Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology
Clinical Research Project Protocol

Project Name	The Role of Mitochondria and Related Genes in the Diagnosis and Prognostic Assessment of Sepsis: A Multicenter, Prospective Cohort Study
Protocol Number	Mit01
Version Number	V2.0
Version Date	2024-09-18
Principal Investigator	Zhang Jiancheng
Specialty Department	Critical Care Medicine
Planned Start and End Date	2024-10～2026-10

Protocol Signature Page

Project Name: The Role of Mitochondria and Related Genes in the Diagnosis and Prognostic Assessment of Sepsis: A Multicenter, Prospective Cohort Study

Version Number/Version Date: V2.0/2024-09-18

I have read and understood this research protocol, confirmed that it contains all necessary content for the implementation of the study, and clarified the investigators' responsibilities associated with this protocol. As the Principal Investigator, I will provide a copy of this protocol and all relevant materials to all researchers participating in the study. I will discuss these materials with them to ensure their full understanding of the protocol implementation procedures. I agree to and will strictly fulfill the relevant responsibilities in accordance with the currently applicable laws and regulations, the Declaration of Helsinki, Good Clinical Practice (GCP) for drug clinical trials, and this research protocol.

Principal Investigator's Signature (in block letters): _____

Principal Investigator's Signature/Date: _____ / _____

Clinical Research Institution: Wuhan Union Hospital Affiliated to Huazhong University of Science and Technology

Protocol Abstract	
Study Name	The Role of Mitochondria and Related Genes in the Diagnosis and Prognostic Assessment of Sepsis: A Multicenter, Prospective Cohort Study
Study Introduction	Sepsis is a severe multiple organ dysfunction syndrome induced by infection, which has become one of the major challenges to global public health. Studies have shown that mitochondrial DNA (mtDNA) may play a crucial role in the pathogenesis of sepsis as a damage-associated molecular pattern (DAMP). mtDNA can promote the spread of inflammatory responses through its interaction with the immune system, and may serve as a potential biomarker for assessing disease severity and prognosis. A number of clinical studies have found that plasma mtDNA levels are significantly elevated in patients with sepsis, which is associated with high mortality. For example, some studies have demonstrated that high mtDNA levels are closely correlated with an increased risk of death in patients admitted to the intensive care unit (ICU). In addition, incorporating mtDNA into clinical prediction models can significantly improve their ability to predict patient prognosis. These studies provide a scientific basis for further exploring the potential of mtDNA as a biomarker for sepsis, and contribute to the improvement of

	early diagnosis and treatment strategies for sepsis.
Research Objective	To clarify and verify the diagnostic value of mitochondrial-related genes in sepsis and their value in judging poor prognosis of sepsis patients.
Study Design	This study plans to collect data from patients diagnosed with sepsis in Hubei Province from September 2024 to September 2025, comprehensively evaluate the role of mitochondrial genes in the diagnosis and prognosis of sepsis, and hope to provide insights for clinical diagnosis and treatment as well as future clinical research.
Total Number of Enrolled Patients	200 cases
Number of Study Groups / Control Groups	100 subjects in the study group, 100 subjects in the control group
Diagnosis	1. Confirmed or suspected infection;2. Change in SOFA score ≥ 2 points
Inclusion Criteria	• Patients with sepsis diagnosed according to Sepsis 3.0, regardless of ethnicity and gender • Aged 16 to 90 years old • Signed written informed consent form
Exclusion Criteria	• Patients over 90 years old or under 16 years old• Pregnant and lactating women • Patients with hematological tumors, post-radiotherapy/chemotherapy tumors, and immune system diseases • Patients with blood-borne diseases (febrile

