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Infections in the intensive care unit

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Infections in the intensive care unit

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METHODS
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Critical Care Database Comprising Patients With Infection

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Patients treated in the intensive care unit (ICU) are closely monitored and receive intensive treatment. Such aggressive monitoring and treatment will generate high-granularity data from both electronic healthcare records and nursing charts. These data not only provide infrastructure for daily clinical practice but also can help to inform clinical studies. It is technically challenging to integrate and cleanse medical data from a variety of sources. Although there are several open-access critical care databases from western countries, there is a lack of this kind of database for Chinese adult patients. We established a critical care database involving patients with infection. A large proportion of these patients have sepsis and/or septic shock. High-granularity data comprising laboratory findings, baseline characteristics, medications, international statistical classification of diseases (ICD) code, nursing charts, and follow-up results were integrated to generate a comprehensive database. The database can be utilized for a variety of clinical studies. The dataset is fully accessible at PhysioNet (https://physionet.org/content/icu-infection-zigong-fourth/1.0/).

Keywords: critical care, database, open access, infections, big data and analytics

BACKGROUND AND SUMMARY

Infection is common in the intensive care unit (1, 2). There are two categories of infections for patients in the intensive care unit (ICU) due to the place where the infection was acquired. One type of infection is the infection present on ICU admission, and most of such patients are transferred to ICU due to the development of sepsis and/or septic shock (3, 4). The other type of infection is the infection acquired after ICU admission, which is also termed the nosocomial infection (1). Critically ill patients are at increased risk of infection because of compromised immunity, use of intravascular catheters, and endotracheal intubation (5, 6). Irrespective of the places where the infection is acquired, the infection can cause systematic inflammatory response (SIRS), sepsis, and septic shock. These complications are associated with a significantly increased risk of mortality (7, 8). Although sepsis has been widely investigated in the literature (4, 9, 10), the raw data are

typically not publicly available due to confidential or legal issues. The restricted data usage created a barrier to reproducing and verifying the results.

Although several open-access critical care databases from western countries have been created to promote data sharing and reuse for the scientific community (11–15), there is a lack of such database comprising Chinese adult patients. Since the Chinese population is the largest in the world, exploring infection/sepsis in the Chinese population is the key to achieving the goal proposed by the surviving sepsis campaign (16). Furthermore, a dataset, especially those generated from electronic healthcare records is large in volume. Secondary analysis of such dataset can generate novel insights into the diseases of interest (13, 17–19). Thus, creating a critical care database relating to patients with infection can help to promote collaborative research across the globe to reveal more insights into the infections in critically ill patients.

The rationales to include all critical patients with infection are 2 folds. First, such a database allows the capturing of longitudinal characteristics before and after infection in critically ill patients. This feature can be explored by restricting patients who acquired infection during ICU stay. A typical example is the subjects with intracranial hemorrhage who developed aspiration pneumonia in ICU. Risk factors for the development of infection can be analyzed. Second, for patients who had the infection before ICU admission, the severity spectrum ranging from infection, systematic inflammatory response syndrome, sepsis, severe sepsis, and septic shock can be captured. Third, the diagnosis of sepsis with international statistical classification of diseases (ICD) code is not accurate because there are many versions of sepsis definition. To include all infection patients allows the exploration of the agreement between these definitions. Clinical studies to develop the sepsis early warning system required the whole spectrum of diseases to be included in the database (20, 21). The critical care database comprises high granularity data including laboratory findings, baseline characteristics, medications, ICD-10 code, and nursing charts, and follow-up results were integrated to make a comprehensive database. The database can be utilized for a variety of clinical study purposes, such as epidemiology of risk factors, predictive analytics, natural language processing, and subphenotype identification.

METHODS

Study Setting and Population

The study was conducted in Zigong Fourth People's Hospital, Sichuan, China from January 2019 to December 2020, and was approved by the Ethics Committee of Zigong Fourth People's Hospital (Approval Number: 2021-014). Informed consent was waived due to the retrospective design of the study. The study complies with the Declaration of Helsinki.

