

Research title:

A study on the microcirculatory characteristics of meridian phenomenon for the heart and lung meridians based on patients with stable angina pectoris

Date: March 25, 2019

Study Protocol

Part1. Study introduction and objectives

(1) Brief introduction

This study will include 40 patients with chronic stable angina pectoris (CSAP), and 40 healthy volunteers. Laser doppler examination will be adopted to assess the microcirculatory characteristics of meridian phenomenon for Heart and Lung meridians. Besides, the relative specificity of the two meridians will be investigated.

(2) Study objects

1) This study aims to assess the microcirculatory characteristics of meridian phenomenon of Heart and Lung in healthy volunteers and patients with CSAP by using laser doppler. Thus, the biological characteristics of meridian phenomenon could be presented objectively in a scientific methodology. Besides, the relative specificity of the two meridians will be investigated.

2) This study aims to build standardized techniques and schemes for detecting the microcirculatory characteristics of meridian phenomenon for Heart and Lung in different conditions, which includes physiology state and pathological state. Besides, it could lead to the formation of a standardized, effective clinical therapeutic regimen for treating diseases relevant to Heart and Lung meridians. The results of this study could provide scientific foundation for traditional meridian theories, even raise the reestablishment of theories.

Part2. Study program

1. Study subjects

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

1.1 Diagnostic criteria

1.1.1 Diagnostic criteria for CSAP

The diagnostic criteria of CASP is based on the “2007 Chronic Angina Focused Update of the ACC/AHA 2002 Guidelines for the Management of Patients With Chronic Stable Angina” by the American College of Cardiology and American Heart

Association (ACC/AHA) in 2007 and “Guidelines for the diagnosis and therapy of patients of chronic stable angina pectoris” by the Chinese Society of Cardiology of Chinese Medical Association.

(1) Region of the pain: The region of typical angina pectoris is often located behind the sternum or in the left anterior chest, and its range is often not limited. It can radiate to the neck, pharynx, jaw, upper abdomen, shoulder, back, left arm and fingers of the left hand, as well as other parts. The region of pain can also be located outside the chest, such as the upper abdomen, pharynx, and neck. The region for each onset of angina pectoris is often similar.

(2) Characteristics of pain: The characteristics of pain is often griplike and squeezing. The symptoms could also be burning or distension in the chest, short breath, or even feeling of fear and impending death. Some patients just complain chest discomfort.

(3) Duration of pain: The pain is paroxysmal. It could last for several minutes, but generally less than 10 minutes. It will not be momentary or last for several hours.

(4) Trigger factors and relieving methods: The onset of pain is related to physical labour or emotional excitement. Fast walking or climbing upstairs could trigger the pain, once stop walking and have a rest, the pain could relieve. Sublingual nitroglycerin will relieve the symptoms in 2 to 5 minutes.

(5) The degree, frequency, characteristics and inducing factors of angina will not change significantly within a few weeks.

(6) Diagnosis is supported by other examinations, such as laboratory examination, electrocardiogram, echocardiography and coronary arteriography.

1.2 Inclusion criteria

1.2.1 Inclusion criteria for CSAP

(1) Patients should meet the diagnostic criteria of coronary heart disease, which includes the following items: 1) confirmed old myocardial infarction (MI), or a history of percutaneous coronary intervention (PCI), or coronary artery bypass grafting; 2) 50% or more luminal stenosis in at least one coronary artery or major branch segment confirmed by coronary angiogram or CT angiography; 3) myocardial

ischemia indicated by exercise stress radionuclide myocardial imaging; 4) treadmill exercise testing is positive (for male patients);

(2) Patients should meet the diagnostic criteria of CSAP and the Canadian Cardiovascular Society (CCS) classification for CSAP is level II or III;

(3) The medical history of angina pectoris ≥ 3 months, with at least 2 episodes per week in the last month;

(4) $35 \leq \text{age} \leq 65$ years, male or female;

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

(6) 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 (in the past 3 months) medical examination report to confirm they have not any cardiovascular, respiratory, digestive, urinary, hematological, endocrine and neurological disease;

(2) $35 \leq \text{age} \leq 65$ 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 CSAP

(1) Patients with acute coronary syndrome (including acute myocardial infarction and unstable angina) and severe arrhythmias (such as severe atrioventricular block, ventricular tachycardia, supraventricular tachycardia, frequent premature beats and premature ventricular contraction);

(2) Patients' chest pain is caused by valvular heart disease, hypertrophic cardiomyopathy and dilated cardiomyopathy;

(3) Patients' chest pain is caused by non-cardiac disease (such as severe neurosis, climacteric syndrome, cervical spondylosis, and esophageal/pulmonary/chest wall

lesions);

(4) Patients have concomitant conditions of lung diseases, such as COPD;

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

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

(7) Pregnant or lactating patients;

(8) Patients are participating in other trials.

1.3.2 Exclusion criteria of health volunteers

(1) Participants have sudden severe diseases during the trial, such as cardiovascular diseases, liver diseases, kidney diseases, urinary diseases and hematological diseases.

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

(3) Pregnant or lactating participants ;

(4) 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 80 participants will be divided into the CSAP group and healthy 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 80 participants, which includes 40 CSAP patients and 40 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 80 participants will be divided into the CSAP group and healthy group, with 40 subjects in each group.

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 Detection and acupuncture method

- (1) Location of the acupoints

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.

