Annex 6.

Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

Patient Informed Consent

(Applicable to prospective studies)

Study Title:	Predictive Role of Early Blood KLK10 and Other Neuroinflammatory Molecules on Clinical Outcomes of Acute Ischemic Stroke
Protocol Number:	2024092
Principal Investigator:	Longxuan Li
Department:	Neurology
Study Period:	April 2024 - March 2025

Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

March 11, 2024

Version Number: 1.0

Patient Informed Consent

Project name: Predictive Role of Early Blood KLK10 and Other Neuroinflammatory Molecules on Clinical Outcomes of Acute Ischemic Stroke

Informed Consent Version Number: 1.0, Version Date: March 11, 2024

Research institution: Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine

Principal Investigator: Li Longxuan

You will be invited to participate in a clinical study. This informed consent form provides you with some information to help you decide whether to participate in this clinical study. Please read it carefully and ask the researcher in charge of the study if you have any questions.

What is the background and purpose of the study?

Research Background:

Studies have found that microglia, as the main immune cells in the brain, are the earliest activated cells after acute ischemic stroke (AIS). They play an important role in accelerating inflammatory responses, increasing or reducing neuronal apoptosis, and regulating the post-stroke inflammatory microenvironment, thereby affecting the prognosis of stroke. Previous studies have shown that kallikrein-related peptidase 10 (KLK10) is expressed on endothelial cells and is involved in regulating endothelial inflammation and atherosclerosis. We recently systematically observed the characteristics of KLK10 in terms of temporal and spatial expression and cellular distribution in the brain after cerebral ischemia, and found that this molecule is expressed on microglia and astrocytes in addition to brain endothelial cells. Although it did not increase significantly in the ischemic penumbra area in the early stage of ischemia, it was significantly highly expressed in the infarct core area at 2 days of ischemia and was significantly co-stained with microglia. It is highly expressed in microglia in specific areas of brain ischemia after AIS, suggesting that KLK10 may be involved in the early pathological damage process after cerebral ischemia. Since the permeability of the blood-brain barrier changes within 6 hours of stroke, KLK10, as a secretory molecule, is likely to be detected in the blood of AIS patients. Whether the level of KLK10 in plasma can reflect the changes in inflammatory pathological damage in the brain of AIS patients and serve as a specific molecular marker for predicting the prognosis of AIS patients is currently unclear and deserves further discussion.

Soluble triggering receptor expressed on myeloid cells 2 (sTREM2) is a natural immune receptor in the brain, which is composed of an extracellular immunoglobulin-like domain, a transmembrane domain, and a cytoplasmic part. After stroke, TREM2 is activated by nucleotides, lipid mediators, and some key factors released by apoptotic neurons and cell/myelin fragments, and plays an important role in regulating neural damage. Literature shows that TREM2 molecules are mainly expressed in microglia, which can reflect their activation state. Elevated plasma sTREM2 levels are closely related to the increased risk of death and cardiovascular events in AIS patients one year after onset.

Astrocytes, which are also involved in regulating inflammatory responses in the brain, are the main glial cells in the central nervous system and are widely distributed in the brain. They provide structural and functional support for neurons in a resting state, regulate synaptic activity by releasing glial transmitters, and participate in the formation and remodeling of synapses. After cerebral ischemia, astrocytes are activated and proliferate, which limits the infiltration of immune cells, repairs the damaged blood-brain barrier, and rebuilds the stability of the brain environment. Our research and that of others have shown that astrocytes are lost in large numbers in the infarct core area after cerebral ischemia, and death peaks 48-98 hours after stroke. The glial fibrillary acidic protein (GFAP) expressed by them can be released into the peripheral blood. The level of GFAP in the blood can reflect the state of cell necrosis and blood-brain barrier damage, and can be used as a molecular marker to distinguish ischemic from hemorrhagic stroke.

Stroke has a very complex pathological damage mechanism. Single neuroinflammatory molecules in plasma, whether sTREM2 or GFAP, are not effective in predicting the prognosis of AIS. Detecting the levels of a group of neuroinflammatory molecules may more accurately reflect the degree of neuropathological damage in AIS. Here, we propose that " early changes in a group of neuroinflammatory molecules such as KLK10, sTREM2 and GFAP in plasma can effectively predict the clinical prognosis of AIS." said the scientist.

Research purposes:

1. To investigate the correlation between changes in a group of neuroinflammatory molecules such as KLK10 in early plasma and clinical prognosis at 3 months after AIS
2. To investigate the correlation between changes in a group of neuroinflammatory molecules such as KLK10 in early plasma and post-infarction hemorrhagic transformation after AIS
3. the correlation between changes in a group of neuroinflammatory molecules such as KLK10 in early plasma and the occurrence of progressive stroke after AIS

If I participate in the research, what do I need to do?

