

Sepsis Multiomic Analysis & Risk sTratification in China (China SMART-1): A Prospective Observational Cohort Study

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SUMMARY

Rationale: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and is listed by the World Health Organization as one of the top 10 causes of death worldwide. Each 1-hour delay in the diagnosis of sepsis increases patient mortality by 7.6%. Although international guidelines (such as the Surviving Sepsis Campaign) emphasize the "golden 6 hours" early intervention concept, clinical practice still faces two core problems: delayed diagnosis and insufficient biomarker efficacy. Therefore, the development of highly sensitive and specific early diagnostic tools is a key breakthrough to improve prognosis.

Objective: The objectives of the present study are 1) Screen sepsis-specific mRNA diagnostic biomarkers via Bulk RNA-seq and establish a molecular risk stratification system for sepsis; 2) Develop an RT-LAMP detection method and validate its diagnostic efficacy; 3) Screen sepsis-specific diagnostic biomarkers in plasma and urine via PRM and metabolomics, and validate them using immunological methods (ELISA).

Study design: Prospective, single-center, observational cohort study at Yuebei People's Hospital. **Study population:** ICU inpatients, divided into sepsis group and non-sepsis group according to Sepsis-3 criteria.

Intervention (if applicable): Not applicable.

Main study parameters/endpoints: Primary outcomes: 1) Diagnostic efficacy of mRNA biomarkers and molecular risk stratification biomarkers; 2) Diagnostic efficacy of RT-LAMP; 3) Diagnostic efficacy of protein/metabolic biomarkers in plasma/urine. Secondary outcomes: 28-day all-cause mortality, ICU length of stay, incidence of new organ dysfunction, subgroup analysis of biomarkers (infection site, pathogen type, organ injury)

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The burden and risks associated with participation in this study are negligible. Whole blood, plasma and urine samples will be collected from patients upon ICU admission. Participants will be contacted via telephone follow-up 28 days after hospital discharge to inquire about their health status. Participation in

this study will not provide direct benefits to individual participants. However, the knowledge gained from this research is expected to benefit patients with suspected infection in the future by enabling rapid evaluation, diagnosis and treatment of sepsis, as well as better identification of patients at high risk of adverse clinical outcomes.

1. INTRODUCTION AND RATIONALE

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and is listed by the World Health Organization as one of the top 10 causes of death worldwide. According to the Global Burden of Disease Study (GBD 2017) ^[1], there are approximately 48.9 million cases of sepsis globally each year, resulting in about 11 million deaths, with a case fatality rate as high as 20%-40% ^[2-3]. In China ^[4], the incidence of sepsis is approximately 240 cases per 100,000 population, and the sepsis-related mortality rate in ICU patients reaches 35.5%, significantly higher than that in developed countries. Although international guidelines (such as the Surviving Sepsis Campaign) emphasize the "golden 6 hours" early intervention concept, clinical practice still faces two core problems: delayed diagnosis and insufficient biomarker efficacy. The current diagnostic criteria (Sepsis-3) ^[5] rely on the SOFA score and evidence of infection, but the SOFA score depends on laboratory indicators (such as platelets and bilirubin), which are difficult to obtain quickly in emergency settings. Commonly used clinical biomarkers such as procalcitonin (PCT) and C-reactive protein (CRP) have significant limitations ^[6-9]: although PCT has certain specificity for bacterial infections, its sensitivity is low (approximately 60%-70%) in local infections (such as abscesses) or infections caused by atypical pathogens (such as viruses and fungi); as an acute-phase reactant, CRP elevation lags behind the inflammatory response (6-12 hours after infection) and is interfered with by non-infectious factors such as surgery and trauma; lactate reflects tissue hypoperfusion, but its elevation mostly indicates that the patient has entered the stage of septic shock, missing the window for early intervention.

Studies have shown that each 1-hour delay in the diagnosis of sepsis increases patient mortality by 7.6%. Therefore, the development of highly sensitive and specific early diagnostic tools is a key breakthrough to improve prognosis.

Although microbial culture is the gold standard for sepsis diagnosis, it takes too long and may delay the diagnosis of sepsis. In addition, microbial culture tests may be negative in approximately 30-50% of sepsis patients ^[10-11]. Current research indicates

that in the intensive care process of sepsis patients, clinical electronic medical record data are increasingly being used to construct multivariate clinical early warning models. Mining these data may accelerate clinical monitoring of sepsis and provide new ideas for the early identification of post-traumatic sepsis ^[12-13].

Peripheral blood mRNA expression levels can directly reflect the host's gene regulatory response to infection and theoretically show detectable systemic changes within hours after infection, having higher early diagnostic potential than protein biomarkers ^[14-15]. With the maturity of RNA sequencing technology and the significant reduction in costs, biomarker screening based on whole transcriptome data has become an important strategy for discovering novel sepsis diagnostic biomarkers. However, existing transcriptome studies are mostly based on small-scale cohorts with limited sample sizes and mainly focus on European and American populations, lacking large-sample prospective studies that systematically screen and validate mRNA diagnostic biomarkers in Chinese ICU populations. In addition to diagnostic dilemmas, sepsis patients show significant differences in response to standardized treatment regimens, suggesting the existence of internal molecular subtypes with different biological characteristics. Transcriptomic studies have confirmed the molecular basis of this heterogeneity: Scicluna et al. ^[16] identified four genomic intrinsic subtypes (MARS classification) in a European multicenter cohort, with significant differences in immune characteristics and 28-day mortality among subtypes; Seymour et al. ^[17] described two robust clinical phenotypes (α -type and β -type), with β -type characterized by immunosuppression and multiple organ injury. These studies have laid the theoretical foundation for the precise stratification of sepsis, but their samples are all from European and American populations, and there are systematic differences in genetic background, pathogen spectrum, and medical practice compared with Chinese patients, limiting the direct extrapolation applicability of the classification results. Currently, there are no large-sample transcriptome-based stratification studies of sepsis in Chinese ICU populations. Therefore, we propose Substudy 1: Screening of early diagnostic molecular biomarkers and molecular

stratification biomarkers for sepsis based on Bulk RNA-seq transcriptome sequencing.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) has the characteristics of high sensitivity, high specificity, and fast detection speed, and has been widely used in clinical testing. In our previous work, we developed relevant detection methods. Therefore, we propose Substudy 2: Validate the diagnostic efficacy of RT-LAMP as an early rapid bedside diagnostic tool for ICU sepsis.

