Project Name

Prospective Case Registry Study of Ischemic Stroke in Shanghai

Clinical Research Unit: Department of Neurology, Huashan Hospital, Fudan University, Shanghai, China

Sponsor: None

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Main text

1. Background of the study

Acute ischemic stroke is the most common type of stroke, accounting for 69.6%-72.8% of new strokes in China. The 3-month disability rate and death rate of Chinese patients with acute ischemic stroke range from 14.6% to 23.1% and 1.5% to 3.2%, while the 1-year disability rate and death rate range from 13.9% to 14.2% and 3.4% to 6.0%; respectively.

The incidence of ischemic stroke in young and middle-aged adults is on the rise globally. In the United States, the incidence of stroke in adults aged 20 ~ 44 years increased from 17/100,000 person-years in 1993 to 28/100,000 person-years in 2015. In a nationwide cohort of more than 15,000 patients in the Netherlands, the incidence of stroke in young adults increased from 14.0/100,000 person-years in 1998 to 17.2/100,000 person-years in 2010 ($p < 0.001$). A French study of more than 4,500 patients over 27 years concluded that for adults aged < 55 years, the incidence of ischemic stroke increased from 11.6/100,000 person-years in 1985-1993 to 20.2/100,000 person-years in 2003-2011.

Worldwide, more than 2 million young people suffer from ischemic stroke each year. Stroke in young and middle-aged people has a considerable socio-economic impact due to high healthcare costs and loss of labor productivity. This emphasizes the need to rapidly identify new risk factors and elucidate the mode of action of traditional vascular risk factors (e.g., hypertension, smoking, and obesity) to reverse this trend. However, there is still a lack of studies on epidemiologic data related to stroke among young and middle-aged people in Shanghai.

Compared with older stroke patients, the causes and risk factors of stroke in young and middle-aged are more diverse and usually involve a number of rare etiologies and specific risk factors, including illicit drug use, pregnancy, arterial entrapment and patent foramen ovale unenclosed (PFO), as well as rare genetic disorders, which often require special differential diagnosis and treatment. In addition, young and middle-aged patients with a life expectancy of several decades have a different clinical prognosis after stroke compared with older patients. In the guidelines published by the American Heart and Stroke Association and the Royal College of Physicians, there are few recommendations on clinical approaches and management of stroke in young and middle-aged people and more evidence is needed to support them. Notably, Fabry disease, a rare inherited metabolic disorder, is a potential cause of stroke in young and middle-aged adults. Currently, with the availability of enzyme replacement therapy, the inclusion of screening for Fabry disease in the evaluation process of young and middle-aged stroke patients is important for achieving early diagnosis and treatment, delaying disease progression, preventing serious complications, and improving quality of life.

The current TOAST typing scheme for ischemic stroke etiology was developed for ischemic stroke patients older than 65 years, which may lead to an unjustified classification of young and middle-aged patients with cryptogenic stroke. The currently used classification groups patients with diverse and rare etiologies and their underlying pathophysiologic mechanisms into 1 other category, thereby ignoring the fact that different etiologies may lead to different long-term prognoses of stroke; for example, carotid artery dissection (CeAD) and antiphospholipid syndrome are grouped into 1 other category; however, antiphospholipid syndrome has been associated with an increased risk of lifelong venous and arterial thrombosis, and there is also research demonstrating that CeAD patients have a higher risk of recurrent stroke in the short term

only. Thus, a category in the TOAST classification contains multiple etiologies but varies widely in terms of prognosis and appropriate secondary prevention strategies. This suggests that the current etiological classification system may not be sufficient and we need to identify and study etiological subtypes in more detail if we want to conduct a more individualized study of the prognosis of young and middle-aged ischemic stroke patients. There is still a lack of large epidemiologic data on the etiology of stroke and its variations in young and middle-aged Shanghai.