	diseases, syphilis, HIV, hepatitis B, hepatitis C) • Patients who have received systematic in-hospital treatment for more than 1 week after the diagnosis of sepsis • Hemoglobin < 40g/L • Subjects who participated in other clinical studies at the time of enrollment or within 3 months before enrollment • Refused to sign the written informed consent form
Study Intervention	No intervention
Evaluation Criteria Primary Endpoints Secondary Endpoints Safety Evaluation	**Primary Endpoints**:(1) To explore the guiding value of mitochondrial-related genes combined with SOFA score and APACHE II score for the prognosis and survival rate of sepsis patients.(2) To compare the differences in the area under the receiver operating characteristic (ROC) curve (AUC) of mitochondrial-related genes, SOFA score, APACHE II score, IL-6, IL-1β, and HMGB1 in predicting death in sepsis patients.**Secondary Endpoints**:To compare the correlations between mitochondrial-related genes and SOFA score, APACHE II score, IL-6, IL-1β, and HMGB1.**Safety Evaluation**:1. Safety of sample collection: Record and evaluate adverse events or complications occurring during peripheral blood sample collection, including blood collection-related reactions (e.g., local bleeding, infection,

	etc.).2. Stability and reliability of biomarker detection: Ensure the consistency and reliability of the detection process of mitochondrial gene expression, and monitor potential problems in the laboratory detection process (e.g., equipment failure, operational errors, etc.).3. Patient safety monitoring: Regularly monitor and record the health status of patients and any study-related adverse reactions during the study to ensure the overall safety of the study for patients.
Statistical Methods： Sample Size Analysis Set Primary Efficacy Endpoint Safety Endpoint	1. **Sample Size**: Based on the expected effect size (moderate correlation between mitochondrial gene expression and patient prognosis, Cohen's d = 0.5), we set the significance level at 0.05 and the statistical power at 90%. Using sample size calculation formulas and statistical software, it is determined that 200 samples are required to ensure sufficient statistical power to detect the effect and reduce the probability of Type II error, thus providing reliable research results.2. **Statistical Analysis Set**: Full Analysis Set (FAS)3. **Primary Efficacy Endpoint**: Evaluate the predictive ability of mitochondrial gene expression on primary efficacy outcomes (e.g., overall survival [OS]), especially to analyze whether the expression level of mitochondrial genes in peripheral blood can independently predict these clinical outcomes.4. **Safety Endpoint**: Refer to the safety evaluation

	content in the above evaluation criteria
Study Duration	2 years
Subject Participation Time	2 years
Study Institution/Location	Multicenter study (Department of Critical Care Medicine, Wuhan Union Hospital)

Principal Investigator's Information

1. Name and Contact Information: Zhang Jiancheng, Tel: 13554105815
2. Qualifications:(1) Obtained the Physician Qualification Certificate on December 18, 2013(2) Obtained the Medical Practitioner Certificate on December 11, 2015, with the practice category of clinical medicine, practice scope of Critical Care Medicine, and practice location at Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology(3) Obtained the GCP Certificate: Training Time (August 2021), Class Hours (32), Training Institution (Senior Executive Training Institute of the National Medical Products Administration), Training Level (National Level), Training Method (Lectures), Certificate Issuance Date (August 30, 2021)

Study Flow Chart

1. Enroll patients with sepsis in the General ICU of Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology in accordance with the inclusion and exclusion criteria, regardless of gender and ethnicity.
2. Collect 5ml of venous blood samples from eligible patients who have signed the informed consent form and store the samples at low temperature.
3. Baseline data collection: Collect clinical data including ventilator use,

vasoactive drug use, routine blood test, biochemistry, coagulation function, myocardial enzymes, CRP, PCT, vital signs, length of hospital stay and clinical outcomes.

4. Laboratory tests: Detect the expression of mitochondrial genes and inflammatory genes in PBMC and bronchoalveolar lavage fluid by PCR, ELISA and WB.

5. Follow-up: Record ICU length of stay, ICU mortality, in-hospital mortality and 28-day mortality.

6. Conduct clinical data collation, laboratory test result analysis and statistical data analysis.

Abbreviation Table	
Abbreviation	Full Name
mtDNA	Mitochondrial deoxyribonucleic acid
APACHE II	Acute Physiology Chronic Health EvaluationII
SOFA	Sequential organ failure assessment
DAMP	Damage associated molecular pattern

1. Introduction

1.1 Research Background

1.1.1 General Overview

Sepsis is a life-threatening multiple organ dysfunction caused by the host's abnormal response to infection, which can lead to various pathological and biochemical abnormalities in the body. With the increasing incidence of sepsis, it has become one of the major global public health problems. According to a 2020 statistical study on sepsis biomarkers, there are currently 258 sepsis biomarkers, among which 69 are mainly used to evaluate diagnostic value, 100 for prognostic value, and 89 for both diagnostic and prognostic values. Exploring effective diagnostic and prognostic biomarkers for sepsis will undoubtedly help us timely and accurately understand the diagnosis, progression and prognosis of the disease.