In addition, we previously used data-independent acquisition (DIA) proteomics and untargeted metabolomics to screen biomarkers with diagnostic potential for sepsis from plasma, such as AHSG, CLU, SERPINA4, etc. (see Appendix 3 for details). Some biomarkers have been validated in cohort studies ^[18-20]. Urine has the same diagnostic value. Therefore, we propose Substudy 3: Validate the diagnostic efficacy of plasma protein/metabolic biomarkers as early diagnostic biomarkers for ICU sepsis. Substudy 4: Validate the diagnostic efficacy of urine protein/metabolic biomarkers as early diagnostic biomarkers for ICU sepsis.

This study intends to rely on the ICU of Yuebei People's Hospital to prospectively enroll 1400 subjects (1000 sepsis cases, 400 non-sepsis controls) and establish the largest comprehensive biobank for sepsis research in Chinese ICUs to date. Bulk RNA-seq, RT-LAMP, parallel reaction monitoring (PRM) proteomics, metabolomics, and ELISA-based immunological detection techniques will be used to detect and analyze whole blood, plasma, or urine samples from enrolled patients.

2. OBJECTIVES

a. Primary Objectives

- 1) Perform Bulk RNA-seq transcriptome sequencing on enrolled samples to screen sepsis-specific mRNA diagnostic biomarkers and evaluate their diagnostic efficacy for early sepsis in the ICU; establish a molecular risk stratification system for sepsis based on mRNA expression profiles, and clarify the immunobiological characteristics, clinical manifestation differences, and prognostic risk levels of each stratification.
- 2) Integrate core indicators screened from transcriptome sequencing, open-source databases, and previous studies to establish and optimize an RT-LAMP rapid detection method for core sepsis targets, validate its diagnostic accuracy, specificity, and reproducibility, and construct a rapid sepsis diagnostic model adapted to bedside scenarios.
- 3) Integrate core indicators screened from open-source databases and previous studies to screen sepsis-specific diagnostic biomarkers in plasma and urine via PRM and metabolomics, complete protein/metabolic level validation using immunological methods (ELISA), construct a combined diagnostic model for sepsis, and complete internal validation and efficacy evaluation.

b. Secondary Objectives

- 1) Clarify the correlation between the mRNA/protein/metabolite expression levels of core diagnostic biomarkers and 28-day all-cause mortality, degree of organ function injury, incidence of septic shock, and different subgroups (infection site, pathogen type, degree of organ injury, immune status, SOFA stratification, etc.) in sepsis patients;
- 2) Analyze the characteristics of immune inflammatory pathways in different molecular stratifications of sepsis to provide a theoretical basis for the subsequent screening of precise therapeutic targets.

3. STUDY DESIGN

This is a single-center, prospective, observational cohort study, serving as an independent cohort for the development of sepsis diagnostic biomarkers. Relying on the ICU of Yuebei People's Hospital, 1000 sepsis cases and 400 non-sepsis cases will be enrolled. Meanwhile, residual samples of whole blood, plasma, and urine from enrolled patients on the day of ICU admission will be collected to establish a large-scale comprehensive biobank for sepsis. Enrolled samples will undergo Bulk RNA-seq transcriptome sequencing, RT-LAMP nucleic acid detection, PRM proteomics quantification, metabolomics quantification, and ELISA quantitative detection according to different substudy projects, achieving cross-validation of multi-dimensional data from the same cohort and the same time point samples, and finally completing the initial construction of diagnostic models and the establishment of rapid detection methods.

4. STUDY POPULATION

a. Inclusion Criteria for Sepsis Group

- 1) Age > 18 years;
- 2) Meet the Sepsis-3 diagnostic criteria for sepsis: confirmed or suspected infection with an increase in SOFA score (Sequential Organ Failure Assessment) of ≥ 2 points from baseline;
- 3) Develop sepsis within 72 hours of ICU admission;
- 4) Voluntarily sign the informed consent form (or signed by the legal representative).

b. Inclusion Criteria for Non-Sepsis Group

- 1) Age > 18 years;
- 2) Hospitalized in the ICU of our hospital during the same period, with no clear evidence of infection and not meeting the diagnostic criteria for sepsis;
- 3) Expected ICU treatment time ≥ 24 hours;
- 4) Voluntarily sign the informed consent form (or signed by the legal representative).

c. Exclusion criteria

- 1) Complicated with end-stage chronic organ failure (end-stage renal disease, decompensated liver cirrhosis (Child-Pugh Grade C), chronic heart failure NYHA Class IV), or malignant tumor;
- 2) Immunocompromised or suffering from autoimmune diseases, or long-term use of glucocorticoids/immunosuppressants within the past 3 months;
- 3) Pregnant and postpartum patients;
- 4) Patients with incomplete data collection;

5) Other conditions deemed unsuitable for participation in the study by the investigator (such as terminal state, refusal to cooperate with sample collection, or inability to cooperate with in-hospital and out-of-hospital follow-up).

d.Sample size calculation

This is a single-center, prospective, observational cohort study aimed at screening early diagnostic and molecular stratification biomarkers for ICU sepsis. The case group consists of ICU patients with confirmed sepsis, and the control group consists of ICU non-sepsis critically ill patients. Set the target AUC ≥ 0.8 , significance level $\alpha = 0.05$ (two-sided), and power $1 - \beta = 0.9$. Based on Obuchowski's diagnostic test sample size formula:

$$n_A = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (1 + 1/k)}{2(\theta_1 - \theta_0)^2}$$

Where n_A is the sample size of the sepsis group, k is the case/control ratio ($k = n_A/n_B = 2$), θ_1 is the expected AUC (taken as 0.8), θ_0 is the null hypothesis AUC (no diagnostic value, fixed at 0.5), $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ are the standard normal distribution quantiles, $Z_{1-\alpha/2} = 1.96$, $Z_{1-\beta} = 1.28$. Substituting into the formula, the minimum number of sepsis cases is calculated to be 220, and the number of non-sepsis controls is 110. To meet the requirements of subgroup analysis such as early sepsis time window (0–6h/6–24h), infection site, and shock status, the sample size of each group needs to be increased by 30%. Exclusion rate and multiple testing: considering a 15% sample exclusion rate and transcriptome multiple testing correction, the sample size is further expanded.

