Microvascular Angina Intervention with Compound Danshen Dripping Pill (MAIDS)

Pre-experimental research plan

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1. Research Background

Coronary microvascular disease (MVD) is a clinical syndrome refers to laboratory evidence of exertional angina or myocardial ischemia caused by structural and/or functional abnormalities of precoronary arterioles and arterioles under the influence of multiple pathogenic factors.. The main symptoms of patients with coronary microvascular disease are exertion-related chest pain attacks. It is difficult to distinguish patients with coronary microvascular disease from patients with severe coronary stenosis based on symptoms alone. However, the following clinical characteristics suggest that patients have coronary microvascular disease. It is more likely. First of all, it is more common in women, accounting for about 56%-79% of patients with coronary microvascular disease. However, most female patients with coronary microvascular disease have their first symptoms after menopause, which is no different from traditional female coronary heart disease patients. Secondly, most of the symptoms are induced by exertion, but it is not uncommon to have patients with silent chest pain. There are fewer patients with coronary microvascular disease who simply present with silent chest pain. Impaired coronary dilatation, increased sympathetic stimulation and sensitivity, and exercise-mediated coronary constriction can lead to the development of coronary microvascular dysfunction and reduced coronary flow reserve. Long-term recurrent angina pectoris attacks affect

the patient's quality of life. Patients with significantly reduced coronary blood flow reserve or myocardial perfusion reserve, especially female patients, may have a higher incidence of adverse cardiovascular events.

Regarding drug treatment, traditional anti-ischemic treatment methods are currently recommended, including β- blockers, calcium ion antagonists and nitrates. If symptoms persist, other drugs include angiotensin-converting enzyme inhibitors, statins, ivabradine , ranolazine , and estrogen drugs. However, there is no specific clinical drug treatment. Basic and clinical studies of the traditional Chinese medicine Compound Danshen Dripping Pill have found that it can improve vascular endothelial function and relieve angina pectoris, and is widely used in clinical practice. However, its effect on coronary reserve function and angina pectoris in coronary microvascular disease needs further clinical research to confirm.

2. Study drug

The main drug studied in this study is Compound Danshen Dripping Pill. Compound Danshen Dripping Pills (Compound Danshen Dripping Pills, produced by Tasly Holding Group Co., Ltd.) is a compound traditional Chinese medicine preparation composed of Salvia miltiorrhiza, Panax notoginseng, and Borneol. In 1994, it was approved as a drug for the treatment of coronary heart disease and angina pectoris. It has good

clinical efficacy and has It has the characteristics of fast onset, low toxicity, small adverse reactions and high safety.

The active ingredients of compound Danshen dripping pills are water-soluble phenolic acids such as salvia miltiorrhiza and Panax notoginseng saponins. During the preparation process, borneol and auxiliary materials are added, and a highly dispersed solid dispersion is made through a specific process. The active ingredients of the drug have high purity, are evenly dispersed, and can be quickly absorbed through the mucous membrane. The dripping pills have good solid dispersion effect. The principle is to melt the drug ingredients and matrix into a solid solution, and the active ingredients of the drug are in the molecular state or in extremely fine form. The crystalline state is highly uniformly dispersed in the matrix, with fast drug dissolution and higher bioavailability. It can be taken sublingually and can quickly relieve angina pectoris and chest tightness.

Compound Danshen Dripping Pill has achieved a full-process quality control method from medicinal materials, intermediates to preparations (GAP-GEP-GMP) through the multi-factor fingerprint quality control system of compound oral Chinese medicine preparations, further improving the full-process quality control system of Compound Danshen Dripping Pill , after testing and analyzing nearly 200 batches of Compound Salvia Miltiorrhizae Dropping Pills Extract and Compound

Salvia Miltiorrhizae Dropping Pills, the similarity is over 90%, and the quality of the products is stable between batches. It has been confirmed through the toxicology study of Compound Danshen Dripping Pill and the clinical use of hundreds of millions of people: it has no toxicity, few adverse reactions, and is safe and reliable for long-term use.

As a Chinese patent medicine for the treatment of stable angina pectoris, Compound Danshen Dripping Pill are included in Category A of the 2018 National Essential Drugs Catalog and the 2019 National Basic Medical Insurance, Work Injury Insurance and Maternity Insurance Drug Catalogue. Under the name of "Compound Danshen Dripping Pill", it has been written into more than 20 national medical guidelines or consensus, 15 of which involve the cardiovascular field, including "Guidelines for Rational Medication in Coronary Heart Disease (2nd Edition)", "Acute Myocardial Medication "Guidelines for the Diagnosis and Treatment of Infarction by Integrated Traditional Chinese and Western Medicine", "Chinese Expert Recommendations on the Clinical Application of Compound Danshen Dripping Pill", "Expert Consensus on the Diagnosis and Treatment of Atherosclerosis by Integrated Traditional Chinese and Western Medicine", etc.

In terms of international evidence-based research, large-sample, randomized, double-blind, placebo-controlled RCT studies were used, and 1,004 patients were included in phase III clinical studies at 127

centers in 9 countries/regions around the world, indicating that the US FDA-Phase III The main clinical efficacy indicators: significantly increase the patient's exercise flat time and improve exercise tolerance. Compared with the increase time of exercise oxygen tolerance of similar drugs, Compound Danshen Dripping Pill has obvious advantages . Secondary efficacy indicators: effectively reduce the number of angina attacks per week and significantly reduce the weekly nitroglycerin dosage.

