ID: 2019ZY004-MERIDIAN

Research title:

A study on the microcirculatory characteristics of meridian phenomenon for the heart and lung meridians based on patients with chronic obstructive pulmonary disease

Date: March 25, 2019

Study Protocol

Part1. Study introduction and objectives

(1) Brief introduction

This study will include 40 patients with chronic obstructive pulmonary disease (COPD), and 80 healthy volunteers. Laser doppler examination will be adopted to assess the microcirculatory characteristics of meridian phenomena and investigate the site specificity for the meridian-visceral association and surface-surface association between two specific meridians.

(2) Study objects

1) This study aims to detect the microcirculatory characteristics of meridian phenomena using laser doppler flowmetry(LDF). Thus, the biological characteristics of meridian phenomenon could be presented objectively in a scientific methodology.

2) This study aims to investigate the site specificity for the meridian-visceral association and surface-surface association between two specific meridians.

Part2. Study program

1.Study subjects

The subjects of this study include two kinds of participants, which includes healthy volunteers and patients with COPD.

1.1 Diagnostic criteria

1.1.1 Diagnostic criteria for COPD

The diagnostic criteria of COPD is based on “The diagnosis and treatment guidelines of chronic obstructive pulmonary disease” by the Thoracic Society of Chinese Medical Association in 2013 and “the Global Initiative for Chronic Obstructive Lung Disease (GOLD)” in 2017. The clinical symptoms include dyspnea, chronic cough and expectoration. Patients often have a history of exposure to various risk factors. And persistent airflow obstruction is indicated by the pulmonary function test (post-bronchodilator FEV1 /FVC＜0.70). In addition,other possible diseases are excluded.

1.2 Inclusion criteria

1.2.1 Inclusion criteria for COPD

(1) Patients should meet the above diagnostic criteria, and the severity of COPD is in the stage of GOLD 2 or 3 based on pulmonary function testing;

(2) COPD patients in the stable phase, who present with mild symptoms of cough, expectoration and short breath;

(3) $35 \leq$ age ≤ 75 years, male or female;

(4) Patients have clear consciousness and could communicate with others normally;

(5) Patients could understand the full study protocol and have high adherence .Written informed consent is signed by themselves or their lineal kin.

1.2.2 Inclusion criteria for health volunteers

(1) Healthy volunteers who could provide a recent medical examination report to confirm they have not any cardiovascular, respiratory, digestive, urinary, hematological, endocrine and neurological disease;

(2) age$\geq$20 years, male or female;

(3) Participants have clear consciousness and could communicate with others normally;

(4) Participants could understand the full study protocol and have high adherence .Written informed consent is signed by themselves or their lineal kin.

1.3 Exclusion criteria

1.3.1 Exclusion criteria for COPD

(1) Patients who fail to meet the diagnostic criteria for COPD, or COPD patients in the phase of acute exacerbation;

(2) Patients have the following complications, such as pneumonia, bronchial asthma, bronchiectasis, active tuberculosis, pneumothorax, chest trauma, tumors of the lung or thorax;

(3) Patients have concomitant conditions of heart diseases, such as chronic stable angina pectoris (CSAP);

(4) Patients have serious concomitant conditions and fail to treat them effectively, such as diseases of the digestive, urinary, respiratory, hematological, and nervous

system;

(5) Patients have mental illness, severe depression, alcohol dependence or history of drug abuse;

(6) Pregnant or lactating patients;

(7) Patients are participating in other trials.

1.3.2 Exclusion criteria of health volunteers

(1) Participants have mental illness, severe depression, alcohol dependence or history of drug abuse;

(2) Pregnant or lactating participants ;

(3) Participants are participating in other trials.

1.4 Participant recruitment

All the participants will be enrolled from the Third affiliated hospital of Zhejiang Chinese Medical university and the First affiliated hospital of Zhejiang Chinese Medical university.