To date, most studies on mitochondria have focused on their role as organelles responsible for energy production, protein synthesis, catabolism and cell death. However, there is a growing interest in mtDNA as a potential damage-associated molecular pattern. Among the known mitochondrial damage-associated molecular patterns, mtDNA has received the most attention. A growing body of literature has shown that mtDNA is not only released in critical illnesses but also can spread inflammatory responses through its interaction with the immune system.

This raises the possibility that mtDNA may serve as a surrogate for disease severity and even a predictor of mortality in critically ill patients. Given the function of mtDNA as a DAMP, there is an increasing interest in using mtDNA as a predictor of disease severity and/or mortality in critically ill patients. In 2013, Nakahira et al. published one of the first clinical trials on this topic. In their study, 200 patients in the medical ICU were enrolled in the main cohort. They found that mtDNA levels were significantly higher in patients who died within 28 days of ICU admission than in survivors. In addition, the odds of death within 28 days of ICU admission increased with the elevation of mtDNA levels. These findings were replicated in a validation cohort of 243 critically ill patients with ARDS. Their data also indicated that mtDNA levels may allow clinicians to distinguish patients with high and low mortality rates, as adding this value to the clinical model increased the AUC of mortality prediction from 0.76 to 0.83.

Since 2013, several studies have attempted to replicate or extend the findings of Nakahira et al. In the past two years alone, five studies have examined mtDNA in critically ill adults admitted to medical or cardiac ICUs. In 2015, Bhagirath et al. published a translational study aiming to clarify the roles of nuclear DNA (nucDNA), mtDNA and bacterial DNA (bacDNA) in sepsis. The clinical part of their study included measuring plasma nucDNA and mtDNA levels in 12 consecutive patients admitted

to the ICU with severe sepsis. In this cohort, plasma nucDNA and mtDNA levels were 200-fold and 50-fold higher than those in the healthy control group, respectively. Recognizing that the quantity and quality of stimulation are not necessarily correlated, Bhagirath et al. conducted subsequent in vivo experiments to measure the effects of different levels of nucDNA, mtDNA and bacDNA on neutrophil viability.

A few months later, Krychtiuk et al. published a prospective observational study investigating mtDNA in 228 critically ill patients; the cohort was highly heterogeneous, including critically ill surgical patients (cardiothoracic) and medical ICU patients (68% of the cohort). They found that plasma mtDNA levels in critically ill patients were significantly higher than those in healthy counterparts. In addition, in the medical population, the highest mtDNA levels were found in patients with sepsis or cardiogenic shock. In contrast, mtDNA levels in surgical patients were almost the same as those in the healthy control group. Although a statistically significant elevation of mtDNA was found, the researchers did not observe an association between mtDNA and vasopressor use, mechanical ventilation requirement, renal insufficiency, or disease severity scores (APACHE II, SAPS II, SOFA). However, they did note that the median mtDNA level was significantly higher in patients who died within 30 days of ICU admission, independent of demographics, initial diagnosis and APACHE II score. They also observed a significant

correlation between mtDNA and mortality in medical patients, but not in surgical patients. Finally, they calculated that the AUC of the ROC curve for mtDNA and the clinical model (involving APACHE II and gender) were 0.6 and 0.79, respectively. When mtDNA was added to the clinical model, the AUC was further increased to 0.81, which was statistically significant.

To explore the relationship between plasma nucDNA, plasma mtDNA and various inflammatory markers (TNF-α, IL-6, IL-8, IL-10 and IL-1RA), Timmermans et al. conducted a prospective observational study by collecting samples on days 1, 3, 5, 6, 9, 14, 21 and 28 of ICU admission in 121 patients with septic shock. Their data showed that nucDNA, mtDNA and inflammatory cytokine levels in sepsis patients were significantly elevated compared with the healthy control group, and remained elevated at all time points.