In addition, a recent prospective cohort study of 544 patients aged 18-50 years with first-ever ischemic stroke or TIA followed for a median of 9.6 years found that the 10-year cumulative risk of bleeding after long-term antithrombotic therapy (21.8%, 95% CI 17.4-26.0) was almost the same as the risk of ischemic events (33.9%, 95% CI 28.3-37.5). The risk of reischemia is low in young and middle-aged stroke patients, so the benefits of their antithrombotic therapy may not always outweigh the risk of bleeding complications. This finding raises the question of whether these patients should be treated with long-term (often lifelong) antithrombotic therapy, while the safety of discontinuing antithrombotic therapy remains unclear and needs to be explored.

The Shanghai Stroke Prevention and Treatment Service System ("4S") was established in 2013, and has formed a stroke service network of 11 municipal stroke centers radiating to 24 district stroke centers and driving 240 community health centers, and has established a hierarchical stroke network construction and standardized treatment training system in a large city. In addition, we have established a system of stroke network construction and training for standardized treatment in large cities, and a system of stroke prevention, first aid and secondary prevention services that are continuous, available and fully covered.

Based on the 4S database, we plan to conduct a prospective cohort study of patients with imaging-confirmed ischemic stroke. The study will focus on the incidence, clinical characteristics, risk factors, and pathogenesis of stroke in middle-aged and young adults, as well as analyze the trend of the etiology of stroke in middle-aged and young adults in recent years in the Shanghai area, including the roles of traditional (e.g., high blood pressure, diabetes mellitus) and emerging factors (e.g., air pollution, lifestyle changes), and explore the relationship between these factors and patients' stroke outcomes. The role of traditional risk factors (e.g., hypertension, diabetes) and emerging factors (e.g., air pollution, lifestyle changes) was analyzed. Meanwhile, the correlation between reperfusion therapy such as intravenous thrombolysis, acute phase management and secondary prevention with clinical prognosis is analyzed. Finally, the specificities and challenges of screening and treatment of Farby patients with hereditary young to middle-aged stroke are discussed in depth, providing comprehensive information on regional stroke in terms of prevention and control strategies for young to middle-aged stroke.

In summary, the aims of this project were to (1) collect clinical data on patients with acute cerebral infarction from several hospitals in Shanghai, analyze the implementation of reperfusion therapy such as intravenous thrombolysis, acute phase management, and secondary prevention, assess the quality of their medical care, and statistically explore the correlation between these factors and patients' prognosis; and (2) analyze the trend of change in the etiology of stroke in recent years in the Shanghai region, including the traditional risk factors (e.g., hypertension, diabetes) and the emerging risk factors (e.g., hypertension, diabetes). (e.g. hypertension, diabetes mellitus) and emerging factors (e.g. air pollution, lifestyle changes); (3) to carry out high-risk screening for Fabry disease in young and middle-aged stroke patients, and to conduct a systematic

survey on the actual prevalence of this disease in stroke patients, so as to fill the gaps in the existing epidemiological data of Fabry disease; (4) to analyze the incidence rate, clinical characteristics, risk factors, and pathogenic mechanisms of Fabry disease for the treatment and prevention of stroke in the middle and youth age group; and to provide a comprehensive and comprehensive study of the causes of stroke in the Shanghai area. (5) To analyze the incidence, clinical characteristics, risk factors and pathogenesis of stroke in young and middle-aged people, and to provide a basis for the development of targeted preventive and treatment measures.

2. Aims of the study

(1) To analyze the annual incidence, prevalence, disability-adjusted life years (DALYs), disability rate, all-cause mortality rate, and its trend in Shanghai;
(2) To compare the proportion of each cause of stroke in young and middle-aged stroke and its changing trend according to TOAST typing in ischemic stroke;
(3) To analyze the proportion of acute cerebral infarction reperfusion therapy such as intravenous thrombolysis and endovascular therapy for young and middle-aged strokes in Shanghai and its relationship with 3-month mRS, the proportion of medication used for secondary prevention of stroke and its relationship with recurrence of ischemic events;
(4) For young and middle-aged stroke patients in Shanghai who were typed as large artery atherosclerosis (LAA) subtype, to analyze the relationship between the risk of hemorrhage and the risk of recurrence of ischemic events of long-term antiplatelet therapy, and to provide a basis for how to use antiplatelet therapy in this type of young and middle-aged stroke patients;
(5) To analyze patients who need anticoagulants for cardiac or other reasons (e.g., antiphospholipid syndrome), analyze the relationship between the proportion of these young and middle-aged stroke patients in Shanghai who use anticoagulants, as well as the risk of bleeding and the recurrence rate of ischemic events, and provide clues for clinical decision-making;