2. Study methods

2.1 Study design

This is a prospective and open-label clinical trial. The time frame of the trial ranges from July 2019 to December 2020. A total of 120 participants will divided into the healthy control group, COPD group and healthy intervention group., with 40 in each group respectively.

2.2 Sample size estimation

This project is a clinical research using laser doppler to assess the biological characteristics of this meridian phenomenon. It belongs to meridian researches. Compared with general clinical trials, there is no unified standard for the sample size estimation. Based on similar studies conducted in China and foreign countries, as well as the consideration of the actual research conditions of this study, we planned to enroll a total of 120 participants, which includes 40 COPD patients and 80 healthy volunteers.

2.3 Participant selection

Prior to the study, the researchers should explain the purpose, contents, benefits

and potential risks of the study to all participants clearly and colloquially. Then the participants or their families should sign the informed consent form, otherwise, they will not be included in the trial.

After signing the informed consent form, all participants will receive the following assessment to determine whether they could be included.

(1) Demographic data and medical history;

(2) Vital signs: respiratory rate, heart rate, blood pressure, and body temperature;

(3)Laboratory examinations: blood routine test, biochemical routine test (including AST, ALT, BUN, Cr, GLU, blood lipid, etc.);

(4) Electrocardiogram.

2.4 Grouping

A total of 120 participants will divided into the healthy control group, COPD group and healthy intervention group., with 40 in each group respectively.

2.5 Blinding

The participants and outcome assessors will not be blinded. In the data analysis stage, blinded statistical analysis will be adopted. Statistical analysis will be conducted by third party statisticians who are blinded to the study protocol.

2.6 Intervention

All the participants are requested to refrain from consuming tea/alcohol/coffee and smoking on the examination day. Besides, exercise and food is also forbidden within one hour before the LDF examination.

2.6.1 Location of the acupoints

(1)LU9: On the anterolateral aspect of the wrist, between the radial styloid process and the scaphoid bone, in the depression ulnar to the abductor pollicis longus tendon.

(2)LU5: On the anterior aspect of the elbow, at the cubital crease, in the depression lateral to the biceps brachii tendon.

(3)HT7: On the anteromedial aspect of the wrist, radial to the flexor carpi ulnaris tendon, on the palmar wrist crease.

(4)HT3: On the anteromedial aspect of the elbow, just anterior to the medial

epicondyle of the humerus, at the same level as the cubital crease.

2.6.2 Examination environment

The environmental temperature is within 24–26◦C during the entire measuring period. The relative humidity will be controlled between 40% and 50%. There is no direct sunlight and obvious air convection in the room.

2.6.3 Procedures for LDF examination and moxibusition intervention

A four-channel LDF (PeriFlux System 5000, Sweden) will be used to measure the microcirculatory characteristics of meridian phenomena for the Heart and Lung meridians, which could monitor 4 measuring sites simultaneously. The participants will be asked to stabilize for 15 minutes in a supine position in the experimental room before formal examination. They will be informed to keep silent and normal breath and avoid limb movement during the whole measuring period. The probes will be left at relevant measuring sites. Blood flow curve will be recorded constantly using Perisoft software (PeriFlux, Sweden). Perfusion units (PU) of microcirculatory flux in the measuring sites will be calculated by the software [PU =concentration of moving blood cells (CMBC)×velocity (V)].

(1)Healthy control group and COPD group

The probes will be left at 4 measuring acupoints, which include Shenmen (HT7) and Shaohai (HT3) of the Heart meridian, Taiyuan (LU9) and Chize (LU5) of the Lung meridian. The blood flow curve and PU will be recorded for 5 minutes.

(2)Healthy intervention group

Two sessions of moxibustion intervention will be performed in the Heart meridian and Lung meridian successively. The washout period between the two sessions is at least one day.