In summary, sepsis is a severe infection-induced multiple organ dysfunction syndrome and a major challenge to global public health. Studies have shown that mtDNA may play an important role in the pathogenesis of sepsis as a DAMP. mtDNA can promote the spread of inflammatory responses through its interaction with the immune system and may serve as a potential biomarker for assessing disease severity and prognosis. Multiple clinical studies have found that plasma mtDNA levels are significantly elevated in sepsis patients and associated with high

mortality. Therefore, understanding and studying the role of mtDNA in sepsis not only helps to reveal its pathogenesis, but also provides potential possibilities for the development of new diagnostic biomarkers and therapeutic targets.

In conclusion, the study of mitochondrial genes, especially mtDNA, in the pathogenesis of sepsis has important biological and clinical significance. In-depth exploration of their role in the regulation of inflammatory responses is expected to contribute to the development of early diagnosis, disease assessment and individualized treatment strategies for sepsis.

1.2 Study Type

Multicenter, prospective cohort study

1.3 Risk/Benefit Assessment

This study involves no clinical intervention. Only 5ml of venous blood will be collected from patients, with minimal risk. The collected venous blood will be used for PCR and WB detection, and the results will be fed back to clinicians in a timely manner to indicate the patient's infection status, but there is no direct benefit for the enrolled patients from this study.

1.3.1 Known Potential Risks

Only 5ml of venous blood will be collected from patients in this study, with minimal risk.

1.3.2 Severity of Harm

Only 5ml of venous blood will be collected from patients in this study, with minimal harm.

1.3.3 Known Potential Benefits

The collected venous blood will be used for PCR and WB detection, and the results will be fed back to clinicians in a timely manner; there is no direct benefit for the enrolled patients from this study.

1.3.4 Potential Risk/Benefit Assessment

No potential risks

1.3.5 Discussion

No potential benefits or risks

2. Research Objectives/Endpoints

The study endpoint is reached when the following conditions are met: complete collection of basic information (e.g., demographics), medical history, vital signs, laboratory tests, disease severity assessment and other data at enrollment and 7 days after enrollment, and completion of follow-up on the survival status of patients at ICU discharge after enrollment.

3. Study Design

3.1 Overall Design

We will strictly enroll sepsis patients in accordance with the inclusion and exclusion criteria, and divide them into a death group and a survival group according to their clinical outcomes. We will compare the

differences in mitochondrial-related genes, SOFA score and APACHE II score at admission between the two groups; analyze the correlations between mitochondrial-related genes and SOFA score/APACHE II score, and compare the differences in the AUC of ROC curve of mitochondrial-related genes in predicting death in sepsis patients. This is a multicenter, prospective study.

3.2 Sample Size

Based on the expected effect size (moderate correlation between mitochondrial gene expression and patient prognosis, Cohen's d = 0.5), we set the significance level at 0.05 and the statistical power at 90%. Using sample size calculation formulas and statistical software, it is determined that 200 samples are required to ensure sufficient statistical power to detect the effect and reduce the probability of Type II error, thus providing reliable research results.

4. Study Population

4.1 Diagnostic Criteria

Sepsis is diagnosed in accordance with the Sepsis 3.0 diagnostic criteria.

4.2 Inclusion Criteria

• Patients with sepsis diagnosed according to Sepsis 3.0, regardless of ethnicity and gender

• Aged 16 to 90 years old

• Signed written informed consent form

4.3 Exclusion Criteria

• Patients over 90 years old or under 16 years old

• Pregnant and lactating women

• Patients with hematological tumors, post-radiotherapy/chemotherapy tumors, and immune system diseases

• Patients with blood-borne diseases (febrile diseases, syphilis, HIV, hepatitis B, hepatitis C)

• Patients who have received systematic in-hospital treatment for more than 1 week after the diagnosis of sepsis

• Hemoglobin < 40g/L

• Subjects who participated in other clinical studies at the time of enrollment or within 3 months before enrollment

• Refused to sign the written informed consent form

5. Evaluation

5.1 Primary and Secondary Endpoint/Outcome Evaluation

Primary Objectives

(1) To explore the guiding value of mitochondrial-related genes combined with SOFA score, APACHE II score, IL-6, IL-1β and HMGB1 for the prognosis and survival rate of sepsis patients.