3. Study design

This study is a prospective cohort study, collected from 2024.12.20-2029.12.20, and the information collected includes patients' general information, past medical history, clinical data in the acute phase, information on reperfusion therapy, information on auxiliary examinations, and information on discharge from the hospital, and 1 follow-up visit at 3 months, with no interventions for the patients. A total of 15,000 cases are expected to be collected over 5 years.

4. Inclusion Criteria, Exclusion Criteria of Study Participants

Inclusion criteria: patients $\geqslant 18$ years of age with a diagnosis of acute or subacute ischemic stroke; inclusion criteria for young and middle-aged stroke were patients with acute or subacute ischemic stroke between $18-55$ years of age; the diagnosis of stroke had to be confirmed by clinical assessment and imaging; in the case of negative or absent imaging, the clinical diagnosis of stroke had to be confirmed by an experienced neurologist; and the patient signed an informed consent form that consent to the collection of information.

Exclusion Criteria:
(1) The amount of missing data was greater than 40% of the data to be entered;
(2)Study participants were found not to meet the inclusion criteria after enrollment or met any of

the exclusion criteria;
(3)Lost visitors;

Withdrawal criteria: study participants who were unwilling or unable to continue in the trial

5. Calculation of the number of cases required to achieve the intended purpose of the study according to statistical principles

5.1 Aims 1-2: aims to analyze patient information through prospective cohort study, for descriptive data, Shanghai 4S database enrolls about 3,000 eligible patients each year, and is expected to have a total of 15,000 cases in 5 years.

5.2 Aim 3: Based on a previous study showing that intravenous thrombolysis was associated with 90-day functional independence (corrected ratio of ratios [aOR] = 1.67,95% CI 1.23-2.28, p = 0.001), with α=0.05 set and validity of 80%, the sample size was calculated as 516 cases/group for a total of 1,032 cases using G power software. Based on previous studies showing that young patients with anterior circulation large vessel occlusion in young and middle-aged stroke patients have a better clinical prognosis than older patients (mRS acOR: 1.8 [95% CI, 1.5-2.2]; functional independence [mRS 0-2] 61% versus 39% [aOR 2.1 (95% CI, 1.6-2.8)], set α = 0.05, the Validity was 80% and the sample size was calculated using G power software as 232 cases/group for a total of 464 cases.

5.3 Aim 4: According to a prospective cohort study of 544 patients aged 18-50 years with a first-ever ischemic stroke or TIA followed up for a median time of 9.6 years, the ten-year cumulative risk of bleeding after long-term antithrombotic therapy in patients (21.8%, 95% CI 17.4-26.0) was almost the same as the risk of an ischemic event (33.9%, 95% CI 28.3-37.5);

5.4 Aim 5: In a study that included patients from eight comprehensive stroke centers in the United States, 1,518 of 2,084 patients with ischemic stroke who had atrial fibrillation had prior anticoagulation and 90-day follow-up, and the results showed that patients with atrial fibrillation who had received anticoagulation were associated with an increased risk of 90-day recurrent ischemic events (aHR 1.50, 95% CI 0.99 - 2.28, p=0.058).

6. Main flow of the study

Study centers entered the 4S Database Registry or the 4S Database Registry combined with the Young and Middle-Aged Stroke Cohort (including Fabry Disease Screening) study as they saw fit.

(1) 4S database registry:

a. The prospective cohort study will be carried out in centers that have participated in the 4S database registration in the past, and each sub-center is a secondary and above hospital that routinely admits and treats acute ischemic stroke in Shanghai. Written informed consent will first be obtained from the patients, and for the included cases, the investigators in each sub-center will enter the relevant data in the web version of the existing 4S database platform (https://stroke.huashan.org.cn:8001/ssss/), including the following information:

General information of patients: name, age, gender, place of residence, date of admission, name of hospitalization institution, level of hospitalization institution, and TOAST typing.