For Substudy 1: Screening of early diagnostic molecular biomarkers and molecular stratification biomarkers for sepsis based on Bulk RNA-seq transcriptome sequencing. The main purpose is to perform sepsis stratification in sepsis samples, so more sepsis samples need to be included. Combined with the actual capacity of our hospital to treat sepsis, we plan to enroll 1000 sepsis patients and 400 ICU non-sepsis patients, which fully meets the requirements of the above formula calculation.

For Substudy 2: Validate the diagnostic efficacy of RT-LAMP as an early rapid bedside diagnostic tool for ICU sepsis. The main purpose is to validate the methodological diagnostic efficacy. We plan to enroll 300 sepsis patients and 150 ICU non-sepsis patients, which meets the calculation according to the above formula.

Substudy 3: Validate the diagnostic efficacy of plasma protein/metabolic biomarkers as early diagnostic biomarkers for ICU sepsis. The main purpose is to validate the diagnostic efficacy of plasma biomarkers. We plan to enroll 300 sepsis patients and 150 ICU non-sepsis patients, which meets the calculation according to the above formula.

Substudy 4: Validate the diagnostic efficacy of urine protein/metabolic biomarkers as early diagnostic biomarkers for ICU sepsis. The main purpose is to validate the diagnostic efficacy of urine biomarkers. We plan to enroll 300 sepsis patients and 150 ICU non-sepsis patients, which meets the calculation according to the above formula.

To construct a comprehensive sepsis biobank with multiple sample types and describe the sepsis response from multiple aspects, each study should have different types of samples. Therefore, residual samples of whole blood, plasma, and urine from all 1400 enrolled patients will be collected in this study.

5.TREATMENT OF SUBJECTS

Not applicable.

6.INVESTIGATIONAL PRODUCT

Not applicable.

7.NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

a.Study parameters/endpoints

I . Main study parameter/endpoint

- 1) Whole-genome mRNA expression profiles of the sepsis group and the non-sepsis group, the list of core diagnostic biomarkers screened by multi-algorithm cross-validation (WGCNA + LASSO + SVM-RFE), and their diagnostic efficacy for early sepsis in the ICU;
- 2) Results of sepsis molecular risk stratification, and differences in clinical characteristics, biological characteristics, and prognosis among different stratifications;
- 3) Methodological performance evaluation of the RT-LAMP rapid diagnostic method and its early diagnostic efficacy for sepsis, reporting AUC, sensitivity, specificity, positive/negative predictive values;
- 4)Early diagnostic efficacy of protein/metabolic biomarkers in plasma/urine for sepsis, and AUC, sensitivity, specificity, positive/negative predictive values of the combined diagnostic model.

II.Secondary study parameters/endpoints

- 1) 28-day all-cause mortality, ICU length of stay, and incidence of new organ dysfunction in patients;
- 2) Correlation between mRNA expression levels and protein expression levels of core biomarkers and 28-day all-cause mortality and SOFA score in patients;
- 3) Comparison results of diagnostic efficacy between novel biomarkers/combined diagnostic models and traditional indicators PCT and CRP;
- 4) Subgroup analysis of biomarkers (infection site, pathogen type, organ injury);

5) Analysis of immune inflammatory pathway characteristics of different molecular stratifications of sepsis.

b. Randomisation, blinding and treatment allocation

Not applicable.

c.Study procedures

I . Screening and eligibility assessment

Screening population: Patients with confirmed sepsis and non-sepsis in the ICU of Yuebei People's Hospital.

Screening process: The research team identifies potential subjects through the electronic medical record system and bedside assessment, checks the inclusion/exclusion criteria, and records key information such as SOFA score, infection site, and antibiotic use time.

Screening timing: Screening is completed during the patient's ICU stay.

Informed consent: Written broad informed consent is obtained from eligible patients.

Baseline assessment: Record demographic characteristics, medical history, SOFA score, infection source, etiological examination, and other laboratory parameters within 24 hours of ICU admission.

II. Sample Collection and Storage

Time Point: Residual samples collected within 24 hours of ICU admission.

1)Whole Blood Collection and Storage. Collect venous blood using PAXgene blood collection tubes. After standing at room temperature for 2 hours, store in a -80°C refrigerator.

2)Plasma Collection and Storage. Collect blood using heparin anticoagulant tubes, centrifuge at 3500 rpm for 10 minutes, separate plasma, and store at -80°C. Avoid repeated freeze-thaw cycles > 3 times.

3)Urine Collection and Storage. Collect clean midstream urine or urine via an indwelling catheter, aliquot into sterile centrifuge tubes, and immediately freeze at -80°C.

III. Laboratory proceduresand Data Analysis

i . Bulk RNA-seq Transcriptome Sequencing and Data Analysis

1)Sequencing Data Standardization, Quality Control and Analysis. For enrolled samples, total RNA is extracted from PAXgene Blood RNA samples using the PAXgene® Blood RNA Kit. mRNA library construction and paired-end sequencing are uniformly completed using the BGI sequencing platform, with a sequencing read length of 150 bp and a sequencing data volume of no less than 6G clean data per sample. Sequencing is performed in batches, and a unified standard RNA is added as an internal reference in each batch to correct batch effects.

2)Sequencing Data Standardization, Quality Control and Analysis. Raw data quality control: Use Trimmomatic software to remove low-quality reads, adapter sequences, and contaminant sequences to obtain clean data; use HISAT2 software to align clean reads to the human reference genome (GRCh38), and complete gene quantification and annotation through StringTie software to generate a gene expression matrix.Differentially expressed gene screening: Perform differential expression analysis between the sepsis group and the non-sepsis control group using the DESeq2 R package, with screening thresholds set to $|\log_2FC| \geq 1$ and adjusted P value (FDR) < 0.05, to obtain the sepsis-related differentially expressed mRNA profile.