In terms of domestic evidence-based research: 1011 studies on Compound Danshen Dripping Pill for the treatment of stable angina, 16 Meta-analyses, with a total enrollment of more than 100,000 people; 137 studies on the treatment of peri-PCI/ thrombolysis period , the total number of participants exceeds 10000 ; there are 36 studies on Compound Danshen Dripping Pill improving coronary microcirculation disorders, filling the gap in Western medicine treatment.

Systematic study of Compound Danshen Dripping Pill combined with Western medicine in the treatment of coronary heart disease angina pectoris: 21 articles were included, with a total number of cases of 2229. Compound Danshen Dripping Pill combined with Western medicine can benefit patients with coronary heart disease angina pectoris, especially in improving clinical symptoms and electrocardiogram . It has outstanding effects on ischemic state and regulating cholesterol levels.

A meta-analysis based on PubMed, EMBASE, CNKI, and Wanfang databases studied the efficacy of aspirin combined with Compound Danshen Dripping Pill in the treatment of coronary heart disease. The analysis included 14 randomized controlled trials with a total of 1,367 patients. The results showed that the combination Compared with aspirin alone, the drug can achieve more significant therapeutic effects in relieving angina pectoris symptoms and lowering blood lipids. Lu Yuhong et al. randomly divided 128 patients with coronary heart disease into groups. The control group took oral aspirin on the basis of conventional treatment, and the observation group took Danshen Dropping Pills on the basis of conventional treatment. After 2 months, the total clinical effective rate of the patients in the observation group was significantly higher than Control group. Compared with before treatment, the maximum platelet aggregation rate and thromboxane B2) levels of the two groups of patients were significantly reduced, and the decrease in the observation group was more obvious than that in the control group . The "Guidelines for the Diagnosis and Treatment of Integrated Traditional Chinese and Western Medicine in Acute Myocardial Infarction", led by Academicians Chen Keji, Academician Ge Junbo, and Professor Zhang Minzhou, were formulated in conjunction with experts in the fields of traditional Chinese medicine, Western medicine, integrated traditional Chinese and Western medicine, and methodology across the country. The

guideline clearly states that Compound Danshen Dripping Pill are recommended for the treatment of acute myocardial infarction. The recommendation level is the highest in the guideline. Compound Danshen Dripping Pill can relieve chest pain, reduce the patient's risk of cardiac death, and improve the patient's cardiac function. and the role of quality of life.

3. Research purpose

A randomized, double-blind, placebo-controlled, multi-center clinical study of Compound Danshen Dripping Pill and blank control was conducted in patients with microvascular angina pectoris .

4. research design

4.1 Study title and registration

The Chinese name of this project is: Microvascular Angina Intervention with Compound Danshen Dripping Pill. English name: Microvascular Angina Intervention with Compound Danshen Dripping Pill. Referred to as MAIDS Study.

4.2 Research design : Randomized, double-blind, placebo parallel-controlled, multi-center clinical study.

4. 3 dosing regimens (double-blind single simulation)

(1) Placebo group: Placebo (the appearance is the same as Compound Danshen Dripping Pill , main ingredient: starch. Production unit: Tianjin Tasly Co., Ltd.), taken orally after meals, 3 times a day, 20 pills each

time .

(2) Treatment group: Compound Danshen Dripping Pill (Tianjin Tasly Co., Ltd.), taken orally after meals, 3 times a day, 20 pills each time .

Note: The batch number on the drug packaging is unified as " 161299 ".

5. Research objects

5.1 Source of patients:

patients with chest pain who had no obvious coronary artery stenosis on coronary angiography or coronary CT examination were admitted to the hospital outpatient clinics and wards .

5.2Selection criteria _

（1） With typical symptoms of exertional angina;

（2） Coronary CTA or angiography with normal coronary artery or <50% stenosis, or <50% residual coronary stenosis after revascularization;

（3） Ischemic downward shift of the ST segment is found in the electrocardiogram at rest or during exercise stress (horizontal or downsloping downward shift behind the J point >0.1mv, lasting 0.08s);

（4） Transthoracic ultrasound before and after intravenous adenosine injection to check the anterior descending coronary artery blood flow reserve test CFR <2.5;

（5） The patient agreed to participate in this study.

5.3 Exclusion criteria

(1) Aged less than 30 years old or older than 75 years old;

(2) Have a history of carotid endarterectomy or stent implantation, and have a history of stroke ;

(3) Myocarditis, pericardial disease, valvular disease, and cardiomyopathy;

(4) Difficult-to-control diabetes (fasting blood glucose >7.0 mmol/L);

(5) Uncontrollable hypertension (SBP>150 mmHg and/or DBP>90 mmHg);

(6) Familial hypercholesterolemia;

(7) Takayasu arteritis;

(8) Those who are pregnant or lactating, or those who intend to have a child within one year, or those who have not taken effective contraceptive measures during the childbearing age;

(9) Abnormal liver function (serum GPT level exceeds 3.0 times the upper limit of normal value) or abnormal renal function (serum creatinine level exceeds 2 mg/dl);

(10) Other clinically significant respiratory, digestive, blood, infection, immune, endocrine, neuropsychiatric, tumor diseases, etc., which may cause serious danger to patients;

(11) Take K channel openers and traditional Chinese medicine

preparations that activate blood circulation and remove blood stasis to improve microcirculation;

(12) Those allergic to intra-arterial injection of contrast media, blood, and blood products;

(13) Patients who are participating in other clinical studies.

