, Euclidean distance.

occurrence and progression of psoriasis is the result of the combined force of a series of inflammatory cytokines and signaling pathways, including IL-6, IL-17, IL-23, IL-27, TNF-α, and INF-γ, but only IL-17 is the core mediator directly involved in the inflammation and disease progression of psoriasis. However, it has been proved that the IL-17 family has been found to play a causative role in tumor progression and autoimmune disease [43]. Therefore, this phenomenon may be correlated with the complex downstream signaling pathways resulting in less specificity.

In order to guarantee the accuracy of unsupervised self-learning, we verified the stability and sensitivity of the system. The large sample sizes increased statistical power that makes the results reliable. With the increase of the sample size, the entropy values were gradually stable, and the numbers of self-learning were also gradually increasing.

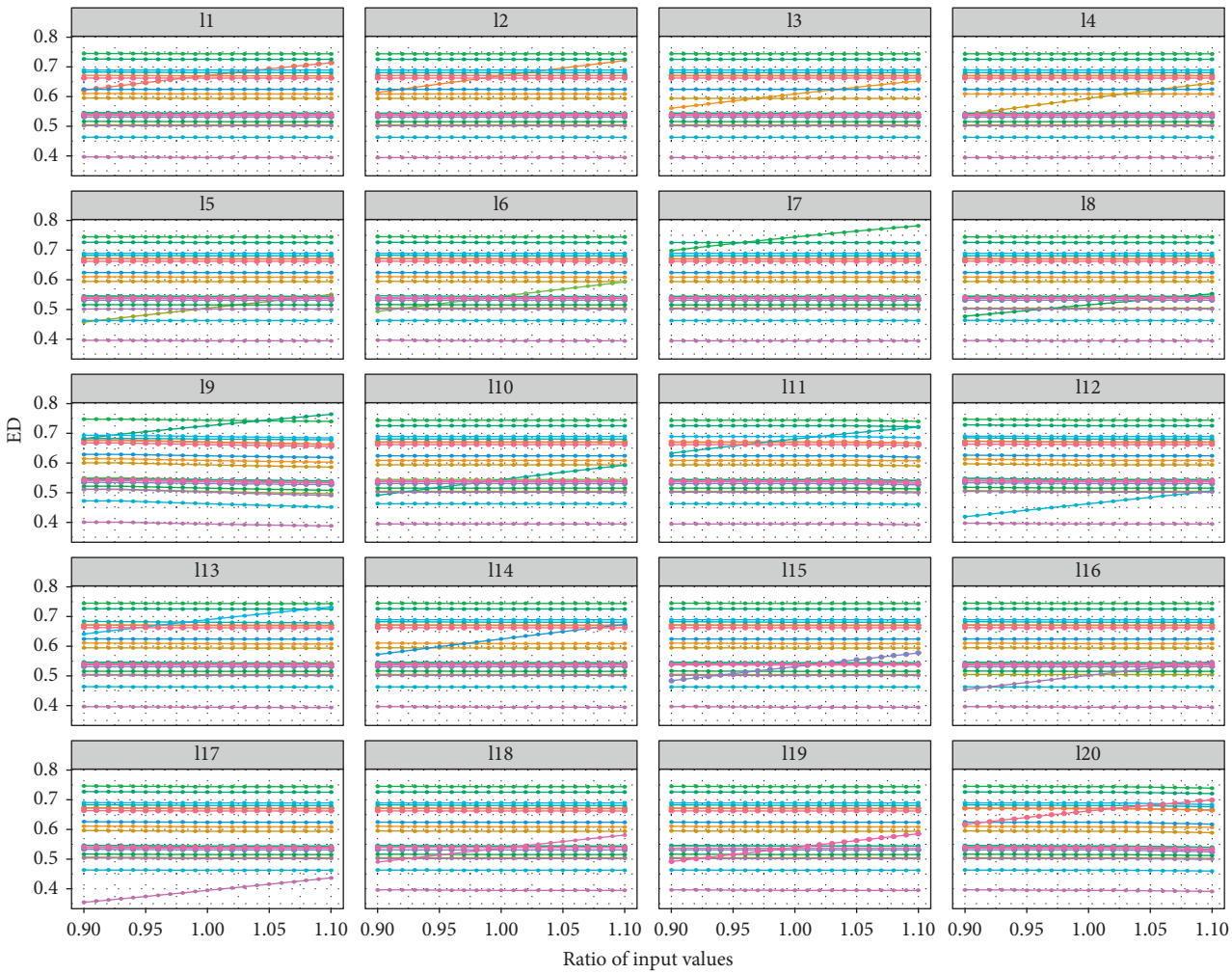

Figure 6: The other patients' ED changed when the input data of a patient were modified.

Moreover, we conducted sensitivity analysis arbitrarily changing the clinical data of patients one by one and found that EPSA results are almost unaffected by the sample size, which indicated that our results were stable.

Compared with the AHP, the present system contains the following characteristic and advantages. The main strength of this study is that we applied a Bayesian network to construct a comprehensive measurement of treatment effectiveness among a variety of symptom indicators. Secondly, the judgment matrix needs to repeatedly modify in the weight's calculation process by the AHP, and the scores assessed by a variety of experts have considerable variation by subjective factors. Therefore, on the basis of the known weights and self-learning method, the optimal weights were output according to the maximum entropy criterion, which has higher objectivity [44]. In addition, SPA or the set pair cloud model all relied on hierarchical classification so that the symptom grades and thresholds were needed during calculation of the CD. In the study, ESPA was an improvement over the SPA, which is applicable at uncertain grades and thresholds.

Despite these promising results, limitations remain. Firstly, the current study only included patients with psoriasis vulgaris, so that one should be cautious when generalizing to other types of psoriasis and the indicators may need to be adjusted. Secondly, a larger sample for model testing may be needed before practical application. Thirdly, considering the large numbers of indicators for psoriasis, other indicators could be included to evaluate the efficacy of psoriasis.

5. Conclusions

Given diversified indicators of psoriasis, we proposed a system that considers multiple indicators and uses more objective weights to evaluate the efficacy, while overcoming the application limitations of SPA. In addition, 100 patients were included to confirm the stability and effectiveness of the system through stability analysis and sensitivity analysis. Theoretically, the system can be applied to other diseases, which requires further research for clarification.

Abbreviations

AHP: Analytic hierarchy process
ESPA: Extended set pair analysis

TCM: Traditional Chinese medicine
DAG: Directed acyclic graph
CPTs: Probability tables
IW: Initial weight
FW: Final weight
CD: Connection degree
SD: Similarity degree
SPA: Set pair analysis
ED: Euclidean distance
PASI: Psoriasis area and severity index
BSA: Body surface area
SCC-Ag: Squamous cell carcinoma antigen
TNF-a: Tumor necrosis factor-α
IL: Interleukin
DLQI: Dermatology Life Quality Index
SAS: Self-Rating Anxiety Scale
SDS: Self-Rating Depression Scale
XQ: Xerostomia Questionnaire
CCS: Cleveland Clinic Score
PSQI: Pittsburgh Sleep Quality Index
C3/4: Complement 3/4
Stds: Standard deviations.

Data Availability

All of the data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

The data from preclinical studies [17] have been approved by the Ethics Committee of the Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, protocol 2019-028.

Consent

All clinical study participants provided written informed consent before the study.

Disclosure

Le Kuai, Xiao-ya Fei, and Jing-si Jiang are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Dr and Bin Li had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Le Kuai and Xiao-ya Fei conceptualized the study;Xin Li curated data; Le Kuai and Xin Li conducted formal analysis; Bin Li, Le Kuai, and Xin Li acquired funding; Jing-si Jiang, Ying Zhang, Ying Luo, and Yi Ru conducted investigation; Jing-si Jiang and Yi Ru formulated the methodology; Wei Li and Jian-kun Song were involved in project administration; Wei Li, Jian-kun Song, and Shuang-yi Yin obtained resources; Xiao-ya Fei and Shuang-yi Yin were responsible for software; Jing-si Jiang, Shuang-yi Yin, Jian-kun Song, and Bin Li supervised the work; Xin Li and Ying Luo performed validation; Shuang-yi Yin performed visualization; Xiao-ya Fei, Jing-si Jiang, and Ying Zhang prepared the original draft; Le Kuai, Xiao-ya Fei, Jing-si Jiang, Xin Li, Ying Zhang, Ying Luo, Yi Ru, Jian-kun Song, Wei Li, Shuang-yi Yin, and Bin Li reviewed and edited the manuscript.

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Supplementary Materials

Supplementary Table S1: experts' judgment on importance of each index. Supplementary Table S2: the data of each index of 100 patients. Supplementary Table S3: expert scores of the indexes. Supplementary Table S4: results of the weights calculated by the AHP and the consistency test. Supplementary Table S5: the SD of 100 patients. (*Supplementary Materials*)

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Review Article

Yanyu Decoction for Aged Patients with Stable Coronary Artery Disease: A Systematic Review and Meta-Analysis

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Background. There was limited evidence of treatments aiming at aged coronary artery disease (CAD) patients. Yanyu decoction (YD) has been used as adjuvant therapy in aged patients with stable CAD and might be a new treatment worthy of recommendation for CAD patients. This study was to evaluate the combined effects of YD plus conventional pharmaceutical treatment (CPT) on senile patients with stable CAD. *Methods*. This review was designed according to the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analysis) recommendations. A literature search was conducted in seven electronic databases from their inception until August 2020. Primary outcomes of interest were adverse cardiovascular events, including cardiac mortality, acute myocardial infarction (AMI), and unstable angina (UA). The secondary outcomes were blood lipids and hemorheology. Studies were pooled to calculate the risk ratio or weighted mean difference and corresponding 95% confidence interval. *Results*. Five studies recruiting 848 aged patients with stable CAD were included. Patients receiving YD as an adjuvant have fewer adverse cardiovascular events, including cardiac mortality, AMI, and UA. Besides, YD plus CPT has a better effect on reducing triglycerides, low-density lipoprotein cholesterol, and improving high-density lipoprotein cholesterol. Moreover, significant effects of YD plus CPT for reducing blood viscosity, plasma viscosity, and platelet aggregation rate were found compared with CPT alone. *Conclusion*. YD plus CPT showed better efficacy than CPT on reducing adverse cardiovascular events and improving hemorheology and blood lipids for aged patients with stable CAD. Our findings may suggest YD as an adjuvant natural-based treatment for CAD. However, more rigorous and larger trials are essential to validate our results, and further consideration of CAD studies specific to aged patients is needed.

1. Introduction

Coronary artery disease (CAD), recognized as a global health threat, is a chronic multifactorial disease and accounts for a high proportion of mortality in the world [1], resulting in 9.48 million deaths in 2016 [2]. Age is the strongest factor related to the development of CAD [3]. The number of people over 60 years old will surpass that of children below 5 years of age by 2020 [4]. With the aging of the global population, better strategies to treat patients of advanced age with CAD are essential. Although stable CAD is not always with an unfavorable prognosis, aged patients with stable CAD are the vulnerable population [5]. Unfavorable prognoses, including cardiac mortality, acute myocardial infarction (AMI), and unstable angina (UA), are not uncommon in aged patients with stable CAD, since aged patients with diminished physical function suffered from a combined CAD-related and age-related effect [6, 7]. Due to an increase in age, hemorheological deterioration emerges in response to aging and decline in organs or tissues [8]. Aged CAD patients usually have hyperviscosity and hyperlipemia and concentrated, aggregating, and adhesive blood, which could induce the anomaly in the vascular morphology and hemorheology in the microenvironment, thus affecting the progression and prognosis of CAD [7, 8].

Recent studies have confirmed the interaction between geriatric diseases and hyperviscosity [8] and demonstrated

that blood viscosity, platelets, and hypercoagulability were related to high neutrophil levels and may increase the severity of CAD [9]. Rheological properties of blood were closely related to serum lipid levels [10]. Research has also shown that total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) were major factors contributing to CAD, while the high-density lipoprotein cholesterol (HDL-C) acted as a major protecting factor in CAD (Cullen et al., 1998). Aged CAD patients were identified with an anomaly in levels of TC, TG, and LDL-C. Treating aged CAD focusing on blood lipids and hemorheology is of paramount importance for public health [8].

Herbal dietary supplements continue to be accepted as an adjuvant medical option in multiple countries on CAD treatments [11]. Yanyu decoction (Yanyu Heji, YD), a formula of traditional Chinese medicine, composed of 60 grams of Stigma Maydis (*Zea mays* L.), 30 grams of Corydalis Rhizoma (*Corydalis* yanhusuo W. T. Wang), 15 grams of Fructus Trichosanthis (*Trichmanthes kiriloxvii* Maxim.), and 15 grams of Allium Macrostemon *(Allium macrostemon* Bge.), has been used in treating aged CAD patients recently. Maydis stigma, the yellowish thread from the stigmas of corn fruits, was the principal herb in YD and has been reported to contain K^+, Ca^{2+}, Mg^{2+}, and Na^+ salts, steroids, alkaloids, polyphenols, tannins, and flavonoids, with certain antioxidant abilities [12]. Some alkaloid compounds in Corydalis Rhizoma have been demonstrated to be the active constituent in the treatment of CAD and exert an antiplatelet aggregation effect [13]. Fructus Trichosanthis and Allium Macrostemon have been proven beneficial for curing CAD as a herb pair [14]. Currently, accumulating clinical evidence confirmed that YD was effective in treating stable CAD in aged patients [15–19] and revealed that YD plus conventional pharmaceutical treatment (CPT) could treat stable CAD with less adverse cardiovascular events and better effect on improving blood lipids and hemorheology.

Nevertheless, there has been no systematic review and meta-analysis available to provide evidence-based guidelines for aged CAD patients in clinical practice. Aged patients with stable CAD were underrepresented in ischemic heart disease trials because they have often been excluded from CAD trials due to higher risk, and many recommendations have been extrapolated from evidence obtained in younger cohorts [20]. Knowledge gaps in CAD have been identified, and efforts are to be paid to fill the gap.

2. Methods

2.1. Search Strategy. This review was conducted according to the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analysis) recommendations [21]. We carried out a systematic literature search of PubMed, Cochrane Library, Web of Science, Scopus, Chongqing VIP Chinese Science and Technology Periodical Database, China National Knowledge Infrastructure Database, and Wanfang database for relevant publications from the start date of the databases until August 31, 2020. Two reviewers independently searched the studies. Any differences were resolved through discussion with another author. The following keywords were used: "Coronary Artery Disease" OR "coronary heart disease" OR "Coronary Arteriosclerosis" OR "Coronary Arterioscleroses" OR "Coronary Atherosclerosis" for CAD, "Yanyu decoction" OR "Yanyu Drug Combination" OR "Yanyu Heji" for YD, and "randomized controlled trial" OR "clinical trial" OR "RCT" for randomized clinical trials (RCTs). There were no restrictions on study time, publication language, gender, location, ethnicity, sample size, blinding methods, or treatment duration. The search results were merged and duplicate records were excluded.

2.2. Selection Criteria. The RCTs eligible for inclusion in the present meta-analysis had to meet the following criteria: (1) Stable CAD patients with age ≥60 years. (2) The YD group should be treated by YD plus CPT, and the control group should receive CPT the same as the YD group. (3) YD was composed of Stigma Maydis (60 g), Corydalis Rhizoma (30 g), Fructus Trichosanthis (15 g), and Allium Macrostemon (15 g). (4) YD and CPT should be given orally. (5) Primary outcomes of interest were cardiovascular events including cardiac mortality, AMI, and UA; the secondary outcomes were effective rate, blood lipids, and hemorheology. (6) No other therapies were used in the two groups.

2.3. Data Extraction and Quality Assessment. Two reviewers selected records and assessed research results for eligible studies independently. The information extracted from eligible studies was as follows: the first author, publication year, patient characteristics (age, gender), total number of cases, study design (interventions and duration of therapy), and reports on adverse effects. Any disagreement was resolved through discussion with another author, and final decisions were made based on consensus. We evaluated the risk of bias of the eligible trials using the Cochrane Handbook, consisting of seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias [22]. Two researchers independently conducted the quality assessment. Another investigator was consulted if a dispute was identified.

2.4. Statistical Analysis. Review Manager software (Review Manager, Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was applied to conduct a meta-analysis, with a mean difference (MD) for continuous outcome and risk ratio (RR) for dichotomous outcomes. The size was expressed with a 95% confidence interval (CI). Heterogeneity among the studies was measured utilizing the chi-square test and the I^2 statistics [23]. If significant heterogeneity was found, a random-effects model was used to compute MD and RR ($I^2 > 50\%$ or $P < 0.1$). Otherwise, the fixed-effects model was applied in the absence of substantial heterogeneity [24–26].

Sensitivity analysis was carried out by omitting a single study and recalculating the pooled estimates. The results were compared with those of meta-analyses before the

exclusion to figure out to what extent the excluded studies would influence the combined effect size and whether those meta-analyses were stable. Publication bias was screened if the number of included studies ≥10.

3. Results

3.1. Study Selection. From a total of 1262 potentially relevant studies searched initially, 1149 records were assessed in this review after 113 duplicates were eliminated. Based on titles and abstracts, 1142 nonconforming pieces of literature were excluded for the following reasons: no control group; not elder CAD population; not YD treatment in the experiment group; combination with other drugs or any other herbs. Furthermore, 7 full-text articles were assessed for eligibility, and we excluded 2 trials because sufficient details on outcomes of interest were not provided. Therefore, a final total of 5 studies, including 424 patients in the YD group and 424 patients in the conventional drug group, were identified in the present study [15–19]. A flowchart of study inclusion is presented in Figure 1.

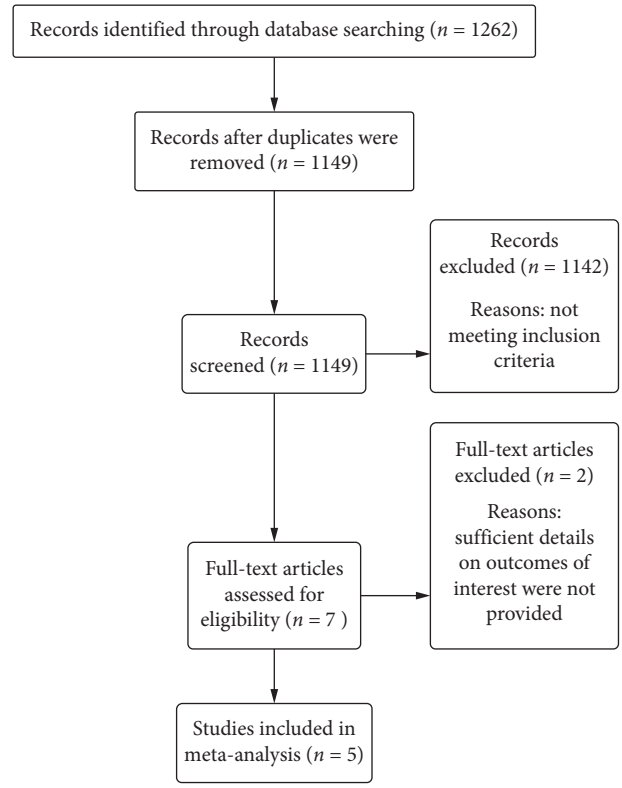


Figure 1: Flow diagram of search and selection process.

3.2. Study Characteristics. Table 1 presented the main characteristics of the included trials. All of the 5 trials were published in Chinese, with the sample size ranging from 36 to 556. The age of the total 848 participants ranged from 60 to 88 years, and the duration of treatment ranged from 28 days to 30 days. All eligible articles compared YD plus CPT with CPT alone. Four of them showed the outcomes of effective rate [16–19], and two reported the whole blood viscosity [15, 19], fibrinogen [15, 17], and platelet aggregation rate [15, 17]. Three [15, 17, 19] reported plasma viscosity, TC, TG, LDL-C, HDL-C. Four [15, 17–19] reported cardiovascular events.

3.3. Methodological Quality and Publication Bias. Two [17, 19] declared the generation of random sequences. None of the trials reported allocation concealment and blinding of patients, investigators, or assessors. A summary of the methodological quality assessment for each study is shown in Table 2. Publication bias was not analyzed since the number of included studies was less than 10.

3.4. Adverse Cardiovascular Events

3.4.1. Cardiac Mortality. Cardiac mortality was mentioned in 4 trials [15, 17–19]. The fixed-effect meta-analyses revealed that YD plus CPT may have less cardiac mortality in aged patients than CPT used alone (RR = 0.05; 95%CI: 0.01 to 0.27, $P = 0.0004$; heterogeneity: $P = 0.38$, $I^2 = 0\%$) (Figure 2(a)).

3.4.2. AMI. AMI events were mentioned in 4 trials [15, 17–19]. Results from the meta-analyses using a fixed-effect model indicated that YD plus CPT can have fewer AMI events in aged patients than CPT used alone (RR = 0.13; 95% CI: 0.03 to 0.54, $P = 0.005$; heterogeneity: $P = 0.93$, $I^2 = 0\%$) (Figure 2(b)).

3.4.3. UA. The cardiovascular events of UA were mentioned in 4 trials [15, 17–19]. The present study may prove YD plus CPT can have fewer UA events in patients than CPT used alone (RR = 0.37; 95%CI: 0.20 to 0.68, $P = 0.001$; heterogeneity: $P = 0.64$, $I^2 = 0\%$, fixed-effect model) (Figure 2(c)).

3.5. Effective Rate. The effective rate was mentioned in 4 studies [16–19] with 292 patients. Since the absence of substantial heterogeneity (chi-square = 3.49, $P = 0.32$, $I^2 = 14\%$), a fixed-effects model was utilized for statistical analysis. Meta-analysis results revealed that compared to CPT, YD significantly improved the effective rate (RR = 1.31; 95%CI: 1.17 to 1.47, $P < 0.00001$) (Figure 3).

3.6. Blood Lipids

3.6.1. Total Cholesterol. Three trials [15, 17, 19] with 740 participants tested the effect of YD on TC. There were 370 patients in the YD group and control group, respectively. The analyzing result was not statistically in favor of YD plus CPT (MD = −0.30; 95%CI: −0.67 to 0.07, $P = 0.11$; heterogeneity: $P < 0.0001$, $I^2 = 91\%$, random-effect model) (Figure 4(a)).

3.6.2. Triglycerides. There were 3 studies [15, 17, 19] that provided relevant data for triglycerides. There were 132 patients in the YD group and 112 patients in the CPT group. Compared with CPT used alone, a noteworthy lowering effect on TG in

Table 1: Characteristics of the included trials.

References	Number of subjects		Intervention		Age: Mean ± SD		Male/Female		Treatment duration
	E	C	E	C	E	C	E	C	
Chen et al. [15]	278	278	YD	CPT	69† (60–82) ∗	72† (61–88) ∗	171/107	163/115	30d
Chen [16]	18	18	YD	CPT	68.54 ± 4.14 (62–78)	68.98 ± 4.76 (61–79)	10/8	11/7	30d
Liu [5]	52	52	YD	CPT	67.5 ± 11.3 (62–78)	68.5 ± 12.6 (60–79)	33/19	35/17	30d
Liu [18]	36	36	YD	CPT	66.5 ± 1.2 (61–77)	67.9 ± 2.5 (60–78)	22/14	20/16	1M
Wang and Yang [19]	40	40	YD	CPT	68.0 ± 4.0 (61–79)	68.2 ± 6.5 (62–77)	22/18	23/17	28d

C = control group, YD = Yanyu decoction, *d* = days, *E* = experiment group, *M* = month, NA = not applicable, CPT = conventional pharmaceutical treatment, SD = standard deviation, *y* = years, †Median, ∗ range. YD: Stigma Maydis 60 g, Corydalis Rhizoma 30 g, Fructus Trichosanthis 15 g and Allium Macrostemon 15 g, 1 dose/day, bid.

Table 2: Methodologic quality of the included trials based on the Cochrane handbook.

References	A	B	C	D	E	F	G
Chen et al. [15]	−	?	?	?	+	+	+
Chen [16]	?	?	?	?	+	+	+
Liu [5]	+	?	?	?	+	+	+
Liu [18]	−	?	?	?	+	+	+
Wang and Yang [19]	+	?	?	?	+	+	+

A = Random sequence generation (selection bias); *B* = Allocation concealment (selection bias); *C* = Blinding of participants and personnel (performance bias); *D* = Blinding of outcome assessment (detection bias); *E* = Incomplete outcome data (attrition bias); *F* = Selective reporting (reporting bias); *G* = Other bias; +, low risk; −, high risk; ?, unclear.

favor of YD therapy was observed after the treatment (MD = −0.52; 95% CI: −0.67 to −0.37; $P < 0.00001$; heterogeneity: $P = 0.97$, $I^2 = 0\%$, fixed-effect model) (Figure 4(b)).

3.6.3. Low-Density Lipoprotein Cholesterol. There were 3 studies [15, 17, 19] evaluating the effectiveness of YD on LDL-C when compared with CPT alone. There were 370 patients in the YD group and 370 patients in the pharmaceutical group. Noteworthy lowering on LDL-C in favor of YD therapy was observed after treatments (MD = −0.31; 95% CI: −0.40 to −0.22; $P < 0.00001$; heterogeneity: $P = 0.25$, $I^2 = 28\%$, fixed-effect model) (Figure 4(c)).

3.6.4. High-Density Lipoprotein Cholesterol. Three trials [15, 17, 19] with 740 patients reported HDL-C. There were 370 patients treated by YD plus CPT and 370 patients treated by CPT. According to the test of heterogeneity (chi-square = 66.04, $P < 0.00001$, $I^2 = 97\%$), a random-effects model was utilized to accommodate heterogeneity. The analyzing result was statistically in favor of YD plus CPT (MD = 0.53; 95%CI: 0.20 to 0.85, $P = 0.001$) (Figure 4(d)).

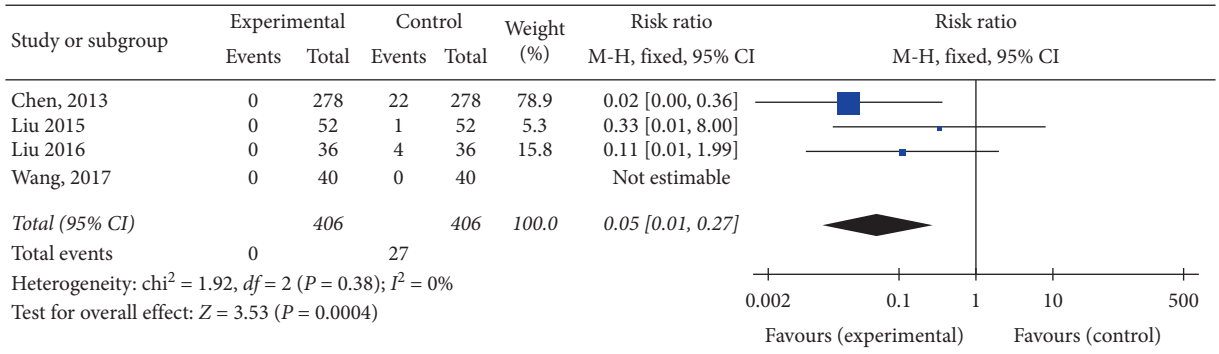
3.7. Hemorheology

3.7.1. Whole Blood Viscosity. Two trials [15, 19] with 636 patients reported blood viscosity. There were 318 patients treated by YD plus CPT and 318 patients treated by conventional pharmaceutical therapy. According to the test of heterogeneity (chi-square = 2.77, $P = 0.10$, $I^2 = 64\%$), a random-effects model was utilized for statistical analysis. The combined effects of these two independent trials might show a significant lowering effect of YD plus CPT on whole blood viscosity in stable CAD patients when compared with CPT alone (MD = −0.67; 95%CI: −0.95 to −0.39, $P < 0.00001$) (Figure 5(a)).

