Structural and **F**unctional **I**mpairment of Multiple **O**rgans in patients with **S**ystemic **S**clerosis（SFIOSS）:

A MR imaging study

Research proposal

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1. Background

Systemic sclerosis (SSc) is a autoimmune disease of unknown etiology with an estimated global incidence of 0.1‰ [1]. SSc characterized by abnormal immune activation, neovascularization, vascular remodeling in pathology, and eventually leads to fibrosis of tissues, skin, and organs [2]. In addition to skin sclerosis, SSc often involved multiple organ involvement, such as finger ulcer, gastroesophageal reflux, interstitial lung disease, pulmonary hypertension, cardiomyopathy, kidney damage, etc. Neuropsychiatric manifestations such as anxiety and depression may also occur in SSc patients [3]. A long-term follow-up study indicated that cardiomyopathy (14%), cardiovascular disease (12%), and kidney injury (4%) were important causes of death in SSc patients [4]. Therefore, early diagnosis and early intervention for multi-organ complications of SSc can significantly prolong the survival time of SSc patients [1].

Previous literature has shown that patients with SSc can suffer from heart and kidney injury at an early stage. [5-6] The incidence of heart and kidney complications is 66.7% [3] and 45-80% [7], respectively. In addition, Patients with SSc more likely occur anxiety (64-72.8%), depression (43-68.4%), electroencephalogram changed (56.3%) and cognitive dysfunction (8.7-86.9%) [8,9]. Anxiety, depression and cognitive dysfunction are the key factors affecting the quality of life and prognosis of SSc patients [1]. The disease pathogenesis is still unclear, the reason why it is so easy to be ignored clinically. A number of studies based on brain imaging have shown that the incidence of white matter lesions, intracranial vascular lesions and intracranial calcification in SSc patients is more than that in healthy people [10], suggesting that SSc may leads organic brain lesions in patients. Previous literature has shown that SSc patients often have multiple organs involved at the same time, by which of some blood biochemical indicators can effectively indicate in SSc patients [11]. Therefore, the more understand of the manifestations of preclinical multiple organ injury in SSc patients can provide more evidence-based medical evidence for clinical diagnosis, treatment and prognosis prediction of this disease. In addition, previous studies lack studies on the nervous system involvement of SSc patients. There is no large sample data research based on comprehensive evaluation, internal correlation and evolution law of multi-organ injury among the heart, brain and kidney.

Magnetic resonance imaging (MRI) is a non-invasive, radiation-free imaging examination method with excellent soft tissue contrast, which can achieve one-stop multi-parameter imaging. At present,

MRI has been widely used to accurately diagnose and evaluate the structure and function of organs such as heart, brain and kidney [12,13]. Previous literature has shown that cardiac MRI can identify cardiac injury in patients with SSc earlier than echocardiography even in the early stage. Meanwhile it can find the abnormalities before clinical symptoms [5]. Therefore, MRI is an effective noninvasive imaging method to evaluate the structural and functional damage of heart, brain and kidney in SSc patients.

A number of studies have suggested that some biomarkers are related to organ involvement in SSc patients [11,14]. Patients with diffuse cutaneous SSc (dcSSc) often have anti-topoisomerase I antibodies positivity, anti-RNAPIII antibodies positivity, and anti-U3 RNP antibodies positivity. It can also be used to predict the occurrence of Interstitial lung disease (ILD), renal crisis, malignant tumor, cardiomyopathy, myopathy and pulmonary hypertension. Anti-TH /To antibodies positivity are more common in localized SSc (lcSSc) patients, which can predict the occurrence of pulmonary hypertension (PAH) and ILD. In addition, CXCL8 and PSE lectin levels are associated with HAQ-DI, and elevated IL-32 may indicate the occurrence of PAH. The study find elevated serum LEVELS of KL-6 and C-reactive protein in SSc patients are associated with the severity of SSC-ILD. Therefore, quest the correlation between biomarkers and organ involvement can be helpful for early detection of organ involvement in SSc patients.

Based on it, this study intends to carry out a prospective, multi-center cohort study based on MRI. We aim to explore the incidence of structural and functional damage of central, brain and kidney in SSc patients and its clinical relevance, and to search for the characteristics of serological markers of structural and functional damage of heart, brain and kidney.

2.Objectives

1. To clarify the patients with SSc whose incidence of structural and functional damage among heart, brain and kidney based on MRI and the damage correlation with clinical features.

2. To explore the correlation between MRI findings of central nervous system and clinical symptoms (anxiety, depression and cognitive impairment) in SSc patients.