Functional enrichment analysis: Perform GO functional enrichment and KEGG signaling pathway enrichment analysis on differential genes using the clusterProfiler

R package to clarify the biological processes and signaling pathways coregulated in sepsis.

Core diagnostic biomarker screening: Integrate algorithms such as WGCNA, LASSO regression, and support vector machine recursive feature elimination (SVM-RFE) to cross-validate and screen core mRNA biomarkers for sepsis diagnosis.

Establishment of sepsis molecular risk stratification: Based on the differential gene expression profiles of 1000 samples in the sepsis group, perform unsupervised molecular stratification using the Consensus Clustering algorithm, and determine the optimal number of stratifications through the cumulative distribution function (CDF) and silhouette width; compare baseline clinical characteristics, degree of organ function injury (SOFA/APACHE II score), inflammatory indicator levels, 28-day all-cause mortality, ICU length of stay, and other prognostic indicators among different molecular stratifications to clarify the risk level of each stratification and establish a sepsis molecular risk stratification system; analyze the immune inflammatory characteristics and pathway differences of different stratifications through gene set enrichment analysis (GSEA) to clarify the biological connotation of the stratifications.

ii. Establishment of RT-LAMP Rapid Detection Method and Construction of Rapid Diagnostic Model

1) RT-LAMP Primer Design and Synthesis. For core mRNA diagnostic biomarkers for sepsis screened in previous studies, use PrimerExplorer V5 software to design specific RT-LAMP primers. Each set of primers includes 2 outer primers (F3/B3), 2 inner primers (FIP/BIP), and 1-2 loop primers (LF/LB). Primers are purified by HPLC, and their specificity is verified by NCBI BLAST alignment to exclude non-specific binding.

2) Optimization of RT-LAMP Reaction System and Conditions. Establish a basic RT-LAMP reaction system, and optimize key reaction parameters by the single-factor variable method: magnesium ion concentration, dNTP concentration, ratio of inner to outer primer concentrations, Bst DNA polymerase dosage, AMV reverse transcriptase

dosage, reaction temperature (60-65 °C), and reaction time (30-60 min). Determine the optimal reaction system and isothermal amplification conditions with amplification efficiency and specificity as core indicators. Amplification products are verified by two methods: a) Real-time fluorescence method: Monitor the amplification curve in real time using SYBR Green I fluorescent dye, and record the amplification start time and fluorescence intensity; b) Agarose gel electrophoresis: Verify the specificity of the ladder-like bands of amplification products, and set no-template negative controls and positive controls simultaneously to exclude non-specific amplification.

3) Methodological Validation and Whole-Sample Detection. Methodological performance validation: a) Sensitivity validation: Serially dilute in vitro transcribed target mRNA to detect the limit of detection (LOD) of the RT-LAMP method and compare it with the gold standard RT-qPCR; b) Specificity validation: Detect the specificity of the method for target genes and exclude cross-reactions with other inflammation-related genes and pathogen nucleic acids; c) Reproducibility and stability validation: Calculate the CV value of detection results through intra-batch and inter-batch repeat experiments to evaluate the reproducibility of the method; test reagents under different storage conditions to evaluate the stability of the method.

Whole-cohort sample detection: Use the optimized RT-LAMP method to perform parallel detection on total RNA from enrolled samples, and record amplification results and quantitative data; simultaneously use RT-qPCR to detect the expression levels of target genes in corresponding samples, and analyze the consistency between RT-LAMP, RT-qPCR, and transcriptome sequencing results through Kappa test.

4) Construction and Evaluation of Rapid Diagnostic Model. Based on the target gene expression data detected by RT-LAMP, combined with core clinical indicators of patients (age, baseline SOFA score), use machine learning to construct a bedside rapid diagnostic model for sepsis; verify the diagnostic efficacy, goodness of fit, and clinical applicability of the model through ROC curves, calibration curves, and DCA analysis, determine the optimal cutoff value of the model, and evaluate its application

value in early rapid screening of sepsis to provide a basis for subsequent bedside detection transformation.

iii. PRM Proteomics Sequencing and Data Analysis

1) Proteomics (PRM) Detection. For enrolled plasma/urine samples, total protein is extracted using a proteolysis kit, and protein quantification is performed using the BCA method. Take an equal amount of protein (50 μ g), reduce with dithiothreitol (DTT), alkylate with iodoacetamide (IAA), add trypsin (Trypsin, Promega) at an enzyme:substrate ratio of 1:50 (mass ratio), and digest overnight at 37 ° C. The digested peptides are desalted using a C18 solid-phase extraction column (Waters Sep-Pak), vacuum-dried, and dissolved in 0.1% formic acid aqueous solution for later use. Based on the results of previous untargeted proteomics screening, an inclusion list of target protein characteristic peptides and corresponding stable isotope-labeled internal standard peptides (SIS, $^{13}\text{C}/^{15}\text{N}$ labeled) is constructed. PRM targeted detection is completed using a Quadrupole-Orbitrap mass spectrometry platform coupled with a nano-liquid chromatography system. Liquid chromatography conditions: C18 reversed-phase analytical column (75 $\mu\text{m} \times 25\text{ cm}$, 1.9 μm particle size), mobile phase A is 0.1% formic acid in water, mobile phase B is 0.1% formic acid in acetonitrile, gradient elution (5%-35% B, 45 min). Mass spectrometry adopts PRM mode: MS1 full scan (resolution 60,000, scan range m/z 350-1,500), followed by high-energy collision dissociation (HCD, NCE 27%) of target parent ions in the inclusion list, with a secondary resolution of 30,000 and an isolation window of 1.6 m/z . SIS internal standard peptides (final concentration 10 fmol/ μL) are added to each sample, and 3-5 characteristic fragment ions are monitored for each target peptide. Each sample is collected with 3 technical replicates, and 1 QC sample (mixed peptides) is inserted every 10 samples to evaluate instrument stability.