3.7.2. Plasma Viscosity. The effectiveness of YD on plasma viscosity rate was evaluated in 3 trials [15, 17, 19]. There were 370 patients in the YD groups and 370 patients in the control groups, respectively. A random-effects model was used for statistical analysis based on the test of heterogeneity (chi-square = 4.48, $P = 0.11$, $I^2 = 55\%$). The combined effects of these 3 independent trials suggested lowering effects of YD plus CPT on plasma viscosity in patients when compared with CPT alone (MD = −0.23; 95% CI: −0.35 to −0.10, $P = 0.0003$) (Figure 5(b)).

3.7.3. Platelet Aggregation Rate. Two studies [15, 17] evaluating the effectiveness of YD on platelet aggregation rate when compared with CPT alone. A sum of 330 patients in the YD group and 330 patients in the pharmaceutical group. A noteworthy lowering effect on platelet aggregation rate in favor of YD therapy was observed after the treatment (MD = −4.70; 95% CI: −6.30 to −3.10; $P < 0.00001$; heterogeneity: $P = 1.00$, $I^2 = 0\%$, fixed-effect model) (Figure 5(c)).

3.7.4. Fibrinogen. Two trials [15, 17] with 660 participants tested the effect of YD on the fibrinogen. There were 330 patients in the YD group and control group, respectively. The combined effects might show a significant improving effect of YD plus CPT on fibrinogen in stable CAD patients when compared with CPT alone (MD = 0.14; 95%CI: 0.01 to 0.28, $P = 0.04$; heterogeneity: $P = 0.79$, $I^2 = 0\%$, fixed-effect model) (Figure 5(d)).



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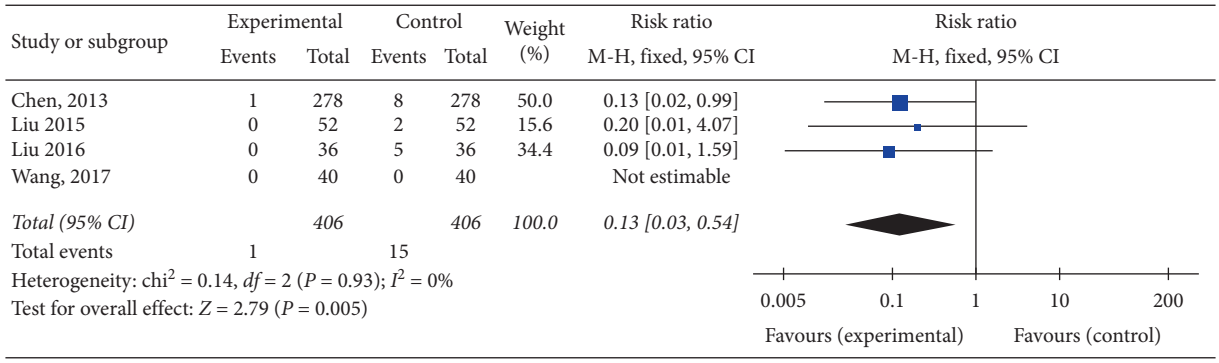

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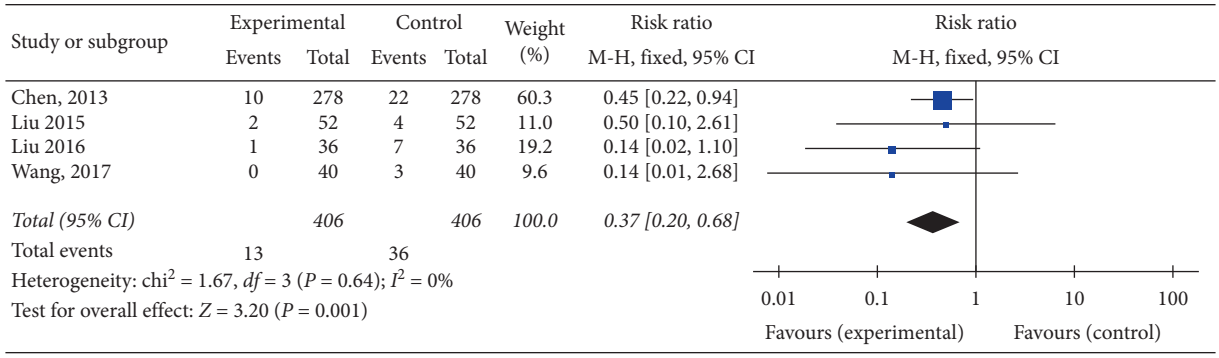
Figure 2: Continued.



(c)

Figure 2: Forest plot of the comparison of Yanyu decoction versus CPT for the outcome of adverse cardiovascular events. (a) Cardiac mortality. (b) AMI. (c) UA.

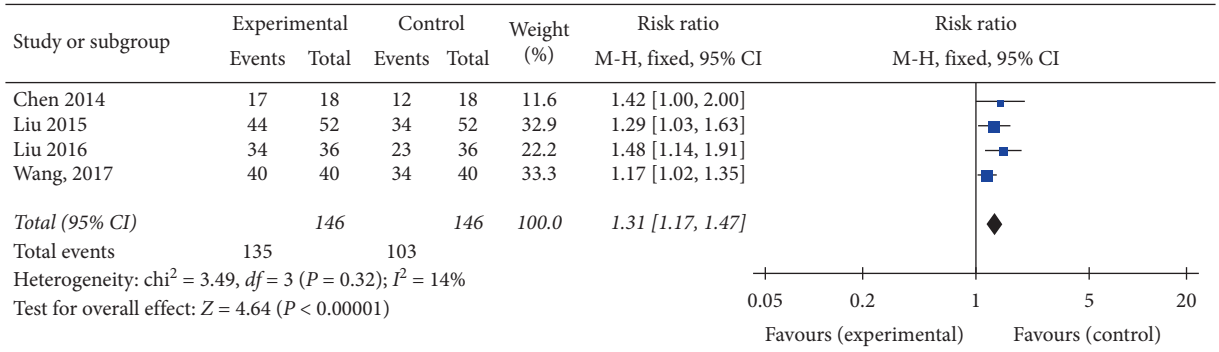
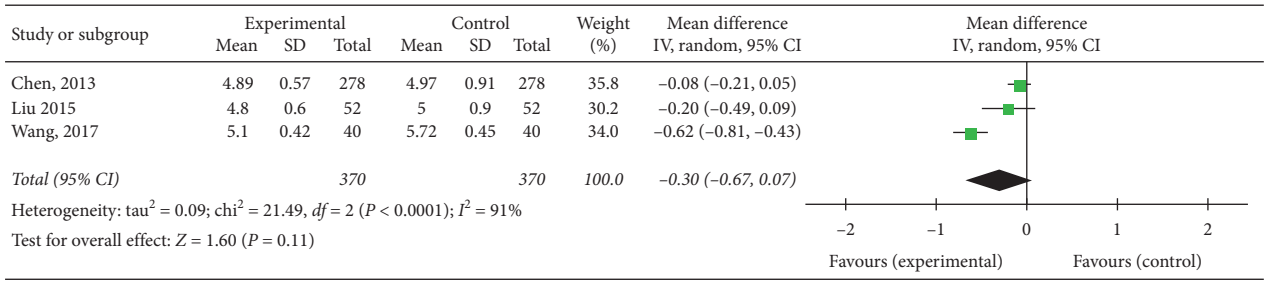


Figure 3: Forest plot of the comparison of Yanyu decoction versus CPT for the outcome of effective rate.



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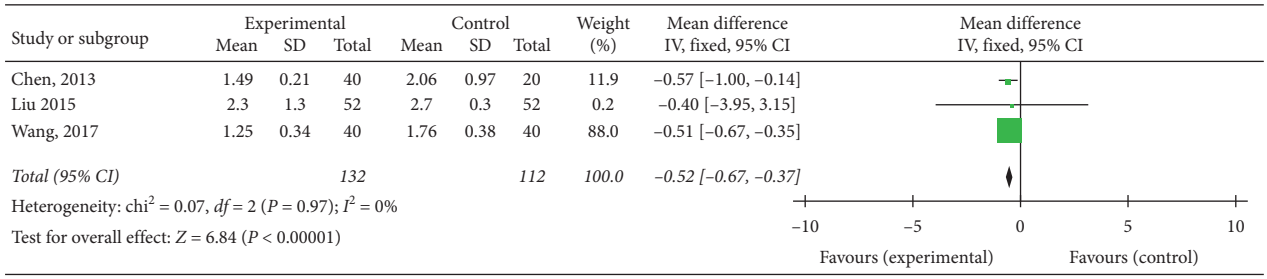

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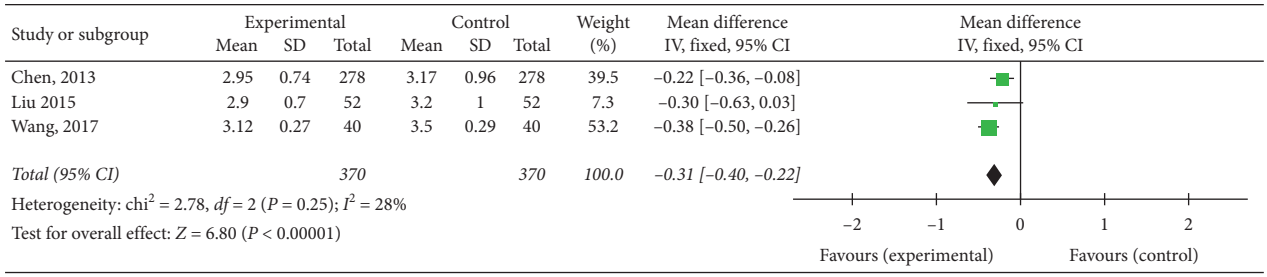

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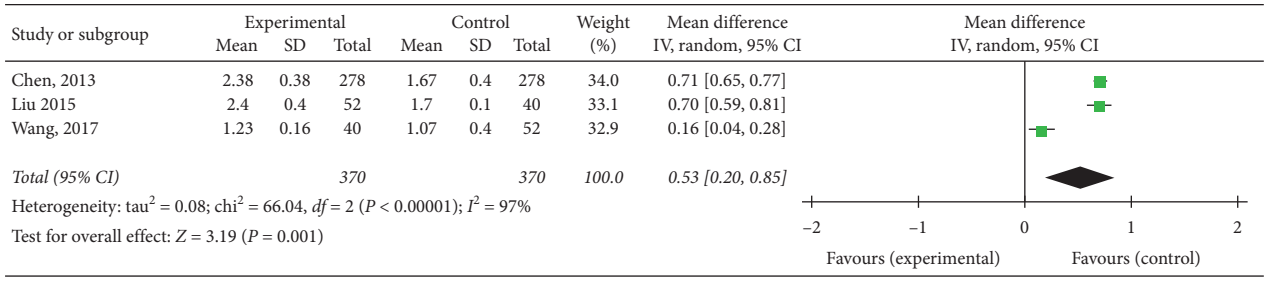

(d)

Figure 4: Forest plot of the comparison of Yanyu decoction versus CPT for the outcome of blood lipids. (a) Total cholesterol. (b) Triglycerides. (c) Low-density lipoprotein cholesterol. (d) High-density lipoprotein cholesterol.

3.8. Generic Adverse Events. Only two articles [15, 16] reported generic adverse events, of which one [15] reported that nausea, dizziness, or palpitations occurred in the YD group but disappeared without any treatment in a short time. However, the other [16] reported that no adverse events occurred in both the YD group and the control group. Whether YD plus CPT has fewer generic adverse events than CPT used alone or not cannot be evaluated.

3.9. Sensitivity Analyses. Sensitivity analysis was conducted to evaluate the robustness of merged results. There was little difference in adverse cardiovascular events, effective rate, and hemorheology between original meta-analyses and reevaluation. The sensitivity of the results about adverse cardiovascular events, effective rate, and hemorheology was relatively low, and the results were stable. However, with regard to blood lipids, the combined effect size was changed when the possible anomalous studies of TC [17, 19] and

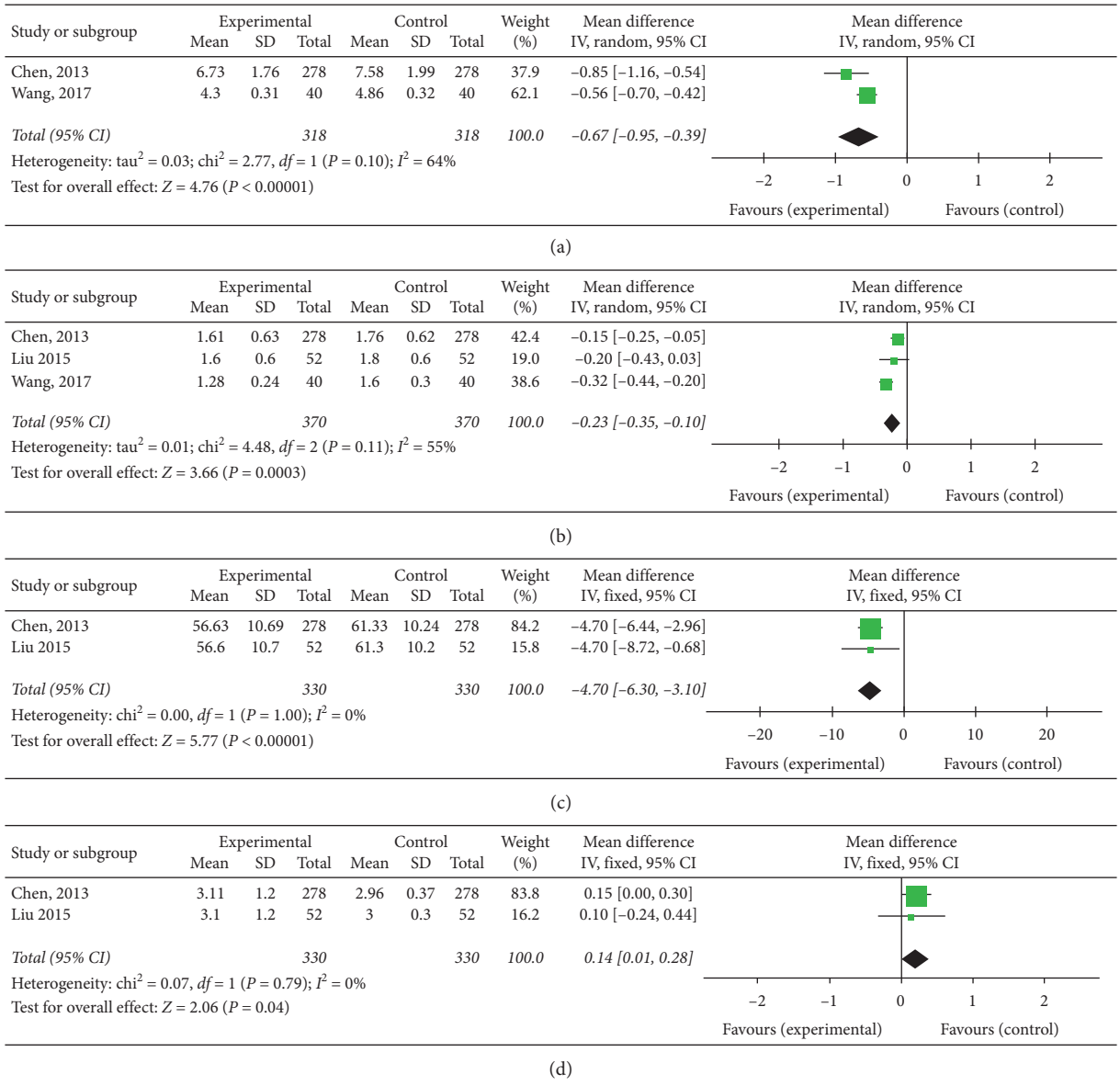

Figure 5: Forest plot of the comparison of Yanyu decoction versus CPT for the outcome of hemorheology. (a) Whole blood viscosity. (b) Plasma viscosity. (c) Platelet aggregation rate. (d) Fibrinogen.

HDL-C [15, 17] were removed, indicating that more information about TC and HDL-C was required.

4. Discussion

4.1. Interpretations. CAD is a major cause of morbidity and mortality in aged patients [27]. Aging is an inevitable part of life, with a remarkable effect on the heart and arterial system, leading to an increase in cardiovascular diseases like atherosclerosis and myocardial infarction [28]. Nonetheless, numerous CAD studies have excluded aged patients, leading to uncertainty about the efficacy and safety of clinical treatments [29]. As aged patients with functional declines are the fastest growing population dying of CAD [30], further improvements in prevention, diagnosis, and treatment of CAD among the aged are needed to address these gaps.

Dyslipidemia is an established risk factor for CAD that is frequent in patients of advanced age [31]. The pathogenesis of CAD in aged patients is closely correlated with the level of blood lipids and hemorheological indicators. Pathways involving lipid metabolism and coagulation play important roles in vascular aging [32]. The adenosine monophosphate-activated protein kinase (AMPK), involved in lipid metabolism, is cardioprotective during ischemia and reperfusion [33]. Mouse models using dominant-negative AMPK exacerbated myocardial infarction [34]. Modifying lipid profiles can reduce coronary morbidity and mortality [35] and more aggressive therapeutics on lowering LDL-C are emerging, e.g., PCSK9 inhibitors [36]. However, high cost and repeated injection reduced its availability for aged patients. YD, a plant-

based regimen, is less costly and shows the beneficial effect for aged patients with CAD. The principal herb of YD is Stigma Maydis, which is also an easily available food with good taste. Stigma Maydis plays a critical role and the dosage of it is the largest in YD. Aged patients are at higher risk for multiple diseases at the same time. In addition to alleviating CAD, Stigma Maydis has a therapeutic effect on hypertension, edema, diabetes, and gastritis, which usually occur in aged CAD patients [12, 37]. Based on traditional Chinese medicine (TCM) theory, Corydalis Rhizoma has been commonly used in clinic for the treatment of angina with the function of activating blood circulation to dissipate blood stasis and relieve pain. According to TCM, the pathological feature of CAD was much phlegm and stasis, which resulted in chest pain. YD, especially suitable for CAD patients with the syndrome of qi stagnation, blood stasis, and phlegm obstruction, could regulate the flow of qi, reduce dampness, and dispel pathogenic factors. However, lack of relevant animal experiments and in vitro cell tests, the pharmacological mechanism of YD has not been investigated, and the potential molecular intervention mechanisms are unclear. Our study may inspire future researches in this direction.

Although YD has been applied in stable CAD treatments in recent years, there was not any systematic review and meta-analysis to summarize the clinical effects of YD on stable CAD. To the best of our knowledge, the present study is the first of its kind to provide an evidence-based approach to the treatments of aged patients with CAD and may suggest YD as a new adjuvant herb treatment for aged patients with stable CAD.

Unfortunately, the heterogeneity of TC and HDL-C was high. We found that the heterogeneity of TC and HDL-C was reduced to 0 when one study [19] was excluded. But when we compared this study with other pooled studies, no significant clinical heterogeneity was found, considering all included studies have the same prescription of Chinese herbal medicine and disease, and the patients and treatment duration were similar among them. Although we used a random-effects model to accommodate heterogeneity, the sources of heterogeneity were not found. Thus, the certainty of evidence relevant to TC and HDL-C may be degraded.

4.2. Strengths and Limitations. There were several strengths related to our study. Firstly, this was the first study systematically reviewing YD in aged stable CAD patients. We used a broad range of search terms and conducted a comprehensive systematic search, including a variety of databases. We founded that only a small number of clinical studies explored pharmaceutical treatments for aged CAD patients, demonstrating the existence of an important gap in the literature. For aged CAD patients, the guidelines providing precise treatment recommendations were not available, and relevant recommendations for treatments can only be based on previous literature. This systematic review and meta-analysis described and evaluated the available clinical trials, providing a comprehensive synthesis of up-to-date evidence of CAD trials focusing on aged patients to fill the gap in the literature. Secondly, independent investigators performed the study selection and quality assessment and demonstrated YD as adjuvant therapy in treating stable CAD among aged patients. YD plus CPT may reduce adverse cardiovascular events, which provided aged CAD patients a new choice in treatments. YD plus CPT might have a better effect on reducing TG, LDL-C and improving HDL-C and hemorheology. YD might address the disorder in hemorheology and dyslipidemia, the key contributors to atherosclerosis in aged patients, which has far-reaching effects. Aged CAD patients may have fewer adverse cardiovascular events using YD as an adjuvant medicine. The improvements in blood lipids and improved blood flow brought by YD may be promising and impressing in CAD treatments. While current studies are still limited, it is expected that the importance of YD for aged patients with CAD will be increasingly appreciated in the coming years. Our research may provide a new direction and focus in future CAD studies.

Some limitations should be considered in our study results. As demonstrated in this review, only five dedicated studies evaluating the treatment of CAD in aged patients were reported, though researches regarding this issue are extremely important. No RCTs about YD in another country out of China have been found up to now. Only five Chinese studies were analyzed, which may induce the potential bias of the study. Whether YD can be used in more ethnic groups needs further studies. In addition, whether YD plus CPT has fewer adverse events or not is still not unequivocal. Moreover, positive results tend to be reported more frequently and the efficacy of YD might be overestimated. Furthermore, it is hard to conduct double-blind clinical trials studying herb decoction. The methodological quality is poor according to the Cochrane Collaboration's tool.

5. Conclusion

In summary, YD plus CPT may have a better effect on reducing cardiac mortality, AMI, and UA in elder stable CAD patients when compared with CPT alone. Besides, it might also be helpful for not only reducing TG, LDL-C and improving HDL-C but lowering blood viscosity, plasma viscosity, and platelet aggregation rate. YD may be beneficial for stable CAD, though some limitations might weaken the validity of these findings. More RCTs of high-quality and more carefully designed clinical trials are recommended to generate a high level of clinical evidence to confirm these findings.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Shihua Shi and Zhenxing Wang are equal contributors and cofirst authors.

Acknowledgments

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Research Article

A Series of N-of-1 Trials for Traditional Chinese Medicine Using a Bayesian Method: Study Rationale and Protocol

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Background. Our previous studies showed that N-of-1 trials could reflect the individualized characteristics of traditional Chinese medicine (TCM) syndrome differentiation with good feasibility, but the sensitivity was low. Therefore, this study will use hierarchical Bayesian statistical method to improve the sensitivity and applicability of N-of-1 trials of TCM. *Methods/Design*. This is a randomized, double-blind, placebo-controlled, three-pair crossover trial for a single subject, including 4–8 weeks of run-in period and 24 weeks of formal trial. In this study, we will recruit a total of 30 participants who are in the stable stage of bronchiectasis. The trial will be divided into three pairs (cycles), and one cycle contains two observation periods. The medications will be taken for three weeks and stopped for one week in the last week of each observation period. The order of syndrome differentiation decoction and placebo will be randomly determined. Patient self-reported symptom score (on a 7-point Likert scale) is the primary outcome. *Discussion*. Some confounding variables (such as TCM syndrome type and potential carryover effect of TCM) will be introduced into hierarchical Bayesian statistical method to improve the sensitivity and applicability of N-of-1 trials of TCM, and the use of prior available information (e.g., "borrowing from strength" of previous trial results) within the analysis may improve the sensitivity of the results of a series of N-of-1 trials, from both the individual and population level to study the efficacy of TCM syndrome differentiation. It is the exploration of improving the objective evaluation method of the clinical efficacy of TCM and may provide reference value for clinical trials of TCM in other chronic diseases. This trial is registered with ClinicalTrials.gov (ID: NCT04601792).

1. Background

1.1. N-of-1 Trials and Traditional Chinese Medicine. Traditional Chinese medicine (TCM) emphasizes treatment based on syndrome differentiation which is indeed the individualized treatment. It is difficult to carry out a standard form of population-based RCTs due to the individualized TCM intervention. The shortage of a reliable and evidence-based clinical efficacy evaluation method of TCM has impeded its globalization and further development [1]. Therefore, it is of great significance to explore and establish a clinical trial method that can fully reflect the individualized characteristics of TCM [2]. N-of-1 trials take the subject him(her)self as the control, and the results will be ultimately used to guide the subject's own treatment. The individualized treatment concept represented by N-of-1 trials is naturally compatible with the principle of (necessarily individually applied) syndrome differentiation of TCM, providing a feasible way for the connection between TCM and western medicine. N-of-1 trials have been listed as "level 1" evidence in the Oxford Centre for Evidence-Based Medicine 2011 levels of evidence [3] and CONSORT extension for reporting N-of-1 trials (CENT) has been published in 2015 [4]; N-of-1 trials are attracting more and more attention from scholars worldwide.

1.2. Bronchiectasis. Bronchiectasis is a common chronic lung disease with inconsistent clinical features and prognosis [5]. In Europe and North America, the prevalence of bronchiectasis is estimated to range from 53 to 566 per 100000 inhabitants, while in China it is approximately as high as 1200 per 100000, making it a serious and growing economic health burden [6–8]. During the stable stage of bronchiectasis, there are still clinical symptoms such as chronic cough, purulent sputum, dyspnea, or extrapulmonary symptoms such as insomnia, fatigue, constipation, or diarrhea. Western medicine lacks effective therapy for stable bronchiectasis, while TCM has good effects in alleviating symptoms and improving the quality of life [9]. At present, there is no standard TCM decoction for the treatment of stable bronchiectasis. Treatment principles are based on TCM syndrome differentiation, including resolving phlegm, clearing lung heat, and strengthening healthy energy [9]. Stable bronchiectasis is a good indication for carrying out N-of-1 trials: (1) not self-limited disease, (2) relatively stable condition, and (3) the treatment needs to be long term [4]. Yet, the requirement of N-of-1 trials is that the therapeutic drug must have a rapid onset and termination of action, and the optimal duration of treatment should be known and practical [4].

1.3. Our Previous Studies and Facing Problems. Since 2012, our team has been exploring the methodology of N-of-1 trials of TCM. We found this method is welcomed by patients because it embodies personalized thinking and is close to the clinical practice of TCM. In the research, we mainly faced the following two problems: (1) because of the long period (usually 3-4 weeks) of N-of-1 trials of TCM, and generally only three cycles were conducted, in terms of individual statistics, its power of statistical analysis was insufficient, which made it difficult for some patients to draw a clear conclusion [10]; (2) through a series of N-of-1 trials, we have proved that the syndrome differentiation decoction was statistically better than the fixed decoction on symptom scores ($P < 0.05$), but the result was not clinically significant. It may be related to the complex composition of TCM and its relatively long onset and failure time (half-life). The nature of TCM may not perfectly satisfy a certain requirement of the classic N-of-1 trials (rapid onset, short half-life, and fast disappearance of efficacy after discontinuation). The possible carryover effect may reduce the sensitivity of the N-of-1 trials of TCM [10]. Other investigators also believe that the carryover effect of TCM is a factor worthy of attention [11, 12].