All patients who transferred to any type of ICU in the hospital from January 2019 to December 2020 were potentially eligible to be included in the database. Electronic healthcare records of consecutive ICU patients with the diagnosis of infection, irrespective of the place where the infection was acquired, were included in the database. Infection was defined according to the diagnosis descriptions that contained keywords such as "infection", "pneumonia" and "-itis". Because the original diagnosis description was recorded in simplified Chinese, in which the above keywords were extracted *via* "Ganran" and "Yan". Some autoimmune or connective tissue diseases such as systemic lupus erythematosis (SLE), multiple sclerosis, rheumatoid arthritis, and Sjögren's syndrome were excluded manually.

Database Development

The critical care database was populated with data that have been acquired during routine clinical practice. Thus, the establishment of the database did not interfere with the clinical practice and was not associated with increased burden on healthcare providers, as well as risks for patients. Data were exported from several information systems including electronic healthcare records (EHR), hospital information system (HIS), laboratory information system (LIS), and critical care nursing chart system. The database was finally organized into seven tables in ".csv" format (**Table 1**). These data tables can be related to each other by patient ID (i.e., INP_NO or PATIENT_ID).

The core table *dtBaseline* contained baseline demographics of included patients, and it can be linked to other tables by either INP_NO or PATIENT_ID. PATIENT_ID was used to identify unique patients and INP_NO was used to identify unique hospital admission.

The *dtOutCome* table was generated by manual entry during follow-up. The *Death_Date* was recorded as hours from admission. The Short-Form Health Survey (SF-36) questionnaire was applied to evaluate the functional outcome of those who survived the critical illness. The Short Form Health Survey is a 36-item, patient-reported survey of patient health, which taps eight health concepts: bodily pain, physical functioning, role limitations due to physical health problems, role limitations due to personal or emotional problems, social functioning, energy/fatigue, emotional well-being, and general health perceptions. It also includes a single item that provides an indication of perceived change in health (22). The long-term mortality followed at 1 to 2 years after discharge was added if the patients' family members were willing to provide such information. In case a patient died after hospital discharge, the date was recorded.

Different from previous similar databases such as MIMIC-III which only contain laboratory values measured during ICU stay, we included all laboratory values during the index hospitalization including those measured outside ICU (11). We believe this can help to capture the full trajectory of pathophysiological changes before and after critical illness. For example, the identification of patients with acute kidney injury (AKI) is usually challenging if baseline serum creatinine (measured before the onset of the critical illness) is not available (23). Some stamp time points of laboratory measurements are earlier than the hospital admission time because these were measured in the emergency room or outpatient visit before hospital admission.

TABLE 1 | Overview of the data tables in the critical care database.

Table name	Description
dtBaseline.csv	This data table contains data on baseline characteristics of individual patients. One line represents one patient entry.
dtDrugs.csv	This data table contains data from the HIS and it is medical order prescribed by physicians. The *datatime* represents the time of the prescription and is not necessarily the time of drug administration.
dtICD.csv	This data table contains ICD-10 code and diagnosis descriptions. The description was translated from Chinese words. The *Status_Discharge* column describes the status of each individual diagnosis. If a patient died on hospital discharge, *Status_Discharge* will be coded as "dead" for all diagnoses. This table can be used to compute hospital mortality.
dtLab.csv	Laboratory variables, as well as the reference range for each item, are listed.
dtTansfer.csv	The data table contains information on transferring between different departments, i.e., from the gastroenterology department to ICU.
dtNursingChart.csv	The nursing chart contains all kinds of recordings by bedside nurses. The progress notes were written in Chinese, which can be used for natural language processing.
dtOutCome.csv	The outcomes of included patients. Especially, it contains the SF-36 questionnaire, which was obtained by follow-up after being discharged home.
datDictionary.csv	Description for the column variables in each table.

SF-36, Short Form Health Survey; ICU, intensive care medicine; ICD, International Statistical Classification of Diseases and Related Health Problems; HIS, Hospital Information System.

TABLE 2 | Comparisons of clincial variables between survivors and non-survivors during hospital say.