2) PRM Data Quality Control and Quantitative Analysis. Raw mass spectrometry data (.raw format) are imported into Skyline software (v21.1, University of Washington). Extracted ion chromatograms (XIC) of each fragment ion are automatically extracted based on the target peptide sequence, parent ion m/z , charge state, and retention

time window. Manual peak-by-peak review is performed, and 2-3 fragment ions with signal-to-noise ratio > 10, symmetrical peak shape, and aligned with the internal standard peptide peak are selected as quantitative ions, with a spectrum matching degree (dotp value) > 0.8 considered a credible signal. The relative quantitative value of each target peptide = endogenous peptide peak area / corresponding SIS internal standard peptide peak area, and the geometric mean of 2-3 peptides for each protein is taken as the final relative protein expression level. The coefficient of variation (CV) of the internal standard peptide peak area in QC samples must be < 15%, otherwise the batch of samples is re-tested. Missing value processing: Only retain proteins that can be quantified in at least 50% of samples in each group.

Differential protein screening: Perform differential expression analysis between the sepsis group and the non-sepsis control group using the limma R package (or t-test), with screening thresholds set to $|\log_2FC| \geq 1$ and adjusted P value (FDR) < 0.05, to obtain the sepsis-related differentially expressed protein profile.

Functional enrichment analysis: Perform GO functional enrichment and KEGG signaling pathway enrichment analysis on differential proteins using the clusterProfiler R package to clarify the biological processes and signaling pathways coregulated in sepsis.

Core diagnostic biomarker screening: Integrate algorithms such as WGCNA, LASSO regression, and support vector machine recursive feature elimination (SVM-RFE) to cross-validate and screen core protein biomarkers for sepsis diagnosis.

iv. Targeted Metabolomics Detection and Data Analysis

1) High-Throughput Targeted Metabolomics Detection. For enrolled plasma/urine samples, take 100 μ L of thawed samples, add an internal standard-containing extraction solution (methanol:acetonitrile = 1:1, volume ratio, containing 0.1% formic acid, internal standard is L-2-chlorophenylalanine, final concentration 5 μ g/mL), vortex to mix, sonicate for 10 min in an ice bath, stand at -20 °C for 30 min to precipitate proteins, centrifuge at 12,000 rpm for 15 min at 4 °C, take the supernatant

and vacuum dry. The dried residue is reconstituted with 100 μ L of acetonitrile:water = 1:1 (volume ratio), centrifuged again, and the supernatant is taken for testing.

High-throughput targeted metabolomics detection is completed using the HM Pro2300 platform coupled with an ultra-high performance liquid chromatography (UHPLC) system. Liquid chromatography conditions: ACQUITY UPLC HSS T3 column (2.1 mm \times 100 mm, 1.8 μ m, Waters), column temperature 40°C, flow rate 0.35 mL/min, mobile phase A is 0.1% formic acid in water, mobile phase B is 0.1% formic acid in acetonitrile. Gradient elution program: 0-1 min, 5% B; 1-10 min, 5%-95% B; 10-11 min, 95% B; 11-11.1 min, 95%-5% B; 11.1-14 min, 5% B. Injection volume is 5 μ L.

Mass spectrometry adopts an electrospray ionization source (ESI), with positive and negative ion modes collected separately, and targeted detection performed in multiple reaction monitoring (MRM) mode. Ion source parameters: curtain gas 30 psi, collision gas Medium, ion spray voltage 5500 V (positive ion mode)/-4500 V (negative ion mode), temperature 500°C. 1-3 ion pairs are monitored for each metabolite, with a dwell time of 30 ms. Qualitative and quantitative analysis is performed using BGI's self-built metabolite database (or commercial standard database, containing more than XX metabolites, covering amino acids, organic acids, fatty acids, bile acids, carbohydrates, nucleotides, etc.). Mixed internal standards (containing multiple stable isotope-labeled internal standards) are added to each sample to correct systematic errors, and 1 QC sample (equal mixture of all test samples) is inserted every 10 samples to evaluate instrument stability. Each sample is tested once, and QC samples are tested repeatedly every 10 samples.

2) Metabolomics Data Quality Control and Differential Metabolite Analysis. Raw mass spectrometry data are imported into MultiQuant software (v3.0, AB Sciex) or BGI's self-developed metabolomics analysis platform, and MRM chromatographic peaks are automatically identified and integrated. Manual peak-by-peak review is performed, and peaks with signal-to-noise ratio < 3, abnormal peak shape, or retention time drift exceeding ± 0.2 min are deleted. The peak area of each metabolite is normalized by

the internal standard peak area to calculate the relative content. The coefficient of variation (CV) of each metabolite peak area in QC samples must be $< 20\%$, and metabolites with $CV > 20\%$ are excluded. Missing value processing: If the missing rate is $< 20\%$, the K-nearest neighbor (KNN) algorithm is used for imputation; if the missing rate is $\geq 20\%$, the metabolite is directly excluded. Data are used for subsequent analysis after Pareto scaling (or logarithmic transformation + centering).

Differential metabolite screening: Orthogonal partial least squares discriminant analysis (OPLS-DA, R package *ropls*) is used to evaluate the separation trend between groups, with variable importance in projection (VIP) ≥ 1 and univariate analysis (t-test or Wilcoxon rank-sum test) P value < 0.05 as screening thresholds, and $|\log_2 FC| \geq 1$ as significantly differential metabolites. Meanwhile, the Benjamini-Hochberg method is used to correct the false positive rate of multiple comparisons (FDR < 0.20 as an auxiliary reference). The differential metabolite profile between the sepsis group and the non-sepsis control group is obtained.

Metabolic pathway enrichment analysis: Import differential metabolites into the MetaboAnalyst 6.0 online platform (or KEGG database), perform hypergeometric distribution test based on the human metabolic pathway library (hsa), and screen significantly enriched metabolic pathways (such as tricarboxylic acid cycle, amino acid metabolism, fatty acid oxidation, etc.) with $P < 0.05$ as the threshold to clarify the core metabolic pathways disturbed in sepsis.