1.4. Hierarchical Bayesian Statistical Method. In order to improve the efficiency of TCM N-of-1 trial design, Chen and Chen [13] tried to provide practice of guidance for the analysis of N-of-1 trials by the comparison of four commonly used models (paired *t*-test, mixed-effects model of difference, mixed-effects model, and meta-analysis of summary data). The conclusion was that mixed-effects model provided an alternative when there was carryover effect for normally distributed data of N-of-1 trials. If the factor of carryover effect is introduced into statistical models, it may improve the efficiency of data analysis of N-of-1 clinical trials for TCM.

Hierarchical Bayesian statistical method has been one of the main statistical methods for N-of-1 trials due to its significant advantages [14, 15]: (1) both individual and aggregate analyses to be simultaneously and coherently undertaken, even when the number of completed cycles between patients is variable; (2) easily introducing confounding variables, such as different patient's constitution, different TCM syndrome type, and potential carryover effect; and (3) in addition, Bayesian methods enable the use of prior available information (e.g., "borrowing from strength" of previous trial results) within the analysis, which may improve the sensitivity of N-of-1 trials [16, 17]. This method is currently rarely applied in N-of-1 trials of TCM, and it is a statistical method worthy of studying and promotion.

In view of the importance of N-of-1 trials for TCM study, we envisaged strategies to improve its sensitivity: applying hierarchical Bayesian statistical method, considering introducing the carryover effect of TCM as confounding variables into hierarchical Bayesian statistics [12, 13], and its special advantage would be used: using the data of similar N-of-1 trials that have been completed in the past as prior information [16, 17], without increasing the cycles of the N-of-1 trials, to improve the reliability and sensitivity of N-of-1 trials of TCM.

2. Hypothesis

The efficacy of TCM based on syndrome differentiation treatment is expected to be significantly better than that of placebo at both the individual and population levels in N-of-1 trials. But there is the possibility of invalid cases, which may be related to some factors (such as the severity of the disease, bacterial strains, and accompanying disease). Introducing carryover effect of TCM as confounding variables into hierarchical Bayesian statistics, and using the "borrowing from strength" [16, 17] function of prior information, can improve the reliability and sensitivity of N-of-1 trials of TCM.

3. Methods

3.1. Study Design. These are a series of randomized, double-blind, placebo-controlled, N-of-1 clinical trials. According to our previous experimental design [10, 18], the brief description is as follows: we will conduct a 4- to 8-week run-in period for patients who meet the inclusion criteria to obtain onset time after drug administration and efficacy maintenance time after drug withdrawal (with the changes of patients' self-rated symptom scores as the primary outcome), so as to determine the length of observation period and the estimation of washout period. Combining the results in the run-in periods and the previous results [10, 18], we fixed the observation periods of the N-of-1 trials to four weeks. Three cycles (pairs) of N-of-1 trials will be conducted in the same individual, with each cycle consisting of two observation periods (the experimental period and the

control period) assigned in random order [18, 19]. The medications will be taken for three weeks and then stopped for one week in each observation period. We will measure the outcomes in the last week of each period and the time before this is supposed to be the washout period (Figure 1). The changes in patients, self-rated symptom scores are the primary outcome (the 7-point Likert scale). Hierarchical Bayesian statistical method will be the key statistical method of this study. Different mathematical models (paired t-test, hierarchical Bayesian, and meta-analysis) for N-of-1 trials of TCM will be compared to improve the sensitivity and applicability of N-of-1 trials of TCM on both individual and population levels. The trial was performed at the clinic of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine. This study started in January 2019 and was expected to end in December 2022.

3.2. Study Participants. According to our previous experimental design [10, 18], the brief description is as follows. For all patients participating in the trial, we will strictly screen according to the following criteria [10].

3.2.1. Diagnostic Criteria

(1) *Diagnostic Criteria of Western Medicine*. Modern medical diagnostic criteria are based on the consensus of domestic experts [5] and combined with the guidelines for the diagnosis and treatment of adult bronchiectasis published by the European Respiratory Society in 2017 [20]. All patients require a definitive diagnosis of bronchiectasis on high-resolution CT. And they should be in the stable stage of bronchiectasis, with stable symptoms such as cough and expectoration.

(2) *Diagnostic Criteria of TCM Syndrome*. The diagnostic criteria of TCM syndrome are based on the "Criteria of Diagnosis and Therapeutic Effect of TCM Diseases" issued by the State Administration of TCM [21] and integrated with the TCM differentiation of bronchiectasis summarized from the literature [22], mainly including lung and spleen deficiency syndrome, qi and yin deficiency syndrome, and phlegm-heat obstructing lung syndrome (including mild phlegm-heat syndrome).

Patients who can be diagnosed with TCM syndrome should have corresponding two primary symptoms or more than two accompanied symptoms with the corresponding tongue and pulse signs.

Clinically, the TCM syndrome differentiation of stable bronchiectasis is mostly a deficiency and excess, mainly of a certain syndrome. Each type of syndrome differentiation of TCM is mixed with a certain degree of phlegm and heat. For example, qi and yin deficiency intermingled with phlegm and heat syndrome or lung and spleen deficiency with phlegm and heat syndrome [9], it is necessary to estimate the severity of intermingled phlegm and heat according to the clinician's experienced syndrome differentiation. In order to ensure the accuracy and high quality of treatment based on syndrome differentiation of TCM, the TCM syndrome of each subject will be discriminated and concluded by two chief physicians. If necessary, it will be decided by a third party (an outstanding veteran doctor of TCM).

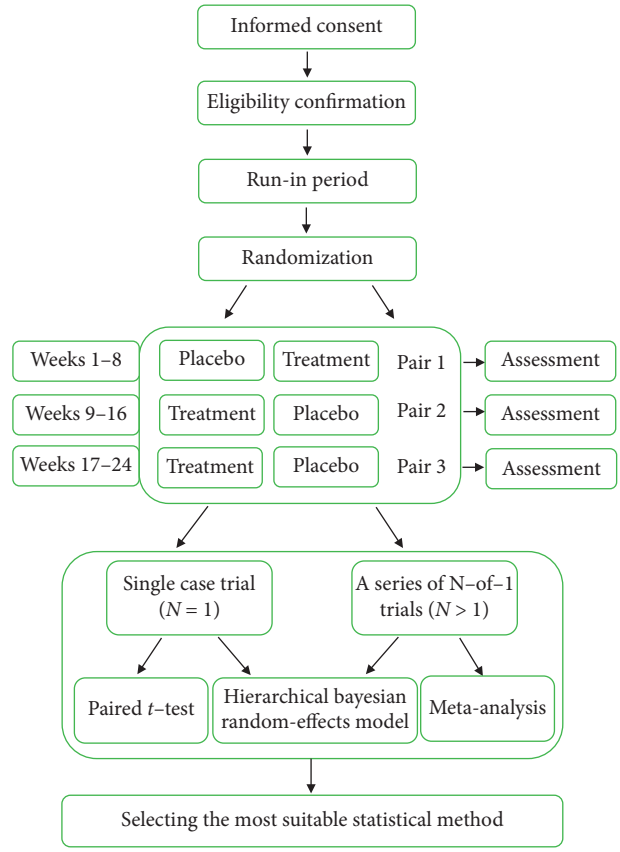

Figure 1: The flowchart of the N-of-1 trials in the treatment of stable bronchiectasis by traditional Chinese medicine based on syndrome differentiation.

3.2.2. Inclusion Criteria. The inclusion criteria are as follows: (1) meeting the above diagnostic criteria of Chinese and Western medicine; (2) male or female, between 18 and 70 years of age; (3) being in the stable stage, and no acute exacerbation of bronchiectasis within the past three weeks; (4) frequency of acute exacerbation of bronchiectasis ≤3 times every year; and (5) signed informed consent.

3.2.3. Exclusion Criteria. The exclusion criteria include the following: (1) patients who do not meet the above diagnostic and inclusion criteria; (2) suffering from respiratory failure with estimated survival time <1 year; (3) complicated with hemoptysis; (4) complicated with active pulmonary tuberculosis; (5) having pregnancy or severe heart, liver, and kidney dysfunctions; and (6) participating in other pharmacological clinical trials within the past 3 months.

3.2.4. Withdrawal Criteria. Patients who meet any of the following conditions will be excluded from the study: (1) patients who voluntarily withdrew from the trial; (2) patients with poor compliance and not taking the medication as required by the study protocol; and (3) patients who are allergic to the medication.

3.2.5. Participant Recruitment. The trial will be set up and coordinated by Yueyang Hospital affiliated to Shanghai University of TCM. Subjects will be recruited from the respiratory outpatient department of the hospital, or they can be recruited online or through advertising.

3.3. Interventions. Basic treatment will continue to be provided to all participants in the study. After assessing patients' TCM syndrome, doctors will prescribe both individualized decoction (syndrome differentiation decoction) and controlled decoction (placebo).

3.3.1. Basic Treatment of Western Medicine. Basic treatment is chest physical therapy [5, 21], mainly, including postural drainage and chest percussion to assist sputum excretion. If acute exacerbation of bronchiectasis occurs, the trial should be suspended; antibiotics and other treatments will be provided conventionally according to the guidelines for noncystic fibrosis bronchiectasis [5, 23]. Other chronic diseases (such as hypertension, coronary heart disease, and diabetes) can be treated at the same time, but the usage should be relatively fixed. Patients should record the medication in detail.

3.3.2. Treatment of TCM

(a) *Syndrome Differentiation Treatment*. The treatment based on syndrome differentiation will be conducted by chief physicians. To ensure the high quality of treatment based on syndrome differentiation, if there is any difficulty or dispute, a third party (distinguished veteran doctor of TCM) will be invited to provide consultation and guidance. It is the highly individualized treatment of TCM, the modification of Bronchiectasis Stabilization Decoction [9] (Rhizoma Fagopyri Cymosi 30 g, Radix Lithospermi 15 g, Radix Ophiopogonis 15 g, Poria 15 g, Radix Astragali 20 g, Rhizoma Bletillae 10 g, Platycodon grandiflorum 10 g, and Semen Coicis 30 g) based on syndrome differentiation. For example, for patients with lung and spleen qi deficiency syndrome, we will add Radix Codonopsis Pilosulae, Pericarpium Citri Reticulatae, and Atractylodes Macrocephala Koidz; for patients with qi and yin deficiency syndrome, we will add Radix Adenophorae, Radix Glehniae, and Radix Rehmanniae; for patients with obvious phlegm-heat syndrome, we will add Radix Scutellariae and Herba Violae. In addition, we can also adjust the individualized decoction according to the patient's other symptoms such as loss of appetite, insomnia, and constipation. In order to reflect the high flexibility of TCM, the prescription based on syndrome differentiation can be adjusted appropriately in accordance with the patient's condition changes throughout the entire study duration. The drug (in the form of TCM granules) which has passed quality inspection in line with the national norms [18] will be provided by the Sichuan New Green Pharmaceutical Technology Development Co., Ltd.

(b) *Placebo*. Placebo is made by dextrin, bitter agent, edible pigment, etc., and adding 5% of the test drug. There will be no difference between the placebo and the test drug in terms of dosage form, appearance, color, specification, label, and so forth [24]. The individualized decoction and placebo will be used in the same way: take one decoction a day in two doses.

3.4. Randomization and Blinding. According to our previous experimental design [10, 18], the brief description is as follows: a specialized pharmacist will use a computer (software SPSS 15.0) to generate random numbers and random number sequences to determine the order of medication for each subject in each observation period, such as BA-AB-BA or AB-BA-BA. The pharmacist will use the method of flipping a coin to determine which of A or B represents individualized prescription or control prescription, record the blind code, and keep it properly. After evaluating the patient's TCM syndromes, the doctor will prescribe both individualized prescription and control prescription. Then, the two prescriptions will be delivered to the specialized pharmacist by the TCM Pharmacy. Then, the pharmacist will prepare the drug following the randomized medication order and the blind code.

3.5. Outcome Measures. The investigators will see the patients and collect data before and after each treatment period. Patients should identify the symptoms that bother them and make a self-administered patient diary or questionnaire. In addition, it is necessary to establish online data collection approaches (WeChat, including personal contact and specialized research group chat) with the subjects in order to obtain their information conveniently and timely. Subjects can report online changes in their condition or adverse reactions during the trial in time. Meanwhile, the personal privacy of the subjects will be protected from disclosure. The following are the outcome measures.

3.5.1. Primary Outcome

Patient Self-Rated Symptom Score. Patients should record their symptoms (cough, expectoration, shortness of breath, chest pain, loss of appetite, fatigue, insomnia, etc.) in a diary and rate the severity of these problems on the 7-point Likert scale supplemented by Visual Analogue Scales (VAS) [10, 25]. The number of questions must be optimized to ensure that the most important aspects of the patient's

problem are examined (usually four to eight items). The higher the score, the more severe the symptom. Take cough as an example.

On average, in comparison with your usual cough, how severe is the cough?

(1) No cough, or as mild as, or milder than they have ever been

(2) Not nearly as severe as usual

(3) Not as severe as usual

(4) As severe as usual

(5) Severer than usual

(6) Very severe, almost as severe as they have ever been

(7) Very severe, as severe as or more severe than they have ever been

An improvement of more than 0.5 points for a problem is considered clinically effective for this problem; if the average improvement of the total score of all symptoms is more than 0.5 points, it can be considered clinically effective for this case [19, 25]. Therefore, the mean difference of 0.5 points is defined as the "Minimal Clinically Important Difference (MCID)" for the 7-point scales.

3.5.2. Other Outcomes

(1) *24 h Sputum Volume*. We will measure the 24 h sputum volume and take the mean value for the 3 consecutive days at the beginning and the end of each trial. To ensure the precision of the measurement, patients are required to spit their sputum into a calibrated collector from 8:00 am to the next 8:00 am. We will use the mean value of the sputum volume for 3 consecutive days as the outcome.

(2) *COPD Assessment Test (Chronic Obstructive Pulmonary Disease Assessment Test, CAT)*. In recent years, foreign scholars [26] have confirmed that the CAT is valid and reliable in patients with bronchiectasis and other chronic respiratory diseases. CAT questionnaire consists of 8 items. Each item is rated on a scale of 0–5 points, thereby making the total score to be 0–40 points. The lower the score, the better the patient's health and quality of life. The "Minimal Clinically Important Difference (MCID)" for the CAT has not been established officially, but it was estimated to be around 2 points [10, 27].

(3) *Safety Outcome*. Blood routine, urine routine, liver and kidney function, electrocardiogram, and other laboratory tests, as well as some vital signs, will be determined and recorded before and after the study. We will observe whether there are any adverse reactions or events related to the test method or drug. If necessary, we will terminate and unblind the trial, provide corresponding treatment measures, and report the adverse event to the ethics committees.

3.6. Data Analysis

3.6.1. Sample Size Calculation. Estimation of the needed sample size was based on the result of the preliminary study. The primary outcome of the study is patient self-reported symptoms scores on a 7-point Likert scale. The mean difference between the symptoms scores of the two groups of the preliminary study was 0.47, with the standard deviation (SD) being 0.53. The basis for estimating the required sample size is that it has at least 80% power ($\beta = 0.20$) to detect the mean difference of 0.47 points, with significance testing at the $\alpha = 0.05$ level and SD = 0.53; using a two-sided test, the ratio of the two groups is 1 : 1, with three cross-overs, assuming no period effect or treatment × time interaction, under the given model parameters [28, 29]. The sample size was calculated by using PASS 11.0 software (NCSS LLC, Kaysville, UT, USA). Preliminary calculations concluded that 21 patients would be needed to meet the requirements. Taking into account the high drop-out rates of N-of-1 trials (30%), the final sample size was determined to be 30.

3.6.2. Statistical Analysis. To avoid or decrease the potential carryover effect of the previously used drug, the values of the outcomes will be measured in the last week of each observation period. All patients with at least one complete treatment cycle will be included in the analysis. The data generated in this study will constitute a dataset, and the database will be established by experts specializing in statistical analysis of N-of-1 trials. For all data in this dataset, carryover effect, period effects, and autocorrelation will be carried out and analyzed by statistical experts [4, 10].

Hierarchical Bayesian statistical method will be the key statistical method of this study. It is based on the Monte Carlo technology of Markov's Bayesian hierarchical model calculation chain and uses WinBUGS software for calculation. The advantages of a Bayesian approach over normal frequentist statistical methods are discussed in detail in Zucker et al., Schluter and Ware, and Nikles et al. [30–32]. We intend to use the advantages of Bayesian analysis: (1) various levels and confounding variables can be introduced, such as the potential carryover effect of TCM, the severity of bronchiectasis, sputum culture (whether *Pseudomonas aeruginosa* is positive), and TCM syndrome differentiation (lung and spleen deficiency syndrome, qi and yin deficiency syndrome, phlegm-heat syndrome (mild, moderate, severe, etc.)), and the difference in efficacy under stratification and confounding variables can be analyzed. Using the data of similar N-of-1 trials that have been completed in the past as prior information, from the Bayesian analysis we will obtain posterior distributions for the mean treatment effect at the population level, as well as posterior distributions for the treatment effects at the individual level, that will exhibit borrowed strength from the population estimates through shrinkage to the population mean. The probability distributions of each model will be evaluated to assess violations and data transformations undertaken, where necessary. Conventional burn-in periods, model convergence and stability diagnostics, and residual checks will be employed.

The forms of hierarchical Bayesian statistics analysis results will be as follows: (1) the mean of the posterior distribution of the mean difference between the outcomes of placebo and the test drug, which gives the best estimate of the difference in the overall effect size between the treatments; (2) the relevant 95% credible region, which gives uncertainty interval of the posterior distribution (2.5 and 97.5 percentiles in this case), and (3) the posterior probability of mean difference that the test drug will be better than the control drug, which describes the likelihood that the patients will tend to actively treat in future cycles. When these estimated values exceed predetermined threshold values (for example, the improvement of the average of the total points of all symptoms is ≥0.5 points), the patient will be defined as a "responder" [33, 34].

To compare the novel methodology of aggregated N-of-1 trials with the traditional analysis methods, the Bayesian method will be compared with other common statistical methods used in N-of-1 trials (including paired *t*-test and meta-analysis) to evaluate its sensitivity and applicability to N-of-1 trials of TCM.

4. Discussion

Our previous studies [10] showed that N-of-1 trials could reflect the individualized characteristics of traditional Chinese medicine (TCM) syndrome differentiation with good feasibility and compliance, but the sensitivity was low. We supposed that the improvement of statistical models may be beneficial in establishing a clinical efficacy evaluation method that meets the characteristics of TCM.

For the past few years, hierarchical Bayesian statistical method has been widely used in this field. Senior et al. [14] concluded that the advantages of Bayesian method over ordinary frequentist statistical methods, such as the following: (1) both individual and aggregate analyses are simultaneously and coherently undertaken, (2) natural hierarchies and serial correlations within the study can be exploited and accommodated (such as clustering by physician, setting, or location), (3) confounding variables can easily be introduced into Bayesian model, and (4) they naturally allow incorporating any relevant trial information that may be sourced from elsewhere [14]. In addition, Bayesian methods allow for the use of prior available information (e.g., previous trial results) within the analysis. In the hierarchical Bayesian model, previous data and new trial data belong to a continuous data chain. When new trial data are generated, the data chain is updated to generate more accurate posterior information [15].

Bayesian methods as yet have not been used in N-of-1 trials of TCM. In this trial design, we consider introducing the carryover effect of TCM as confounding variables into hierarchical Bayesian statistics [12, 13] and using the data of similar N-of-1 trials that have been completed in the past as prior information, without increasing the cycles of the trials, by "borrowing from strength" from the results of other similar N-of-1 trials to improve the reliability and sensitivity N-of-1 trials of TCM. In addition, the Bayesian method will be compared with other common statistical methods used in N-of-1 trials (including paired *t*-test and meta-analysis) to evaluate its sensitivity and applicability to N-of-1 trials of TCM.

It should be mentioned that this trial protocol has other characteristics: (1) a relatively reasonable observation and washout period were determined through the run-in period combined with investigators' clinical experience [10, 18]; (2) placebo control used in the study has high test sensitivity, can detect the absolute effectiveness and safety of the test drug, and has a high ability to reduce bias; the carryover effect of TCM can also be calculated from it; (3) highly individualized treatment based on syndrome differentiation: not only can the investigational drug be individualized, but it can also be adjusted according to the patient's condition and syndrome changes during the whole process of N-of-1 trials, which conforms to the principle of "do what is proper according to the circumstances" in TCM. Meanwhile, the control drug will be always unchanged. Our ultimate goal is to explore the establishment of an objective evaluation method of therapeutic effects reflecting the essence of TCM treatment based on syndrome differentiation in line with the trend of international medical development. In a broad sense, it may have reference value for clinical trials of TCM for other chronic diseases.

Data Availability

The data used to support the rationale and protocol of this study are included within the article and the protocol has been registered in ClinicalTrials.gov (no. NCT04601792). All data contained in this study can be obtained by contacting the corresponding author upon request.

Ethical Approval

The protocol has been approved by the Ethics Committee of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine (Ethical review approval number: 2019-038). The study will be introduced to the patients in detail. Before inclusion into the study, the investigators should obtain their written informed consent.

Disclosure

Lizhi Lu and Jiaqi An are the co-first author. The data used in this study are disclosed for the first time in clinicaltrials.gov (identifier: NCT03147443).

Conflicts of Interest

All authors declare no conflicts of interest.

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Review Article

Investigation on the Efficiency of Tonic Chinese Herbal Injections for Treating Dilated Cardiomyopathy Based on Bayesian Network Meta-Analysis

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Introduction. This network meta-analysis investigated the efficacy of six tonic Chinese herbal injections (Huangqi injection, Shenfu injection, Shengmai injection, Shenmai injection, Shenqi Fuzheng injection, and Yiqifumai injection) compared to Western medicine for the treatment of the deteriorating state associated with dilated cardiomyopathy. *Methods*. PubMed, the Cochrane Library, Embase, the Chinese Biological Medicine Database, China National Knowledge Infrastructure, the Wanfang Database, and the Chinese Scientific Journal Database were searched from their inception to October 15, 2020, to retrieve randomized controlled trials (RCTs). Study selection and data extraction conformed to a priori criteria. The risk of bias of the included RCTs was determined, and GRADE was used to evaluate outcomes. The network meta-analysis was calculated using WinBUGS 1.4.3 and Stata 13.0 software. The clinical effective rate, left ventricular ejection fraction, 6-minute walk test, left ventricular end-diastolic dimension, heart rate, and cardiac output were deemed outcomes. All outcomes were summarized as odds ratios or mean differences with their 95% credible intervals. The ranking probability of the interventions across various outcomes was also presented. *Results*. Forty RCTs and 2970 patients were enrolled. Integration of the outcome results revealed that a combination of Shenfu injection and Western medicine ranked ahead of the other injections in most outcomes, especially in the clinical effective rate (OR = 0.21, 95% CI: 0.12–0.34), left ventricular ejection fraction (MD = 7.43, 95% CI: 2.41–12.38), and 6-minute walk test (MD = 50.39, 95% CI: 25.78–76.33). Shenmai injection plus Western medicine ranked ahead of the other injections in left ventricular end-diastolic dimension (69.5%) and cardiac output (60.9%). The cluster analysis suggested that Shenfu injection plus Western medicine was the most effective intervention for dilated cardiomyopathy. *Conclusions*. Shenfu injection plus Western medicine may be a preferable treatment in dilated cardiomyopathy. Clinicians should also consider the specific patient's various conditions when making diagnostic decisions. Due to an insufficient network meta-analysis, more high-quality RCTs need to be implemented to support our conclusions.

1. Introduction

Dilated cardiomyopathy (DCM) is the most common cardiomyopathy worldwide, and it refers to a heart muscle disease that is characterized by left ventricular and biventricular dilatation and systolic dysfunction without volume overload or coronary artery disease. These conditions result in decreased cardiac output and stroke volume, increased end-diastolic pressure, and ultimately malignant arrhythmia, heart failure, and even sudden death [1–4]. DCM patients suffer from a lower quality of life and heavy economic pressure with a high hospitalization rate and mortality [1, 5]. The estimated prevalence of DCM was 40 cases in 100,000 individuals with an annual incidence of 7 cases in 100,000 individuals [1, 2]. Several factors may contribute to DCM, such as persistent infection, autoimmunity, gene mutation, and genetic factors [4, 6, 7]. The mechanisms underlying the deterioration in cardiac

function are largely unknown [4, 6–8], but therapies in most cases concentrated on the symptoms of heart failure and related complications [4, 6, 7]. Angiotensin-converting enzyme inhibitors, β blockers, digoxin, and diuretics are standard treatment options.

With the promotion of traditional Chinese medicine, its utilization is becoming increasingly instrumental to the treatment of DCM. Traditional Chinese medicine theories consider DCM a "heart impediment," "chest obstruction," and "edema" that emerges in the heart and then affects the lung, spleen, and kidney [7, 9]. The clinical principle is primarily directed at strengthening the body's resistance to eliminate pathogenic factors. As an indispensable part of traditional Chinese medicine, Chinese herbal injections (CHIs) are vital for the treatment of DCM with Western medicine (WM) treatment [10–16]. Tonic CHIs refer to injections whose main components are ginseng, astragalus, and *Ophiopogon japonicus*, which have the functions of replenishing qi, nourishing blood, nourishing yin, and assisting yang, and are primarily used to treat various deficiency symptoms. The present network meta-analysis (NMA) incorporated six common tonic CHIs that are used in the treatment of DCM, namely, Huangqi injection, Shenfu injection, Shengmai injection, Shenmai injection, Shenqi Fuzheng injection, and Yiqifumai injection, to determine their efficacy. The China Food and Drug Administration authorized all of these tonic CHIs.

We retrieved the relevant RCTs, and most of the clinical trials compared the efficacy between one of the aforementioned six tonic CHIs plus WM and WM alone rather than a head-to-head comparison of the CHIs. NMA is a technique that allows researchers to meta-analyse more than two interventions simultaneously according to a combination of direct and indirect evidence [16–18]. We performed an NMA to provide an overview of the efficacy of tonic CHIs and more guidance in the selection of DCM treatment.

2. Methods

This NMA was performed according to the PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions [19]. A completed PRISMA checklist is included in the supplementary material (available here) (PRISMA Checklist).

2.1. Data Sources and Searches. The included studies were acquired after comprehensive database searches of PubMed, the Cochrane Library, Embase, the Chinese Biological Medicine Database, China National Knowledge Infrastructure, the Wanfang Database, and the Chinese Scientific Journal Database from their inception to October 15, 2020. Additional relevant studies were retrieved from the reference lists of previous meta-analyses and the included studies to avoid omission. For example, two reviewers developed the following search strategy in PubMed: (huangqi [Title/Abstract] OR astragalus [Title/Abstract] OR shenfu [Title/Abstract] OR shengmai [Title/Abstract] OR shenmai [Title/Abstract] OR shenqi fuzheng [Title/Abstract] OR yiqifumai [Title/Abstract]) AND (dilated cardiomyopathy [MeSH terms] OR dilated cardiomyopathy [Title/Abstract]) AND (randomized controlled trial [Publication Type] OR controlled clinical trial [Publication Type] OR randomized [Title/Abstract] OR placebo [Title/Abstract] OR clinical trials as topic [MeSH Major Topic] OR randomly [Title/Abstract] OR trial [Title/Abstract]) More details on the strategy of CHIs are provided in the supplementary material (available here).