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

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

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)Matters needing attention

During the 2-week study period, patients in the CSAP group will maintain their previous treatment regimen. However, if additional medications or other treatments are used during the study period due to any reasons, patients should report it to the researchers to enable them to keep a detailed record. Besides, participants in the healthy group should not take any medications during the entire study period. If drugs or other treatments are used due to sudden diseases, the researchers should record the information and evaluate whether they should be withdrawn from the study.

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 1 hour before the detection.

(3)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.

2.6.1 Laser doppler examination

(1) Experimental device: Four-channel laser doppler flowmetry (PeriFlux System 5000, Sweden).

(2) Experimental process: The participants will be allowed to stabilize for 15 minutes in a supine position in the experimental room before formal examination. They are asked to keep silent and normal breath and avoid limb movement during the whole measuring period. The probes will be left at 4 measuring sites. Blood flow curve will be recorded constantly using Perisoft software (PeriFlux, Sweden). Microcirculatory flux in the measuring sites will be calculated [Perfusion units (PU) =concentration of moving blood cells (CMBC)×elocity (V)]. The acupoint microcirculation will be recorded for 5 minutes.

(3) Measurement sites: Shenmen (HT7) and Shaohai (HT3) of the Heart meridian, Taiyuan (LU9) and Chize (LU5) of the Lung meridian

3. Outcome measurement

(1) Microcirculatory characteristics: blood flow curve and blood perfusion units (PU).

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-2019-039, 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.

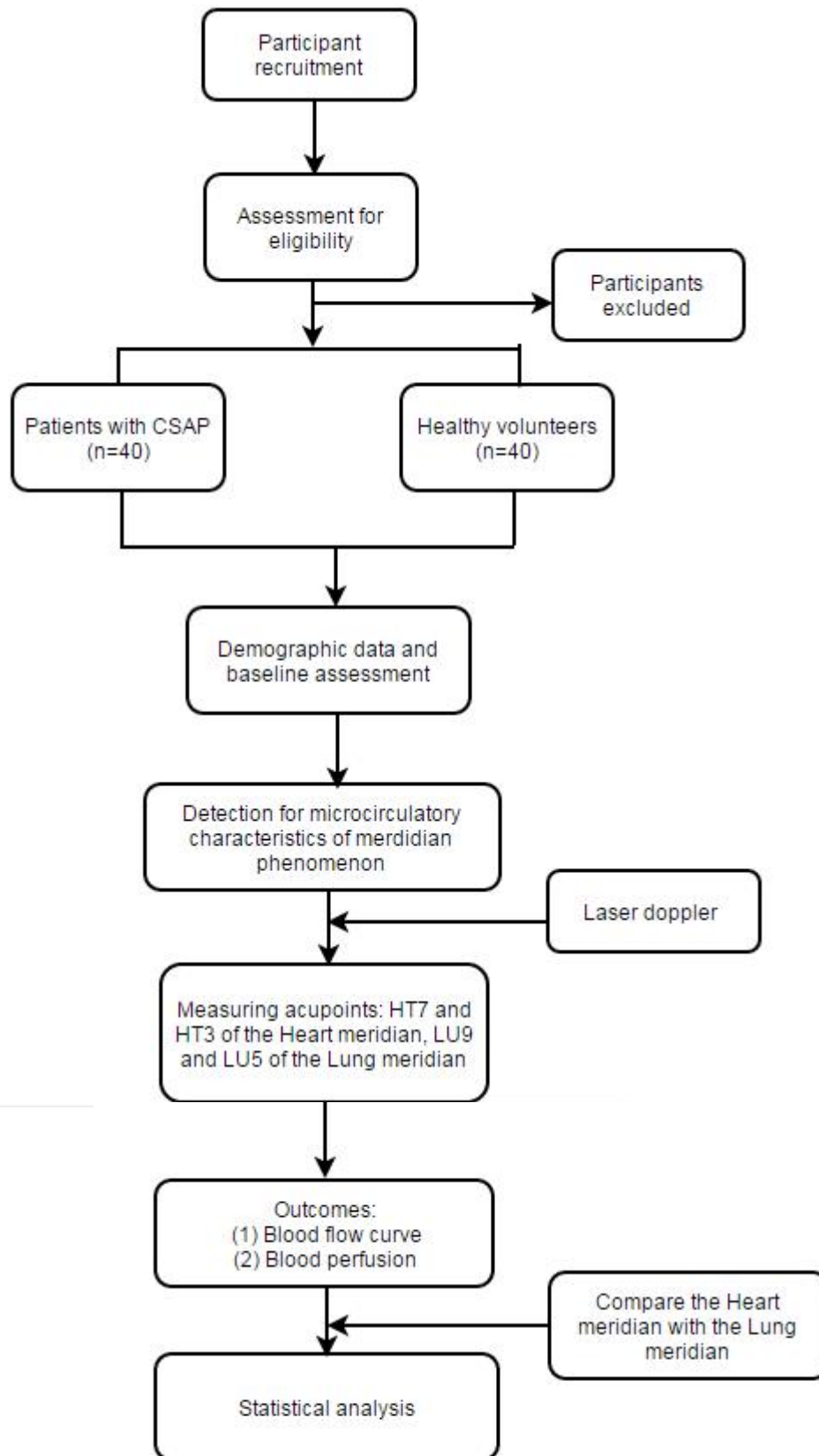
(4) Data collection will be performed in accordance with the pre-approved protocols. All assessors will be trained uniformly to record outcomes and fill in the case report forms. One independent supervisor will regularly verify the consistency of the raw data and the recorded data. Data management and 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 (χ^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.