Variables	Total (n = 2,790)	Survivors (n = 2,629)	Non-survivors (n = 161)	p
Age, median (Q1,Q3)	69.2 (56, 78.8)	69.3 (56.1, 78.8)	67.8 (54.9, 79.6)	0.768
Sex, *n* (%)				0.014
Female	1,114 (40)	1,065 (41)	49 (30)	
Male	1,676 (60)	1,564 (59)	112 (70)	
InfectionSite, *n* (%)				0.003
Abdomen	180 (6)	178 (7)	2 (1)	
Biliary	74 (3)	73 (3)	1 (1)	
Brain	22 (1)	21 (1)	1 (1)	
Intestine	40 (1)	40 (2)	0 (0)	
Liver	32 (1)	31 (1)	1 (1)	
Mediastinum	3 (0)	3 (0)	0 (0)	
Others	325 (12)	306 (12)	19 (12)	
Pancreatitis	63 (2)	60 (2)	3 (2)	
Pelvic	3 (0)	3 (0)	0 (0)	
Pneumonia	1,876 (67)	1,745 (66)	131 (81)	
Soft Tissue	71 (3)	71 (3)	0 (0)	
UTI	101 (4)	98 (4)	3 (2)	
ICU LOS (days), median (Q1,Q3)	4 (1.8, 10.1)	4 (1.8, 10.2)	2.8 (0.9, 9.9)	0.012
Hospital LOS (days), median (Q1,Q3)	11 (2.9, 22.5)	11.7 (3.2, 22.9)	3.3 (0.9, 10.4)	< 0.001

Q1, first quartile; Q3, third quartile; ICU, intensive care unit; LOS, length of stay; UTI, urinary tract infection.

Deidentification

The data were deidentified before incorporating into the critical care database. The Health Insurance Portability and Accountability Act (HIPAA) protected health information identifiers including patient name, cell phone/telephone numbers, address, and any other variables that could uniquely identify the individual in structured data sources. The key variables PATIENT_ID and INP_NO were randomly assigned a unique number and the original patient ID and hospital ID were removed. Event time points were replaced with an offset value measured in hours from the hospital admission time (i.e., hospital admission time was the zero point). The original time points were removed from the dataset. Patients older than 89 years were assigned a random number from 90 to 120 for the age variable.

DATA RECORDS

The study generated a relational database consisting of seven tables (**Table 2**). The database integrated comprehensive information of 2,790 patients in ICU with infection from January 2019 to December 2020. Tables are linked by identifiers such as INP_NO or PATIENT_ID. INP_NO refers to unique hospital admission and PATIENT_ID refers to a unique subject.

High-granularity charted events such as progress notes, fluid intake, consciousness, vital signs, mechanical ventilator parameters, Richmond Agitation-Sedation Scale (RASS), and critical-care pain observation tool (CPOT) scores are recorded in the nursing chart table. Information from different sources might be inconsistent. For example, a drug may be prescribed by the physician as recorded in the *dtDrugs* table. However, the drug is actually not administered and thus will not be found in the *dtNursingChart* table. Our approach is to keep these tables independent for clarity because these tables reflect different sources of information and contain information for prognostic or predictive analytics. For example, the physician may prescribe analgesics for a patient on admission, but this patient actually does not experience pain or agitation and the analgesics are not actually administered. However, the presence of medical order reflects the physician's expectation and thus