2.2. Eligibility Criteria. This NMA included randomized controlled trials (RCTs) that reported the efficacy of six CHIs combined with WM for treating DCM. The predesigned study inclusion criteria for this NMA were as follows: (1) RCTs with a minimum sample size of 15 patients in each group. No limitations on language, publication year, or publication status were applied. (2) The patients were diagnosed with DCM according to standard diagnostic criteria. No restrictions on gender, race, and nationality were applied. (3) The interventions met the standard framework of "CHI + WM versus WM" or "CHI + WM versus CHI + WM." WM consisted of angiotensin-converting enzyme inhibitors, β-blockers, diuretics, and cardiotonics. Patients received other relevant therapies if they had complications during the therapeutic process. (4) The RCTs reported the clinical effective rate or left ventricular ejection fraction. Cardiac function classification conformed to the standard issued by the New York Heart Association in the United States. The clinical effective rate was calculated using this formula: (number of remarkable recovery patients + number of basic recovery patients)/total number of patients $*$ 100%. Patients in whom clinical symptoms disappeared and cardiac function improved at least 2 levels were classified as remarkable recovery, and patients with relieved clinical symptoms and an increase in cardiac function of 1 level were regarded as basic recovery. If patients' clinical symptoms and cardiac function were unaltered or worse, they were classified as patients showing deterioration. The aforementioned outcomes were set as primary outcomes, and the 6-minute walk test, left ventricular end-diastolic dimension, heart rate, cardiac output, and adverse drug reactions/adverse drug events (ADRs/ADEs) measures were evaluated as secondary outcomes. (5) The full text of the RCTs could be assessed.

2.3. Study Selection and Data Extraction. Two reviewers screened the title, abstract, and keywords of every retrieved study to determine which studies required further assessment. The full text of potential studies was examined in detail. In cases of disagreement between reviewers, a consensus was obtained via discussion or consultation with a third reviewer. A standardized data extraction form was designed using Microsoft Excel 2016 to collect data from the included RCTs. The data extraction items included the first author's name, publication year, country, patient characteristics (sample size, gender, age, patients' baseline cardiac function classification, and disease duration), details of the

intervention, outcomes, the RCT design, and the domains for a risk of bias assessment.

2.4. Quality Assessment. The Cochrane Collaboration risk of bias tool was used to evaluate the risk of bias of the included RCTs. The evaluation items included sequence generation (selection bias), allocation concealment (selection bias), blinding of patients and personnel (performance bias), blinding of outcome assessors (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. Each item was appraised as "low risk," "unclear risk," or "high risk" as a description for the included RCTs. Any conflicts were resolved via discussion or consultation.

Based on the results of the systematic review and network meta-analysis, this study adopted GRADE (Grading of Recommendations Assessment, Development, and Evaluation) to evaluate the evidence quality of outcomes. In GRADE system, RCTs are set as the highest level of evidence at the starting point, and its down rating indicators include limitations (risk of bias), inconsistency, indirectness, imprecision, and publication bias. Up rating indicators are only applied to observational studies, which include effect size, confounding factors, and dose-effect relationship.

2.5. Data Analysis. This NMA was performed for each outcome in a Bayesian hierarchical framework using WinBUGS 1.4.3 software (MRC Biostatistics Unit, Cambridge, UK). Other correlative graphical representations were processed using Stata 13.0 software. The random effects model in WinBUGS was selected. The number of iterations was set to 200,000, and the first 10,000 were used for the annealing algorithm to eliminate the impact of the initial value. For binary outcomes, an odds ratio (OR) with a 95% credible interval (95% CI) was produced. A mean difference (MD) with its 95% CI was generated for continuous outcomes. A 95% CI of OR that did not include 1.00 or a 95% CI of MD that did not include 0.00 was considered statistically significant.

To obtain the rank of each intervention for the various outcomes, this NMA calculated the surface under the cumulative ranking (SUCRA) value to estimate the ranking probabilities for each intervention. The SUCRA value was interpreted as a percentage. The SUCRA value of the best intervention was 100%. Otherwise, the SUCRA value was 0% [20, 21]. To determine the most efficacious injection in treating DCM, a cluster analysis for primary outcomes was performed [20, 22]. The consistency of direct and indirect evidence is an essential assumption of NMA [23, 24]. Therefore, if the closed loop of interventions was available, a loop-specific approach was examined to check the inconsistency of evidence. An inconsistency factor and its 95% CI was calculated to estimate the presence of inconsistency in each loop. It was deemed a consistency between direct and indirect evidence when $p > 0.05$ [25]. A funnel plot was also created to assess the publication bias [26, 27]. The network graph, the forest plot, and the contribution plot are also depicted [26, 27].

This NMA depended on previously published studies. As a result, ethical approval was not necessary.

3. Results

3.1. Study Selection. Of 558 potential studies scanned, 215 studies were further assessed. From these studies, 175 studies were excluded for the following reasons: (1) was not an RCT ($n = 2$); (2) was a retrospective study ($n = 2$); (3) was a case report ($n = 2$); (4) was not in accordance with the diagnostic criteria ($n = 115$); (5) did not conform to the requirements of the intervention ($n = 46$); (6) did not contain the relevant outcomes ($n = 4$); (7) contained repetitive data ($n = 4$). Forty-two-armed RCTs were ultimately included and analysed. All included RCTs were published and implemented in China from 1998 to 2017 (Figure 1).

3.2. Study Characteristics and Quality Evaluation. A total of 2970 patients and six CHIs were included, including 1503 patients in the treatment group and 1467 patients in the control group. Male patients were approximately 59.5% of the total, and the age of patients ranged from 20 to 81 years. Thirty-nine included RCTs compared the efficacy between one of the included CHIs plus WM and WM, and 1 RCT compared Shenmai injection plus WM with Huangqi injection plus WM. WM included angiotensin-converting enzyme inhibitors, β-blockers, cardiotonics, and diuretics. CHIs were used in the following proportions in the 40 RCTs: Huangqi injection (7 RCTs), Shenfu injection (12 RCTs), Shengmai injection (7 RCTs), Shenmai injection (12 RCTs), Shenqi Fuzheng injection (1 RCT), and Yiqifumai injection (1 RCT). All included CHIs were intravenously injected. Patients in most RCTs received CHIs once a day, and patients in only 1 RCT received a CHI twice a day. The treatment course in most trials was 14 days. According to outcomes, 90% of the RCTs reported the clinical effective rate, and 70% of RCTs investigated the left ventricular ejection fraction. Six RCTs adopted the dialectical theory based on traditional Chinese medicine in the treatment of DCM. The main characteristics of the included RCTs are shown in Table 1, and the network graphs for various outcomes are illustrated in Figure 2.

Overall, the quality of the included RCTs was general. For selection bias, 7 RCTs were deemed "low risk" because they used a random number table method to generate the sequence. Five RCTs were deemed "high risk" because they generated the sequence using some rule based on hospital or date of admission. Only 1 RCT used a single-blind method and was evaluated as "high risk" in performance bias because the colour and usage of CHIs suggested the formulation. All included RCTs reported complete outcome data and were subsequently evaluated as "low risk" in attrition bias. Two RCTs did not report all outcomes in its design and were deemed "high risk" in reporting bias. Four RCTs did not refer to whether there was comparability between the treatment group and the control group, which may broaden the difference between the results and influence the outcome data. Therefore, these 4 RCTs were assessed as "high risk" in

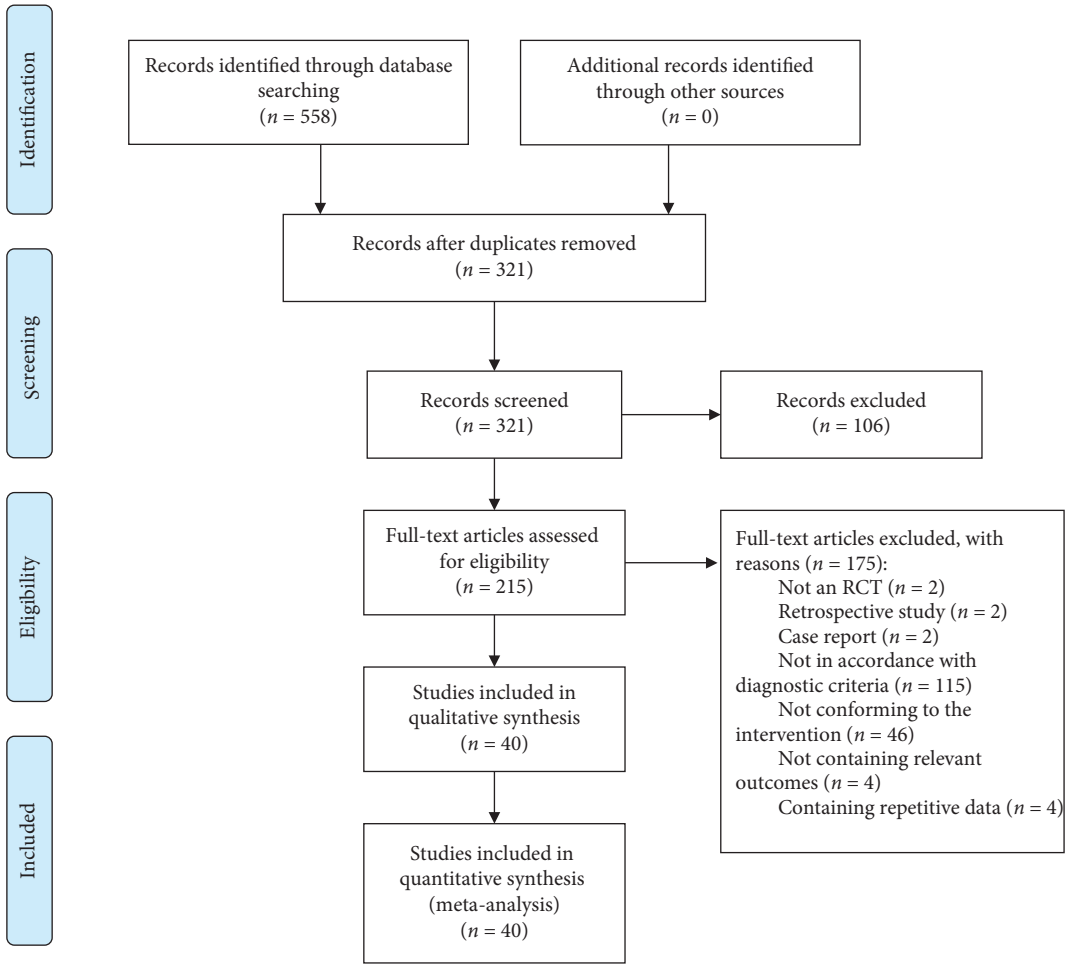


Figure 1: PRISMA flow diagram (*n*, number of articles).

other biases. The remaining items were considered "unclear risk" due to insufficient information (Figure 3).

The four outcomes including the clinical effective rate, left ventricular ejection fraction, 6-minute walk test, and left ventricular end-diastolic dimension were evaluated by GRADE classification. The other two outcomes were not graded due to the insufficient amount of literature included in their comparison. We came to the following conclusions. (1) In the NMA results of the clinical effective rate, HQI + WM vs WM, SFI + WM vs WM, SMI + WM vs WM, and HQI + WM vs SMI + WM were evaluated as low quality, and other comparisons were evaluated as very low. (2) In the results of left ventricular ejection fraction, only the comparison of YQFMI + WM vs WM was rated as low quality, and others were very low. (3) In the results of the 6-minute walk test, the comparison of SFI + WM vs WM and SI + WM vs WM was rated as medium. And the low quality was rated for SFI + WM vs SI + WM, SFI + WM vs YQFMI + WM, SI + WM vs YQFMI + WM, and YQFMI + WM vs WM. The other comparisons were rated as very low. (4) In the rating results of the left ventricular end-diastolic dimension, SFI + WM vs SMI + WM, SFI + WM vs YQFMI + WM, SFI + WM vs WM, SMI + WM vs YQFMI + WM, SMI + WM vs WM, and YQFMI + WM vs WM were all rated as low, and the rest were rated as very low. Detailed information about the result of GRADE is shown in supplementary material (available here).

3.3. Outcomes

3.3.1. The Clinical Effective Rate. The clinical effective rate was the primary outcome because it directly reflects the efficacy. There were 36 RCTs that investigated this outcome (Huangqi injection, 7 RCTs; Shenfu injection, 10 RCTs; Shengmai injection, 7 RCTs; and Shenmai injection, 12 RCTs). As shown in Table 2, all included CHIs were associated with a significantly higher improvement in the clinical effective rate. Huangqi injection + WM (OR = 0.28, 95% CI: 0.16–0.48), Shenfu injection + WM (OR = 0.21, 95% CI: 0.12–0.34), Shengmai injection + WM (OR = 0.26, 95% CI: 0.15–0.43), and Shenmai injection + WM (OR = 0.24, 95% CI: 0.16–0.37) were more effective in promoting the clinical effective rate compared to WM and had statistically significant differences. The ranking analysis suggested that Shenfu injection + WM was more efficacious for the clinical effective rate with a probability of 78.5% (Table 3). Other beneficial interventions were Shenmai injection + WM

Table 1: Characteristics of the included randomized controlled trials.

Study ID	Sample size (T/C)	Sex (M/F)	Average age (year)	Disease duration (year)	NYHA(T/C)			Treatment group	Solution of Chis	Control group	Course of treatment (day)	Outcomes
					II	III	IV					
Yang XL 2009	30/30	NR	NR	NR		NR		HQI20 ml + WM	5%GS250 ml	WM	14 d	①
Wu XH 2005	26/26	28/24	E:39.10 ± 17.92 C:41.20 ± 16.81	NR	—	14/15	12/11	HQI30 ml + WM	5%GS250 ml	WM	15 d	①②⑤
Chen DM 2005	60/60	84/36	E:42.5 C:41.7	NR		NR		HQI40 ml + WM	5%GS250 ml	WM	14 d	①
Shang WM 2007	36/36	43/29	59 ± 12	NR				HQI40 ml + WM	5%GS250 ml	WM	14 d	①②
Luo HM 2007	36/32	36/32	E:46 ± 3 C:48 ± 4	E:8.4 ± 0.3 C:8.5 ± 0.2	12/14	14/10	10/8	HQI40 ml + WM	5%GS250 ml	WM	15 d	①⑦
Gao XJ 2010	18/15	24/9	E:42 C:40	E:3–15 C:0.4–15	5/4	7/6	6/5	HQI20 ml + WM	5%GS250 ml/0.9% NS250 ml for diabetic	WM	14 d/course, 2 courses	①②
Lei HF 2011	30/30	60/0	41.5 ± 7.2	NR		NR		HQI20 ml + WM	0.9%NS250 ml	WM	15 d, some patients may be extended to 20~30 d	①⑦
Liang CC 2001	37/37	42/32	E:56 C55	NR	—	23/14	23/14	SFI20 ml + WM	5%GS100 ml	WM	—	①②④⑥
Que HX 2003	32/28	37/23	E:35–76 C:33–75	E:0.8–15 C:0.8–15	8/7	21/19	3/2	SFI60 ml + WM	5%GS250 ml or 0.9%NS250 ml	WM	14 d	①②⑥
Tang BN 2005	29/29	40/18	E:48.8 C:49.8	NR	1/2	15/17	13/10	SFI60 ml + WM	5%GS250 ml	WM	14 d/course, 2 courses	①②
Yang Y 2009	30/30	42/18	E:42.50 ± 16.81 C:40.52 ± 15.98	NR	—	8/12	22/18	SFI60 ml + WM	5%GS250 ml	WM	14 d	①②⑤⑦
Chen ZG 2009	30/27	42/15	E:21–75 C:20–76	NR	—	20/18	10/9	SFI50 ml + WM	5%GS250 ml/plus insulin for diabetic	WM	14 d	①②④⑤
Lv G 2010	31/30	44/17	E:41.5 ± 10.2 C:40.3 ± 12.5	NR	—	10/11	21/19	SFI50 ml + WM	5%GS250 ml/plus insulin for diabetic	WM	14 d	①②④⑤
Wang CK 2011	50/50	66/34	E:31 ± 9 C:32 ± 9	E:6.1 ± 1.8 C:6.5 ± 1.5	23/21	19/23	8/6	SFI1 ml/(kg·d) + WM	NR	WM	14 d	②③
Nie YJ 2012	38/40	52/26	58.5 ± 10.5	NR	13	45	20	SFI2 ml/(kg·d) + WM	5%GS250 ml	WM	10 d	①②⑤
Yu M 2013	39/40	40/39	40 ± 13	0. 5–12		NR		SFI50 ml + WM	NR	WM	14 d	②③④⑤
Zhang F 2014	40/40	44/36	E:52.1 ± 12.6 C:49.9 ± 13.8	E:6.5 ± 4.6 C:6.2 ± 5.1		NR		SFI50 ml + WM	5%GS250 ml/0.9% NS250 ml	WM	14 d	①
Wang L 2014	42/38	49/31	E:61.8 ± 10.3 C:60.8 ± 9.9	NR	—	12/30	21/17	SFI50 ml + WM	5%GS250 ml	WM	10 d	①②
Qi CH2015	60/60	82/38	E:63.00 C:62.70	NR	—	31/28	29/32	SFI60 ml + WM	5%GS250 ml	WM	14 d	①②⑥
Wu XH 2001	28/28	33/23	E:40.3 ± 15.0 C:41.5 ± 16.8	E:0.1–4.0 C:0.1–4.2	—	16/15	12/13	SI40-60 ml + WM	5%GS250 ml	WM	15 d/course, 2 courses	①②⑤⑦

Table 1: Continued.

Study ID	Sample size (T/C)	Sex (M/F)	Average age (year)	Disease duration (year)	NYHA(T/C) II	NYHA(T/C) III	NYHA(T/C) IV	Treatment group	Solution of Chis	Control group	Course of treatment (day)	Outcomes
Zhang YC 2002	50/50	59/41	E:58 ± 15 C:60 ± 16	E:0.5–8.0 C:0.6–8.0	10/10	35/36	5/4	SI60 ml + WM	5%GS250 ml/0.9% NS250 ml	WM	14d	①②⑥
Wang H 2006	30/20	30/20	E:41.0 ± 15.0 C:39.5 ± 13.5	E:0.5–6.0 C:0.4–5.5	11/9	14/9	5/2	SI40-60 ml + WM	5%GS250 ml	WM	14 d/course, 2 courses	①④
Li W 2006	30/22	35/17	E:39.5 C:37.6	E:4.8 C:4.2	13/10	11/8	6/4	SI60 ml + WM	5%GS250 ml/0.9% NS250 ml	WM	14 d	①②④⑥
Wu XL 2009	30/30	42/18	E:45–81 C:41–79	E:0.7–7 C:0.6–8	8/8	16/18	6/4	SI60 ml + WM	5%GS250 ml	WM	14 d	①
Li BH2015	30/30	40/20	53 ± 6	NR		NR		SI50 ml + WM	5%GS500 ml	WM	10 d	①②③④⑦
Shi L 2017	38/38	43/33	E:45.05 ± 7.15 C:46.08 ± 7.12	E: 9.31 ± 3.51 C:9.16 ± 3.37	14/12	15/16	9/10	SI40 ml + WM	5%GS250 ml	WM	14 d/course, 2 courses	①②⑤⑥⑦
Wang NX 2006	15/15	25/5	E:36–59 C:39–58	NR		NR		SMI60 ml + WM	5%GS250 ml/0.9% NS250 ml	WM	15 d	①②
Wang X 2008	30/30	34/26	E:39.6 C:41.2	NR	9/8	15/16	6/6	SMI50 ml + WM	5%GS250 ml	WM	7 d/course, 4 courses	①
Cao Y 2011	111/111	140/82	E:36–59 C:39–58	NR		NR		SMI60 ml + WM	5%GS250 ml	WM	15 d	①
Wang AC 2011	30/30	—	—	NR		NR		SMI100 ml + WM	NR	WM	28 d	①②③⑦
Chen XY 2012	34/34	38/30	42.2	NR	18/19	14/11	2/4	SMI60 ml + WM	5%GS250 ml	WM	14 d	①⑦
Cao L 2012	24/22	31/15	E:42.6 ± 9.8 C:40.1 ± 15.2	NR	—	18/16	6/6	SMI60 ml + WM	5%GS150 ml	WM	14 d	①②⑦
Tian HM 2012	30/30	34/26	E:48.6 ± 5.8 C:49.8 ± 2.5	NR	8/12	14/12	8/6	SMI50 ml + WM	5%GS250 ml	WM	7 d/course, 3 courses	①
Wu JJ 2013	30/30	41/19	—	E:0.3–10 C:0.3–11		NR		SMI60 ml + WM	5%GS250 ml/0.9% NS250 ml for diabetic	WM	14 d	①②⑦
Yan GQ 2014	58/58	62/54	40.6	NR	22/16	28/33	8/9	SMI60 ml + WM	5%GS250 ml	HQI40 ml + WM	15 d	①
Zhao XR 2015	50/50	57/43	E:54.2 ± 4.5 C:54.7 ± 4.2	E: 5.2 ± 1.9 C:5.1 ± 1.5	6/5	25/26	19/19	SMI60 ml + WM	5%GS150 ml	WM	14 d	①②③④⑦
Wang JY 2016	30/30	34/26	E:59.3 ± 8.6 C:60.7 ± 10.2	NR	6/24	10/20	—	SMI100 ml + WM	NR	WM	14 d	①②③⑥
Song CH 2017	50/50	52/48	E:35.25 ± 4.61 C:36.06 ± 4.05	NR	12/10	29/26	9/14	SMI40 ml + WM	5%GS250 ml	WM	14d	①②④⑥⑦
Duan Y 2006	40/42	39/43	E:63.8 ± 4.5 C:59.9 ± 5.2	E:12.6 ± 6.9 C:13.4 ± 7.2	3/4	18/20	19/18	SQFZI250 ml + WM	—	WM	14d	②
Li GK 2014	41/39	44/36	E:67 ± 8 C:66 ± 7	NR	28/25	13/14	—	YQFMI2.6 g + WM	5%GS250 ml	WM	14d	②③④

C, control group; F, female; GS, dextrose solution; HQI, Huangqi injection; M, male; NR, not related; NS, normal saline; SFI, Shenfu injection; SI, Shengmai injection; SMI, Shenmai injection; SQFZI, Shenqi Fuzheng injection; T, treatment group; YQFMI, Yiqifumai injection. Outcomes: ① the clinical effective rate, ② left ventricular ejection fraction, ③ 6-minute walk test, ④ left ventricular end-diastolic dimension, ⑤ heart rate, ⑥ cardiac output, ⑦ ADRs/ADEs.

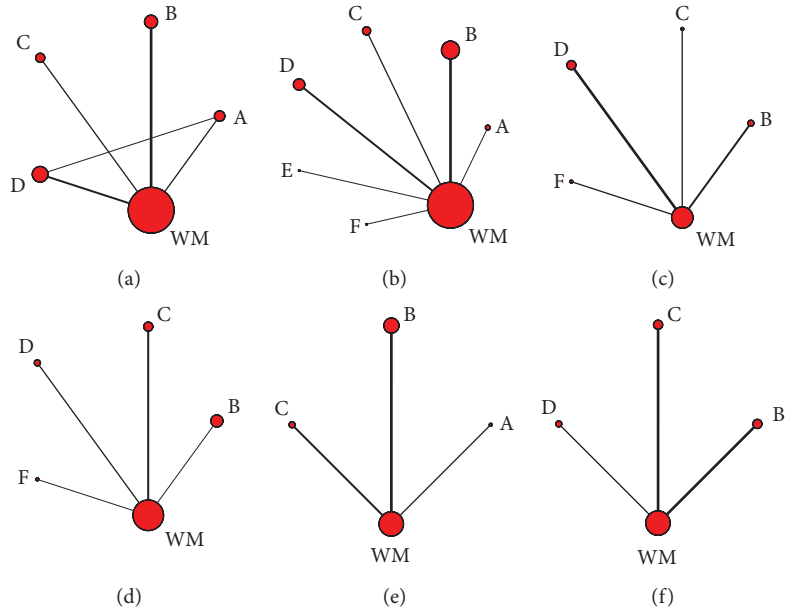


Figure 2: Network graphs for different outcomes. A, Huangqi injection + WM; B, Shenfu injection + WM; C, Shengmai injection + WM; D, Shenmai injection + WM; E, Shenqi Fuzheng injection + WM; F, Yiqifumai injection + WM. (a) The clinical effective rate. (b) Left ventricular ejection fraction. (c) 6-minute walk test. (d) Left ventricular end-diastolic dimension. (e) Heart rate. (f) Cardiac output.

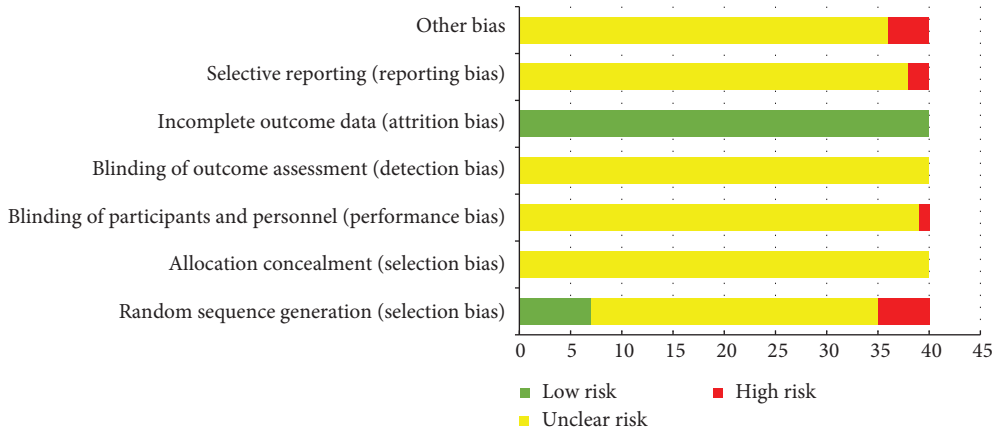


Figure 3: Assessment of risk bias.

(64.0%) and Shengmai injection + WM (58.5%) (Figure 4). The forest plot and the contribution plot for the clinical effective rate are shown in Figures 5 and 6.

3.3.2. Left Ventricular Ejection Fraction. The left ventricular ejection fraction was also deemed a primary outcome. DCM is often accompanied by heart failure, and the left ventricular ejection fraction is a diagnostic index of heart failure and a reflection of patient prognosis [3]. Twenty-eight RCTs identified the left ventricular ejection fraction (Huangqi injection, 3 RCTs; Shenfu injection, 11 RCTs; Shengmai injection, 5 RCTs; Shenmai injection, 7 RCTs; Shenqi Fuzheng injection, 1 RCT; and Yiqifumai injection, 1 RCT). Shenfu injection + WM (MD = 7.43, 95% CI: 2.41–12.38) and Shengmai injection + WM (MD = 3.88, 95% CI: 1.10–8.05) were associated with a significantly higher mean difference in left ventricular ejection fraction than WM (Table 2). Based on its SUCRA, Shenfu injection + WM was the best in increasing left ventricular ejection fraction with a probability of 71.9%, Shenqi Fuzheng injection + WM (62.5%) was ranked second, and Yiqifumai injection + WM (59.7%) was ranked third (Table 3, Figure 4). The forest plot and the contribution plot for the left ventricular ejection fraction are shown in Figures 5 and 6.