Core diagnostic biomarker screening: Integrate OPLS-DA VIP ranking, LASSO regression, random forest, and other algorithms to cross-validate and screen core metabolite biomarkers for sepsis diagnosis. Evaluate the diagnostic efficacy of single and combined biomarkers (calculate AUC, sensitivity, specificity) through receiver operating characteristic (ROC) curves.

v. ELISA Detection and Construction of Combined Diagnostic Model

1) Determination of Indicator List to Be Detected. Integrate two types of core indicators: a) Markers screened by targeted proteomics and high-potential sepsis-related diagnostic markers reported in open-source databases such as GEO and TCGA; b) Differentially expressed factors related to sepsis discovered in our team's previous studies (see Appendix 3) to determine the ELISA detection list.

2) Whole-Sample ELISA Detection. For plasma samples from enrolled patients, use commercial high-specificity ELISA kits to quantitatively detect the protein expression levels of the indicators to be tested in strict accordance with the kit instructions and standardized SOPs; standard curve duplicate wells, blank controls, negative controls, and positive controls are set in each batch of experiments to draw standard curves, requiring the standard curve $R^2 \geq 0.99$, intra-batch coefficient of variation (CV) < 10%, and inter-batch CV < 15% to ensure stable and reliable detection data.

3) Construction and Validation of Combined Diagnostic Model. Biomarker screening: Perform univariate logistic regression analysis to screen indicators significantly associated with the occurrence of sepsis ($P < 0.05$), which are included in multivariate logistic regression analysis to construct a multi-indicator combined diagnostic model for sepsis.

Model evaluation and validation: Visualize the diagnostic model using a nomogram; calculate the area under the curve (AUC), sensitivity, specificity, positive predictive value, and negative predictive value of the model through receiver operating characteristic (ROC) curves to evaluate the diagnostic efficacy of the model; complete internal validation of the model using Bootstrap or 5-fold cross-validation, evaluate the goodness of fit of the model through calibration curves, and evaluate the clinical net benefit of the model through decision curve analysis (DCA).

Comparative analysis: Compare the diagnostic efficacy of the initial diagnostic model constructed in this study with that of traditional indicators PCT and CRP alone to

clarify the clinical advantages of the model; subgroup analysis verifies the diagnostic stability of the model in different infection sites, different molecular stratifications, and different age subgroups.

vi. Follow-up

Follow-up is divided into in-hospital follow-up and out-of-hospital follow-up. In-hospital follow-up mainly records laboratory tests and patient outcomes during the first 7 days of hospitalization. Out-of-hospital follow-up is conducted at 28 ± 7 days after discharge, mainly inquiring about the patient's health status. Fill in the follow-up record.

d. Withdrawal of individual subjects

Patients or its legal representatives are free to withdraw consent at any time without providing a reason. Patients or legal representatives who wish to withdraw consent for the study will have anonymized data and samples collected up to the point of that withdrawal of consent included in the analyses. The patient will not contribute further data to the study.

Data up to the time of withdrawal will be included in the analyses unless the patient explicitly states that this is not their wish.

I. Specific criteria for withdrawal (if applicable)

Not applicable.

e. Replacement of individual subjects after withdrawal

If a patients withdraws informed consent, he or she will be replaced by a new subject.

f. Follow-up of subjects withdrawn from treatment

Not applicable.

g. Premature termination of the study

Not applicable, given the observational nature of the study.

9. SAFETY REPORTING

a. Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

b. AEs, SAEs and SUSARs

I . Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to trial procedure. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

II. Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

As we propose an observational study, we will only report AEs and SAEs that are related to blood sampling. Standard clinical care will not be affected or hampered by participation in the study. We do not expect AEs and SAEs as the result of blood sampling.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

III. Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

c. Annual safety report

Not applicable.

d. Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

e.Data Safety Monitoring Board (DSMB) or Safety Committee

Not applicable due to negligible risks of our study.

10. STATISTICAL ANALYSIS

a. Statistical Software

Statistical analysis is performed using IBM SPSS Statistics 26.0, GraphPad Prism 9.0, and R 4.2.0 software.

b. Basic Principles

All statistical inferences use two-sided tests with a significance level of $\alpha = 0.05$, and 95% confidence intervals are used for parameter estimation.

c. Descriptive Statistics

Measurement data: Normally distributed data are described as mean \pm standard deviation ($\bar{x} \pm s$), and skewed distributed data are described as median (interquartile range) [M (P25, P75)].

Count data: Described as frequency (percentage) [n (%)].

d. Intergroup Comparisons

Measurement data: t-test is used for normally distributed data with equal variance, and corrected t-test is used for data with unequal variance; Wilcoxon rank-sum test is used for skewed distributed data.

Count data: χ^2 test or Fisher's exact test is used.

e. Diagnostic Efficacy Analysis

ROC curves are drawn, and AUC, sensitivity, specificity, PPV, NPV, positive likelihood ratio, and negative likelihood ratio (95% CI) are calculated.

DeLong's test is used to compare the AUC differences between different biomarkers (or combined biomarkers and single biomarkers).

Machine learning models are constructed to establish a multi-biomarker combined diagnostic model.

f. Prognostic Analysis

Cox proportional hazards regression model is used to analyze the association between biomarker concentrations (baseline and dynamic changes) and 28-day all-cause mortality, and calculate hazard ratio (HR) and 95% CI.

Logistic regression model is used to analyze the association between biomarkers and 72-hour mortality and incidence of septic shock.

Kaplan-Meier method is used to draw survival curves, and log-rank test is used to compare survival differences between groups with different biomarker concentrations.

g. Subgroup Analysis

Stratify according to preset subgroups (infection site, pathogen type, organ injury), calculate the diagnostic efficacy indicators (AUC, sensitivity, specificity) and prognostic association indicators (HR, OR) of biomarkers in each subgroup respectively, and evaluate the stability of biomarker efficacy.

h. Missing and Abnormal Data Processing

Missing data: Data missing due to changes in the subject's physical condition preventing enrollment or follow-up will not be imputed; when the missing rate of key data is > 10%, multiple imputation method is used for sensitivity analysis.

Abnormal data: Outliers are identified using the three-standard-deviation method, and the impact of excluding outliers on the results is explained before analysis; recheck the data distribution after exclusion to ensure data rationality.