This project intends to select 182 patients with anterior circulation AIS admitted to the Department of Neurology of the Northern Campus of Ruijin Hospital for the first time as observation subjects, and 91 healthy subjects as the control group. 10 ml of venous blood from the elbow of AIS patients was collected at the time of admission, 24h and 7d after stroke, and 10 ml of fasting venous blood from the elbow of healthy subjects was collected at one time to observe the dynamic changes and differential expression characteristics of a group of neuroinflammatory molecules such as KLK10, sTREM2, and GFAP in plasma, and to compare and analyze the correlation between the changes of this group of neuroinflammatory molecules in plasma in the early stage (at admission and 24h after the disease) and the severity of neurological deficits in the acute phase after stroke (NIHSS score), cerebral infarction volume, brain edema, hemorrhagic transformation, the occurrence of progressive stroke, and clinical prognosis at 3 months (mRS score), and to evaluate the predictive value of this type of molecules for clinical prognosis after AIS.

If you agree to participate in this study, we will number you and create a medical record. During the study, we need to collect your head CT or head MRI report, and retrieve your blood routine and blood biochemistry reports. At the same time, we need to collect some of your specimens. Professionals will take samples for you and draw 10 ml of venous blood from your arm for a total of 3 times. Your samples are only used to detect the expression of neuroinflammatory molecules in the blood.

Disposal of biological samples and information after the study: After the study is completed, the remaining biological samples will be destroyed directly. Imaging materials and other data will be stored in electronic files, which should be password -protected and stored in computers. Paper files are placed in locked cabinets. All data can only be accessed by researchers. No research information can be disclosed to unauthorized third parties without approval. All data and original documents of the study will generally be retained permanently, and permission should be obtained before destruction.

As a research subject, you need to provide truthful information about your medical history and current physical condition; tell the research doctor any discomfort you experience during this study; tell the research doctor whether you have recently participated in other studies or are currently participating in other studies.

Are there risks in the research?

Your blood sample will be collected in strict accordance with sterile requirements. There may be some very small risks in the collection of specimens, including brief pain, local bruising, mild dizziness in a few people, or extremely rare needle infection. If this occurs, we will take appropriate symptomatic treatment.

How might participating in research help me?

This study has no direct benefit to individual subjects, but the new findings in the study will help guide future stroke treatments.

Is there any cost or compensation for participating in the study?

Cost: Blood draw for testing the expression of neuroinflammatory molecules such as KLK10, sTREM2 and GFAP in the blood is free of charge.

Compensation: No special compensation for this project

What if I am harmed by participating in research?

If any damage occurs related to this clinical research , you can receive free treatment and/or corresponding compensation .

Is my information kept confidential?

If you decide to participate in this study, your participation in the study and your personal information in the study will be kept confidential. Your biological specimens will be identified by the study number instead of your name. Information that can identify you will not be disclosed to members outside the research team unless you allow it. All research members and research sponsors are required to keep your identity confidential. Your files will be kept in a locked filing cabinet and only accessible to researchers. To ensure that the research is conducted in accordance with regulations, if necessary, members of the government management department or the ethics review committee can access your personal information at the research unit as required . When the results of this study are published, no personal information about you will be disclosed.

Do I have to attend?

You voluntarily choose to participate or not participate in this study, or notify the researcher at any time to withdraw from the study. Your data will not be included in the research results, and any of your medical treatment and rights will not be affected.

The study physician may terminate your participation in this study if you require additional treatment, if you do not comply with the study protocol, if you develop a study-related injury, or for other reasons where continued participation may increase your risk of harm from participating in the study.

Who should I contact for more information?

You can keep up to date with the information and research progress related to this study. If there is any new safety information related to this study, we will notify you in a timely manner. If you have any questions related to this study, or if you experience any discomfort or injury during the study , or if you have any questions about the rights of participants in this study, you can contact us through 13611649930 and Longxuan Li connect .

This study has been reviewed **by the Human Research Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine** . If you have any questions or concerns about your rights and health when participating in this study, you can contact the Ethics Committee of this institution at 54661789; Contact person: Professor Wang.

Informed consent signature page

I have read this Informed Consent Form.

I had the opportunity to ask questions and all of them were answered.

I understand that participation in this research is voluntary.

I may voluntarily choose to participate or not to participate in this study, or withdraw after notifying the researcher at any time without being discriminated against or retaliated against, and any of my medical treatment and rights will not be affected.

The study physician may terminate my participation in this study if I require additional treatment, if I fail to comply with the study protocol, if a study-related injury occurs, or for other reasons where continued participation may increase my risk of harm from participating in the study .

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

Subject’s Name: _____

Subject’s Signature: _____

Date : _____ / _____ / _____

Name of legal representative: _____

Signature of legal representative: _____

Date : _____ / _____ / _____

Witness’ Name: _____

Witness Signature: _____

Date : _____ / _____ / _____

(*Note: If the subject is illiterate, a witness' signature is required; if the subject is incapable of acting/has limited capacity for acting, a legal representative's signature is required*)

I have accurately informed the subject of this document and asked him/her to read this informed consent form carefully and answer any questions or doubts raised carefully.

Researcher Name: Longxuan Li

Investigator’s Signature: 

Date : March 11, 2024