(2) To compare the differences in the AUC of ROC curve of mitochondrial-related genes, SOFA score, APACHE II score, IL-6, IL-1β and HMGB1 in predicting death in sepsis patients.

Secondary Objectives

(1) To compare the correlations between mitochondrial-related genes and SOFA score, APACHE II score, IL-6, IL-1β and HMGB1.

(2) To explore the effect of mitochondrial-related genes combined with APACHE II score and SOFA score on the total hospital stay and infection-related complications during hospitalization in ICU sepsis patients.

5.2 Safety Evaluation

Safety of sample collection: Record and evaluate adverse events or complications occurring during peripheral blood sample collection, including blood collection-related reactions (e.g., local bleeding, infection, etc.).

Stability and reliability of biomarker detection: Ensure the consistency and reliability of the detection process of mitochondrial gene expression, and monitor potential problems in the laboratory detection process (e.g., equipment failure, operational errors, etc.).

Patient safety monitoring: Regularly monitor and record the health status of patients and any study-related adverse reactions during the study to ensure the overall safety of the study for patients.

6. Adverse Events and Serious Adverse Events

Patients only need to undergo a single 5ml blood collection throughout the study with minimal risk, and there is no possibility of adverse events or serious adverse events occurring.

7. Statistical Analysis and Statistical Methods

SPSS 20 software will be used for statistical analysis. Descriptive statistics will be adopted to summarize the baseline characteristics of patients. Continuous variables will be described by mean, median, standard deviation, minimum and maximum values; all categorical variables will be described by frequency and percentage. All statistical tests will be two-tailed unless otherwise specified, and a two-tailed P value < 0.05 will be considered statistically significant. Binary logistic regression analysis will be used to analyze the correlation between mortality and various factors.

8. Medical Care and Protection of Subjects

8.1 Assessment of Risks to Subjects in the Study and Risk Disposal Measures & Contingency Plans

Patients only need to undergo a single 5ml blood collection throughout the study with minimal risk. Aseptic operation will be strictly performed before blood collection, and blood infection indicators will be closely monitored after blood collection.

9. Supporting Documents and Notes

9.1 Privacy Protection

To protect patients' privacy, all case report forms, research reports and study-related communications will use patient code and enrollment number as identification marks instead of real names. In accordance with the relevant national and local laws and regulations, investigators shall allow the sponsor or designated personnel to access relevant medical records within the permitted scope to verify the data collected in the case report forms and audit the data collection process. Regulatory authorities may also request access to all study records. In accordance with the relevant requirements of the informed consent form, clinical data shall not be disclosed without the patient's written permission.

9.2 Collection and Use of Specimens and Data

The data used in this study are derived from the hospital's case management system, which will be exclusively used for this study and strictly kept confidential.

9.3 Quality Control and Quality Assurance

(1) There will be 1 principal investigator and 3 fixed research team members, who will strictly implement the clinical trial protocol.

(2) All experimental detection items must adopt the national statutory measurement units. Experimental test reports must include complete items, including test date, detection items, test results and their normal value ranges, and be signed by the relevant personnel.

(3) Retained test specimens shall be properly stored. The kits purchased for laboratory detection must be products with international or domestic quality assurance, and the detection shall be strictly carried out in accordance with the kit instructions.

9.4 Data Processing and Record Keeping

9.4.1 Data Collection and Management

Paper case report forms will be used to record clinical data, laboratory results, various scores, adverse events, follow-up information and other data. After the completion of paper data collection, all data will be entered into electronic case report forms, and other members of the research team will recheck the collected data.

Data collection will be conducted by clinical researchers under the supervision of the principal investigator, who is responsible for ensuring the accuracy, completeness and timeliness of the reported data.

9.4.2 Retention of Research Data

All study materials and data, including ethical approval documents, signed informed consent forms, research protocols and completed case report forms, will be stored in a locked location in the General ICU of Union Hospital, and retained for at least 5 years.

9.5 Conflict of Interest Statement

No conflict of interest.