3.3.3. Secondary Outcomes. Data on the 6-minute walk test were available from 7 RCTs (Shenfu injection, 2 RCTs; Shengmai injection, 1 RCT; Shenmai injection, 3 RCTs; and Yiqifumai injection, 1 RCT). The MD results demonstrated that Shenfu injection + WM (MD = 50.39, 95% CI:

Table 2: Odds ratio/mean difference (95% CIs) of various interventions for all interventions.

Intervention	The clinical effective rate	Left ventricular ejection fraction	6-minute walk test	Left ventricular end-diastolic dimension	Heart rate	Cardiac output
Huangqi injection + WM versus						
Shenfu injection + WM	1.32 (0.63, 2.77)	−3.03 (−14.61, 9.05)	—	—	9.73 (30.64, 51.04)	—
Shengmai injection + WM	1.10 (0.52, 2.31)	0.33 (−11.01, 11.62)	—	—	8.08 (−31.68, 48.92)	—
Shenmai injection + WM	1.14 (0.62, 2.11)	−0.06 (−12.70, 13.15)	—	—	—	—
Shenqi Fuzheng injection + WM	—	−3.07 (−23.08, 16.11)	—	—	—	—
Yiqifumai injection + WM	—	−2.59 (−23.88, 18.61)	—	—	—	—
WM	**0.28 (0.16, 0.48)**	4.35 (−6.35, 15.39)	—	—	2.33 (−36.19, 48.92)	—
Shenfu injection + WM versus						
Shengmai injection + WM	0.83 (0.40, 1.72)	3.43 (−3.04, 9.22)	3.30 (−43.76, 50.75)	−0.82 (−9.73, 8.41)	−1.75 (−19.17, 15.46)	−0.06 (−2.50, 2.49)
Shenmai injection + WM	0.87 (0.44, 1.64)	3.04 (−5.68, 11.62)	23.68 (−60.90, 117.00)	1.22 (−10.09, 12.33)	—	−0.18 (−3.33, 2.85)
Shenqi Fuzheng injection + WM	—	−0.11 (−17.75, 16.76)	—	—	—	—
Yiqifumai injection + WM	—	0.27 (−18.05, 19.07)	25.02 (−47.42, 101.60)	−3.03 (−20.58, 14.69)	—	—
WM	**0.21 (0.12, 0.34)**	**7.43 (2.41, 12.38)**	**50.39 (25.78, 76.33)**	−3.37 (−10.95, 4.39)	−7.55 (−20.73, 5.86)	0.48 (−1.22, 2.51)
Shengmai injection + WM versus						
Shenmai injection + WM	1.05 (0.54, 2.07)	−0.37 (−7.75, 7.38)	20.95 (−71.87, 122.00)	1.99 (−7.90, 11.50)	—	−0.12 (−3.24, 2.78)
Shenqi Fuzheng injection + WM	—	−3.42 (−20.29, 13.08)	—	—	—	—
Yiqifumai injection + WM	—	−3.06 (−20.89, 15.47)	22.42 (−60.02, 105.10)	−2.29 (−18.64, 14.20)	—	—
WM	**0.26 (0.15, 0.43)**	**3.88 (1.10, 8.05)**	**46.43 (5.27, 88.48)**	−2.47 (−7.92, 2.27)	−5.79 (−16.50, 5.00)	0.51 (−1.04, 2.41)
Shenmai injection + WM versus						
Shenqi Fuzheng injection + WM	—	−3.10 (−20.97, 14.20)	—	—	—	—
Yiqifumai injection + WM	—	−2.74 (−20.97, 14.20)	1.80 (−114.00, 109.70)	−4.03 (−22.14, 13.23)	—	—
WM	**0.24 (0.16, 0.37)**	4.36 (−2.47, 11.31)	26.47 (−65.86, 109.60)	−4.53 (−12.86, 3.91)	—	0.65 (−1.62, 3.38)
Shenqi Fuzheng injection + WM versus						
Yiqifumai injection + WM	—	0.60 (−23.54, 24.96)	—	—	—	—
WM	—	7.48 (−8.75, 24.20)	—	—	—	—
Yiqifumai injection + WM versus						
WM	—	7.13 (−10.87, 24.71)	24.37 (−46.95, 95.41)	−0.31 (−16.01, 15.41)	—	24.37 (−46.95, 95.41)

Bold results indicate statistical significance.

25.78–76.33) and Shengmai injection + WM (MD = 46.43, 95% CI: 5.27–88.48) had statistically significant differences compared to WM in the 6-minute walk test (Table 2). The ranking analysis indicated that Shenfu injection + WM was the most favourable intervention in promoting a 6-minute walk test with a probability of 75.2% (Table 3, Figure 4).

Data on the left ventricular end-diastolic dimension were available from 10 RCTs (Shenfu injection, 4 RCTs; Shengmai injection, 3 RCTs; Shenmai injection, 2 RCTs; and Yiqifumai injection, 1 RCT). The MD results suggested no statistically significant decreases between the included interventions for the left ventricular end-diastolic dimension (Table 2). The ranking analysis demonstrated that Shenmai injection + WM was best at decreasing left ventricular end-diastolic dimension with a probability of 69.5% (Table 3, Figure 4). A total of 8 RCTs that included three CHIs

Table 3: Ranking probability of the various interventions among all interventions.

Intervention	The clinical effective rate		Left ventricular ejection fraction		6-minute walk test		Left ventricular end-diastolic dimension		Heart rate		Cardiac output	
	SUCRA (%)	Rank	SUCRA (%)	Rank	SUCRA (%)	Rank	SUCRA (%)	Rank	SUCRA (%)	Rank	SUCRA (%)	Rank
Huangqi injection + WM	49.0	4	48.1	5	—	—	—	—	37.9	3	—	—
Shenfu injection + WM	78.5	1	71.9	1	75.2	1	60.8	2	70.9	1	54.9	3
Shengmai injection + WM	58.5	3	46.5	6	69.9	2	56.7	3	64	2	60.4	2
Shenmai injection + WM	64.0	2	49.0	4	46.7	3	69.5	1	—	—	60.9	1
Shenqi Fuzheng injection + WM	—	—	62.5	2	—	—	—	—	—	—	—	—
Yiqifumai injection + WM	—	—	59.7	3	44.7	4	39.8	4	—	—	—	—
WM	0.00	5	12.2	7	13.5	5	23.1	5	27.2	4	23.8	4

(Huangqi injection, 1 RCT; Shenfu injection, 5 RCTs; and Shengmai injection, 2 RCTs) contributed to the analysis of heart rate. No significant differences were observed between the various interventions (Table 2). Based on the ranking analysis, Shenfu injection + WM attained the highest rank in heart rate relief with a probability of 70.9% (Table 3, Figure 4). Eight RCTs also tested cardiac output (Shenfu injection, 3 RCTs; Shengmai injection, 3 RCTs; and Shenmai injection, 2 RCTs). None of the included interventions produced significant improvements in cardiac output (Table 2). The ranking analysis showed that Shenmai injection + WM had the best impact on boosting cardiac output with a probability of 60.9% (Table 3, Figure 4).

3.3.4. Cluster Analysis and Radar Presentation. A cluster analysis was performed for the primary outcomes to evaluate the best intervention for the treatment of DCM. Shenfu injection + WM was the farthest from zero relative to the other interventions, which suggested that it was more effective than the other treatments for DCM (Figure 7).

As a way to synthesize the SUCRA results of the various interventions across outcomes, this NMA created a pictorial presentation for the outcomes via a radar map. If the injection exhibited outstanding efficacy relative to other treatments for a certain outcome, it would appear on the outermost side of the corresponding line in the radar map. Figure 8 shows that the Shenfu injection excelled at increasing the clinical effective rate, left ventricular ejection, 6-minute walk test, and calming heart rate.

3.3.5. Publication Bias. Publication bias was detected using funnel plots for the primary outcomes. Visual inspection showed asymmetry in the clinical effective rate, but the funnel plot of the left ventricular ejection fraction did not display any asymmetry (Figure 9).

3.3.6. Inconsistency Test. Loop-specific analysis did not find any inconsistency in the clinical effective rate. The p-value of the loop of Huangqi injection + WM-Shenmai injection + WM-WM was above 0.05, and the inconsistency factor was 1.072 (0.00, 2.41), which determined that the direct and indirect evidence were consistent.

3.3.7. ADRs/ADEs. Nine RCTs (Huangqi injection, 2 RCTs; Shenfu injection, 1 RCT; Shengmai injection, 1 RCT; and Shenmai injection, 5 RCTs) reported that there were no ADRs/ADEs during the treatment, and 3 RCTs reported that ADRs/ADEs had occurred. The remaining RCTs did not report ADRs/ADEs in their publication. The interventions resulting in the ADRs/ADEs in the 3 RCTs were the control group WM in one RCT and the treatment group Shengmai injection + WM in two RCTs. In Li's study [28], 1 case of hypotension occurred in the treatment group, and 7 cases of hypotension and palpitation occurred in the corresponding control group. In Shi's research [29], the treatment group experienced 2 cases of rash, 3 cases of insomnia, 2 cases of arrhythmia, and 1 case of flush, and the corresponding control group experienced 1 case of rash, 2 cases of insomnia, 2 cases of arrhythmia, and 1 case of flush. One RCT [30] reported 2 cases of mild gastrointestinal reaction in the Shenmai injection + WM treatment group. All of the symptoms were treated and did not affect the results.

4. Discussion

DCM has received increasing attention due to its prevalence and high mortality, and with the adoption of tonic CHIs in the treatment of DCM based on WM, its efficacy has been perfected. Traditional Chinese medicine theory generally believes that the cause of DCM is congenital deficiency and acquired disorders. With the pathogenesis of "ben xu biao shi," i.e., low physical fitness and severe disease symptoms, it is necessary to use WM to relieve symptoms and use tonic drugs to regulate the foundation of the innate condition. Although most RCTs and pairwise meta-analyses investigated CHIs' efficacy, the number of RCTs that directly compared tonic CHI treatments was insufficient. Based on

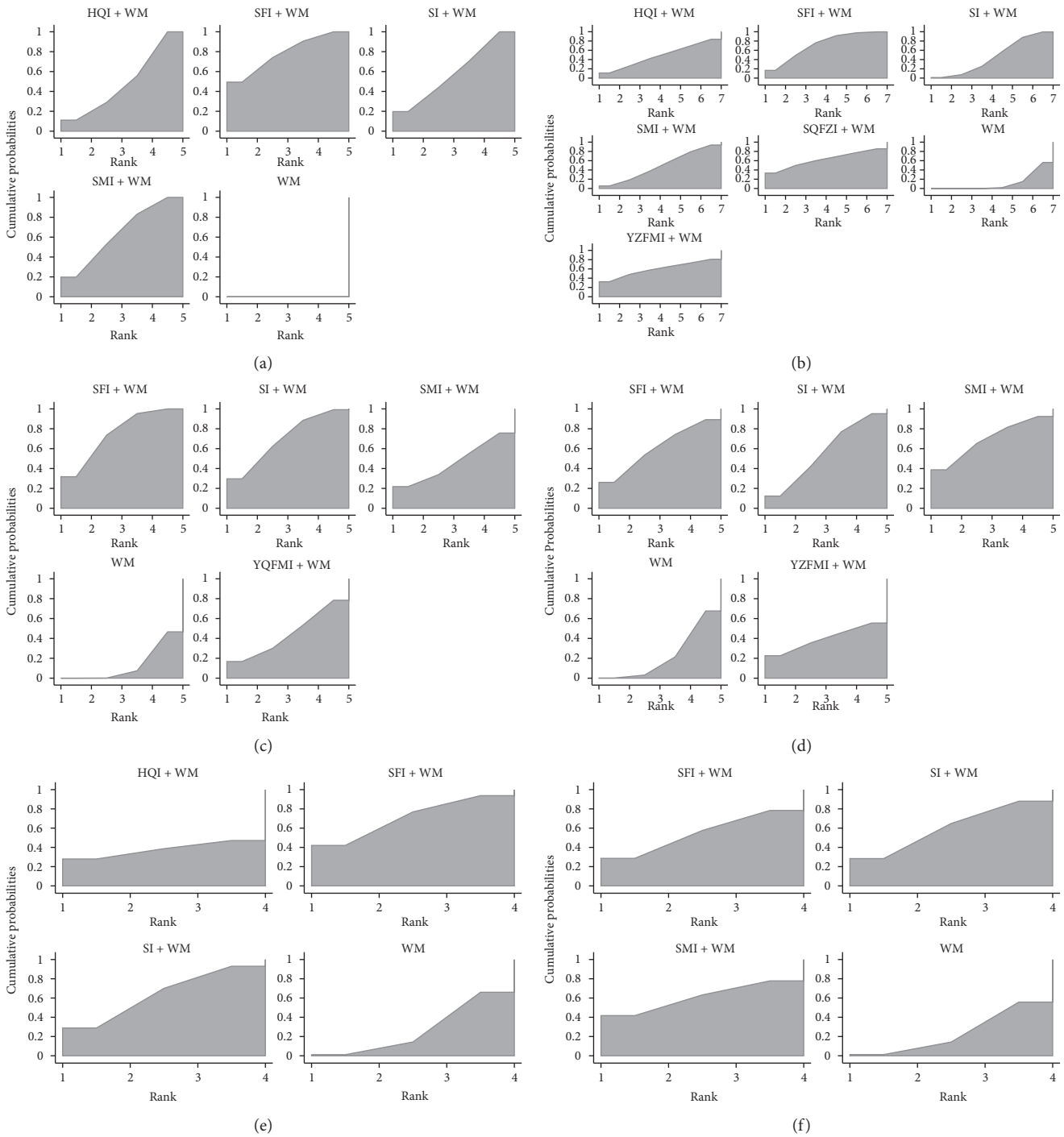
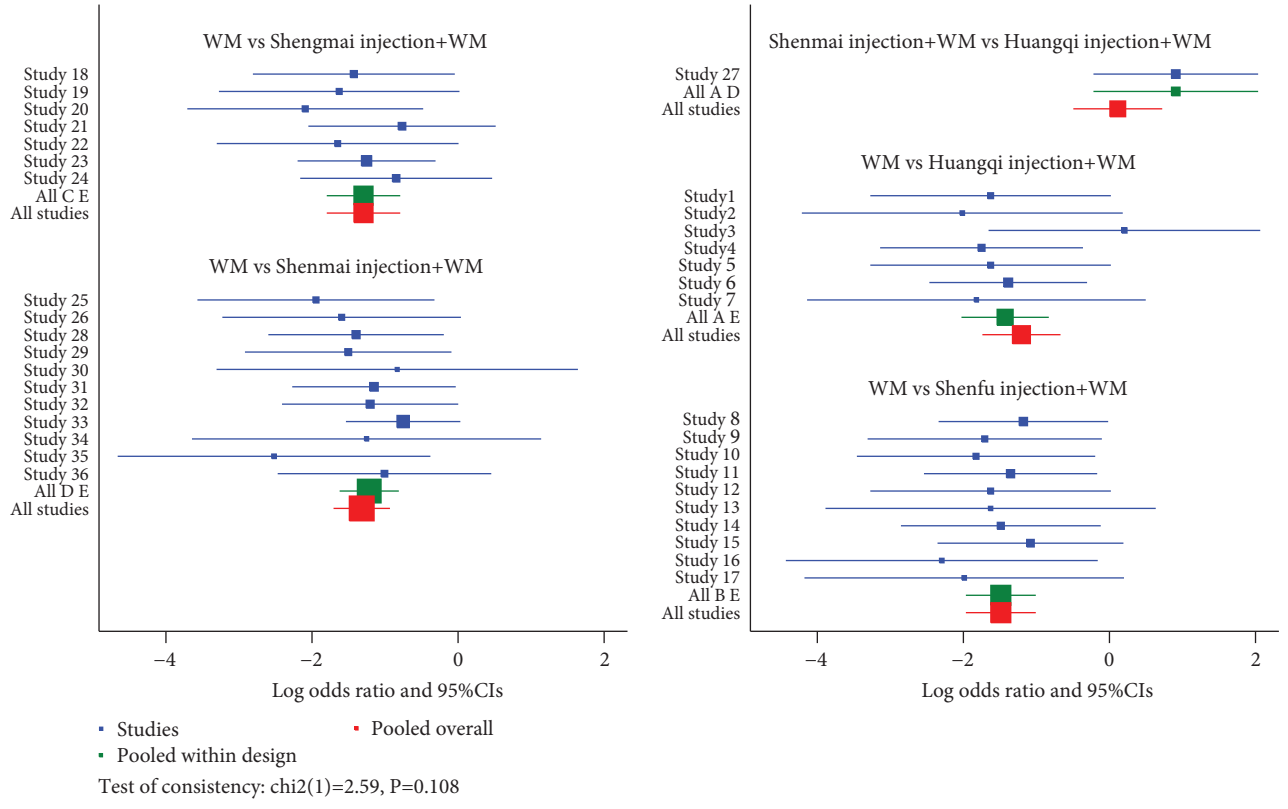


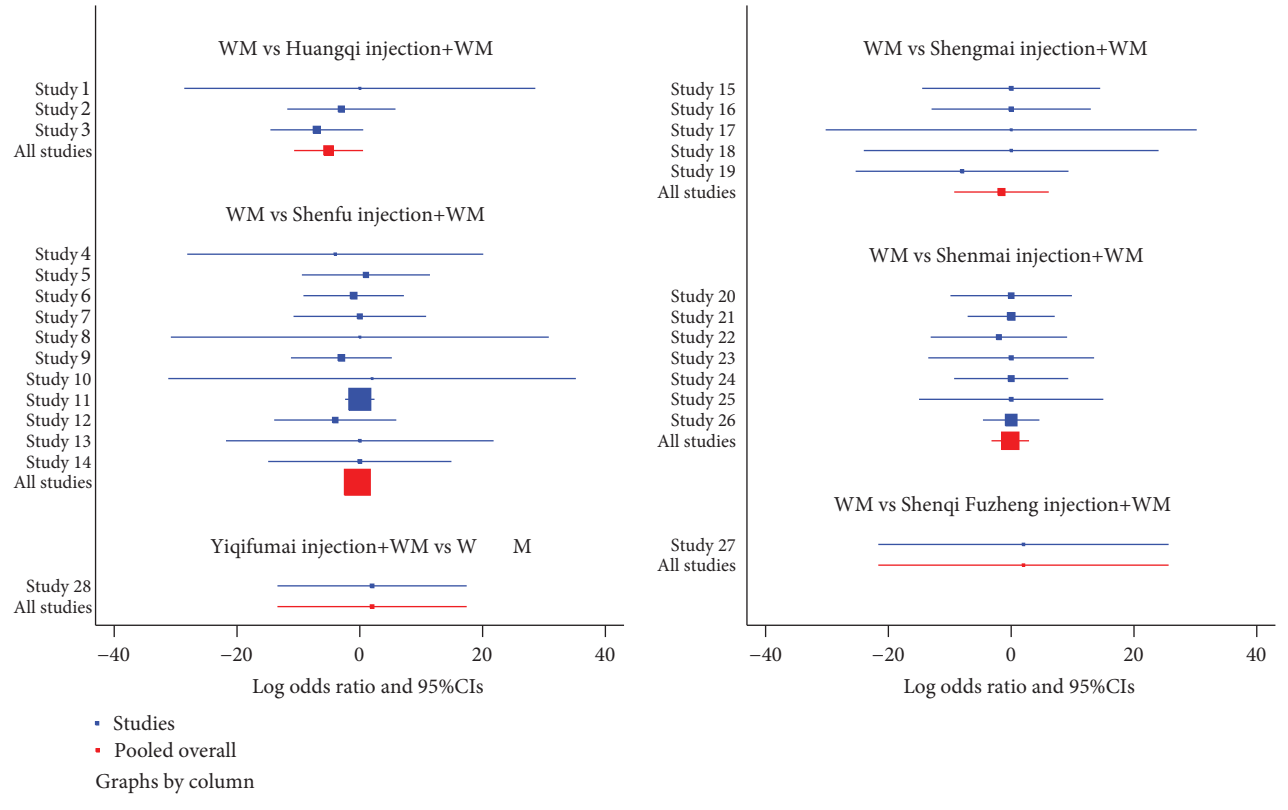
Figure 4: Plot of the surface under the cumulative ranking curves for all interventions for different outcomes. (a) The clinical effective rate. (b) Left ventricular ejection fraction. (c) 6-minute walk test. (d) Left ventricular end-diastolic dimension. (e) Heart rate. (f) Cardiac output.

this lack of studies, clinicians cannot acquire an overview of the efficacy of various tonic CHIs. However, an NMA may address this void because it is useful to have comparative efficacies in the absence of head-to-head comparisons. The ranking analysis of the CHIs in the treatment of DCM was verified [31, 32]. We performed an NMA to examine the efficacy of these seven interventions: Huangqi injection + WM, Shenfu injection + WM, Shengmai injection + WM, Shenmai injection + WM, Shenqi Fuzheng injection + WM, Yiqifumai injection + WM, and WM.

There were three principal findings that provided new evidence on the efficacy of tonic CHIs for the treatment of DCM: (1) relative to other treatments, Shenfu injection + WM resulted in a significantly greater increase in the clinical effective rate, left ventricular ejection fraction, and 6-minute walk test; (2) with respect to left ventricular end-

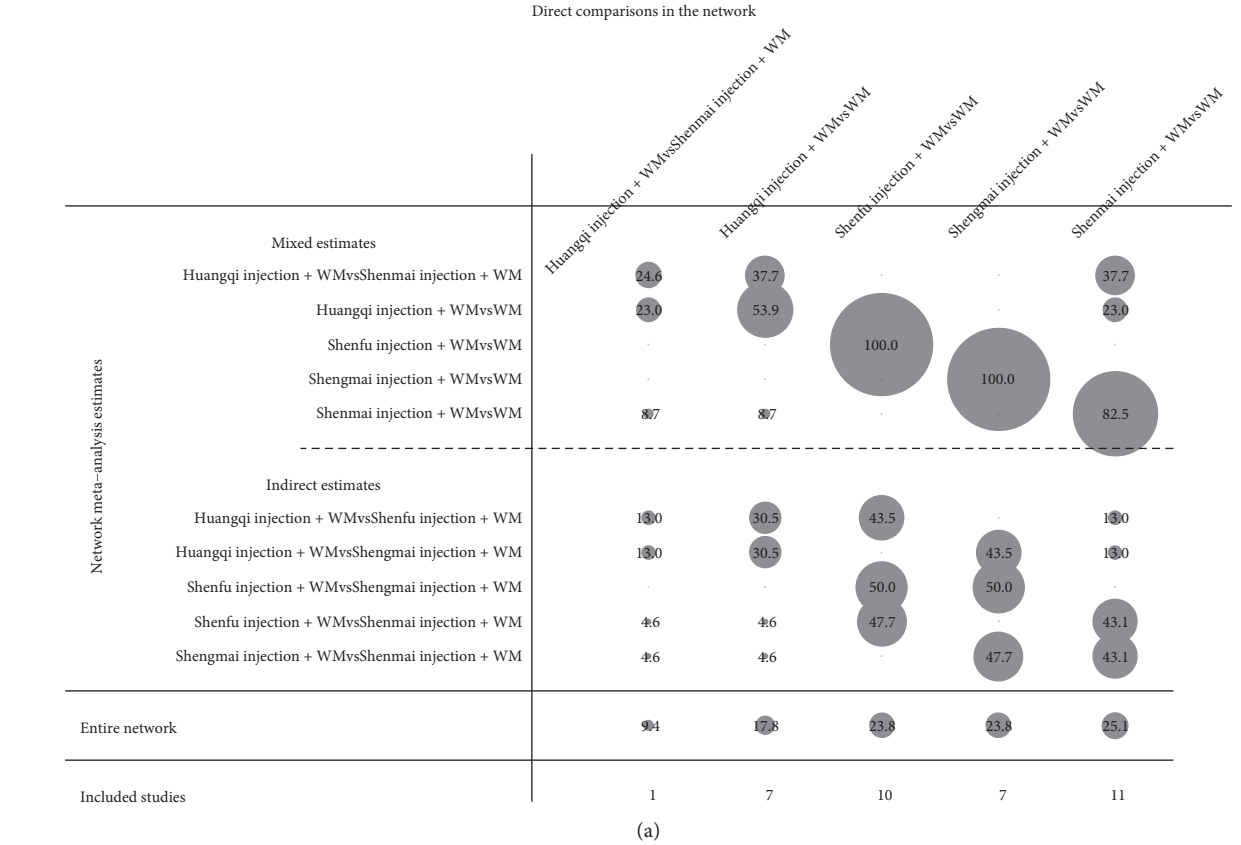


(a)

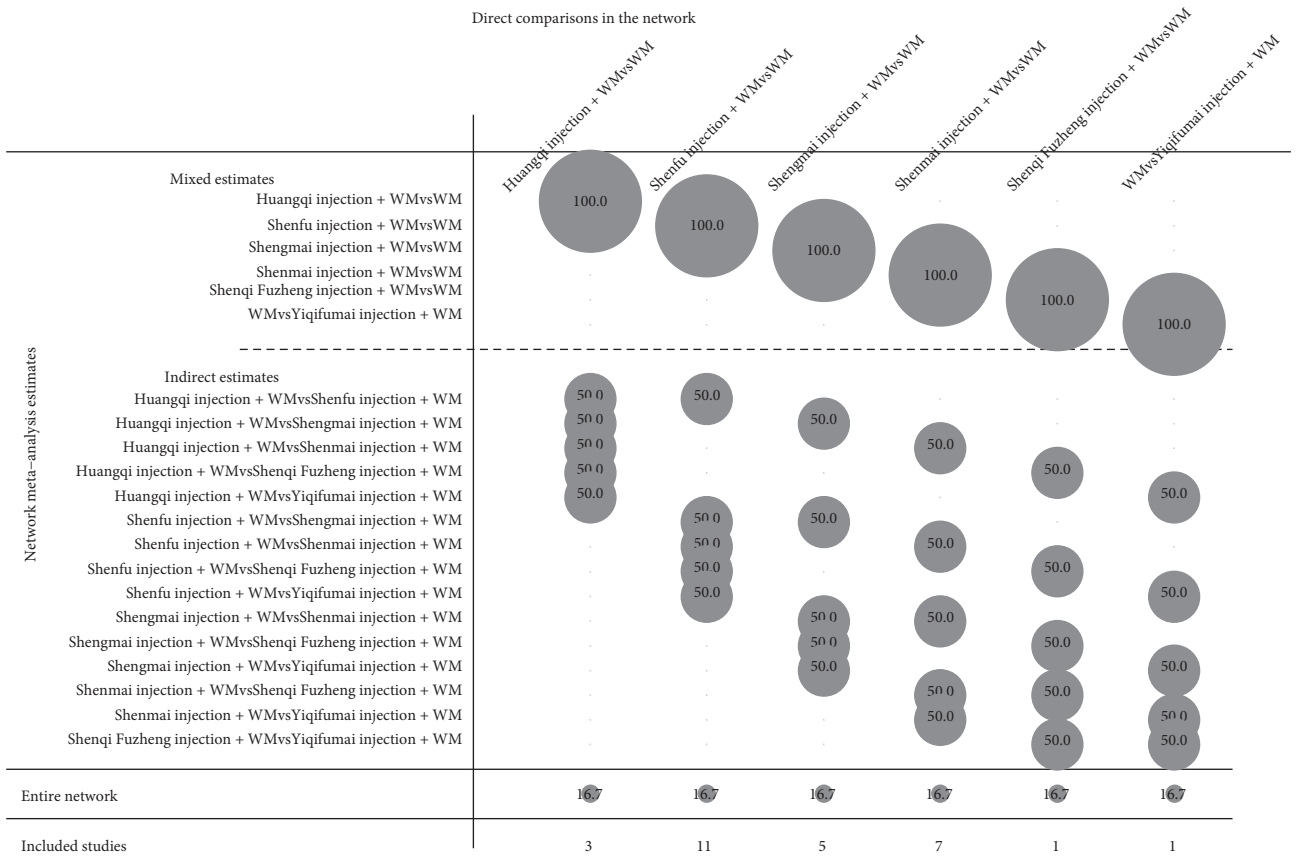


(b)

FIGURE 5: Network forest plot for the clinical effective rate and left ventricular ejection fraction. (a) The clinical effective rate. (b) Left ventricular ejection fraction.