Medical history:

Risk factors: smoking, hypertension, atrial fibrillation, hyperlipidemia, diabetes mellitus, previous history of cerebral infarction, myocardial infarction, other cardiac disease, dementia, chronic

obstructive pulmonary disease, history of or predisposition to bleeding

History of pre-morbid medications: antihypertensive drugs, hypoglycemic drugs, hypolipidemic drugs, anticoagulants
Acute phase clinical data: baseline NIHSS score, mRS score prior to stroke admission, last normal time, time of detection of abnormality, time of arrival at hospital
Reperfusion therapy: intravenous thrombolysis or not, endovascular treatment or not
Discharge data: date of discharge, discharge mRS score, discharge diagnosis.
c. Data collected by each sub-center will be summarized to the principal investigator and members of the research team.

(2) Midlife Stroke Cohort Registry:
a. Patients were enrolled in the Young and Middle-Aged Stroke Cohort if they met the criteria for enrollment in the 4S database (age 18-55 years);
b. Entry of relevant data in the 4S database platform for the included cases, including the following.
Patient general information: name, age, gender, height, weight, BMI, date of admission, TOAST typing

Medical history:
Risk factors: smoking, alcohol consumption, hypertension, atrial fibrillation, hyperlipidemia, diabetes mellitus, previous history of cerebral infarction, previous history of cerebral hemorrhage, migraine, ischemic heart disease, family history of stroke-related illnesses
History of pre-morbid medications: aspirin, clopidogrel, warfarin, new oral anticoagulants, other antithrombotic drugs, statins, antihypertensive drugs, hypoglycemic drugs, birth control pills, HRT (hormone replacement therapy)

Acute phase clinical data: baseline NIHSS score, mRS score before stroke admission, other clinical manifestations

Reperfusion therapy: intravenous thrombolysis or not, endovascular therapy or not

Ancillary tests: baseline CT scan, baseline MRI, cerebral small-vessel disease, large-vessel occlusion/stenosis and degree of stenosis, foam test results, cardiac ultrasound results, electrocardiogram results, Holter results

Discharge information: date of discharge, discharge NIHSS score, discharge mRS score, discharge with medications

c. Follow-up at 90 days (±7 days), recorded by the Acute Stroke QC administrator at each center via telephone follow-up including 3-month mRS score, recurrence of cardiovascular disease or not, current secondary prevention medications, EQ/5D score;
d. Completed data collected at each subcenter were summarized to the principal investigator and study team members.

7. Criteria for study termination

(1) Significant errors in the clinical study protocol or serious deviations in the implementation of the protocol that make it difficult to assess the utility of the study;

(2) During the course of the study, treatment-related adverse events in the study participant population are analyzed and the investigator confirms that continuation of the study is not appropriate.

8. Assessment criteria, including method of rating parameters, duration of observation, recording and analysis

(1) Incidence rate of stroke in young and middle-aged: the numerator was the number of first strokes in the 4S database of 18-55 years old in Shanghai, and the denominator was the total population of this age group in Shanghai in the current year; the annual incidence rate of stroke in young and middle-aged was calculated, and a line graph was plotted to observe the trend;

(2) Disability-adjusted life years (DALYs) for young to middle-aged stroke: the sum of years of life lost to early death (YLL) and years of life lost to disability (YLD); YLL=N× L (N: number of deaths, L: standardized life expectancy to death); YLD=I × DW × L (I: number of cases of incidence; DW: disease weighting; L: average number of years of disability);

(3) All-cause mortality rate of stroke in young and middle-aged people: the numerator is the number of all deaths per year, and the denominator is the total population of Shanghai each year;

(4) Changes in etiology were analyzed based on the percentage of patients with ischemic stroke TOAST subtypes in young and middle-aged stroke patients in each year of the database, and line graphs were plotted to observe trends;

(5) Reperfusion therapy status: calculate the annual intravenous thrombolysis rate and arterial thrombolysis rate of young and middle-aged stroke patients in Shanghai from the 4S database data;

(6) Secondary prevention: to calculate the percentage of patients on secondary prevention at 3-month follow-up;