6. Research methods

6.1Sample size

The experimental drugs and control drugs in this clinical trial will be selected according to the number of cases in a ratio of 1:1. 100 patients were enrolled in the preliminary experiment .

6.2 Random grouping

The randomization method adopts the central randomization method. The central randomization system uses DAS2.1.1 software to generate random numbers (00 1 -100) and drug packaging numbers (B ZH001-100). The random numbers and drug packaging numbers are separated in the system . The researcher passes the Apply to the system to obtain the drug packaging number for the subject. The random number and drug packaging number of the same subject are different, but their corresponding treatment plans are consistent within the system.

6.3 Research process

After signing the informed consent form, the selected patients were

divided into the Compound Danshen Dripping Pill treatment group and the placebo group according to a random, double-blind, placebo-controlled method. Dosage of Compound Danshen Dripping Pill or placebo: 3 times a day, 20 pills each time, for a total of 6 months . Follow-up visits were conducted every 2 months, and the follow-up period was 6 months in total .

6.4 Screening Phase

（1）**CTA or CAG examination:** Coronary artery CTA or angiography examination shows that the coronary arteries are normal or stenotic <50%, and coronary epicardial vasospasm is excluded;

（2）**Electrocardiogram:** Resting or exercise stress test shows ischemic downward shift of ST segment, horizontal or downward shift behind J point >0.1mv, lasting 0.08s.

（3）**Laboratory tests:** Fasting for more than 6 hours, blood routine, urine routine, fasting blood glucose, alanine aminotransferase, aspartate aminotransferase, troponin, serum creatine phosphokinase, urea nitrogen, creatinine , plasma total cholesterol, triglycerides, hypoglycemia The existing data on density lipoprotein cholesterol, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, and prothrombin time during the screening phase can no longer be reviewed.

（4）**Echocardiogram:**

After signing the informed consent form, the following inspections will be performed: See Appendix 1 for inspection methods.

1) Routine ultrasound examination: two- dimensional dynamic images of left ventricular parasternal long axis, apical four-chamber, apical two-chamber, apical long axis and left ventricular apex level, papillary muscle level, and mitral valve level short-axis sections. At the same time, pulse wave Doppler was used to obtain the diastolic mitral valve orifice blood flow spectrum, and tissue Doppler was used to obtain the mitral valve annular motion spectrum. See Appendix 1 for details.

2) Acoustic contrast adenosine test: First, observe the distal left anterior descending coronary artery under color Doppler guidance, measure the blood flow velocity with pulsed Doppler ultrasound, and then administer the contrast agent SonoVue (add 8.5ml sterile saline to one bottle) bolus injection, followed by slowly injecting 5 ml of normal saline over 20 seconds, and measuring the blood flow velocity of the distal left anterior descending coronary artery again . Finally, adenosine 140 $\mu g.kg^{-1}.min^{-1}$ was given for 6 minutes by continuous intravenous micropump injection. During the injection process, the coronary blood flow velocity was monitored. Pulse Doppler ultrasound measured the maximum coronary blood flow velocity in the distal left anterior descending artery. This could be repeated at the

same time. Acoustic contrast enhances spectral signals.

3) The adenosine test simultaneously records a 12-lead electrocardiogram.

4) After recording the coronary blood flow in the contrast-enhanced ultrasound examination, the two-dimensional short-axis sections of the left ventricular parasternal long axis, apical four- chamber heart, apical two-chamber heart, apical long axis and left ventricular apex level, papillary muscle level, and mitral valve level were recorded. dynamic images.

6.5 Enrollment and follow-up phase

（1）Case grouping

100 patients were selected and randomly divided into Compound Danshen Dripping Pill group and control group, 50 cases each. Compound Danshen Dripping Pill: 20 pills, three times a day; Placebo: 20 pills, three times a day.

（2）Follow-up observation

Follow-up observations were conducted at 2 months, 4 months, and 6 months after inclusion, including clinical conditions, laboratory examinations, and ultrasound examinations. Safety assessment.

7. Efficacy evaluation

7.1 Main observation indicators

Difference in left anterior descending coronary flow reserve (CFR) measured by ultrasound at 6 months compared with baseline.

7.2 Secondary observation indicators

1) Difference in left anterior descending coronary flow reserve (CFR) measured by ultrasound at 2 months and 4 months compared with baseline.

2) Number of angina attacks per week;

3) The time when angina pectoris occurs in exercise test;

4) The time when ischemic ST segment depression occurs during exercise testing.

8.Safety assessment

Including clinical adverse reactions, laboratory indicators, etc.

9. Adverse event observation and reporting

9.1Definition of Adverse Events

Adverse events refer to any adverse medical event that occurs to a patient in this clinical trial from the time the patient signs the informed consent form and is selected to participate in the trial to the last follow-up visit, regardless of whether this event is causally related to the above-mentioned drugs. .

NOTE: An adverse reaction may be any discomfort and unconscious signs (including abnormal laboratory test results), symptoms, or disease

(new or worsening) related to the medication.