10. References

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Informed Consent Form

Project Name	The Role of Mitochondria and Related Genes in the Diagnosis and Prognostic Assessment of Sepsis: A Multicenter, Prospective Cohort Study
Protocol Number	Mit01
Version Number	V2.0
Version Date	2024-09-18
Principal Investigator	Zhang Jiancheng
Specialty Department	Department of Critical Care Medicine

Dear Patient and Family Members,

You are invited to participate in a clinical study entitled *The Role of Mitochondria and Related Genes in the Diagnosis and Prognostic Assessment of Sepsis: A Multicenter, Prospective Cohort Study*, initiated by Union Hospital. The following sections describe the research background, objectives, methods, potential benefits, possible risks or inconveniences to you during the study, and your rights and interests. Please read this information carefully before deciding to participate. This informed consent form is designed to help you make an informed decision about participation. If you have any questions, please ask the investigator in charge of the study to ensure you fully understand the content. Your participation is entirely voluntary. If you agree to take part, please sign the statement at the end of this form.

This study has been approved by the Ethics Committee of Union Hospital Affiliated to Huazhong University of Science and Technology.

Research Background

Sepsis is a systemic inflammatory response syndrome caused by severe infection, which can lead to multiple organ dysfunction and even death in severe cases. In recent years, increasing research has focused on the role and importance of mitochondrial genes in the pathogenesis of sepsis. Mitochondria are vital organelles within cells, primarily responsible for energy production, cellular signal regulation, and involvement in life

processes such as cell death. Beyond these traditional functions, mitochondria contain their own DNA, known as mitochondrial DNA (mtDNA). Under normal conditions, mtDNA stably exists within mitochondria; however, when cells are damaged or undergo death, mtDNA can be released outside the cell and act as a damage-associated molecular pattern (DAMP) signaling molecule.

In the pathophysiological process of sepsis, a large number of inflammatory mediators are activated and released, triggering abnormal immune and inflammatory responses in the body. These responses may cause mitochondrial damage and dysfunction within cells, subsequently leading to the release of mtDNA. Once released into the bloodstream, mtDNA acts as a potent immunologically active molecule, promoting the spread and exacerbation of inflammatory responses by activating Toll-like receptors and other inflammatory signaling pathways of the immune system.

Studies have shown that plasma mtDNA levels are typically significantly elevated in patients with sepsis, and this elevation is closely associated with disease severity and prognosis. For example, high mtDNA levels in sepsis patients are linked to higher mortality and more severe organ damage. Therefore, mtDNA, as a potential biomarker, can assist clinicians in assessing the severity of a patient's condition and predicting the likelihood of disease progression. In addition, researchers have found

that mtDNA release is closely associated with the activation of inflammatory responses and may be involved in regulating the role of the immune system during sepsis.

Understanding and investigating the role of mtDNA in sepsis not only helps to reveal its pathogenesis but also provides potential opportunities for the development of new diagnostic biomarkers and therapeutic targets.

In summary, research on mitochondrial genes, especially mtDNA, in the pathogenesis of sepsis holds significant biological and clinical importance. In-depth exploration of their role in the regulation of inflammatory responses is expected to contribute to the development of early diagnosis, disease assessment, and individualized treatment strategies for sepsis.

Research Objective

To clarify and verify the diagnostic value of mitochondrial-related genes in sepsis and their value in determining poor prognosis.

Eligibility for Participation

Inclusion Criteria

Adult patients (≥18 years old) diagnosed with sepsis or septic shock according to the Sepsis 3.0 criteria.

Exclusion Criteria

1. Patients over 90 years old or under 16 years old;
2. Pregnant and lactating women;

3. Patients with hematological tumors, post-radiotherapy/chemotherapy tumors, or immune system diseases;
4. Patients with blood-borne diseases (febrile diseases, syphilis, HIV, hepatitis B, hepatitis C);
5. Patients who have received systematic in-hospital treatment for more than 1 week after a confirmed diagnosis of sepsis;
6. Hemoglobin < 40g/L.

Study Brief

This is a multicenter, prospective study with the objective of *clarifying and verifying the diagnostic value of mitochondrial-related genes in sepsis and their value in determining poor prognosis*. The study requires only a single collection of 5ml venous blood upon admission to the ICU. The collected blood will be used for PCR and WB testing, and the results will be promptly fed back to clinicians to indicate the patient's infection status and guide clinical treatment.