(7) Calculation of the percentage of patients on antiplatelet therapy and anticoagulation per year and the percentage of recurrent cardiovascular events at follow-up in young and middle-aged stroke patients in Shanghai;

9. Treatment of adverse events

(1) Methods of recording and reporting adverse events:

Any adverse medical event that is related to any medical intervention (clinical data collection, blood sample collection) in the study is considered an adverse event (AE). If a serious adverse event occurs in a subject during the study, regardless of whether it is related to the medical measures performed, the investigator will immediately take appropriate therapeutic measures for the subject, safeguard the subject's safety, and report the event to the hospital's Ethics Committee and the relevant administrative department within 24 hours of the event.

(2) Expected adverse events and contingency plans:

This study is an observational study without any intervention for the study participants. Adverse events will not occur in this study as subjects undergo clinical data collection. For this reason, we will take measures in the following areas: explain in detail the purpose of the

diagnostic and therapeutic operation and possible discomfort before the operation, and ensure that the subjects give informed consent. In case of any discomfort, necessary medical support will be provided immediately.

All adverse events will be recorded in detail and reported to the Research Ethics Committee in a timely manner. The study team will regularly review the occurrence of adverse events to ensure the safety of the subjects and the smooth running of the study.

10. Statistical analysis plan, definition and selection of data sets for statistical analysis:

GENERAL METHODS: Statistical analyses were performed with Prism7 using descriptive statistics demographic characteristics and clinical indicators, using means ± SD for continuous variables and percentages for categorical variables. Correlations between intravenous thrombolysis rate, arterial thrombolysis rate and 3-month mRS score were analyzed using ordered regression (ordinal regression). Hazard ratios (HRs) for the risk of any vascular event were determined for each predefined factor (e.g., TOAST classification, age, sex, stroke severity, cardiovascular risk factors, etc.) using univariate analyses (chi-square test) as well as multivariate analyses (logistic regression, COX proportional modeling, etc.).

11 Data management and information confidentiality

(1) Data entry

Data entry should be done by the investigator, and the data should be sourced from and consistent with the original documents, such as the original record sheet and/or laboratory test report form, and any observation or test result in the study should be entered into the study record sheet in a timely, correct, complete, clear, standardized, and truthful manner. The principal investigator and study members are responsible for reviewing and managing the entered data. For any doubts about the data, the study members will send appropriate queries to the subunit investigators, who will respond to the queries sent by the principal investigator in a timely manner, and the principal investigator may re-challenge them if necessary. The data collected by each center will be summarized to the principal investigator and team members for unified analysis, and each center will not be able to access each other's data.

(2) Confidentiality program for study participants' information

All study participants' information must be kept strictly confidential, and personal data from participation in the study and during the study will be kept confidential. Study participant information and study data will be identified by study number digits rather than by name. Information that identifies them will not be disclosed to members outside the study team unless permission is obtained from the study participant. All study members are asked to keep the identity of study participants confidential. Research participants' files will be kept in locked filing cabinets and will be accessible only to researchers. To ensure that the study is conducted in accordance with the regulations, members of the government administration or the Ethical Review Committee will have access to the personal data of the study participants at the research unit when necessary and as required. No information on individual research participants will be disclosed when the results of this study are published.

(3) Confidentiality program of the study data

The research data are also confidential, and all research members are required to keep the

research data confidential, not to inform members outside the subject group of the research data without the permission of the principal investigator, not to transfer the research data to outside organizations without the permission of the hospital, and not to transfer the research data involving human genetic resources to outside organizations, or domestic organizations containing foreign capital without the approval of the permission of the National Office of Human Genetic Resources, except for the normal case of Except for the publication of research results that meet the requirements of regulations.

12. Quality control and quality assurance of clinical research

The Principal Investigator will organize relevant training to ensure that the study is conducted in a standardized manner and that the completion of study record forms and other reports follow GCP principles and the study protocol. All data and information must be verifiable. Quality control will be applied at every stage of the study to ensure that all data are reliable and study procedures are accurate. Ensure that informed consent is obtained from all subjects prior to the start of the study. Any breach or deviation from the protocol should be promptly reported to the Ethics Committee.