1)Intervention in the Heart meridian: By igniting the moxa stick and inserting it into a homemade moxibustion holder to adjust the appropriate angle and height, moxibustion will be performed above Shaohai (HT3) of Heart meridian for 15 minutes. During moxibustion, the probes will detect the blood flow curve and PU in four sites in two meridians, which include Shaohai (HT3) of the Heart meridian, the midpoint of the Heart meridian along the left forearm (i.e. the midpoint of the line

between HT3 and HT7), Chize (LU5) of the Lung meridian, and the midpoint of the Lung meridian along the left forearm(i.e. the midpoint of the line between LU5 and LU9). The measuring time points include 5 minutes before moxibustion, 15 minutes during moxibustion and 5 minutes after stopping moxibustion.

2)Intervention in the Lung meridian: The moxibustion acupoint is Chize (LU5) of the Lung meridian. The LDF examination, moxibusition procedure, measuring sites and time points are the same as 1).

2.6.4 Concomitant treatments

During the study period, all the participants in the COPD group will maintain their previous treatment regimen. If additional medications or other treatments are used during the study period due to any reasons, the details (e.g. the name, administration time and dosage of the medication) should be documented.

Besides, participants in the healthy control group and healthy intervention group should not take any medications during the full study period. If drugs or other treatments are adopted due to sudden diseases, relevant information should be documented and researchers will evaluate whether they should be withdrawn from the study.

3. Outcome measurement

Primary outcomes will be blood flow curve and blood perfusion units (PU) of relevant sites along the Heart and Lung meridians.

4. Safety evaluation

Adverse events (AEs) that occur during the trial will be recorded and assessed by the investigators during each examination session and at each visit next time. If serious AEs occur, the researchers should report them to the principal investigator and ethics committee immediately, who will make a decision on whether the participant should be withdrawn from the study.

5. Ethical approval and study registration

Ethics approval (approval document No: ZCMU-KY-2019-042, April 29, 2019) was obtained from the Ethics Committee of Zhejiang Chinese Medical University. The purpose, contents and potential risks of the research will be fully explained to the

participants and their families. All participants will complete the informed consent form before participating in the study. All participants' personal and disease information will be kept confidential.

6. Quality control

(1) The trial protocol has been modified according to suggestions from experienced acupuncturists.

(2) Before the trial, all researchers who enroll participants and collect data must attend a series of training sessions. These training sessions will ensure that all research staff involved fully understand the trial protocol and standard operating procedures (SOP).