Biological Sample Collection

A single 5ml venous blood sample will be collected from the patient for this study.

Detection, Storage and Disposal of Biological Samples

The collected venous blood will be used for PCR and WB testing and stored for 48 hours. Testing will be conducted at the Experimental Center

of Tongji Medical College, Huazhong University of Science and Technology, and the results will be promptly fed back to clinicians. After the completion of the study, the remaining blood samples will not be used for other related or future research. They will be treated by high-temperature and high-pressure sterilization and then transferred to a designated unit for centralized incineration disposal.

Subject's Obligations

The subject shall cooperate with a single venous blood collection for the study.

Potential Risks of Participation

Only a 5ml venous blood sample will be collected for this study, with minimal risk.

Potential Benefits of Participation

The collected venous blood will be used for PCR and WB testing, and the results will be promptly fed back to clinicians to indicate the patient's infection status and guide clinical treatment.

Management of Study-Related Injuries

Only a single venous blood collection is required upon admission to the department, with an extremely low risk of injury.

Associated Costs

In addition to routine ICU tests, the costs of PCR, WB and ELISA testing will be borne by the investigators.

Notification of New Clinical Study Information

The collected venous blood will be used for PCR and WB testing, and the results will be promptly fed back to clinicians.

Circumstances for Study Termination

After the patient consents to enrollment, only a single venous blood collection is required. The clinical study will be terminated if the patient experiences vital sign fluctuations that make blood sample collection impossible.

Duration of Participation

Patient enrollment and screening will be conducted from September 1, 2024 to September 1, 2025. Case files will be established, the study will be completed in October 2025, and data statistics will be performed thereafter.

Sample Size

A total of 200 patients are expected to participate in this study.

Privacy and Confidentiality

Your personal information and health status will be strictly kept confidential in this study. No information about you will be disclosed to any third party except authorized regulatory authorities.

Voluntary Participation and Right to Withdraw

You may choose not to participate in this study, or withdraw from the study at any time by notifying the investigator, without facing

discrimination or retaliation. Your medical treatment and rights will not be affected in any way.

The investigator may terminate your participation in the study if you require additional diagnosis/treatment, fail to comply with the study protocol, or for any other reasonable cause. You may access information and progress related to the study at any time.

How to Obtain Assistance

If you have questions about trial information, study progress, or subject rights and interests, or experience any trial-related discomfort or harm, you may contact the investigator Zhang Jiancheng at 13554105815 (24-hour hotline), or contact the Ethics Committee of our center: Medical Ethics Committee of Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Tel: 027-85726375.

Subject's Statement

I have carefully read this informed consent form, have had the opportunity to ask questions, and all my questions have been answered. I understand that my participation in this study is voluntary. I may choose not to participate, or withdraw from the study at any time by notifying the investigator, without facing discrimination or retaliation, and my medical treatment and rights will not be affected in any way.

The investigator may terminate my participation in this clinical study if I require additional diagnosis/treatment, fail to comply with the study protocol, or for any other reasonable cause.

I voluntarily agree to participate in this clinical study and will receive a signed copy of this informed consent form.

Subject's Full Name (in block letters): _____Contact Phone Number: _____Subject's Signature: _____Date: _____ Year _____ Month _____ Day

If the subject is incapable of signing the informed consent form (e.g., due to lack of capacity) or is a minor, the guardian shall sign on their behalf.

Guardian's Full Name (in block letters): _____Contact Phone Number: _____Guardian's Signature: _____Date: _____ Year _____ Month _____ DayRelationship with Subject: _____Reason the Subject Cannot Sign the Informed Consent Form: _____

If the subject or their guardian is illiterate, an impartial witness shall sign the form.

Impartial Witness's Full Name (in block letters): _____Contact Phone Number: _____Impartial Witness's Signature: _____Date: _____ Year _____ Month _____ Day

Investigator's Statement

I have accurately informed the subject of the content of the informed consent form, answered all the subject's questions, and the subject has voluntarily agreed to participate in this clinical study.

Investigator's Full Name (in block letters): _____Contact Phone Number: _____Investigator's Signature: _____Date: _____ Year _____ Month _____ Day