(a)



(b)

Figure 6: Network contribution plot for the clinical effective rate and left ventricular ejection fraction. (a) The clinical effective rate. (b) Left ventricular ejection fraction.

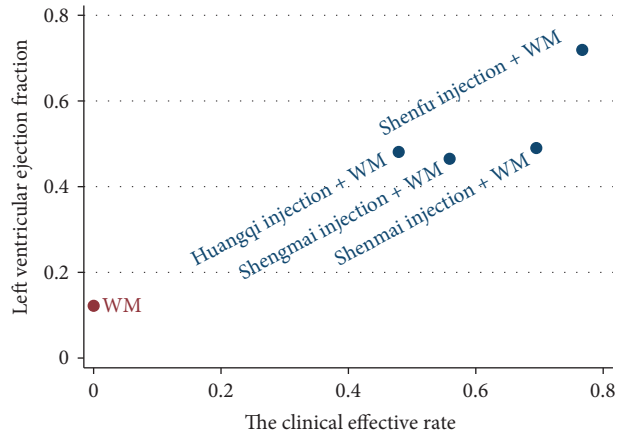


Figure 7: Cluster analysis plot for four outcomes.

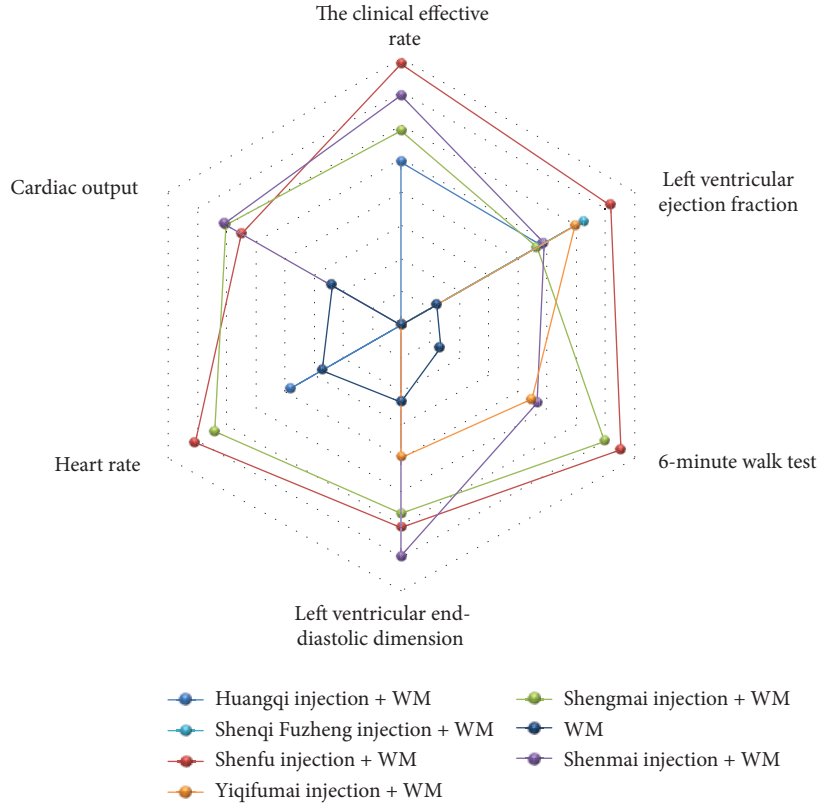


Figure 8: Radar map of different outcomes.

diastolic dimension, heart rate, and cardiac output, Huangqi injection + WM, Shenfu injection + WM, Shengmai injection + WM, Shenmai injection + WM, Shenqi Fuzheng injection + WM, and Yiqifumai injection + WM presented no significant differences between treatments and had a good impact on these outcomes; and (3) the safety of the included CHIs could not be ascertained due to insufficient information. Although most comparisons revealed no significant differences in the NMA, the SUCRA values demonstrated the ranking probability of the included interventions. Shenfu injection + WM obtained a high probability of becoming the most efficacious intervention for the clinical effective rate, left ventricular ejection fraction, 6-minute walk test, and heart rate. Shenmai injection + WM ranked ahead of the other treatments in left ventricular end-diastolic dimension and cardiac output. Based on the SUCRA value, the cluster analysis suggested that Shenfu injection + WM was the most effective intervention for DCM. Shenfu injections stemmed from Shenfu Tang, which is generally used for cardiovascular diseases in China [33]. Its major ingredients are the extracts of Ginseng Radix et Rhizoma Rubra and Aconm Lateralis Radix Praeparata, and Shenfu injections tonify qi, restore yang, and prevent exhaustion [34]. Pharmacological research revealed that

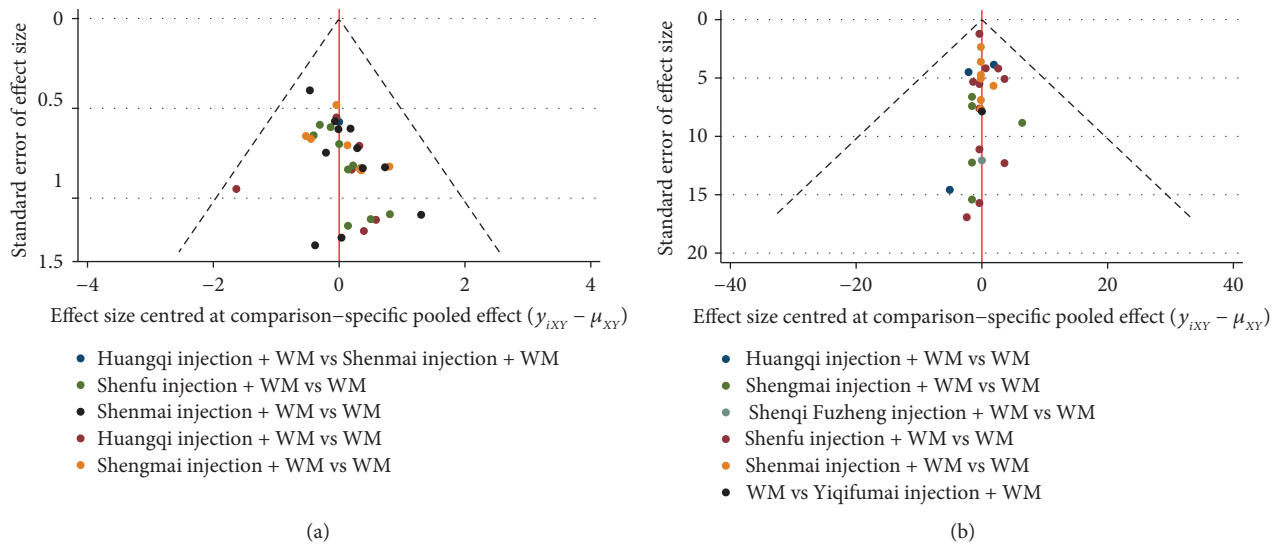


Figure 9: Funnel plot for the clinical effective rate and left ventricular ejection fraction. (a) The clinical effective rate. (b) Left ventricular ejection fraction.

Shenfu injection had a positive inotropic effect [35]. It promotes ventricle contractility, reduces blood viscosity, and optimises myocardium blood flow [33]. Several pairwise meta-analyses showed that Shenfu injection plus WM exhibited a superior capability to increase efficacy in a 6-minute walk test and a lower left ventricular end-diastolic dimension [12].

The safety of the interventions is also an essential consideration for clinicians. Approximately two-thirds of the included RCTs did not report ADRs/ADEs, which meant clinicians paid less attention to this aspect. However, CHIs are delivered via intravenous administration, and their ADR/ADE risk is higher than other administration routes [36]. Therefore, it is the responsibility of the clinicians to focus on its safety, and particularly on its dose and the solution [37]. In this NMA, three of the included RCTs exceeded the specified dose, and 2 RCTs injected CHIs based on the patients' weight, which may have also exceeded the regulated dose. Only 1 of these RCTs showed no ADRs/ADEs in the treatment process. However, the remaining RCTs did not mention the safety situation due to lower concentrations. The Huangqi injection specification did not mention the solution, and Shenqi Fuzheng injection did not need a solution. The remaining CHIs in the included RCTs abided by the specifications. Although most RCTs used solutions that conformed to the specification, it is necessary to provide a more detailed description. For example, the specification should state whether 0.9% sodium chloride or insulin was used for diabetes.

Based on the design and contents, three merits enhanced the credibility of this NMA. First, this NMA based on a Bayesian analysis included a comprehensive literature search and examined the comparative efficacy of six CHIs for the first time. The Bayesian model was the most applicable approach for addressing the multiple interventions in this NMA, and its posterior probability provided a ranking of the different treatments [23, 38]. Second, a strict eligibility criterion was formulated before implementing the NMA to ensure the baseline of the included RCTs and reduce the clinical heterogeneity. Third, in addition to the clinical effective rate and left ventricular ejection fraction as primary outcomes, it was pivotal to analyse the 6-minute walk test, left ventricular end-diastolic dimension, heart rate, and cardiac output to assess multiple aspects of the patients' cardiac condition.

Several limitations are worthy of being mentioned. First, only RCTs performed in China were included, which leads to a potential publication bias. The imbalance in the clinical status of the patients between the included RCTs may have affected the results due to patients with various cardiac function classifications and disease durations. Second, the sample size for a few of the outcomes could have been more comprehensive. When calculating continuous outcomes, it was necessary to calculate the data before and after the RCTs. However, several RCTs merely reported the latter, and they could not be included in this NMA. Third, the insufficient methodological information provided for the RCTs restricted the credibility of results and directly led to the low rate of GRADE results in this study. Previous research demonstrated that an RCT that does not apply allocation concealment and blinding to its implementation may overrate the efficacy. Most RCTs did not conceal the allocation or use complete blinding, which resulted in an overestimation of the effects of the included CHIs.

Because of the foregoing shortcomings, we propose several suggestions for future RCTs on CHIs. It is imperative to register in advance and be congruent with the CONSORT standard for RCTs as a way of being retrospective [39]. In an attempt to reveal the objective efficacy of CHIs, researchers should better implement RCTs using allocation concealment and blinding when possible. Although blinding methods are difficult for RCTs of CHIs, it is possible to blind assessors to lower detection bias. Furthermore, clinicians should present dialectical theory in the treatment of DCM, which is a

notable feature of traditional Chinese medicine theory. If a syndrome differentiation was determined for patients when diagnosed, meta-analysis researchers could analyse the clinical data from various types of patients and provide specific advice.

5. Conclusions

The present NMA compared 6 tonic CHIs in the treatment of DCM and revealed several findings. The results demonstrated that Shenfu injection plus Western medicine exerted a positive effect on improving the overall efficacy of DCM treatment, especially in the clinical effective rate, left ventricular ejection fraction, and 6-minute walk test. Shenmai injection plus Western medicine had a good curative effect in left ventricular end-diastolic dimension and cardiac output. However, it is necessary for clinicians to make diagnostic decisions relying on the efficacy of the CHIs and the patients' situation. Further evidence with larger sample sizes and higher quality is needed to boost the conclusions of this NMA.

Abbreviations

95% CI:	95% credible interval
ADRs/ ADEs:	Adverse drug reactions/adverse drug events
CHIs:	Chinese herbal injections
DCM:	Dilated cardiomyopathy
GRADE:	Grading of Recommendations Assessment, Development, and Evaluation
MD:	Mean difference
NMA:	Network meta-analysis
OR:	Odds ratio
RCTs:	Randomized controlled trials
SUCRA:	The surface under the cumulative ranking
WM:	Western medicine.

Data Availability

The datasets generated during the analysis are not publicly available because the analysis process is the core of the results and cannot be made public. Requests to access the datasets of included studies can be met by checking out them in databases. The study gathered data from Embase, PubMed, the Cochrane Library, the China National Knowledge Infrastructure Database (CNKI), the Wanfang Database, the CQVIP Database (VIP), and the China Biology Medicine disc (SinoMed) database.

Disclosure

Kaihuan Wang and Haojia Wang should be regarded as the co-first authors.

Conflicts of Interest

The authors declare the there are no conflicts of interest.

Authors' Contributions

Kaihuan Wang contributed to conceptualization, methodology, formal analysis, original draft preparation, and visualization. Haojia Wang contributed to writing of original draft, reviewing and editing, and supervision. Jiarui Wu contributed to conceptualization, funding acquisition, and resources. Xiaojiao Duan contributed to formal analysis and supervision. Xinkui Liu contributed to formal analysis. Dan Zhang contributed to methodology. Shuyu Liu and Mengwei Ni contributed to reviewing and editing. Ziqi Meng wrote the original draft. Xiaomeng Zhang contributed to methodology. Kaihuan Wang and Haojia Wang contributed to the work equally.

Acknowledgments

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Supplementary Materials

This file contains five parts, which include items regarding the PRISMA checklist for network meta-analysis and corresponding pages of this study, the search strategy of traditional Chinese medicine injections in Chinese databases and PubMed database, information of included RCTs, details about the product information of CHIs, and the outcome and rate in GRADE of each outcome. (*Supplementary Materials*)

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Research Article

Evaluating the Long-Term Efficacy of Acupuncture Therapy for Subacute Poststroke Aphasia: Study Protocol for a Randomized, Blinded, Controlled, Multicentre Trial

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Background. Poststroke aphasia (PSA) is a disabling condition that decreases the quality of life, and the duration of the disease harms the quality of life of PSA patients. Acupuncture has been widely employed for PSA. There is some evidence for the immediate treatment efficacy of acupuncture for PSA; however, long-term results after acupuncture may be poorer. *Methods*. This is a multicentre, randomized, blinded, nonacupoint (NA) acupuncture controlled, multimodal neuroimaging clinical trial. A total of 48 subjects with subacute PSA will be randomly assigned to an acupoint group or an NA control group. The acupoint group will receive acupuncture with normal needling at DU20, EX-HN1, HT5, GB39, EX-HN12, EX-HN13, and CV23. The NA control group will receive acupuncture in locations not corresponding to acupuncture points as sham acupoints. Both groups will receive identical speech and language therapy thrice a week for four weeks. The primary outcome will be the change in the aphasia quotient (AQ) score measured by the Western Aphasia Battery (WAB) test during the 12th week after randomization. Participants will be blindly assessed at prerandomization (baseline) and 4 weeks, 12 weeks, and 24 weeks after randomization. The secondary outcomes include the Boston Diagnostic Aphasia Examination (BDAE) score, the Disease Prognosis Scale score for ischaemic stroke, etc. Magnetic resonance imaging (MRI) and electroencephalogram (EEG) will also be performed at 4-time intervals as secondary outcomes. All scores and image evaluations will be taken at the same point as the linguistic evaluation. The multilevel evaluation technique will be used to assess the long-term efficacy of acupuncture therapy. MRI scans and EEG will be used to assess acupuncture-related neuroplasticity changes. *Discussion*. The results from our trial will help to supply evidence for the long-term acupuncture effects for PSA over a long follow-up period. It will provide valuable information for future studies in the field of PSA treatment. The trial was registered at the Chinese Clinical Trial Registry on 16 March 2020 (ChiCTR2000030879).

1. Introduction

Stroke is a leading cause of mortality and disability globally [1]. Poststroke aphasia (PSA) is one of the most devastating symptoms in stroke survivors [2], who rarely spontaneously recover in the ensuing time. Approximately 30% of stroke patients suffer from aphasia [3], 50% of stroke survivors are still aphasic one year after stroke, and residual symptoms may persist for many years [4]. It can impact an individual's ability to speak, comprehend spoken language, read, and

write [5]. Basic requirements of daily life that rely on communication are affected, and social participation can be dramatically impaired. A large-scale survey investigated the relationship between the presence or absence of 75 diseases and the quality of life scores. The highest negative correlation is aphasia [6]. Aphasia rehabilitation has been listed as one of the top 10 research priorities related to life after aphasia [2]. Patients with PSA experience longer hospitalization stays and need more healthcare support, so studying the long-term curative effect of acupuncture treatment on PSA is conducive to maximizing of medical resources.

A review of clinical trials for PSA over the past 5 years revealed that a multitude of interventions can be beneficial in improving language and functional outcomes for patients with PSA [5], with the majority of high-quality clinical research focusing on the chronic phase of aphasia [7–9]. Language disorders are diverse and can change over time. The clinical symptoms may be different in the subacute phase as well as in the chronic phase [10]. Only a few randomized studies ($n = 12–30$) have examined the efficacy of PSA treatment in the subacute phase [11–13]. Research on the subacute period is therefore relatively scarce.

Effective therapies focusing on improving speech and language in patients with PSA are essential. At present, considerable evidence has suggested that treatment with speech and language therapy (SLT) is effective in improving communication and quality of life in individuals with aphasia. These studies have provided evidence of the effectiveness of SLT for people with aphasia following stroke in terms of improved functional communication, reading, writing, and expressive language compared with no therapy [14]; however, the effect sizes of SLT are moderate, potentially reflecting a physiological limit of training-induced progress, the treatment is costly, and progress is often slow. One of the studies also stated that SLT for more than 2 hours a day provided no added value on PSA [15]. It is certain that the various stages of PSA are associated with varying degrees of language recovery, but recovery requires a large number of therapy sessions. It remains a challenge to optimize the effect of aphasia therapy. As a result, an increasing amount of research has been devoted to alternative methods to improve the effectiveness of aphasia treatment, generally by increasing the total amount of treatment achieved [16].

Acupuncture is an easy-to-use, low-cost adjunct to traditional SLT to enhance language outcomes in individuals with PSA. It is one of the main treatments of traditional Chinese medicine (TCM) and has been used for thousands of years. During the past decade, the results of several meta-analyses have concluded that acupuncture after stroke seems to be effective in improving PSA functional communication and language function [17, 18]; however, most of the studies did not assess therapeutic effects over extended periods. For acupuncture treatment, long-term efficacy is considered one of the most important therapeutic effects, which has been suggested by numerous clinical studies [19–25]. It represents the cumulative effect of positive achievements [16]. Long-term efficacy will allow the patient's therapeutic effects to accumulate ("more is better"). Our previous studies on the effects of acupuncture on PSA have shown that participants in the acupuncture group had lower severity than the control group in week 4, but the difference was not significant; however, a significant reduction in PSA severity during weeks 5 to 12 was noted [26]. In addition, we explored the traditional Chinese medical theory and acupuncture technique of this acupuncture programme [27, 28]. Acupuncture has also been shown to have long-term efficacy in chronic pain and tinnitus, which has shown effective therapeutic results [29, 30]. Therefore, we speculate that the long-term therapeutic effect for PSA will also be well maintained; however, the long-term efficacy of acupuncture compared with the placebo effect in patients with PSA has not been investigated.

In terms of the current state of evaluation of aphasia, the requirements for a comprehensive evaluation of PSA have not yet been fully implemented. One of the important means was to evaluate the language characteristics of PSA, which is the major part of the evaluation; however, to date, this single evaluation approach is often insufficient. As such, neuropsychological tests in conjunction with in vivo measures may be more sensitive than neuropsychological tests alone in the assessment of brain structure and electrophysiology [31–33]. We introduce MRI and EEG for evaluation of the objective characteristics of PSA because the multimodal assessment of brain structure and function allows for a more objective evaluation of the recovery characteristics of patients with aphasia. Based on the previous study [34], we conducted a TCM syndrome evaluation of PSA patients and confirmed the relationship between linguistic features and TCM syndrome. Therefore, in this study, we have also introduced the evaluation of TCM syndrome to provide more comprehensive evidence of efficacy based on the combined evaluation of Chinese and Western medicine. For the reasons above, we propose to investigate whether acupuncture treatment on PSA has the value of maintaining long-term efficacy through this randomized controlled trial (RCT) with multimodal evaluation.

We designed an assessor- and participant-blinded RCT with PSA patients. Using NA acupuncture as a control, the trial aims to identify the efficacy of acupuncture by answering two questions: (1) what is the long-term efficacy and reliability of acupuncture as an intervention for PSA compared with the placebo effect? and (2) what interactions will be observed through an integrated evaluation of linguistic features, brain function, and TCM syndrome?

2. Methods and Analysis

2.1. Study Design. This is a multicentre, randomized, assessor- and participant-blinded, NA acupuncture controlled, multimodal neuroimaging clinical trial. It aims to compare the long-term efficacy of the acupoint group and NA control group (in locations not corresponding to acupuncture points). In all groups, participants will receive identical SLT but will be permitted to use medications for the basic internal medicine treatment of stroke. The type, dose, and time of administration of the agent will be recorded in the case report form. The trial design is depicted by the flow diagram

in Figure 1. The timeline for study enrolment, intervention, and assessment is illustrated in Table 1. We designed this acupuncture research at full length following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement [35]. The design and reporting of the study will follow the Consolidated Standards of Reporting Trials (CONSORT) statement for non-pharmacological interventions [36] (http://www.consort-statement.org/home/).

2.2. Randomization and Blinding. Eligible patients will be randomly assigned to the acupoint group or NA control group with a 1 : 1 ratio. The randomization sequence will be generated by a third-party professional statistician using a computer-generated randomization digital table using SAS V.9.4 software (SAS Institute Inc., North Carolina, USA). An independent assessor will interview the participants and carry out the screening. Random numbers and group assignments will be confirmed immediately through short message service to the practitioners who conduct acupuncture. All participants will be blinded to the types of acupuncture. An independent, blinded assessor who does not know the group assignment will conduct the outcome evaluation after the treatment. The researcher who will oversee the statistical analysis will also be blinded, with the treatment for each group remaining unknown; however, it is impossible to blind practitioners who conduct acupuncture. The practitioner will be forbidden from discussing the type of acupuncture with the participants. We will endeavour to ensure that subjects begin the trial with similar expectations of efficacy by informing them that the provided treatments are effective. In this study, participants, assessors, and statisticians will be blinded to treatment allocation. Participants will receive treatments alone at different times to avoid communication with each other. An eye patch will be applied to patients during the acupuncture treatment. To minimize the unintentional physical cues and bias in this trial, acupuncturists will be required to emulate the same procedure for the nonacupoint control group. In addition, participants will be asked to answer the following question during week 4 to test the blinding effect: "Do you think you have received real acupuncture treatment?" The participants can choose "yes" or "no" as an answer [37, 38]. The percentage of participants who answered "yes" in both groups after the final treatment will be analyzed. If the results show no significant difference in the response to this question between the two groups, they could suggest that the blinding effect is sufficient.

2.3. Setting and Recruitment. Participants who meet the inclusion and exclusion criteria will be recruited from the inpatients in the Dongzhimen Hospital affiliated to Beijing University of Chinese Medicine (BUCM), China Rehabilitation Research Center, and Peking University Third Hospital. Participating institutions and the level of the institution are listed in Table 2. The study will be advertised through the Internet and posters in communities and hospitals. The inpatients and potential participants will call an investigator and be prescreened for eligibility and will learn how to participate in this clinical trial through visits or telephone calls to our hospital. During visits to the clinical research centre of Dongzhimen Hospital affiliated to BUCM, China Rehabilitation Research Center, and Peking University Third Hospital, the accessors will explain the study to the patients, who will be asked to sign an informed consent form before voluntary participation. The participants of this study will be selected from the applicants who meet all the inclusion criteria but do not meet any of the exclusion criteria. To facilitate participation in this study, the accessors will properly adjust the evaluation and treatment schedule of the enrolled participants. Therefore, the enrolled participant will be able to complete the treatment and evaluation. Every time the enrolled participant visits, the accessors will inform the participant of the next scheduled visit, and the day before the visit, the accessors will remind the enrolled participant of the schedule by telephone.

2.4. Eligibility Criteria

2.4.1. Inclusion Criteria. Participants meeting all of the following criteria will be included in this trial: (1) diagnosed as stroke through computed tomography (CT) or MRI, 1 to 6 months after stroke onset; (2) 30 to 80 years of age (After the expert discussion and the actual situation of our country, the age range has been adjusted), the native language is Chinese, and right-handed; (3) primary school and above education with no serious heart, liver, or kidney diseases; (4) clear consciousness and no cognitive impairment; (5) normal language function before the stroke onset and dominant language dysfunction with mild limb dysfunction; (6) specific aphasia syndrome diagnosed as motor aphasia by the WAB; (7) BDAE score of 2 to 4; and (8) able to cooperate for the 30-minute MRI examination.

2.4.2. Exclusion Criteria. The exclusion criteria are as follows: (1) received pacemaker surgery, coronary intervention, or coronary artery bypass surgery or have other metal products in the body; (2) language dysfunction caused by congenital or childhood diseases; (3) language dysfunction caused by mental disturbance and normal mental retardation; (4) severe dysarthria and hearing impairment; (5) superficial sensation abnormalities in the neurological examination, and (6) participation in other studies.