3. To explore serological markers related to structural and functional damage of heart, brain and kidney.

4. To preliminarily explore the relationship between structural and functional damage of heart,

brain and kidney.

3.Methods

3.1. Main endpoint: the incidence of structural and functional disorders of heart, brain and kidney in SSc patients.

3.2. Secondary study endpoints

1. The relationship between MRI findings of central nervous system and clinical symptoms (anxiety, depression and cognitive impairment) in SSc patients.

2. Relationship between SSc autoantibodies and serological markers and structural and functional damage of heart, brain and kidney.

3. Correlation between structural and functional damage of heart, brain and kidney in SSc patients.

3.3 Definition of endpoint indicators

1. Cardiac structure and dysfunction: MRI confirmed abnormalities in left ventricular end-diastolic diameter, ejection fraction, end-systolic volume, cardiac volume, cardiac output, myocardial mass, end-diastolic maximum ventricular wall thickness, and myocardial T1 mapping and T2 mapping.

2. Brain structure and functional disorders: MRI confirmed that there were white matter lesions, brain microhemorrhage, special deposition and microcalcification brain structure damage; The quantitative indexes of Diffusion tensor imaging (DTI) are abnormal. Abnormal signal of brain-blood -oxygen level dependent (BOLD) was found. Abnormalities in brain function and connectivity.

3. Renal structure and dysfunction: MRI confirmed that there are abnormal renal blood perfusion, renal structure changes, and ABNORMAL T2* mapping;

4. Neuropsychiatric dysfunction: the presence of anxiety, depression or impaired cognitive function. Anxiety: Scored below 24 on the Hamilton Anxiety Scale; Depression: Score below 24 on the Hamilton Depression Scale. Impaired cognitive function: below 26 on the Montreal Cognitive Assessment Scale.

4. Statistical methods and sample size calculation

This study is based on a multicenter prospective cohort study to investigate the morbidity of

SSc patients with concurrent structural and functional impairment among heart, brain, and kidney. The sample size was calculated in light of the previous prevalence reported in the literature. About 38.8% of SSc patients had obvious cardiac complications, and about 20.5% had white matter lesions. Considering 15% dropped out cases, the sample size needed to be 95.

Parametric or nonparametric T test was used to compare the hematologic indexes and complications of heart, brain and kidney in SSc patients. Spearman rank test was used to calculate the correlation coefficients of hematological indexes and structural and functional damage of heart, brain and kidney. Receiver Operator Curve (ROC) was used to evaluate the predictive value of hematological characteristics for cardiac, cerebral and renal complications. The risk prediction model of structural and functional damage of heart, brain and kidney based on hematological indexes was established by random forest.

5. Inclusion and exclusion criteria

5.1 Patient inclusion and exclusion criteria

(I) Inclusion criteria:

1. Age > 18;

2.Fulfilled the SSc diagnostic (classification) criteria established by ACR in 1980; or Diagnostic (classification) criteria for SSc developed by ACR/EULAR in 2013;

3. Able to sign informed consent independently;

4. Right-handed.

(II) Exclusion criteria

1. Other autoimmune tissue diseases;

2. Claustrophobia;

3. Contraindications for MR examination (stent implantation, pacemaker, presence of metal or magnetic implants);

4. Heart or respiratory failure;

5. Diseases that affect MR findings (e.g. rapid atrial fibrillation);

6. Complicated with definite heart, brain and kidney diseases not caused by SSc, such as diabetic nephropathy and congenital heart disease;

7. Pregnant;

8. Severe disturbance of consciousness (coma, etc.);

9. Can not perform the scale assessment.

5.2 Inclusion and exclusion criteria of healthy subjects

(I) Inclusion criteria:

1. Age and sex were matched with experimental group;

2. Able to sign informed consent independently;

3. Right-handed;

(II) Exclusion criteria

1. Have a clear history of cardiovascular and cerebrovascular diseases and kidney diseases;

2. A history of immune system diseases;

3. Claustrophobia;

4. Contraindications for MR examination (stent implantation, pacemaker, presence of metal or magnetic implants);

5. Pregnant.

6.The research process

6.1 Clinical information collection (Flow chart as presented in Fig. 1)

1. Demographic data collection

2. Clinical data (course of disease, complications, clinical symptoms, laboratory indicators, etc.), treatments, mRSS score and PGA of the case group were collected.