Events that meet the definition of adverse events include:

- Exacerbation of an existing chronic or intermittent disease, including increased frequency and/or intensity.
- A disease that is newly detected or diagnosed after taking the investigational drug, even though it may have been present before the trial began.
- Signs, symptoms, or clinical sequelae suspected of being caused by an interaction.
- Signs, symptoms or clinical sequelae caused by suspected overdose of the investigational drug or concomitant medication (overdose itself is not an adverse event / serious adverse event).
- " Lack of efficacy " or " failure to achieve the expected pharmacological effect " itself is not reported as an adverse event or serious adverse event. However, signs, symptoms, and/or clinical sequelae due to lack of efficacy will also be reported as adverse events or serious adverse events if they meet the definition of an adverse event or serious adverse event.

Events that do not meet the definition of an adverse event include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy);

the event leading up to the procedure is an adverse event.

- Situations in which adverse medical events would not occur (social security and / or admission facilitation).
- There was no worsening of pre-existing diseases or conditions that were present or detected at the start of the trial, only expected day-to-day fluctuations.
- / disease being studied , or the expected progression, signs, or symptoms of the disease being studied. Unless the situation is more serious than expected.

Definition of serious adverse event

" serious adverse event " is an adverse event that can occur at any dose and will:

- cause death;
- threatening life;

  Note: " Life-threatening " means that the subject is in danger of death when the event occurs, not that death could theoretically result if the event were more serious.
- Requires hospitalization or prolonged hospitalization;

  Note: Typically hospitalization refers to a subject staying in the hospital or emergency room (usually at least overnight) to receive observation and/or treatment that cannot be completed in the

doctor's office or outpatient clinic. Complications that occur during hospitalization are considered adverse events. An event is classified as a serious adverse event if the complication prolongs hospitalization or meets the criteria for any other serious adverse event. When it cannot be determined whether " hospitalization " occurred or was necessary, it will be treated as a serious adverse event.

Elective hospitalization that does not worsen a pre-existing condition compared with baseline is not an adverse event.

- Causes disability, or affects the ability to work and live;

  Note: “ Disability ” refers to a substantial impairment of an individual’s ability to carry out normal life. It does not include discomforts of little clinical significance, such as common headaches, nausea, vomiting, diarrhea, influenza, and accidental trauma (such as sprained ankles), which may affect the quality of daily life but will not constitute a substantial disruption.

- Cause congenital malformations;

In other cases, medical or scientific judgment should determine whether adverse event reporting is appropriate. For example, some important medical events may not be immediately life-threatening, leading to death or hospitalization, but may harm subjects or require drug or surgical intervention to avoid the serious adverse events listed above.

These events should also be considered serious adverse events, such as invasive or malignant cancer, allergic bronchospasm requiring close monitoring in the emergency room or at home, hematologic cachexia, or convulsions that do not result in hospitalization, drug dependence, or drug abuse.

9.2 Adverse drug reactions

Definition: Refers to harmful reactions that occur under normal usage and dosage of qualified drugs and have nothing to do with the purpose of medication.

Causal judgment indicators for adverse reaction judgment:

Item 1 : Is there a reasonable sequence between the time of starting medication and the time when the suspicion appears?

Item 2 : Whether the suspected adverse reaction conforms to the known adverse reaction type of the drug;

Item 3: Whether the suspected adverse reaction can be explained by the patient's pathological condition, concomitant medications, concomitant therapies, or previous therapies;

Item 4 : After stopping the drug or reducing the dose, whether the suspected adverse reactions are reduced or disappeared;

Item 5 : Whether the same reaction reappears after using the suspected drug again.

Causality judgment criteria: Judgment based on the order of the

above five judgment indicators.

It is up to the investigator to evaluate possible associations between adverse events, trial drugs, and concomitant medications according to the table below.

Adverse reaction cause and effect judgment table

critical result	Judgment index				
	Project 1	Project 2	Project 3	Item 4	Item 5
Definitely related	+	+	-	+	+
probably related	+	+	-	+	?
may be relevant	+	+	±	±	?
suspicious	+	-	±	±	?
Impossibly related	+	-	+	-	-

Explanation: + Affirmation, - Negation, ± Difficult to affirm or deny,? The situation is unknown.

1. Based on the table above, determine the relationship between the following grade 5 adverse events and the drug.

(1) Definitely related, (2) Probably related, (3) Possibly related, (4) Suspicious, (5) Impossibly related.

2. The incidence rate of adverse reactions is calculated using the total number of 1 + 2 + 3 + 4 cases as the numerator, and all selected cases that can be evaluated for adverse reactions as the denominator.

9.3Recording of adverse events

on the basis of comprehensive consideration of comorbidities and concomitant medications, their relevance to the experiment should be evaluated. The relevance of the drug and documented in detail by the physician.

If an adverse event is discovered, the observing physician can decide whether to terminate the observation based on the condition. Cases of drug discontinuation due to adverse events should be followed up and investigated, and the results should be recorded in detail. If any abnormality in safety test indicators (blood, urine, stool routine, electrocardiogram, liver function, kidney function) occurs during or after treatment , the adverse event form should be filled in in a timely manner, reviewed at an appropriate time, and communicated with the subject A

comprehensive analysis will be conducted on the onset, treatment, etc. to determine whether it is related to the experimental drug.