2.5. Interventions. Acupuncture will be performed by registered acupuncturists with over 2 years of experience who will be trained in the standardization of the acupuncture scheme. Only sterile, stainless steel, disposable acupuncture needles (size 0.25 mm × 40 mm, product no. 20182270011; ANDE Acupuncture, Guizhou ANDE Medical Equipment, China) will be used. Both acupuncture and sham acupuncture points will be located in the limbs and head. All the participants will receive 3 treatment sessions per week (alternate days) for 4 consecutive weeks, resulting in a total of 12 sessions. Each treatment will be administered for

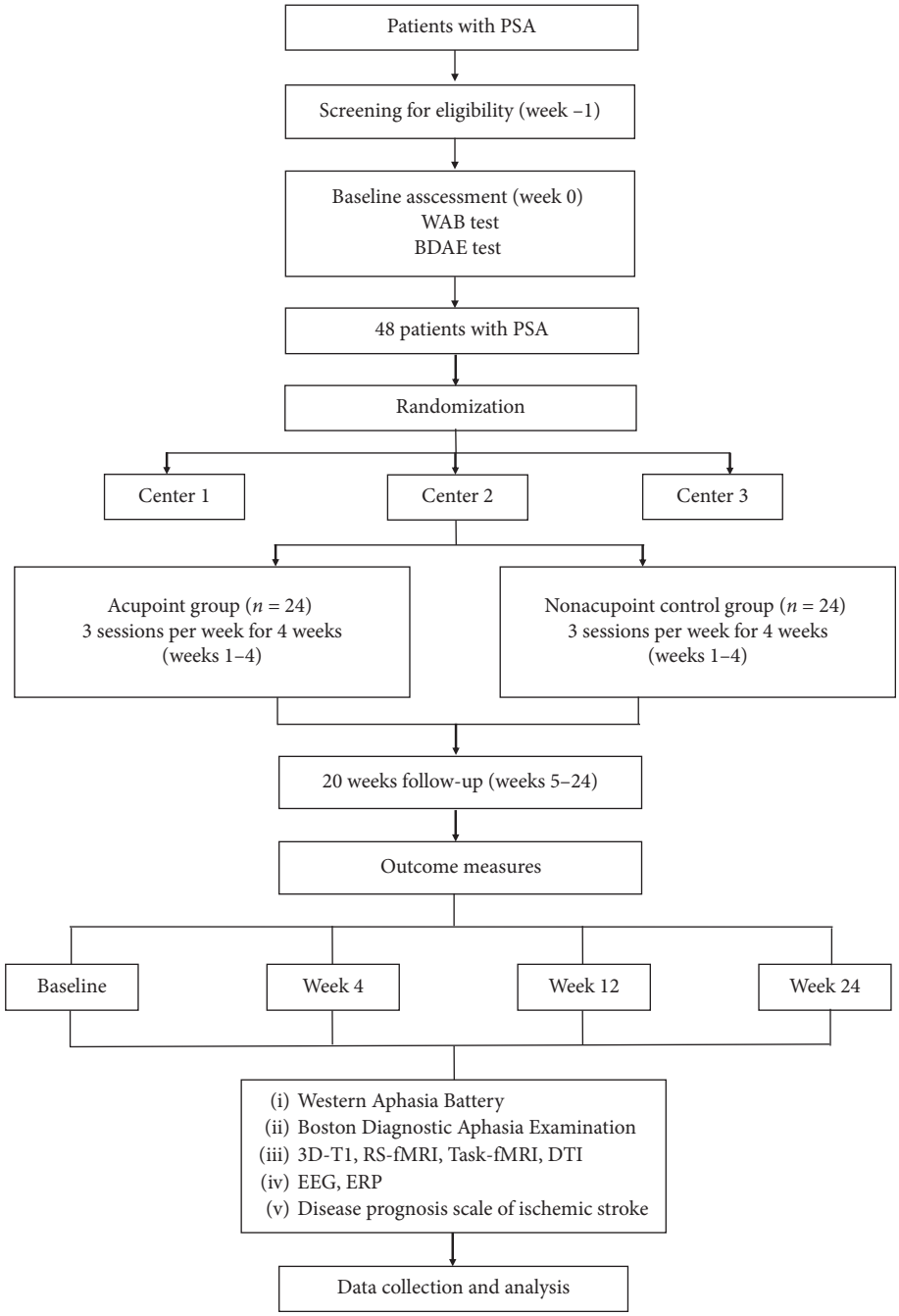

Figure 1: Trial flow chart. WAB, Western Aphasia Battery, BDAE, Boston Diagnostic Aphasia Examination; RS-fMRI, resting-state functional magnetic resonance imaging; Task-fMRI, task functional magnetic resonance imaging; DTI, diffusion tensor imaging; EEG, electroencephalogram; ERP, event-related potential.

30 minutes. The acupuncture and sham acupuncture interventions will be performed by a consensus of acupuncture experts. To ensure strict adherence to the study protocol, the experts will receive training together and use the same techniques. The details of the acupuncture treatment are described in the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA [39]) checklist in Table 3.

2.5.1. Acupoint Group. Participants in the acupoint group will receive acupuncture at bilateral Tongli (HT5) and Xuanzhong (GB39) limb acupuncture points. After skin disinfection, acupuncture needles will be inserted through the skin and approximately 0.5 cun [≈10 mm] into the skin. Needle insertion will follow an angle of 90° in an inferomedial direction for the two points. Following needle insertion, small, equal manipulations of twirling and thrusting

Table 1: Study design schedule.

Period	Screening and baseline	Treatment (w1–4)				Follow-up (w5–12)	Follow-up (w13–24)
Week (W)	W −1	W1	W2	W3	W4	W12	W24
Eligibility	×						
CT or MRI	×						
General information	×						
Physical examination	×				×	×	×
Medical history and demography	×						
Informed consent	×						
WAB		×			×	×	×
BDAE		×			×	×	×
Disease Prognosis Scale of ischaemic stroke		×			×	×	×
MRI (3D-T1, RS-fMRI, Task-fMRI, DTI)		×			×	×	×
EEG		×			×	×	×
ERP		×			×	×	×
Discomfort and acceptance of acupuncture		×	×	×	×	×	×
Assessment of blind method		×					
Adverse event			×	×	×	×	×
Compliance			×	×	×	×	×

CT, computed tomography; MRI, magnetic resonance imaging; WAB, Western Aphasia Battery; BDAE: Boston Diagnostic Aphasia Examination; RS-fMRI, resting-state functional magnetic resonance imaging; DTI, diffusion tensor imaging; EEG, electroencephalogram; ERP, event-related potentials.

Table 2: List of participating institutions and level of the institution.

Participating centre	Level of the institution
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine	Tertiary A hospital
China Rehabilitation Research Center	Tertiary A hospital
Peking University Third Hospital	Tertiary A hospital

will be performed on all needles to reach de qi (a composite of sensations including numbness, distention, soreness, and heaviness that is an important indicator of successful acupuncture treatment), which is believed to be an essential component for acupuncture efficacy. The needles placed in HT5 and GB39 will be manually stimulated every 10 minutes. The acupuncture points on the head are Lianquan (RN23), Jinjin (EX-HN12), Yuye (EX-HN13), Baihui (DU20), and Sishencong (EX-HN1). For RN23, acupuncture needles will be inserted through the skin and approximately 1 cun [≈20 mm] into the skin. Following needle insertion, small, equal manipulations will be performed on the needles to reach numbness. EX-HN12 and EX-HN13 will be quickly inserted for bloodletting. DU20 and EX-HN1 will be inserted through the skin approximately 0.5 cun. Needle insertion will follow an angle of 15° in an inferomedial direction for the two points (for details, see Table 4 and Figure 2).

2.5.2. NA Control Group. Participants in the NA control group will receive sham acupuncture with real needles on an NA. NA 1 will be 0.5 cun (≈10 mm) lateral to HT5. NA 2 will be 0.5 cun (≈10 mm) horizontal to GB39, and NA 3 will be 0.5 cun (≈10 mm) lateral to ST8 (for details, see Table 5 and Figure 3). Procedures and other treatment settings will be the same as in the acupoint group but with no needle manipulation for de qi. In both groups, the needles will be retained for 30 minutes for each treatment session. The participants will be treated with acupuncture three times a week, on alternate days, for 4 successive weeks, resulting in a total of 12 sessions for each patient.

2.5.3. Permitted and Prohibited Concomitant Treatments. All the participants will receive identical SLT. The treatment period is 4 weeks (3 treatment sessions per week, alternate days), resulting in a total of 12 sessions. Speech therapy will be performed by an experienced language therapist who received standardized training in language rehabilitation.

The language rehabilitation training method will be as follows: first, the patients will be assessed for their language function and scored, and then a training plan and training principles will be formulated according to the type of aphasia and language ability; the training is progressively followed by targeted strengthening exercises. The main content includes motor training of pronunciation organs, oral pronunciation training, naming, intonation, etc. (1) Mouth shape and voice training: at the beginning of the training, patients will be taught to control their lip and tongue movements through mouth shape and voice control to practice pronunciation. The patients will practice the rhymes and consonants first and then gradually transition to the differentiation of approximate sounds. (2) Use of language training equipment (cell phones, iPads, etc.): patients can use phrases and sentences from daily life to make audio files that are suitable for reading. The patients will practice the phrases first, and then, the sentences. (3) Training the pronunciation muscles:

Table 3: Revised standards for reporting intervention in clinical trials of acupuncture (STRICTA [39]]).

Item	Item criteria	Description
(1) Acupuncture rationale	(1a) Style of acupuncture	Traditional Chinese medicine therapy
	(1b) Reasoning for treatment provided, based on historical context, literature sources, and/or consensus methods, with references where appropriate	(i) Reasoning for treatment provided—based on historical context, literature sources, and traditional Chinese medicine (consensus) (ii) Reasoning for treatment provided—based on historical context, literature [27,28], selection of treatment regions based on related papers, expert experience, and textbooks
	(1c) Extent to which treatment varied	Standardized treatment
(2) Details of needling	(2a) Number of needle insertions per subject per session (mean and range where relevant)	10 or 12
	(2b) Names (or location if no standard name) of points used (unilateral/bilateral)	DU20 (Baihui), EX-HN1 (Sishencong), HT5 (Tongli), GB39 (Xuanzhong), EX-HN12 (Jinjin), EX-HN13 (Yuye), CV23 (Lianquan)
	(2c) Depth of insertion, based on a specified unit of measurement or a particular tissue level	Needle insertion will follow an angle of 90°in an inferomedial direction for the two points (HT5, GB39). Depth: 0.5 cun [≈10 mm]. Needle insertion followed an angle of 15° in an inferomedial direction for the two points (DU20, EX-HN1). Depth: 0.5 cun. For RN23, the angle is 90° and the depth is 1 cun [≈20 mm]. EX-HN12 and EX-HN13 were quickly inserted for bloodletting
	(2d) Responses sought	Following needle insertion, small, equal manipulations of twirling and thrusting will be performed on all needles to reach de qi
	(2e) Needle stimulation	Small, equal manipulations of twirling and thrusting will be performed on the needles of HT5 and GB39
	(2f) Needle retention time	30 min per session
	(2g) Needle type	Sterile, stainless, disposable acupuncture needles (size 0.25 mm × 40 mm, product no. 20182270011; ANDE Acupuncture, Guizhou ANDE Medical Equipment, China)
(3) Treatment regimen	(3a) Number of treatment sessions	12
	(3b) Frequency and duration of treatment sessions	3 times/week, 30 min per session, on alternate days, for 4 successive weeks
(4) Other components of treatment	(4a) Details of other interventions administered to the acupuncture group	None
	(4b) Setting and context of treatment, including instructions to practitioners, and information and explanations to patients	The study will be conducted in the Dongzhimen Hospital affiliated to BUCM, China Rehabilitation Research Center, and Peking University Third Hospital, and all information will be provided to the subjects
(5) Practitioner background	(5a) Description of participating acupuncturists	The chief physician of Dongzhimen Hospital, Ph.D., 11 years of formal university training in traditional Chinese medicine, with qualifications for practising doctors stipulated in the law
(6) Control or comparator interventions	(6a) rationale for the control or comparator in the context of the research question, with sources that justify the choice	The nonacupoint control group will receive sham acupuncture with real acupuncture needles at nonacupoint locations. Through such an approach, the self-perception of placebo effects in the nonacupoint control group is difficult to distinguish from the real acupoints
	(6b) Precise description of the control or comparator; details for items 1–3 with the use of sham acupuncture or any other type of acupuncture-like control	Participants in the nonacupoint control group received sham acupuncture with a pragmatic placebo needle on a sham acupoint. The needles used are the same as the acupoint group. Procedures and other treatment settings will be the same as in the acupoint group but with no needle manipulation for de qi

patients with aphasia may have different degrees of wasting atrophy of the pronunciation-related muscles, which results in slurred speech. During training, patients are instructed to practice tonal movements of the tongue and oral muscles to promote accurate pronunciation. (4) Regular check-ups and weakness reinforcement exercises: weaknesses in

Table 4: Location of acupoints used in the acupuncture group.

Acupoints	Location
DU20 (Baihui)	On the median line of the head, 5 cun superior to the anterior hairline, at about the middle on the connecting line between the two auricular tips
EX-HN1 (Sishencong)	On the vertex, 1 cun from the front, back, left, and right to DU20 (Baihui), common 4 points
HT5 (Tongli)	On the palmar side of the forearm, 1 cun superior to the transverse crease of the wrist, at the radial border of the ulnar carpal flexor muscular tendon
GB39 (Xuanzhong)	On the lateral side of the leg, 3 cun directly above the tip of lateral malleolus at the anterior border of the fibula
EX-HN12 (Jinjin)	In the mouth, EX-HN12 (Jinjin) is located with tongue furled, on the vein on the left side of the frenulum of the tongue
EX-HN13 (Yuye)	EX-HN13 (Yuye) is located on the vein on the right side of the frenulum of the tongue
CV23 (Lianquan)	In the neck, on the anterior midline, above the laryngeal protuberance, in the depression of the superior border of the hyoid bone

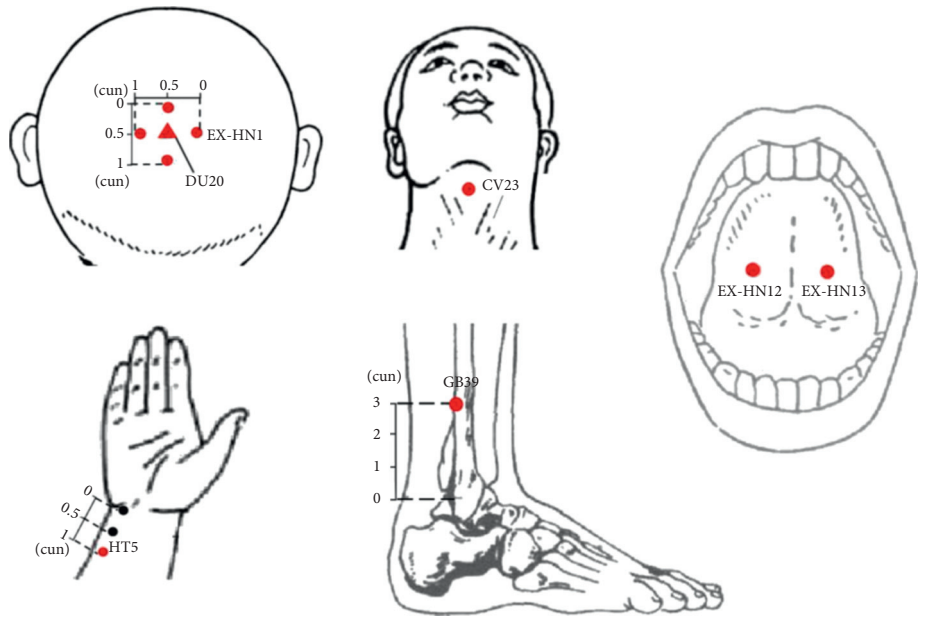

Figure 2: Locations of acupoints.

Table 5: Location of sham acupoints used in the NA group.

Nonacupoint (NA)	Location
NA 1	0.5 cun lateral to HT5 (Tongli)
NA 2	0.5 cun horizontal to GB39 (Xuanzhong)
NA 3	0.5 cun lateral to ST8 (Touwei)

pronunciation for targeted exercises will be identified, and individual reinforcement training for patients will be provided if necessary.

2.6. Sample Size Calculation. No large RCTs have been carried out within the field examining the long-term effects of acupuncture on PSA. Previous studies have used interventions not applicable to the current protocol and have been of varying quality and used small sample sizes. We were, therefore, unable to calculate the prior accurate sample size. Thus, our RCT will support a more accurate sample size estimate and inform a definitive trial examining the long-term effectiveness of acupuncture for aphasia. The study involves the experimental study of functional magnetic resonance imaging (fMRI) and EEG for language tasks, which falls under the scope of experimental psychology. Taking into account individual psychological differences, it is expected that the PSA patients to be included will be divided into 2 groups. The number of patients to be included in the statistics in each group will be 20. Considering a drop-out rate of 20%, the target recruitment number is 48 participants (24 per group).

2.7. Multimodal Data Acquisition

2.7.1. MRI Data Acquisition. Patients will undergo brain MRI at prerandomization (baseline) and 4 weeks, 12 weeks, and 24 weeks after randomization. MRI scans will be performed with a 3.0-T MR scanner (Siemens AG, Germany) in the Dongzhimen Hospital affiliated to BUCM. The parameters of the sequences to be employed in this study are provided by the China Association of Brain Imaging. Sagittal structural images will be acquired using a magnetization-prepared rapid gradient-echo three-dimensional (3D) T1-

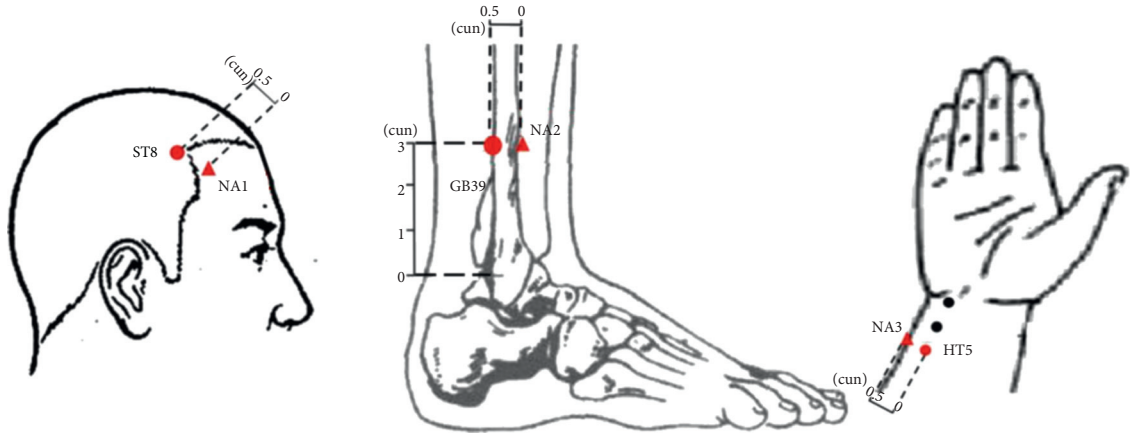

Figure 3: Locations of nonacupoints. NA, nonacupoint.

weighted sequence with the following parameters: repetition time (TR)/echo time (TE) = 1900/2.13 ms, flip angle = 9°, inversion time = 1100 ms, resolution = 256 × 256, voxel size: 1.0 × 1.0 × 1.0, 1-mm slice thickness without slice gap.

RS-fMRI and task-fMRI will be performed using an echo-planar imaging (EPI) sequence with the following parameters: TR/TE = 2000/30 ms, flip angle = 90°, resolution = 64 × 64, FOV = 225 × 225, bandwidth = 2520, slice thickness = 3.5 mm with 0.7 mm slice gap, 31 axial slices, and voxel size: 3.5 × 3.5 × 3.5.

Diffusion tensor imaging (DTI) will be acquired with a diffusion-weighted, single-shot, spin-echo, echo-planar imaging sequence that uses 30 directions with $b = 0\ \text{s/mm}^2$ and $b = 1000\ \text{s/mm}^2$, slice thickness: 2 mm, gap = 0 mm, slices = 65,TR: 11000 ms, TE: 94 ms, matrix: 128 × 128, FOV: 256 × 256, and phase encode direction: $A >> P$.

All scans will be qualitatively reviewed by two radiologists to screen for possible brain lesions or structural abnormalities. fMRI data will be collected during the word-picture judgement task and at rest. fMRI data will be performed with the SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) for MATLAB. Brain activation and connectivity changes will be compared between the two groups.

2.7.2. EEG Data Acquisition. The 64-channel EEG recording and analysis system produced by the Neuroscan of Australia, E-prime 2.0 stimulation display software, scan data analysis software, SYNAMPS EEG amplifier, Fastrak 3D imaging digital instrument, Quick-Cap electrode cap, recording electrode, electrode paste, and Curry multichannel neuroimaging software will be used to collect EEG signals. EEG data in the resting state will be collected for 8 minutes, and event-related potentials (ERPs) will be collected during language task assessment for 8 minutes. The same word-picture judgement task used for the MRI will be applied to ERP collection. Patients will undergo an EEG at a different time on the same day as the MRI scan.

2.7.3. Word-Picture Judgement Task. The patients will be trained before entering the fMRI scanner. They will complete a practice version of the word-picture judgement task paradigm in the computer. They need to perform the task and reach an accuracy criterion of 90% to ensure that the patients understand how to do the task in the scanner. Patients will view black and white line pictures and Chinese high-frequency nouns. The patients are required to press a mouse button when the picture and the noun appear on a white background. The patients are asked to press the left button if the picture and the noun express the same meaning; otherwise, they should right-click. The stimuli are presented in 60 blocks on a computer using E-prime 2.0. In the ERP experiment, there will be 120 blocks. Trial types within blocks are presented in a pseudorandomized order. During the MRI scanning, all patients are asked to lie quietly in the scanner with their eyes open, trying to avoid systematic thinking, and moving as little as possible. In task-state fMRI scanning, the patients are instructed to maintain a central view and try to not think of other things. During ERP testing, patients are asked to sit in front of the computer, and the surroundings will be kept quiet (Figure 4).

2.8. Outcome Measures

2.8.1. Clinical Outcome Assessments. The primary outcome will be the change in the AQ score during the 12th week after randomization. For the assessment of language functioning, the WAB with the subtests spontaneous speech, auditory comprehension, repetition, and naming is included in the test battery. The WAB test will be conducted based on a previous study [40]. Secondary outcomes include four items, the details of which are listed in Table 1.

2.8.2. Neuroplasticity Assessments. In this study, neuroplasticity changes between the two groups will be measured by MRI. Before acupuncture treatment, patients will complete the fMRI scan within 3 days. They will also have a follow-up fMRI scan within 3 days after the completion of their intervention and at 8 weeks and 16 weeks after the intervention (Figure 1). Brain activity and functional connectivity will be assessed under a resting state and a word-picture judgement task. Group differences in white matter integrity will be assessed using DTI.

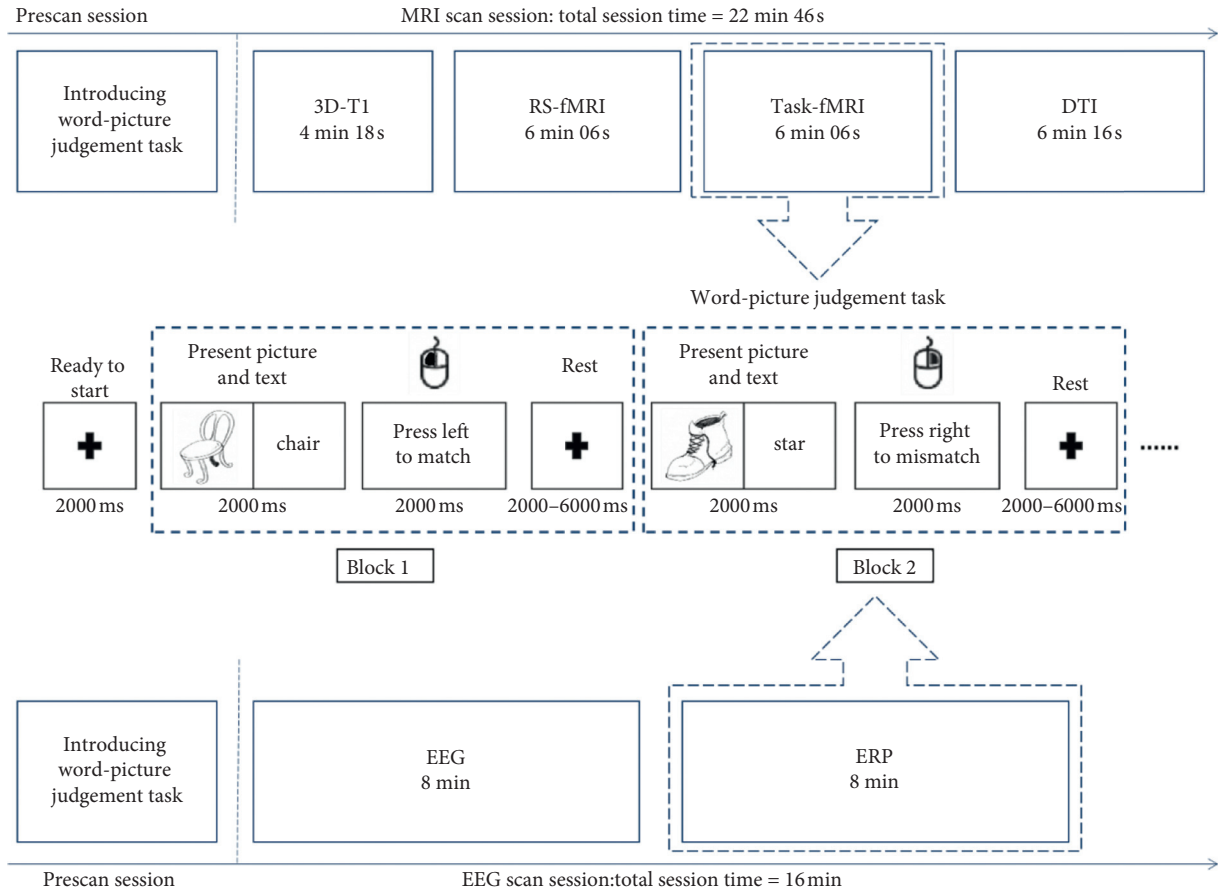

Figure 4: MRI experimental paradigm and an illustrative diagram of the word-picture judgement task. For fMRI, there were 60 blocks. For ERP, 60 blocks. When presenting the task, the word is displayed in Chinese.

2.8.3. Assessment of Acupuncture Safety. Acupuncture is considered to be a generally safe procedure [41–43]; however, all adverse events will be recorded in every detail. In this study, acupuncture-related adverse events would mainly refer to broken needles, fainting due to the needling procedure, local infection, hematoma, and other events that can be caused by acupuncture (such as headache, dizziness, and insomnia).

2.8.4. Quality Control. All practitioners will conduct the study according to the standard operating procedure of the study. We will conduct several simulations for our volunteers before the start of the study. During the study, regular meetings will be held to discuss issues raised by researchers or participants, the need to improve protocols, the side effects, and participant recruitment.

2.9. Statistical Analysis

2.9.1. Clinical Data Analysis. All statistical analyses will be performed by a statistician from the Clinical Evaluation and Analysis Centre of Dongzhimen Hospital affiliated to BUCM using Statistical Package for the Social Sciences (SPSS) V.22.0. The statistician will be blinded to the allocation of groups. The level of significance is established at $\alpha < 0.05$ with a two-tailed test. The main objective is to compare the change in the AQ score at week 12 from baseline between the acupoint group and the NA control group. The null hypothesis is that the acupoint group will show the same change as the NA control group, while the alternative hypothesis is that the acupoint group shows a greater improvement. Categorical data will be represented by percentages, whereas continuous data will be represented by the average, standard deviation, median, minimum value, and maximum value. For comparison with the baseline, a *t*-test or nonparametric test will be used for continuous data and nonparametric tests for categorical data. For comparison of two independent samples, if the residuals are normally distributed, the analysis of covariance (ANCOVA) will be used for the primary outcome and subgroup analysis stratified by aphasia severity, *t*-tests for other continuous data, and chi-square tests for categorical data; if the residuals are non-normally distributed, a nonparametric test will be used for both the continuous and categorical data. The results of the intention-to-treat (ITT) analysis will be used to assess the validity of the study as a whole. The ITT analysis will collect data from all the participants in this trial, and for those lost to follow-up, the last observation carried forward method will be implemented.