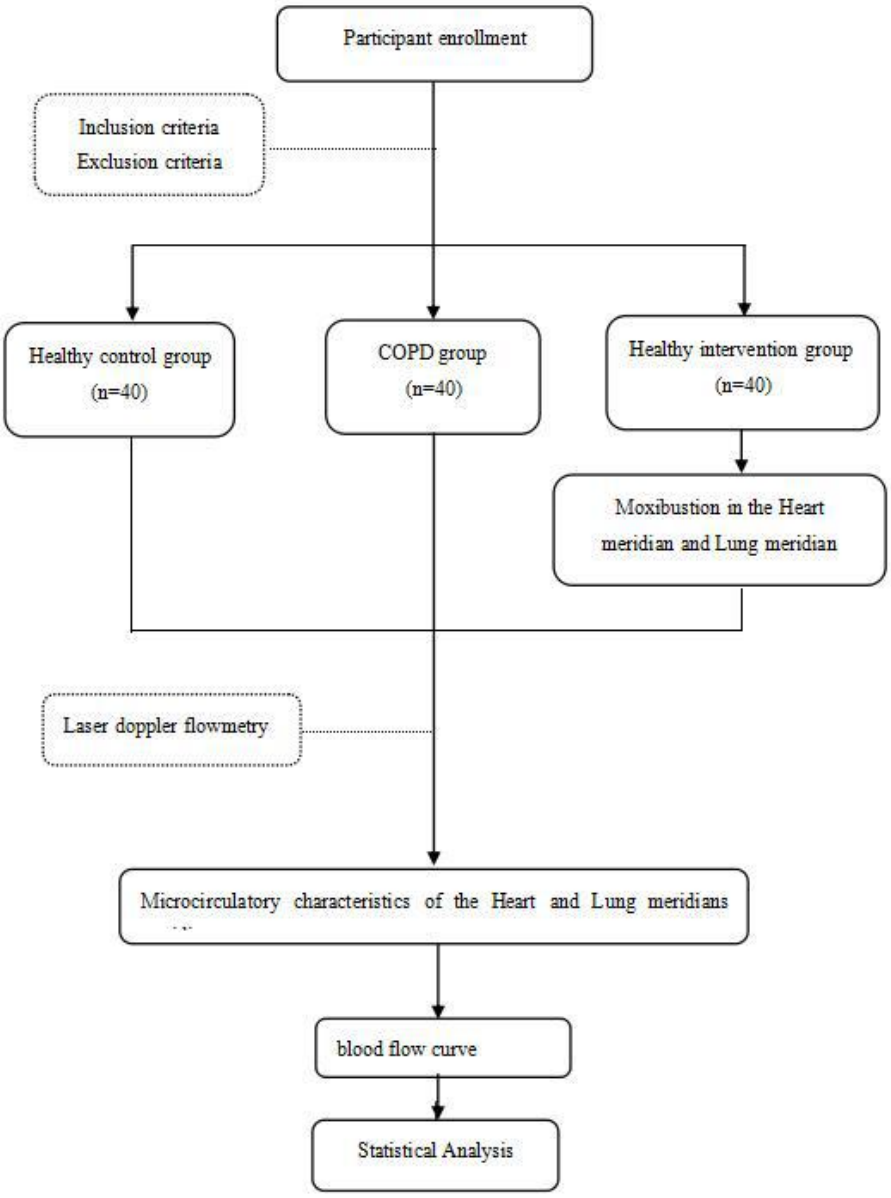
(3) SOP of the examination method is designed before the trial.

(4) Data collection will be performed in accordance with the pre-approved protocols. All assessors will be trained uniformly to record outcomes and fill in the case report forms. One independent supervisor will regularly verify the consistency of the raw data and the recorded data. Data management and monitoring will also be performed by using ResMan Research Manager (http://www.medresman.org).

(5) During the trial, clinical supervisors will guide and supervise the operators regularly (once every three months).

(6) Economic compensation is adopted to improve compliance and reduce dropouts and withdrawals of participants.

7. Flow diagram of the study design



Part3. Statistical Analysis Plan

1. Data entry and storage

1.1 Data entry

(1) The original data collection

All observation scales will be measured on a "one to one" basis according to the unified standard, and the subject will be completed independently under the guidance of the investigators to ensure complete and correct completion. In the spot, check whether the filling quantity is accurate. If any omission or blurring is found in the case report forms, the assessors should verify the original data in time.

(2) Data entry and statistics

The Excel spreadsheet is used to record the original data and ensure it is accurate and reliable. A blinded statistician is employed to carry out the statistical analysis and the research managers should verify whether the statistical methods are appropriate.

1.2 Data storage

(1) The special person is responsible for the management of various documents, and there are special folders for storage in dedicated files, so that the test researcher can view it, and have access and access records.

(2) The test documents shall be protected strictly in accordance with the confidential management principles.

(3) Test file is available for test researchers and relevant researchers view, and other irrelevant personnel should not be entitled to refer to.

(4) The equipment of storing test files has safety measures such as insect repellent, fire prevention, moisture-proof and anti-theft.

2. Statistical processing

2.1 Statistical software

SPSS v20.0 (SPSS, Chicago, IL, USA).

2.2 Data description

Measurement data is described as mean standard±deviation, median, maximum, minimum and quartile, and counting data is presented as percentage (%).

2.3 Statistical analysis

All data in this study will be analyzed by a blinded statistician. Independent sample T test and Chi-square test ($\chi 2$ test) will be used for numerical variables and categorical variables, respectively. When the distribution of variables is abnormal, a non-parametric test will be selected. A P value < 0.05 will be considered statistically significance.