6.2 Patient screening and baseline assessment

1. Patients should undergo routine examination and evaluation during screening period, baseline period and follow-up period: complete routine blood and urine examinations, as well as blood biochemical and immune indexes, and draw 10ml blood for IL-2/6/9, 5Ht and PON-1 tests at baseline period.

2. Complete ECG, chest HRCT and echocardiography.

3. Assessment of disease activity, including: PGA (physician and patient).

6.3 Scale Scoring

1. Health group received baseline treatment in Tsinghua University:

A) Hamilton Depression Scale

B) Hamilton Anxiety Scale score

C) Montreal Cognitive Assessment Scale

2. The case group was required to visit each center during the baseline period (0 weeks) and follow-up period (52 weeks):

A) Disability Index Scale (HAQ-DI) score

B) Hamilton Depression Scale

C) Hamilton Anxiety Scale score

D) Montreal Cognitive Assessment Scale

6.4 MRI imaging scheme

All enrolled patients underwent MRI examinations at baseline (0 months) and follow-up (12 months). The healthy control group only had MRI at baseline. The case group and the control group were examined by MRI in Tsinghua University with Philips Ingenia 3.0T whole-body MR. MRI examination was performed at two intervals of 30 minutes, as follows:

1. Whole brain imaging: The patient was placed in supine position and the signal was received by the standard 8-channel head receiving coil. The imaging sequences included: 3D T1-weighted (T1W), 3D T2-FLAIR, 3D TOF, Diffusion tensor imaging (DTI), 3D pCASL, and magnetic sensitivity weighted imaging (SWI) resting functional magnetic resonance imaging (RS fMRI). The imaging time was about 45 minutes.

2. Combined cardio-kidney imaging: The patient was placed in supine position, and signals were received by 16-channel abdominal coil. Ecg gating was adopted during the imaging process. Imaging sequences include CINE, HEART T1 mapping, heart T2 mapping, kidney artery spin labeling (ASL), kidney diffusion imaging (DWI), and kidney T2* mapping. Except for cardiac CINE, which required five breath-holding cycles, all the sequences were free breathing sequences and the imaging time was about 32 minutes.