2.9.2. MRI Data Analysis. For imaging, data will be analyzed using the Data Processing & Analysis for (Resting-State) Brain Imaging (DPABI) toolbox [44] performed on MATLAB V.8.6 (MathWorks) to detect any changes in brain function due to acupuncture treatment. Lesion symptom mapping was demarcated on T1-weighted images manually using MRIcroGL (http://www.cabiatl.com/mricrogl/) in native space by neurologists who were blinded to the participants' language scores. DTI images will be analyzed using MATLAB (https://www.mathworks.com/) and FMRIB Software Library (http://www.fmrib.ox.ac.uk/fsl). After data preprocessing, some data-driven approaches will be performed to investigate neuroplasticity between the two groups, such as the amplitude of the low-frequency fluctuation, regional homogeneity, and voxel-wise degree centrality. A two-sample *t*-test will be conducted to investigate the differences in brain regions between the acupoint group or NA control group in the DPABI software. Multiple comparisons will be used to better control for a highly inflated false positivity rate. Pearson's correlation analysis will be performed to examine the association between the fMRI data and clinical variables.

2.9.3. EEG Data Analysis. EEG data will be preprocessed by NeuroScan software, and electrocardiogram, ophthalmic, and myoelectric artefacts will be removed by independent component analysis (ICA). eLORETA software will be used to extract the characteristic indexes of the brain network. The electric current sources in different brain regions will be accurately and intuitively calculated, and the functional network characteristics of the brain in different frequency bands will be analyzed.

2.9.4. ERP Data Analysis. Using the threshold selection method based on a small-world network across different thresholds to build a functional brain network, analyses will be conducted to examine the network topological structure of the brain under the different thresholds, build different threshold brain networks, and use the theory of the complex network diagram analysis method to calculate the weighted aggregation coefficient, weighted characteristic path length, and small-world network features.

3. Discussion

PSA has proven to be difficult to treat. There is a degree of spontaneous recovery in the subacute phase, so there are few studies on the subacute phase of PSA. However, the subacute phase also requires treatment. The extant literature shows that acupuncture is probably effective for PSA [18, 45–47]. We intend to evaluate the long-term efficacy of acupuncture in maintaining speech production function. One common problem is the lack of standardization of acupoint selection, needle retention time, number of needles used, needling depth, and needle manipulation in acupuncture research.

From 2006 to now, we attempted to explore the general acupuncture scheme based on syndrome differentiation. In our PSA acupuncture treatment programme, DU20 is located in the area at the top of the head and is particularly closely related to the regulation of brain speech activity. The EX-HN1 are the acupuncture points around the DU20, both of which are used together to benefit the brain and promote speech rehabilitation. The treatment of PSA by EX-HN12 and EX-HN13 bloodletting has a theoretical basis in TCM and unique clinical efficacy. CV23 is mainly used for the treatment of speechlessness, stabbing into the skin towards the root of the tongue, and has the effect of restoring speech. The acupuncture theory of HT5 and GB39 to be used in our study is different from the traditional meaning of these points. FMRI can help us evaluate the changes of needle-related neuroplasticity, so we have conducted fMRI experiments based on this pair of effective points for aphasia. Electroacupuncture at HT5 and GB39 may modulate language and cognition function through a complex network formed by an extensive area of the brain cortex. It is beneficial to the recovery of language function [47]. In previous studies, we have demonstrated that this acupuncture protocol has shown significant improvements in auditory comprehension, reading, and dictation in patients with subacute PSA after 4 weeks of treatment. Therefore, we would like to know more about whether this treatment programme has long-term effects in these areas and observe the association with overall linguistic changes.

Regarding the study design, we developed an assessor- and participant-blinded design to minimize bias. The specific effects are thought to be generated by needling the acupoint with the appropriate manipulation. The nonspecific effects are due to other aspects of the therapy, such as the expectations of the patient, which might influence the treatment outcome. To recognize the specific effects, a placebo is needed. Among acupuncture research, placebo controls for acupuncture studies have been difficult to select. To maximally exclude the placebo effect, rigorous methodological designs are followed. In our study, control conditions involve being punctured with real acupuncture needles at NA locations. Thus, the self-perception of placebo effects in the NA control group is difficult to distinguish from the real acupoints. Additional needle manipulations will not be used in the NA control group. De qi is a characteristic constellation of sensations felt by patients during acupuncture needling. It has been regarded as an important factor related to clinical effects; however, we do not pursue this kind of sensation in the NA control group. By following these methods, participants can be successfully blinded, and the efficacy of acupuncture could be confirmed if the results of the acupoint group prove superior to those of the NA control group.

We will assess patients with PSA on multiple levels, including multiple neuropsychological tests, functional and structural brain alterations, and TCM syndrome evaluation. This multidomain assessment will be used to identify possible biomarkers involved in the effects of acupuncture in PSA.

It is worth mentioning that functional and structural brain alterations will be used as outcome measures. MRI allows for noninvasive evaluations of neural functional changes [48]. fMRI studies use two modalities, task-related and resting-state methods [49]. Task-related fMRI has

revealed functional disturbances in individuals with aphasia. MRI may be more sensitive to smaller treatment effects. It can be used as a tool to assess the efficacy of acupuncture in treating aphasia. These analyses may identify whether neural efficiency is improved or the brain connectome is reorganized to achieve language enhancement. Electrophysiological methods, such as intracranially recorded EEG or ERP, are particularly promising to offer a mechanistic understanding of language formation processes. Compared with the language scales alone, the combination of in vivo measures of brain alterations in this study will be more sensitive in detecting acupuncture efficacy [50].

Therefore, this study can provide clinical evidence on the long-term efficacy of our acupuncture programme in patients with PSA and explore biomarkers for the recovery of function and the efficacy of acupuncture in patients with PSA through a multidimensional evaluation. This trial will fill the gaps in the evidence on the long-term efficacy of acupuncture for aphasia and provide a model for the multimodal evaluation of PSA.

Abbreviations

PSA: Poststroke aphasia
AQ: Aphasia quotient
WAB: Western Aphasia Battery
BDAE: Boston Diagnostic Aphasia Examination
MRI: Magnetic resonance imaging
EEG: Electroencephalogram
SLT: Speech and language therapy
TCM: Traditional chinese medicine
RCT: Randomized controlled trial
SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials
CONSORT: Consolidated Standards of Reporting Trials
BUCM: Beijing University of Chinese Medicine
CT: Computed tomography
STRICTA: Standards for Reporting Interventions in Clinical Trials of Acupuncture
NA: Nonacupoint
RS-fMRI: Resting-state functional magnetic resonance imaging
DTI: Diffusion tensor imaging
ERP: Event-related potentials.

Data Availability

All data are available from the corresponding author upon reasonable request.

Ethical Approval

This RCT was approved by the IRB of Dongzhimen Hospital affiliated to Beijing University of Chinese Medicine (certificate number DZMEC-KY-2018-36).

Consent

All participants gave their written informed consent to the research assistant before joining the RCT. The purpose, procedures, confidentiality, and potential risks of the RCT were explained clearly to the participants.

Disclosure

The trial results will be published through publication in scientific papers and posters or oral presentations at conferences. The funding source has no role in the study design and do not have any role during the execution, analyses, interpretation of the data, or decision to submit results.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

The trial was designed and developed by JLC and YG. The manuscript was prepared by XLL. CZ and SRL revised carefully the protocol and provided methodological support. JLC and XLL registered the protocol in the Chinese Clinical Trial Registry and obtained ethical approval. XLL and CML prepared the tables and figures. QSZ and XYX contributed to recruitment. ZJT and BLZ are responsible for image parameter setting and data acquisition. RWF, XH, MJX, XS, HMY, and QK are the coordinators and responsible for the screening and enrolment of patients. All authors read and approved the final manuscript.

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Supplementary Materials

SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents. (*Supplementary Materials*)

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Research Article

A Survey of Clinical Evidence Evaluation Systems for Traditional Chinese Medicine

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Objectives. We investigated the cognition and application of the "Evaluation System of Traditional Chinese Medicine Clinical Evidence" among populations with disparate backgrounds. *Methods*. We performed an online survey using a self-designed questionnaire. *Results*. Of 307 returned questionnaires, 284 were noted to be valid, and the effective recovery rate was 92.5%. Our analyses showed that the respondents demonstrated a better understanding of clinical evidence-based evaluation systems and that they used these occasionally. For both the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) and the traditional Chinese medicine (TCM) evidence evaluation systems, the respondents generally showed poor overall understanding, rendering the systems impractical. Among the respondents who were exposed to the existing evidence evaluation system for TCM. More than 70% of the respondents remarked that it was difficult to obtain high-quality evidence using any existing methods to evaluate TCM clinical evidence, that there was a lack of clear evaluation criteria, and that it was difficult to grasp the evaluation process. *Conclusions*. The evaluation systems of TCM clinical evidence have gained a certain degree of recognition among practitioners, who show a great willingness to use it, but practical applications are limited. In addition, it is also expected that an evaluation system would be more in line with the clinical characteristics of TCM.

1. Introduction

Evidence provides the all-important basis for evidence-based decision-making, and it needs to be evaluated appropriately to create a reliable foundation for further study [1]. High-quality evaluation of clinical evidence with respect to traditional Chinese medicine (TCM) requires scientific evaluation criteria with TCM characteristics. Many domestic scholars have explored classification and recommendation systems with respect to TCM evidence over the past 20 years. Based on the characteristics of TCM clinical theory and practice, such as treatment according to syndrome differentiation and inheritance of ancient medical literature and famous experts' experience, there are two developmental directions: the first is to adapt to the existing international evaluation standards to include the evidence types with TCM characteristics for evaluation and the second is to establish independent evaluation standards based on TCM theories [2]. At the same time, with the rapid development of evidence-based medicine in China and the wide application of the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) worldwide, many researchers have investigated the application of this system to evaluate the evidence regarding TCM [3]. With respect to the field of acupuncture, several research teams have combined the GRADE system with historical documents of TCM and well-known expert experience to establish an evidence evaluation system for clinical research on acupuncture. To better understand and apply the TCM clinical evidence evaluation system to the healthcare industry and to better promote the development of TCM clinical evidence-based evaluation, we organized and implemented the current survey questionnaire.

2. Methods

2.1. Survey Methodology. The questionnaire was developed by the project team of Shanghai TCM Evidence-based Medicine Research Center. Its content includes basic information on the respondents and cognition and application of the evidence evaluation system (including general information, the GRADE system, and the characteristic evidence evaluation system of TCM), as well as the current situation of clinical evidence evaluation of TCM, making for a total of 25 questions. Before the questionnaire was issued, we consulted with experts and conducted a presurvey to optimize the survey questions and to evaluate these for scientific approach and reasonableness. Based on the presurvey results, the Raosoft sample size calculator (http://www.raosoft.com/samplesize.html) was used to ensure the appropriate number of participants, given the selected margin of error (5%), confidence interval (90%), and approximate population size (20,000 individuals). In addition, the minimum sample size of this survey was 267. We used a 5-point Likert scale to investigate the cognition and usage of evidence evaluation systems and assigned points when data were collated: 1 for very well known/very frequently used, 2 for relatively well known/frequently used, 3 for moderately/occasionally used, 4 for not well known/rarely used, and 5 for not at all known/never used.

The questionnaire was designed using the questionnaire website (https://www.wjx.cn), conducted in the form of a network survey, and distributed through WeChat and the questionnaire website. The survey was open to all people through social networks, regardless of age, sex, year of experience, or educational background, and respondents completed questionnaires according to their interest in this subject. The survey was filled out anonymously and completed independently by the respondents.

2.2. Data Analysis. The questionnaire was collected from the questionnaire website, and the data were downloaded in the form of an Office Excel spreadsheet. Office Excel software was then used to sort the data and eliminate any invalid questionnaire data, and the sorted data were imported into SPSS 24.0 software for statistical analysis. Questionnaires with missing data and inconsistent answers were considered invalid. The effective recovery rate was the ratio of effective questionnaires to total recovery questionnaires [4]. The Cronbach alpha reliability coefficient method was used to test the questionnaire's reliability, and we utilized the factor analysis method for the construction-validity test, with differences with $p < 0.05$ considered statistically significant.

3. Results

The questionnaire survey was conducted between June 15 and July 15 of 2020. A total of 307 questionnaires were collected, of which 284 were considered valid, constituting an effective recovery rate of 92.5%. The calculated Cronbach's alpha reliability coefficient was 0.95, the KMO value for the validity test was 0.905, and the Bartlett spherical test resulted in $p < 0.01$. The questionnaire showed high construct validity and scale reliability.

3.1. Characteristics of the Participants. In terms of occupation, the respondents were mainly clinicians (49.6%), followed by scientific researchers (15.1%) and nurses. Organizations were mainly higher medical institutions, accounting for 54.9%, followed by colleges and universities (26.1%). Chinese medicine (57.7%) and integrated medicine (29.2%) dominated the professional fields. Of the respondents, 74.6% had acquired a master's degree or above, and 24.6% possessed a bachelor's degree. In terms of professional titles, the percentage of senior titles was the highest (36.3%) (see Table 1 for details). Statistically, there were significant differences among the occupations, educational backgrounds, organizations, and professional fields of the respondents ($p < 0.01$), which may be related to the distribution of questionnaires and respondent professions.

3.2. Cognition and Application of Evidence Evaluation System. Respondents showed a better understanding of the "clinical evidence evaluation system"; however, they only used it infrequently or occasionally. For the GRADE and TCM evidence evaluation systems, cognition ranged from moderate to a low level of understanding, and the use status was at the no-use level (Figure 1). The difference was statistically significant compared with the three points of normal/occasional use ($p < 0.05$).

Further analysis of respondents' occupations, education, titles, and professional fields revealed significant differences in cognition and usage ($p < 0.05$). In terms of cognition and use of the clinical evidence system, GRADE system, and TCM evidence system, researchers showed a better understanding and more frequent use. There were also significant differences in cognition and usage across educational levels ($p < 0.05$); i.e., the respondents with doctorate and master's degrees were more familiar with the evidence evaluation system and more typically used the evaluation system than individuals with a bachelor's degree. However, those with intermediate and advanced titles were inclined to know more and use TCM clinical evidence evaluation more often, and there was no significant difference in the cognition and application of the GRADE system. In terms of professional field, respondents in the fields of Western medicine and integrated medicine used the "clinical evidence evaluation system" more often, and their cognition and use score of the GRADE system were higher than those of respondents in the field of TCM. In the field of integrated medicine, the TCM clinical evidence system was applied more often.

In terms of the application of evidence evaluation in certain situations, more than 70% of the respondents indicated that they would apply the existing evidence evaluation system (such as the Oxford system, GRADE system, or other evaluation criteria) when encountering clinical problems, conducting research design, and writing professional/scientific manuscripts.

Table 1: General information of the respondents.

Project		Count	Percentage (%)
Occupation	Clinician	141	49.6
	Nurse	7	2.5
	Management	18	6.3
	University teacher	26	9.2
	Researcher	43	15.1
	Student	40	14.1
	Others	9	3.2
Educational background	Doctorate	98	34.5
	Master	114	40.1
	Bachelor	70	24.6
	Junior college or below	2	0.7
Professional title	Senior title	103	36.3
	Intermediate title	83	29.2
	Junior title	58	20.4
	Others	40	14.1
Organization	College or university	74	26.1
	Scientific research institution	12	4.2
	Government sector	6	2.1
	Pharmaceutical/medical device enterprise	7	2.5
	Higher medical institution	156	54.9
	Primary medical institution	18	6.3
	Others	11	3.9
Work experience	Less than 5 years	94	33.1
	5–10 years	58	20.4
	11–20 years	68	23.9
	21–30 years	51	18.0
	More than 30 years	13	4.6
Professional field	Traditional Chinese medicine	164	57.7
	Western medicine	37	13.0
	Integrated medicine	83	29.2

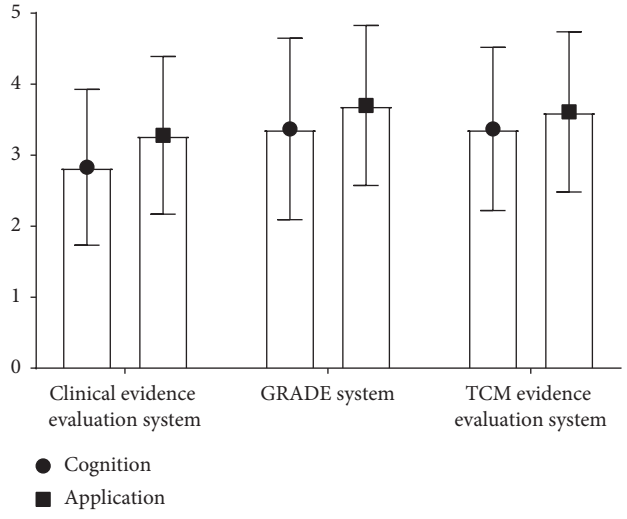

Figure 1: Respondents' cognition and use of evidence evaluation system.

3.3. Evaluation of TCM Clinical Evidence. Of the 284 valid questionnaires, 54 respondents had no knowledge of the "TCM clinical evidence evaluation/TCM clinical evidence evaluation system." Of the 230 individuals who exhibited knowledge regarding the evaluation of clinical evidence for TCM, over half of the respondents knew the "Composition of traditional medical evidence body and classification of evidence" proposed by Liu [5] or Wang et al. [6] "Evidence-based Chinese medicine clinical practice guidance evidence grading system." Some interviewees had also read the "TCM clinical evidence grading and scoring system" proposed by Wang et al. [7], the "TCM clinical evidence evaluation method" proposed by Zhong [8], the "Acupuncture clinical research evidence evaluation system" proposed by Ren et al. [9], or other self-created evaluation systems (see Table 2).

Of all respondents, 267 indicated that they had used existing methods to evaluate the clinical evidence for TCM. Over one-third (34.5%) of the respondents had used the "Five-level system of TCM version (Jianping version [4])," and 28.5% had used the GRADE system to evaluate the TCM evidence. Some respondents indicated that they had evaluated the TCM evidence using the "Oxford system" (16.5%), the self-created evaluation method (13.5%), or other methods (28.1%) (Table 3).

Over 70% of the respondents indicated that when using any existing method to evaluate the clinical evidence underlying TCM, they encountered difficulties in obtaining high-quality evidence, lacked clear evaluation criteria, or faced an unclear evaluation process (Table 4). There were also many other problems, including too many evaluation systems from which to choose, large disparities in the levels of the same evidence in different systems, opaque evaluation

Table 2: Cognitive situation of common TCM evidence evaluation systems.

Project	Count	Percentage (%)
Jianping—Composition and classification of evidence in traditional medicine	92	40
Shouchuan—Evidence-based Chinese medicine clinical practice guidance evidence grading system	69	30
Jie—TCM clinical evidence grading and scoring system	51	22.2
Fanrong—Acupuncture clinical research evidence evaluation system	29	12.6
Jingbai—TCM clinical evidence evaluation method	26	11.3
Others	64	27.8

Table 3: Application of the TCM evidence evaluation method.

Project	Count	Percentage (%)
Five-level system of TCM version (Jianping version)	92	34.5
GRADE system	76	28.5
Other methods	75	28.1
Oxford system	44	16.5
The self-created evaluation method	36	13.5

Table 4: Difficulties in evaluating TCM clinical evidence.

Project	Count	Percentage (%)
Hard to obtain evidence, especially high-quality evidence	126	47.2
Lack of clear evaluation criteria	101	37.8
Difficult to perform evaluation process	94	35.2
Little clinical significance and poor practicability	55	20.6
Low credibility and no reference value	54	20.2
Others	45	16.8

processes, and a lack of involvement in the formation of recommendations (Table 5).

3.4. Application in the Evaluation of TCM Evidence Using the GRADE System. Of all the respondents, 218 (76.8%) had heard of the GRADE system, while 66 (23.2%) had no knowledge of it at all. Among the 218, 110 (39.1%) had used the GRADE system to evaluate TCM evidence or had read articles published by others using the GRADE system. More than half (56.7%) of all respondents believed that the GRADE system could be used to evaluate TCM clinical evidence but that an appropriate modification was needed first to reflect the clinical characteristics of TCM. Only 3.9% of the respondents believed that the GRADE system was fully applicable for use without any modifications, while others thought that it was not applicable at all, that an independent evaluation system of TCM was needed, or that because it was not applied, respondents did not make a statement.

When we surveyed the opinions of the respondents who had used or read that the GRADE system being used by others to evaluate TCM evidence (Table 6), the practitioners indicated the following: "generally lower level of evidence limited the usage of GRADE" (88.2%); "the characteristics of treating the same disease with different treatments in TCM lead to higher risk of consistency bias" (74.5%); "higher-priority evidence, such as ancient TCM case reports, rated lower in GRADE" (51.8%); and "it can be well applied and improve the decision-making level" (38.1%). A small number of respondents believed that the results of other evaluation systems were better, or they did not take a position.

Most of the respondents thought that the level of characteristic TCM evidence such as classical TCM or expert experience should be improved (60.5%), and 16 of them thought that it should be decided according to the specific evidence.

3.5. Suggestions for the Evaluation of Clinical TCM Evidence. To promote research and application into a clinical evidence evaluation system for TCM, an open question was designed at the end of the questionnaire to collect the respondents' suggestions on an evaluation system of clinical evidence regarding TCM. We ultimately collected 31 suggestions. After classifying similar opinions, we determined the order of frequency (Table 7).

4. Discussion

With the development of modern Chinese medicine, clinical studies have been widely conducted, accumulating a large amount of clinical evidence for Chinese medicine. TCM clinical evidence and its evaluation methods are the foundation of TCM clinical practice guide formulation, TCM diagnosis, and treatment plan formation, evidence management, and other associated efforts. Therefore, research on the evaluation of TCM clinical evidence plays a vital role in promoting its production and utilization.

Table 5: Problems in the evaluation of TCM clinical evidence.

Project	Count	Percentage (%)
Same evidence may greatly vary among different systems	138	51.7
The evaluation process is opaque and subjective	86	32.2
Lots of evaluation systems and difficulty choosing	72	27
Most methods do not involve recommendations	67	25.1
Little help for clinical decision-making	58	21.7
Others	50	18.7
Evaluation process is complicated and difficult to apply in practice	43	16.1

Table 6: Evaluation of TCM evidence using the GRADE system.

Project	Count	Percentage (%)
Generally lower level of evidence limited the usage of GRADE	97	88.2
"Treating the same disease with different methods" leads to higher risk of consistency bias	82	74.5
Higher-priority evidence such as ancient TCM case reports rated lower in GRADE	57	51.8
It can be well applied and improve the decision-making level	42	38.1
Others	8	7.3

Table 7: Suggestions on the evaluation of clinical evidence for TCM.

Project	Count
Hope to be able to promote and provide more help for clinical decision-making of grassroots TCM doctors	10
Pay attention to the characteristics of TCM diagnosis and treatment and construct an evaluation system in accordance with its own laws	7
The evaluation system should be operable, objective, and simple for practical use	6
If a system is recognized worldwide, then international tools should be used	3
There should be a unified evaluation standard	3
International evidence-based norms should be followed, and the characteristics of TCM differentiation and treatment should be integrated	2

Through an electronic questionnaire, the present study investigated the cognition and application status of TCM clinical evidence evaluation systems in the medical industry. The results showed that the respondents possessed a better understanding of clinical evidence evaluation systems and used them occasionally. However, with respect to the TCM evidence and evaluation system, they possessed only a moderate understanding, with the use aspect rated at the "infrequently" level. More than half of the respondents who had been exposed to TCM evidence evaluation knew and used Liu Jianping's or Wang Shouchuan's versions of the "Five-level system of TCM improved version," which may relate to the development of evidence-based medicine in China and the formulation and promotion of TCM clinical practice guidelines in recent years. In one study [10], the authors reviewed the evidence-based clinical practice guidelines of TCM published within the past five years. Of the 24 guidelines, 11 applied the "Five-level system of TCM improved version" by Wang Shouchuan, and 11 applied the Jianping version; the majority of medical practitioners learned and used the guidelines in clinical practice [11]. The results of the survey also showed that most practitioners used the existing evidence evaluation system for clinical problems, research design, and manuscript writing, indicating that it was necessary to improve and popularize the evaluation method of TCM clinical evidence.

When they applied any existing methods to evaluate the clinical evidence for TCM, most respondents said that they encountered difficulties in obtaining high-quality evidence and that clear evaluation criteria were lacking. The most common complaint of the respondents was that the evaluation standards of various evaluation systems were inconsistent, and the grades for the same evidence in disparate evaluation systems were quite different. Research on the evaluation of TCM clinical evidence is currently primarily performed by medical colleges and evidence-based medical centers at all levels in China. The lack of recognized evaluation standards, especially the evaluation standards of ancient and modern literature evidence of TCM, leads to the same type of evidence, such as the experience of ancient doctors, and is simply evaluated as the highest or lowest level of evidence and thus may create problematic issues regarding the formation of recommendations and even clinical decision-making. In addition, the lack of dissemination also affects the application of evidence evaluation systems [12].

The proportion of respondents who used the GRADE system to evaluate TCM evidence was not large: over half of the respondents believed that the GRADE system was suitable for TCM clinical evidence evaluation but that appropriate modifications needed to be made to reflect the clinical characteristics of TCM (such as improving the level of TCM evidence). Some investigators [13] believe that the

introduction of the GRADE system is significant in constructing the evidence evaluation system for TCM. In addition, they recommend that we should pay attention to TCM syndrome differentiation and treatment, the improvement of TCM clinical research quality, and the evaluation of historical TCM literature and expert experience. The GRADE system has the characteristics of strong practicality, exhibiting a transparent evaluation process and ease of operation, and being used widely internationally. Combined with the suggestions of respondents, the evaluation of TCM clinical evidence may ultimately be unified based upon the GRADE system.

The survey was mainly designed for individuals in the medical and related industries who were interested in this topic. Although voluntary response bias cannot be ruled out in these voluntary and anonymous questionnaires, the results do not reflect a large number of strong opinions. While the sample size outnumbered the calculated minimal value, it may still be limited, which might engender some biases in the results. However, in the absence of relevant data in this field, we believe that the conclusion based on 284 valid samples is meaningful. In addition, due to the strong professionalism of the questionnaire, the distribution of the respondents was not uniform, and the investigated population was relatively concentrated; thus, this questionnaire had some limitations in sampling representativeness. The use of the limited media of questionnaires, WeChat, and web links, and the accessibility of the Internet may also have influenced the results.

5. Conclusions

In conclusion, practitioners exhibited a certain degree of awareness regarding the evaluation of clinical evidence for TCM. Although they showed a substantial willingness to use it, its practical application was not acceptable in its current situation. Practitioners expected to possess an evidence-based evaluation system that would be more congruent with the clinical characteristics of TCM. The principal obstacles to its application appear related to its own development, including the numerous evaluation systems, variable standards, an opaque process, a lack of training and promotion, and other external factors. It may be an effective method to establish a unified evaluation system on the basis of the GRADE system that is widely used, but with additional in-depth research, improvement, and application according to the characteristics of clinical practice and the current standards of TCM. Standardized evidence-based evaluation of TCM would provide strong support for the development of clinical practice guidelines for TCM and for evidence management and information services. Scientific classification and recommendation systems of clinical evidence for TCM would promote the development of evidence-based Chinese medicine and internationalization of TCM.

Data Availability

The data used to support the findings of this study are available from the corresponding author on request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Supplementary Materials

Questionnaire on clinical evidence evaluation of traditional Chinese medicine. (*Supplementary Materials*)

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