9.4 Handling of serious adverse events

For any serious adverse events that occur during the trial (including events requiring hospitalization, prolonged hospitalization, disability, affecting work ability, endangering life or death, causing congenital malformations, etc.) during the clinical trial , the researcher shall immediately respond to the patient's request. In addition to taking emergency measures, the subject must also immediately report to the ethics committee of the National Drug Clinical Trial Institution of Shandong University Qilu Hospital and the main research unit Tianjin Tasly Co., Ltd., the unit responsible for the research. The content of the report of serious adverse events should include: patient's name, random number, length of study participation, start date and stop date of serious adverse events, maximum intensity of serious adverse events, possible relationship between serious adverse events and study drugs, due to serious Whether the adverse event required a change in the study drug, the treatment given to the patient as a result of the serious adverse event, concomitant medications if the serious adverse event occurred, and the outcome of the serious adverse event.

10. Data management and statistical analysis

10.1Data management

(1) Establish database: The data administrator establishes an EXCEL database based on the research plan and CRF, and sets up logical verification according to the data verification plan (DVP). Release for use after passing the test.

(2) Data entry: Data entry personnel conduct independent double entries and double checks. Inconsistent results will be checked and corrected item by item against the CRF until the results are completely consistent.

(3) Data questions and answers: After data entry is completed, the data administrator conducts question screening according to DVP's manual verification plan, opens database access to researchers, and answers questions remotely. The data administrator responds to questions and can issue questions again if necessary until the data is "clean".

(4) Database locking: After the main researcher, statistical analysts and data managers jointly sign the "database lock record", the data administrator locks the database.

(5) Database submission: The data administrator submits the database to the statistician.

10.2 Statistical analysis data set

(1) Full analysis set (FAS): a set of all randomly enrolled cases that used the study drug at least once.

(2) Per-protocol set (PPS): It is a data set generated by subjects who are fully compliant with the trial protocol. Compliance includes the treatment received, the availability of primary endpoint measurement, and no major impact on the trial protocol. Violation etc. PPS analysis was used for the primary efficacy outcome measure.

(3) Safety Data Set (SS): Actual data that received at least one treatment and had post-treatment safety indicator records. The incidence of adverse reactions was based on the number of SS cases as the denominator.

10.3Statistical methods

9.3.1 Subject distribution analysis

(1) List the number of subjects selected and completed the trial, and determine three analysis data sets (FAS, PPS, SS).

(2) Conduct a classification analysis on the reasons for not entering PPS and calculate the number of subjects in different categories.

(3) Make a detailed list of group classifications, including the reasons for not being included in PPS/FAS/SS.

(4) Draw a flow chart of subject distribution.

10.3.2 Demographic data and baseline analysis

Descriptive statistics Demographic information and other baseline characteristic values:

(1) Calculate the number of cases, mean, standard deviation, 95%CI, minimum and maximum values for continuous variables.

(2) Count and grade data calculation frequency and composition ratio.

(3) Inferential statistical results (P values) are listed as descriptive results.

10.3.3 Medication compliance and concomitant medication analysis

(1) Calculate the percentage of subjects with medication compliance in the range of 80%-120%, and use $^{the}\chi^2$ test or Fisher's exact probability method to compare differences between groups.

(2) Medication exposure, use t test to compare differences between groups.

(3) Calculate the percentage of subjects with combined medications, and use the $\chi 2$ test or Fisher's exact probability method to compare ^{differences} between groups.

10.3.4 Efficacy analysis

(1) Analysis of main efficacy indicators

FR measured by ultrasound at 6 months and the baseline between the two groups was compared using t test.

(2) Analysis of secondary efficacy indicators

FR measured by ultrasound at 2 months and 4 months and the baseline was compared between the two groups using t test;

② For the number of angina attacks per week, t test was used to compare the differences between groups;

③For the time of occurrence of angina pectoris in exercise test, t test was used to compare the differences between groups;

④The time when ischemic ST segment downward shift occurs in exercise test, and the t test is used to compare the differences between groups;

10.3.5 Security analysis

(1) Calculate the incidence of adverse events/reactions, serious adverse events/reactions, and adverse events/reactions leading to dropout.

(2) List a detailed list of cases of various adverse events/reactions, serious adverse events/reactions, and adverse events/reactions leading to dropout.

(3) List the crosstabs of laboratory tests and electrocardiograms before and after medication.

(4) Descriptive statistics of changes from baseline in laboratory examinations, electrocardiograms, and vital signs, and actual measured values.

(5) Make a detailed list of abnormal values in laboratory tests, electrocardiograms, and physical examinations.

10.3.6Statistical software

(1) Analysis using S PSS software.

(2) All statistical tests adopt two-sided tests, and a P value less than or equal to 0.05 will be considered as statistically significant.

(3) Detailed statistical methods will be provided in the statistical analysis plan.

Standard operating procedures for echocardiography

1. Preparation before inspection

1. Rest for 5 minutes before examination.

2. Connect the synchronized ECG monitoring electrodes to

determine the phase of the cardiac cycle. The end of the T wave of the electrocardiogram is used to define the end of ventricular systole, and the peak of the R wave of the QRS wave is used to define the end of ventricular diastole.

3. Position **:** The left lateral decubitus position is used as the position for echocardiographic parasternal and apical views.

2. Parameter settings of echocardiography equipment and technical requirements before image acquisition

1. For two-dimensional echocardiography grayscale images, the frame rate of observing and recording images should be greater than or equal to 30 frames/second; for color tissue Doppler velocity images, the frame rate of observing and saving images should be greater than or equal to 80 frames. /second to facilitate subsequent functional parameter analysis.