11. ETHICAL CONSIDERATIONS

a.Regulation statement

This clinical study must be conducted in accordance with the Declaration of Helsinki and relevant Chinese clinical research regulations. Before the start of the study, materials such as the clinical study protocol and informed consent form must be submitted to the ethics committee for approval, and the study may be implemented only after approval. Modifications to materials such as the clinical study protocol and informed consent form during the clinical study must be implemented after approval by the ethics committee. Investigators ensure the rights and safety of subjects and protect the privacy of subjects.

b.Recruitment and consent

The attending physician will obtain consent from patients on whether their residual blood samples can be used for scientific research purposes. Written broad informed consent will be obtained from willing participants. Biological samples collected within 24 hours of ICU admission will be stored at -80°C. Eligible patients will be enrolled according to the clinical diagnosis made by attending physicians.

c.Objection by minors or incapacitated subjects (if applicable)

When the patient is unable or incapable of giving informed consent (e.g. due to loss of consciousness), informed consent will be asked from the legal representative of the patients, preferably in person, but if necessary by telephone. The researchers will contact the patient or next-of-kin to provide information about the study, its aim and the burden to the patient. When the patient regains consciousness, he/she will also be asked informed consent.

d.Benefits and risks assessment, group relatedness

Patients have no benefit of participation in the study. Patients receive their regular treatment as was intended and this will not be influenced by the study. The burden of

the blood samples taken is negligible. There is no additional risk in participating in the study.

e.Compensation for injury

Not applicable.

f.Incentives (if applicable)

Not applicable.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

a. Handling and storage of data and documents

Data will be handled confidentially and coded. To ensure data security and to protect privacy, data on individual subjects will be encoded according to a subject identification code list. The key to the code will be safeguarded by both the executive and coordinating investigators. They will have access to the data at any time. The research file will be password protected and stored for 15 years. The samples will be stored for 5 years. The file and the electronic data from the electronic Case Report Form (eCRF) will be stored for 15 years. All information, data, and results that originate from this study may not be disclosed without the written permission of the principal investigator.

b. Future use of samples

Samples collected will be used for the purpose of China SMART-1 and stored for a maximum of 5 years for future analyses that are relevant to the study questions. Any proposed plans to use samples other than for those relevant to the study question will be submitted to the relevant ethics committees prior to any testing.

c. Monitoring and Quality Assurance

The study will be monitored by the clinical monitoring center.

d. Amendments

Amendments are changes made to the research after a favourable opinion by the accredited medical research ethics committee has been given. All amendments will be notified to the medical research ethics committee that gave a favourable opinion.

All substantial amendments will be notified to the medical research ethics committee and to the competent authority.

Non-substantial amendments will not be notified to the accredited medical research ethics committee and the competent authority, but will be recorded and filed by the sponsor.

e. Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited medical research ethics committee once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

f. Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited medical research ethics committee of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the medical research ethics committee immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited medical research ethics committee within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited medical research ethics committee.

g. Public disclosure and publication policy

The results of this study will be disclosed unreservedly and published in a peer reviewed medical journal.

13. STRUCTURED RISK ANALYSIS

Not applicable.

14. REFERENCES

- [1]. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet*. 2020 Jan 18;395(10219):200-211.
- [2]. Fleischmann C, Scherag A, Adhikari NK, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am J Respir Crit Care Med*. 2016;193(3):259-272.
- [3]. Adhikari NK, Fowler RA, Bhagwanjee S, et al. Critical care and the global burden of critical illness in adults. *Lancet*. 2010;376(9749):1339-1346.
- [4]. Xie J, Wang H, Kang Y, et al. The epidemiology of sepsis in Chinese ICUs: A national cross-sectional survey. *Crit Care Med*. 2020;48(3):e209-e218.
- [5]. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810.
- [6]. Lindner HA, Balaban U, Sturm T, et al. An Algorithm for Systemic Inflammatory Response Syndrome Criteria-Based Prediction of Sepsis in a Polytrauma Cohort. *Critical care medicine* 2016, 44(12):2199-2207.
- [7]. Wang HE, Donnelly JP, Griffin R, et al. Derivation of Novel Risk Prediction Scores for Community-Acquired Sepsis and Severe Sepsis. *Critical care medicine* 2016, 44(7):1285-1294.
- [8]. Gouel-Cheron A, Allaouchiche B, Floccard B, et al. Early daily mHLA-DR monitoring predicts forthcoming sepsis in severe trauma patients. *Intensive Care Med*. 2015 Dec;41(12):2229-30.
- [9]. Eriksson J, Gidlof A, Eriksson M, et al. Thioredoxin a novel biomarker of post-injury sepsis. *Free radical biology & medicine* 2017, 104:138-143.

- [10]. Zhang AQ, Gu W, Zeng L, Zhang LY, Du DY, Zhang M, Hao J, Yue CL, Jiang J: Genetic variants of microRNA sequences and susceptibility to sepsis in patients with major blunt trauma. *Annals of surgery* 2015, 261(1):189-196.
- [11].David VL, Ercisli MF, Rogobete AF, et al. Early Prediction of Sepsis Incidence in Critically Ill Patients Using Specific Genetic Polymorphisms. *Biochem Genet.* 2017 Jun;55(3):193-203.
- [12]. Wei S, Gonzalez Rodriguez E, Chang R, et al. Elevated Syndecan-1 after Trauma and Risk of Sepsis: A Secondary Analysis of Patients from the Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) Trial. *J Am Coll Surg.* 2018 Dec;227(6):587-595.
- [13]. Halldorsdottir HD, Eriksson J, Persson BP, et al. Heparin-binding protein as a biomarker of post-injury sepsis in trauma patients. *Acta Anaesthesiol Scand.* 2018 Aug;62(7):962-973.
- [14]. Sweeney TE, Shidham A, Wong HR, et al. A comprehensive time-course-based multicohort analysis of sepsis and sterile inflammation reveals a robust diagnostic gene set. *Sci Transl Med.* 2015;7(287):287ra71.
- [15]. Maslove DM, Tang BM, McLean AS. Identification of sepsis subtypes in critically ill adults using gene expression profiling. *Crit Care.* 2012;16(5):R183.
- [16]. Scicluna BP, van Vught LA, Zwinderman AH, et al. Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. *Lancet Respir Med.* 2017;5(10):816-826.
- [17]. Seymour CW, Kennedy JN, Wang S, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. *JAMA.* 2019;321(20):2003-2017.
- [18]. Ren X, Wen W, Fan X, et al. COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. *Cell.* 2021 Apr 1;184(7):1895-1913.e19. doi: 10.1016/j.cell.2021.01.053IF: 42.5 Q1 . Epub 2021

Feb 3. Erratum in: Cell. 2021 Nov 11;184(23):5838. doi: 10.1016/j.cell.2021.10.023. PMID: 33657410; PMCID: PMC7857060.