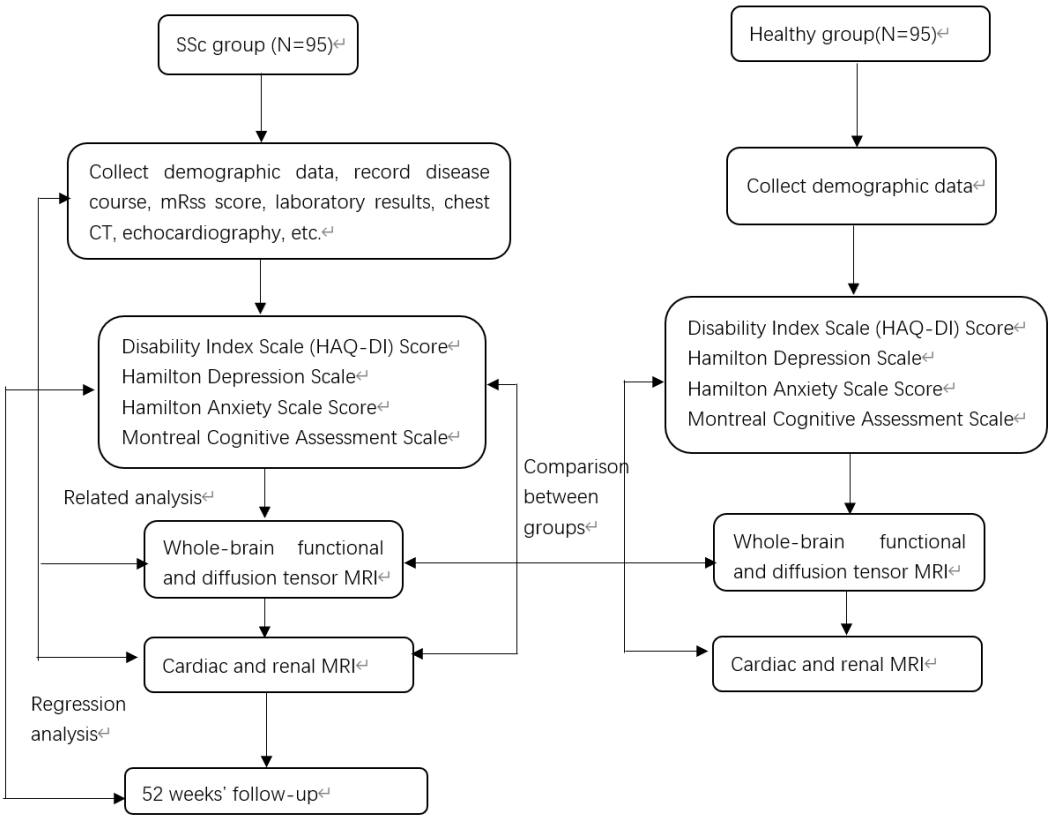

Fig. 1 Flow chart

7.Quality control of research

Patients with systemic sclerosis are rare diseases, and the case-control method of multi-center cooperation can save time, cost and be easy to manage. During the research, each center should select cases and follow up strictly according to the requirements of research design. Documented management method is applied to the enrolled patients, and the research data are recorded in detail, accurately and objectively by using the research notebook to ensure the authenticity, integrity, reliability and comparability of the data. Statistical science of experimental data, true and rigorous writing of research reports, and proper archiving and preservation of research materials. Tsinghua University Biomedical Imaging Research Center will evaluate the image quality. Those who pass the evaluation can be enrolled in the group, while those who fail the evaluation need to be rescanned.

8.Follow up

Outpatient/telephone follow-up was conducted in the 24th week, asking whether there were any new complications, and whether the clinical symptoms had improved or deteriorated or died. We

would tell the patients to visit the outpatient clinic at any time when their condition changed. Outpatient follow-up was conducted for 52 weeks. See "Annex 2: Frequency Table of Inspection Items in the Study Phase" for the follow-up contents, including vital signs, skin score, blood and urine routine, blood biochemistry, immunological indicators, echocardiography, whole brain imaging, combined cardio-renal imaging, MR imaging protocol and Consistent indicators were maintained at baseline; disease assessment scales: PGA (physician and patient), HAQ-DI score, Hamilton Depression Scale, Hamilton Anxiety Scale, Montreal Cognitive Assessment Scale; adverse event records.

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Magnetic Resonance Imaging Study of Multiple Organ Structural and Functional Damage in Patients with Systemic Sclerosis

Informed Consent Form

Version Number：1.0　Version Date：2021-11-30

Dear subject：

After the examination, we are obliged to inform you that you have been diagnosed with systemic sclerosis. We cordially invite you to participate in a clinical study: Magnetic Resonance Imaging (MRI) Study of Multiple Organ Structural and Functional Damage in Patients with Systemic Sclerosis. This research protocol has been reviewed by the Medical Ethics Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and agreed to conduct this clinical study.

Please try to read the following carefully before you decide whether to participate in the study. It can help you understand the study and why it is being done, the process and duration of the study, and the possible benefits, risks and discomforts in the study. If you want, you can also discuss it with your family, friends, or ask your doctor for explanations and help you make a decision.

1. Purpose and content of the study

Systemic sclerosis (SSc), also known as scleroderma, is an autoimmune diffuse connective tissue disease, which is characterized by the pathological changes and fibrosis of multi organ vascular. The disease induces phenotypic changes of immune cells, vascular cells and interstitial fibroblasts through the interaction of genetic factors and environmental influences, resulting in multiple organ involvement. Since SSc is a complex disease with multiple organ involvement and has a high rate of disability and death, early detection and treatment of organ involvement is the best way.

In our study, subjects with SSc voluntarily participating in the study are selected as research objects. We observed the incidence of organ structural and functional damage through imaging examination (MRI) and investigate its correlation with clinical manifestations, laboratory tests and prognosis.

Our study can provide valuable evidence for early detection of organ damage in patients with SSc, to guide patients for early treatment and follow-up and to evaluate the prognosis of patients. Your participation will make an important contribution to obtaining such evidence so that other patients can benefit from your contributions.

2. People who are not suitable for the study

There are strict inclusion and exclusion criteria in this study, mainly including right-handed adults with a clear diagnosis of SSc as our subjects. Patients with other autoimmune diseases or who cannot undergo MRI examinations (such as claustrophobia, stent, etc.) cannot be included. Patients who do not meet the inclusion criteria are not suitable to participate in the study. In

addition, there may be other reasons that researchers think are not suitable.

3. What will you need to do if you participate in the research?

Before you are enrolled in the study, the doctor will first obtain your consent and you should sign an informed consent form, and then ask and record your medical history, physical examination and disease evaluation, including previous laboratory test results and imaging results. If necessary, additional laboratory and imaging tests would be supplemented.