2. In order to avoid the artificial shortening effect of the long axis of the left ventricle during apical observation, the left lateral decubitus position should be used, avoid using too soft mattresses, avoid overly trusting the apical pulse position palpable by palpation, adjust the probe position and angle, and select Measurements were made on the section with the largest inner diameter of the long axis of the left ventricle.

3. When the endocardium of two adjacent ventricular segments is

unclearly displayed, appropriate ultrasound emission frequency, gain, dynamic range, side sound shadow control, and grayscale color coding can be selected to enhance the display and visualization of the endocardial boundary. Identify.

4. In order to accurately determine the end-diastole and end-systole of the ventricle, mitral valve motion and chamber diameter changes should be referenced at the same time to avoid over-reliance on electrocardiography to determine the phase.

The sampling line for inner diameter measurement in two-dimensional and M-mode echocardiography should be perpendicular to the interface of bilateral anatomical structures. The distance parallel or nearly parallel to the direction of the sound beam is measured from the echo front edge to the leading edge (leading edge to leading edge). The distance perpendicular or nearly perpendicular to the direction of the sound beam is measured from the black and white interface. Method to detect black-white interface to black-white interface. The area is measured using the black and white interface depiction method.

6. The pulse wave Doppler sampling frame is set to 1mm. Adjust the filter appropriately to avoid spectral distortion caused by too low or too high filtering (too high filtering will cause low-speed frequency shift signals to be filtered out, and too low filtering will cause excessive noise signals).

7. After using high MI (0.8) to destroy the microbubbles , use ultra-low or low MI (0.1-0.3) mode for continuous real-time contrast imaging to observe coronary blood flow .

8. When measuring coronary blood flow velocity, use pulse wave Doppler technology to measure and pay attention to the sonic blood flow angle to be as small as 40°.

3. Image acquisition and image saving

1. Save 5 consecutive cardiac cycle dynamic images of each cardiac section in DICOM format.
2. Save one static image of each observation section parameter after measurement in JPED format.

4. General echocardiographic examination methods

1. Routinely collect two-dimensional dynamic images of patients' left ventricular parasternal long axis, apical four-chamber heart, apical two-chamber heart, apical long axis and left ventricular apex level, papillary muscle level, and mitral valve level short-axis sections, and adjust the depth, width, Gain and other parameters, adjust the frame rate between 60 and 110 frames / s to obtain a clear and complete left ventricular image.

2. At the same time, pulse Doppler is used to obtain the diastolic mitral valve orifice blood flow spectrum, and tissue Doppler is used to obtain the mitral valve annular motion spectrum.

3. The images were analyzed offline using QLAB software. Enter a - 2DQ mode, select the patient's apical four-chamber and two-chamber dynamic images respectively, the software automatically outlines the endocardial border, and fine-tunes the tracing points to make the tracing trajectory completely consistent with the endocardial border. After calculation, the software automatically obtains EDV , ESV , LVEF . Then enter the a - CMQ mode, select the apical four-chamber, three-chamber, and two-chamber dynamic images respectively. The software automatically traces the endocardium and epicardium and tracks the area of interest. It can appropriately fine-tune the tracing points to make the tracking more accurate. Determine the aortic valve closing time in the cavocardiac view, and the software calculates the overall long-axis peak strain (GLS) of the left ventricle during systole; use the same method to track the left ventricular apex level, papillary muscle level, and mitral valve level short-axis views, and the software Automatically obtain the global peak circumferential strain (GCS) of the left ventricle during systole and the rotation angle change curves of the apex and base during systole. During systole, the apex rotates counterclockwise, which is a positive curve; the base rotates clockwise, which is a negative curve.

Read the peak apical rotation angle during systole (Peak apical rotation , Prot - A) and the peak basal rotation angle (Peak basal rotation , Prot - B) on the curve. Calculate the peak systolic torsion angle (PTW) : PTW = Prot - A - Prot - B.

2. measurement standard

of cardiac chambers by two-dimensional echocardiography of the American Society of Echocardiography . The left ventricular end-diastolic diameter (LVIDd), left ventricular end-systolic diameter (LVIDs), and ventricular end-diastolic diameter (LVIDs) are measured at the level of the mitral valve chordae tendineae in the parasternal long-axis view. Septal end-diastolic thickness (SWTd), left ventricular posterior wall end-diastolic thickness (PWTd)

(2) Indicators of changes in left ventricular systolic function

1) Left ventricular ejection fraction (LVEF , %);

2) Global long-axis peak strain of left ventricular systole (GLS , %);

3) Global peak circumferential strain of left ventricular systole (GCS , %);

4) Peak systolic torsion angle (PTW , degrees).

(3) Left ventricular diastolic function parameters

1) Ratio of peak velocity of early and late diastolic mitral valve orifice blood flow (E / A);

2) The ratio of peak velocity of mitral valve orifice blood flow to peak velocity of mitral valve annulus in early diastole (E / e ').