If necessary, the research team will develop standard operating procedures and implement quality control procedures in all aspects of study implementation and data processing to ensure standardization and reliability of study implementation and data manipulation.

13. Study-related ethics

This study is a prospective cohort study, which requires the participating units to collect patients' clinical data, but will not disclose patients' personal information, and the follow-up items will be the routine follow-up contents for patients with acute or subacute ischemic stroke.

14. Study participant recruitment methods and the process of obtaining informed consent

Each subcenter screened patients who met the enrollment criteria from the patients they received and obtained their written informed consent.

15. Anticipated progress and completion date of the clinical study

2024.12.20-2025.1 Recruitment of centers, issuance of invitation letters, signing of collaboration agreement, etc;

2025.1-2026.1 Patient recruitment, data entry at each center, academic members/QCs will communicate registration progress after monthly data verification;

2025.03-2025.05 Data compilation and analysis for Fabry disease screening;

2025.06-2025.12 Write up the paper;

2026.2-2026.4 Perform data compilation and analysis on the topic of stroke in young and middle-aged adults;

2026.5-2026.6 Write-up of paper;

2026.7-2027.7 Evaluate the safety and efficacy of treatment for elderly and very elderly patients;

2027.8-2027.12 Write-ups;

2028.1-2029.1 Analysis of the clinical quality of acute cerebral infarction reperfusion therapy such as intravenous thrombolysis, acute management of stroke, and secondary prevention of stroke in

Shanghai in the past 5 years, and its correlation with prognosis;
2029.1-2029.6 Data analysis and writing of the paper;
2029.7-2029.12 communication and feedback from the centers

Information for Study Participants

Protocol name: Shanghai Ischemic Stroke Prospective Case Registry Study

Protocol Number: KY2024-1385

Protocol version number: 02, January 16, 2025

Informed Consent Version No.: 02, January 16, 2025

Institution: Department of Neurology, Huashan Hospital, Fudan University, China

Dear Study Participant:

You are being invited to participate in a clinical research study. This information sheet provides you with information to help you decide whether or not to participate in this clinical study. Please read it carefully and ask the investigator in charge of the study if you have any questions. If you agree to participate in this study, you will be asked to provide information about your disease, including the onset of the disease, your family history, previous visits to the doctor, and the results of any tests you have had, and you will be assigned a number to create a medical record.

Your participation in this study is voluntary. This study has been reviewed by the Ethics Review Committee of our research organization.

1. **Study Overview**:The disability and mortality rates of acute stroke are very high. This study plans to conduct a prospective cohort study of ischemic stroke patients in Shanghai based on the Shanghai Stroke Prevention and Treatment Service System ("4S") database. By collecting and analyzing clinical data and follow-up data of ischemic stroke patients, we hope to better understand the incidence, etiological characteristics, risk factors, and treatment effects of local ischemic stroke patients, and to provide a scientific basis for the subsequent development of individualized prevention and treatment measures. You have been hospitalized for ischemic stroke. We would like to analyze the epidemiology, etiology, and prognosis of ischemic stroke by using your clinical findings and hematology samples during your hospitalization.
2. **Objective of the study**:To collect clinical data from patients with acute cerebral infarction in several hospitals in Shanghai, to understand the pathogenesis, risk factors and treatment effects of stroke, and to provide a basis for future prevention and treatment strategies.
3. **Research process**: This is an observational study. If you agree to participate in the study, we will obtain your clinical information collection during your hospitalization through the medical history system after you are discharged from the hospital. In addition, you will receive a telephone visit approximately 90 days after stroke onset to ask about your recent ability to live, any related recurrence of cardiovascular events, and medication use.
4. **Risks and Discomfort**: This is an observational study and subjects undergoing clinical data collection will not experience adverse events. In the event of discomfort, you may inform the research team at any time and the research team will provide appropriate treatment and medical

support.

5. **Benefits**: This study may have the following benefits for you:

(1) Understanding your condition: By participating in this study, you will receive systematic health information collection. The research team will assess your health based on the results of the tests and help you understand your current condition and potential risk factors. This information will not only help you understand your health, but also provide a scientific basis for personalized treatment and disease prevention strategies.