If you are an eligible participant, you will undergo an MRI of the brain, heart and kidneys at the Biomedical Imaging Research Center of Tsinghua University, Haidian District, Beijing, which will take about 90 minutes. And you will undergo a scale evaluation on the quality of life and the level of depression, anxiety as well as cognition, which will take approximately 50 minutes. In addition to these routine examinations, you need to draw an additional 10ml of blood when participating in this study to conduct free examinations such as IL-2/6/9, 5-HT and PON-1 in our center. You will not take additional medications or increase the number of visits because of your participation in the study. During this period, you need to have follow-up visits by clinic or telephone every 24 weeks or so, and at 52 weeks, MRI of brain, heart and kidney and the scale evaluation will be carried out again in addition to routine examinations

4. Possible benefits of participating in the study

If you participate in the study, the results of the study will have great significance to the clinical decision-making of all patients with the disease, and through the examination, it is possible to find preclinical lesions, so as to carry out early treatment follow-up. This program does not include any examinations other than the routine diagnosis and treatment, which will not increase your costs. The MRI in the study is free for you.

5. Possible risks, adverse reactions, discomfort and inconvenience of participating in the study

The imaging examination of this project is a non-ionizing radiation MRI commonly used in clinical practice. Common adverse reactions include: some discomfort caused by entering a narrow space, such as palpitation, shortness of breath, chest tightness, sweating, dizziness and headache due to prolonged supine position and noise, etc.

During the study, You will receive telephone visits every 24 weeks or so, which will take up some time and may be troublesome or inconvenient to you.

6. Relevant expenses and compensation

This study is observational and does not increase your treatment costs. Therefore, all treatment costs and routine examinations of the disease shall be paid by the patients. The additional blood drawing examinations and the MRI of the head, heart and kidneys in the study will be free. There is no interventional treatment in this study and the treatment of patients is not affected so no compensation is involved.

7. Is personal information confidential?

Your medical records (Case Report Form/CRF, laboratory tests, etc.) will be kept completely

at the hospital where you were treated. The doctor will record the results of laboratory tests and other examinations on your medical record. Researchers and Ethics Committees will be allowed to refer to your medical records. All your personal information, including name, telephone, email, address, etc., will not be recorded in the electronic database, and any public report on the results of the study will not mention your personal identity or information. We will make every effort to protect the privacy of your personal medical information within the law.

8. How to get more information?

You can ask any questions about the study at any time and receive answers. Consultation telephone number (researcher's mobile phone number): And you can consult on issues about your rights or related risks. Consultation telephone (Ethics Committee's telephone number): 69156874

During the study, your doctor will inform you of any important new information that may affect your willingness to continue participating in the study.

9. Free choice of participating in the research and withdrawing from the research midway

Whether to participate in the study depends entirely on you. You may refuse to participate in the study or withdraw at any time during the study, which will not affect your relationship with the doctor or cause your loss of medical or other benefits.

Out of your best interests, the doctor or researcher may discontinue your participation in the study at any time during the study.

If you withdraw from this study for any reason, you may also be required to undergo laboratory tests and physical examinations if the doctor thinks it clinically necessary.

10. What to do now?

Decide whether to participate in the study by yourself (and your family)

Ask your doctor all your questions before making your decision whether to participate in the study.

Thank you for reading the content above. If you decide to participate in the study, please inform your doctor and he/she will arrange everything for you about the study. Please keep this form.

Consent Statement

I have read the introduction to the study above and had the opportunity to discuss it with the doctor and ask questions about it.

I am aware of the possible risks and benefits of participating in the study. I know that participation in the study is voluntary, confirm that I have had sufficient time to consider it, and understand that:

- I can ask my doctor for more information whenever I want.

- I can withdraw from the study at any time without discrimination or retaliation, and my medical treatment and rights will not be affected in any way

I also know that if I withdraw from the study, it would be beneficial to the study if I tell my doctor about the changes in my condition and completed the corresponding physical and chemical examinations.

I agree that representatives of Ethics Committee can refer to my research data.

I will receive a signed and dated copy of the Informed Consent Form.

Finally, I decide to participate in the study and promise to follow the doctor's advice as much as possible.

Subject Name (Block Letter) ____ Subject Signature ____ Date____

If the subject has appointed a legal representative (if applicable, and has signed the entrustment agreement):

Legal Representative Name（Block Letter）____

Legal Representative Signature____ Date____

I confirm that I have explained the details of the study to the subject, including the rights and possible benefits and risks, and gave the subject a copy of the signed Informed Consent Form.

Researcher Name____ Signature____ Date____