5. Transthoracic coronary Doppler ultrasound

Transthoracic Doppler ultrasound (TTDE) can be used to measure epicardial coronary blood flow velocity (CBFV), which is positively correlated with coronary volume flow. The distal end of the left anterior descending artery (LAD) was selected for CBFVR measurement because the LAD is close to the chest wall and easier to observe than other branches. At the same time, intravenous contrast agent is used, and its reliability is close to 100%. Measurement of Coronary Blood Flow Velocity Reserve (CBFVR):

1. For routine echocardiography, select images with clear coronary blood flow;
2. Pulse wave Doppler is used to measure the coronary blood flow velocity in the resting state. When the image is complete and the maximum blood flow velocity can be measured, a contrast -enhanced ultrasound examination is performed and the coronary blood flow velocity is recorded again.
3. Then, adenosine 140 μg.kg $^{-1}$ .min $^{-1}$ was given for 6 minutes by continuous intravenous micropump injection . At the end of the injection , a contrast-enhanced ultrasound

examination was performed. Record coronary blood flow velocity.

4. CBFVR refers to the ratio of the average blood flow velocity or peak blood flow velocity in the diastolic period of the coronary arteries during hyperemia to the resting blood flow velocity of the coronary arteries.

6. Myocardial Contrast Echocardiography (MCE)

1. Select a clear image of the coronary arteries and activate the low mechanical index (MI<0.3) or ultra-low mechanical index (MI<0.2) real-time contrast-enhanced ultrasound examination mode of the ultrasound equipment. Place the focus point on the area of interest , adjust the gain to make the image have a light noise background, adjust the sector size and depth, and keep the image frame rate > 25Hz.

2. Bolus injection of the contrast agent SonoVue (add 8.5ml of sterile physiological saline to one bottle) or use a special micro-input pump to inject the contrast agent SonoVue 0.8 – 0.9ml/min . The pump can be manually oscillated to maintain uniform distribution of microbubbles , and then Slowly push 5ml of normal saline into the right brachial vein for more than 20 seconds .

3. After the contrast agent is injected, it takes at least 30 seconds for the left ventricle to start imaging , and the coronary blood flow signal is dynamically recorded. Acquisition of dynamic images includes: 2 cardiac

cycles, followed by triggered high mechanical index "flash" images (usually 3-7 frames, MI 0.9) and 15 cardiac cycles of ultra-low or low mechanical index reperfusion images. Continuous dynamic image acquisition should at least include coronary blood flow signals.

4. After the angiography is completed, the venous access is interrupted or retained according to the diagnosis and treatment conditions of different patients.
5. The UCA intravenous injection speed should be slow, and vital signs (blood pressure, heart rate, heart rhythm, respiration), ECG, allergic reactions , etc. need to be monitored for about 30 minutes after the angiography.
6. The laboratory needs to be equipped with corresponding first aid drugs and equipment.

Use ultrasonic technology to observe the backscattered signal of microbubbles. Since the diameter of microbubbles is smaller than that of red blood cells, they can freely pass through the myocardial capillaries together with red blood cells and can be regarded as tracers of red blood cells. Therefore, MCE can evaluate the activity of coronary microcirculation. After intravenous injection of this contrast agent containing microbubbles similar in size and rheology to red blood cells, the dynamic data of microbubble destruction and filling can repeatedly

and quantitatively measure microvascular blood flow velocity and capillary volume. With the trigger interval t as the abscissa and the visual score of myocardial imaging as the ordinate, the imaging time-intensity curve of the myocardium at this location is drawn. This curve conforms to the exponential function: $y = A(1 - e^{-\beta t})$. A is the maximum intensity of myocardial imaging that rises to the plateau phase, and β is the average slope of the curve rising to A (that is, the average microbubble velocity). Myocardial blood volume fraction can be calculated based on the A value, myocardial blood volume fraction × myocardial blood flow velocity = myocardial blood flow (MBF). The decrease in MBF may reflect a decrease in myocardial blood volume (seen after myocardial infarction, when there is a clear culprit vessel), or a decrease in blood flow velocity (seen in severe vascular stenosis), or both (seen when the infarcted myocardium is supplied by collateral circulation or is blocked by collateral circulation). supply of a blood vessel with severe stenosis and limited blood flow).

7. Drug injection methods and precautions

Adenosine:

1. Record the blood flow spectrum distal to the LAD in the resting state, and then inject adenosine intravenously. The adenosine injection dose was initially 50μg·kg $^{-1}$ min $^{-1}$ for 1 min, and then gradually increased to 75μg·kg -1 min $^{-1}$, 100μg·kg $^{-1}$ min $^{-1}$ and 140μg·kg $^{-1}$ min $^{-}$ at 1-min intervals. ^{1} . The

maximum dose is 140μg·kg $^{-1}$ min $^{-1}$ for 5 min. CFVR is the ratio of the maximum mean peak blood flow velocity after adenosine injection to the resting state mean peak blood flow velocity. The coronary arteries reach maximum dilation at a dose of adenosine of 140μg·kg $^{-1}$ min $^{-1}$ · The maximum blood flow velocity can also be measured by directly injecting 140μg·kg $^{-1}$ min $^{-1}$ adenosine intravenously and observing for 5 minutes.

2. Clinical studies have shown that the blood flow velocity of the coronary arteries has reached the maximum when adenosine is injected for 2 minutes . To reduce side effects, CFVR can be measured 2-3 minutes after adenosine is injected . During the CFVR measurement, 12-lead cardiogram monitoring was performed and blood pressure was measured every minute at rest and after adenosine injection until 5 minutes after stopping adenosine injection.