(2) Advancement of stroke prevention and treatment strategies: Your participation will provide an important scientific basis for the prevention and control of stroke in young people, especially for the identification and management of rare causes of stroke, laying the foundation for more effective prevention and treatment strategies in the future.

6. **Alternative therapies to the current study**: As this is an observational study and does not involve additional interventions, participants who choose not to participate in this study can continue to be seen and treated according to existing standard clinical pathways and physician recommendations. Existing treatment options include routine treatment of stroke, acute phase management, and secondary prevention measures such as antiplatelet or anticoagulant medications, and lifestyle modifications. For patients who choose to participate in this study, this study will also not interfere with your usual treatment.

7. **Cost**: This study will not cost you anything.

8. **Compensation**:There is no compensation for this study.

9. **Study Participant Responsibilities**: As a participant in this study, you have the following responsibilities to ensure the smooth running of the study and the accuracy of the data:

(1) Provide truthful information: Please provide truthful information about your current physical condition, medical history, medication history, and other health-related information to help the research team fully understand your situation.

(2) Report discomfort: If you experience any discomfort, unusual symptoms or physical changes during the study, please inform the study doctor promptly so that timely assessment and appropriate measures can be taken.

(3) Inform of other research participation: If you have recently participated in other research studies or are currently participating in other research projects, it is important that you talk to the study doctor so that the impact on the study can be assessed and your safety can be ensured.

(4) Know and follow the study procedures and precautions: Before the study begins, please read and understand the instructions for participation and be clear about the precautions to be taken; please follow the arrangements of the research team and be punctual in attending the follow-up visits, examinations, and related interviews so that the research team can obtain accurate data records.

10. **Privacy issues**: If you decide to participate in this study, your participation in the study and your personal information during the study will be kept confidential. Your study data/biological samples will be identified by the study number and not by your name. Information that identifies you will not be disclosed to members outside the research team unless you give your permission. All members of the research team and the research sponsors are asked to keep your identity confidential. Your file will be kept in a locked filing cabinet and will be accessible only to researchers. To ensure that the study is conducted in accordance with the regulations, members of the government regulatory authority or the ethical review board are required to have access to

your personal data at the research unit when necessary. No information about you will be disclosed when the results of this study are published.

11. **If you are harmed as a result of participating in this study**: This is an observational study involving only data collection and blood sample collection, and all operations will be conducted in strict accordance with the requirements for sterility, and therefore will not cause any harm to you. Since the study does not involve any experimental treatment or drug intervention, the risk of participating in this study is extremely low. In case of damages related to this clinical study, you will be entitled to free treatment and appropriate financial compensation/reimbursement.

12.**Disposal of biological samples and information at the end of the study**: At the end of the study, the biological samples collected will be temporarily stored for necessary follow-up analysis. The sample storage period will be strictly limited to the time required for the study analysis (2025.1-2030.1) and will ensure destruction within an appropriate time frame after the study is completed to protect your privacy. During storage, the use of these biological samples will be limited to this study and will not be made available to the public or used in other unrelated studies. The study data will be stored for at least 10 years after the completion of the study as required by our regulations.

13. **Feedback on study results**: Study results are usually presented in the form of aggregate data for academic research and disease prevention decision-making, and will not involve specific feedback from individuals.

14. **Other**: You may choose not to participate in this study, or you may request to withdraw from the study at any time by notifying the researcher. To ensure the scientific validity of the results of the study, data collected prior to your withdrawal from the study will be included in the results of the study. Any of your medical treatments and rights will not be affected as a result.

The research physician may terminate your continued participation in this study if you need other treatment, if you do not follow the study plan, if you have a study-related injury, or for any other reason.

You will be kept informed of information and research progress related to this study, and you will be notified of new information about safety related to this study as it becomes available. If you have questions about this study, if you experience any discomfort or injury during the study, or if you have questions about your rights as a participant in this study, you may contact ***** at ******.

If you have any questions or claims regarding the rights and health of participants in this study, you may contact the Institutional Ethics Committee at *****.