[19]. Zhou F, Chen M, Liu Y, et al. Serum mitochondrial-encoded NADH dehydrogenase 6 and Annexin A1 as novel biomarkers for mortality prediction in critically ill patients with sepsis. Front Immunol. 2024 Nov 14;15:1486322. doi: 10.3389/fimmu.2024.1486322. PMID: 39611143; PMCID: PMC11602424.

[20]. Chen J, Liu Z, Zhou F, et al. Assessment of S100A8/A9 and resistin as predictive biomarkers for mortality in critically ill patients with sepsis. Front Cell Infect Microbiol. 2025 Jun 3;15:1555307. doi: 10.3389/fcimb.2025.1555307 IF: 4.8 Q1 . PMID: 40568710; PMCID: PMC12188459.

15. Supplementary file

Supplementary table 1. SOFA (Sequential Organ Failure Assessment) Score

Supplementary table 2. qSOFA (Fast Sequential Organ Failure Assessment) Score

Supplementary table 3. Previously Screened Protein Biomarkers

Supplementary table 4. Follow-up Schedule

Supplementary table 1. SOFA (Sequential Organ Failure Assessment) Score

Score	0	1	2	3	4
Central Nervous System(Glasgow Coma Scale)	15	13-14	10-12	6-9	<6
Cardiovascular System(MAP OR Vasopressors Required)	MAP \geq 70 mmHg	MAP < 70 mmHg	Dopamine \leq 5 μ g/kg/min OR Dobutamine (any dose)	Dopamine > 5 μ g/kg/min OR Epinephrine \leq 0.1 μ g/kg/min OR Norepinephrine \leq 0.1 μ g/kg/min	Dopamine > 15 μ g/kg/min OR Epinephrine > 0.1 μ g/kg/min OR Norepinephrine > 0.1 μ g/kg/min
Respiratory System (PaO ₂ /FiO ₂ [mmHg (kPa)])	\geq 400	< 400	< 300	<200 AND Mechanically ventilated (including CPAP)	<100 AND Mechanically ventilated (including CPAP)
Coagulation (Platelets $\times 10^3/\mu$ l)	\geq 150	<150	<100	<50	<20
Liver (Bilirubin [mg/dl (μ mol/L)])	<20	20-32	33-101	102-204	\geq 204
Renal Function (Creatinine [mg/dl (μ mol/L)] OR Urine Output)	<110	110-170	171-299	300-400 OR< 500 ml/day	<440 OR< 200 ml/day

Supplementary table 2. qSOFA (Fast Sequential Organ Failure Assessment) Score

Clinical Variable	Criterion	Score
Respiratory rate	≥ 22 breaths/min	1
Mental status	Altered mentation (GCS ≤ 14)	1
Systolic blood pressure	≤ 100 mmHg	1

Supplementary table 3. Previously Screened Protein Biomarkers

Serial Number	Protein Name	Serial Number	Protein Name	Serial Number	Protein Name
1	mt-ND6	22	FGB	43	F11
2	AHSG	23	FGG	44	F12
3	CLU	24	ACTA2	45	F2
4	SERPINA4	25	ADAMTS1	46	PLG
5	ANXA1	26	ADAMTS2	47	PROC
6	ActivinA	27	ADAMTS4	48	SERPIND1
7	ActivinB	28	ADAMTS9	49	PON1
8	ICAM-1	29	ADAMTS13	50	TRAIL
9	VCAM-1	30	ADAMTS15	51	IP-10
10	uPAR	31	VCL	52	OLFM4
11	ENO1	32	PKM	53	GSDMD-CT
12	PFN1	33	RDX	54	Fibulin2
13	YKL-40	34	KLKB1	55	ACBP
14	RASGRP2	35	APOA1	56	HRG
15	ITIH1	36	APOC2	57	Presepsin

16	ITIH2	37	APOC3	58	sTREM-1
17	I-FABP1	38	APOH	59	Gelsolin
18	L-FABP1	39	APOA2	60	Syndecan-1
19	ACTB	40	KRT10	61	N-GSDMD
20	FCGR3A	41	LCAT	62	SLPI
21	FGA	42	KRT9	63	

Supplementary table 4. Follow-up Schedule

Visit Phase	Screening Period	Enrollment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
	V _s	V ₀	V ₁	V ₂	V ₃	V ₄	V ₅	V ₆	V ₇	V ₈
Visit Time (Days)	0	0	1	2	3	4	5	6	7	28±7
Informed Consent Form	*									
Demographic Information ^A	*									
Medical History ^B	*									
Vital Signs ^C	*									
Laboratory Tests ^D	*									
Outcome Variable Collection ^E			*	*	*	*	*	*	*	*
Previous/Concomitant Medications ^F	*									

Notes:

A: Demographics: including patient gender and age.

B: Medical history: including past and current medical history of diabetes, hypertension, chronic kidney disease, liver disease, respiratory disease, cardiovascular disease, and immune diseases.

C: Vital signs: including blood pressure, heart rate, respiratory rate, and body temperature.

D: Laboratory tests: including WBC, CRP, PCT, platelets, creatinine, total bilirubin, oxygenation index, lactate, and Glasgow Coma Scale score.

E: Outcome variable collection: patient clinical outcomes.

F: Previous/concomitant medications: antibiotics, hormones, or immunosuppressants.