Standard operating procedures for exercise load testing

1. Personnel and equipment requirements

In order to ensure the quality of data collection and standardization of analysis and measurement , experienced physicians from the central laboratory - Qilu Hospital of Shandong University first conducted guidance and demonstrations of exercise stress testing and analysis in

accordance with the research plan . In the screening stage , researchers from each research unit need to submit the collected data and analysis results to the central laboratory for review, and the central laboratory will evaluate and provide feedback . All personnel involved in collection and analysis have no interest in this study. Each operator needs to have corresponding qualifications and be trained to better conduct exercise load test inspections and analyze measurements .

2. How to operate

exercise stress testing before enrollment, 12 months and 24 months after treatment . Patients should fast and refrain from smoking for 3 hours before the test, and do not drink caffeinated beverages; wear comfortable shoes and loose clothes; and try to stop taking drugs that may affect the test results, such as digitalis drugs, before the test; 12 Strenuous physical exercise must be avoided within hours; before the exercise test, 12-lead electrocardiograms of the subjects in lying and standing positions should be recorded and blood pressure should be measured as a control.

Subjects should exclude the contraindications of the following conditions before conducting exercise stress testing: (1) acute myocardial infarction or myocardial infarction combined with ventricular aneurysm; (2) unstable angina pectoris; (3) heart failure; (4) moderate and Severe valvular disease or congenital heart disease; (5) Acute or severe chronic disease; (6) Severe hypertension; (7) Acute pericarditis or myocarditis; (8)

Pulmonary embolism; (9) Severe aortic stenosis; (10) Those who are severely disabled and unable to exercise.

The main instrument models used are General Medical's Series 2000 Teaadmill treadmill and the corresponding Case exercise testing system. Let the subject walk on the movable tablet. According to the selected exercise plan, the instrument automatically increases the speed and slope of the tablet in order to adjust the load until the heart rate reaches the subject's expected heart rate. Analyze the results before, during and after exercise. ECG changes to determine the results. Recent studies have shown that no matter what kind of exercise program, the optimal exercise time to reach the maximum oxygen consumption value is 8-12 minutes. Extending the exercise time does not increase the clarity of diagnosis. It is emphasized that the choice of exercise program should be based on the specific conditions of the subjects . It depends.

Before the exercise test, the 12-lead electrocardiogram of the subject should be recorded in the lying and standing positions and the blood pressure should be measured as a control. During exercise, the heart rate, rhythm and ST-T changes were monitored through the monitor, and the electrocardiogram and blood pressure were recorded every 3 minutes according to the predetermined plan. After reaching the expected submaximal load, maintain the expected maximum heart rate for 1-2 minutes before terminating the exercise. After the exercise is terminated,

an electrocardiogram is recorded every 2 minutes and is generally observed for at least 6 minutes. If the ischemic changes in the ST segment have not returned to the pre-exercise pattern after 6 minutes, observation should continue until recovery. During the test, subjects should be observed for angina pectoris, dyspnea, fatigue, paleness, clammy skin, claudication and lower limb joint pain.

Exercise testing is often terminated when the patient reaches the maximum predicted heart rate. In addition, during the exercise test, if the subject experiences the following adverse reactions, timely termination of the test should be considered: ①ST segment elevation ≥1.0mm; ②Systolic blood pressure decrease >10mmHg, accompanied by other signs of myocardial ischemia; ③Medium Severe angina; ④ Gradually worsening neurological symptoms (such as ataxia, dizziness or presyncope); ⑤ Signs of hypoperfusion (cyanosis or pallor); ⑥ Sustained ventricular tachycardia; ⑦ Difficulty monitoring ECG or Blood pressure; ⑧The subject requested to terminate exercise. After the exercise test is terminated, the test conditions and data are recorded and analyzed.

1. Analysis content: including analysis of exercise capacity, clinical symptoms, hemodynamics and electrocardiogram changes.

2. Positive criteria for exercise test: The most important positive electrocardiogram manifestation is ischemic downward shift of the ST

segment, horizontal or downslope downward shift behind the J point >0.1 mv, lasting 0.08 s; upslope ST segment depression should be considered A critical status or negative result. Ischemic chest pain during exercise testing, especially angina that leads to termination of exercise testing, has important clinical significance. Abnormal exercise capacity, systolic blood pressure response and heart rate response during exercise are also important positive manifestations.

3. Data Storage and Analysis

All data including exercise capacity, clinical symptoms, hemodynamic and electrocardiographic changes and corresponding analysis results should be recorded and saved in detail. Create a folder for each patient , including the data, time and operator of each exercise stress test. The data information will be uniformly sent to the central laboratory by the person in charge of each center for summary and storage, and then two experienced doctors will follow the two-way process. Based on the principles of blindness and randomization , the situation and data of each patient were analyzed . Data under special circumstances will be re-examined by a team of professionals and analyzed multiple times to determine the final analysis results .

4. Intra-observer variation and inter-observer variation observation

In order to ensure the reproducibility of the analysis, this study randomly selected 50 subjects for repeated analysis . Inter-observer variability is the

comparison of the differences between measurements made by two physicians , and intra-observer variability is the comparison of the differences between two analyzes performed by the same physician at different times . Difference comparisons were analyzed using the Bland-Altman method, scatter plots were drawn, and correlation coefficients were calculated.

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