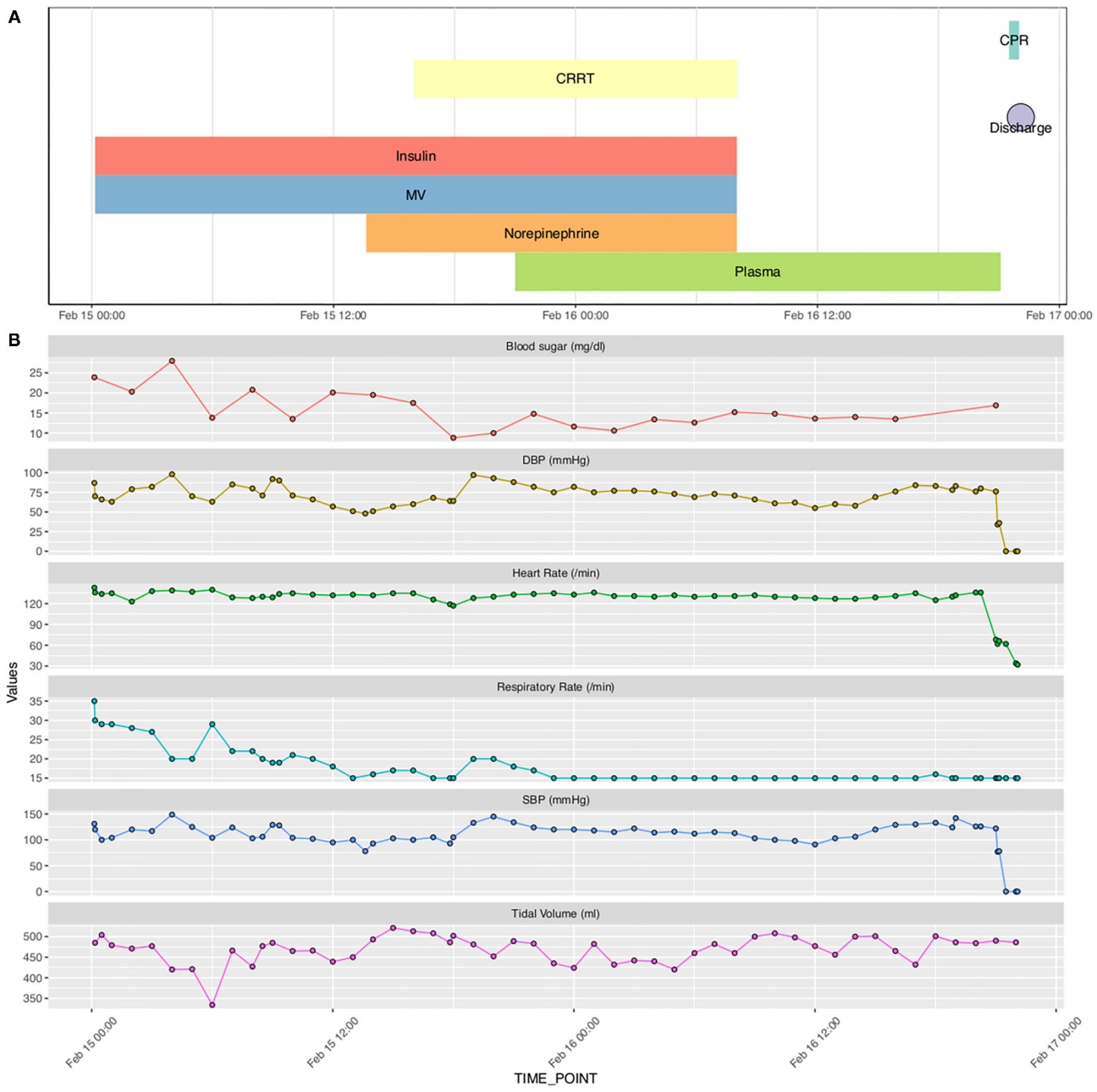

FIGURE 1 | Sample data for a single patient stay in the ICU. **(A)** Chart events extracted from free texts recorded in the nursing chart; **(B)** Structured numeric data extracted from the nursing chart. CRRT, continuous renal replacement therapy; MV, mechanical ventilation; CPR, cardiopulmonary resuscitation; SBP, systolic blood pressure; DBP, diastolic blood pressure.

may contain prognostic information. The dataset is available at PhysioNet (https://physionet.org/content/icu-infection-zigong-fourth/1.0/).

Technical Validation

Data were retrospectively extracted from the information systems in the Zigong Fourth People's Hospital. Firstly, the required data were exported from an electronic healthcare database with the assistance of an information technology technician (Zhou). The exported data were then reviewed by three expert critical care physicians (PX, LC, and ZZ). Most variables recorded in Chinese such as diagnosis description, laboratory item, and department name were translated into English. However, the progress notes from the nursing chart remained in Chinese because such information can be used for natural language processing. Some embedding features might be lost or modified when they are translated into other languges (24). In the meantime, some impossible date entries (follow-up date earlier than the discharge date), impossible values from the nursing chart (i.e., respiratory rate = 2), and outliers (i.e., tidal volume = 30) were either removed or updated after a manual check. Data were finalized and fully anonymized on August 20, 2021.

USAGE NOTES

Data Access

The critical care database is provided as a collection of comma-separated value (CSV) files. Such files can be easily processed with popular languages scripts such as PostreSQL, MySQL, R (version 4.01, The R Foundation for Statistical Computing), and MonetDB. In particular, the relational database can be easily managed with the *tidyverse* pipeline. In *tidyverse* pipeline, all packages can be fit together seamlessly and users do not need to worry about compatibility issues between different functions from different sources, and *tidyverse* scripts are easier to write, read, and understand than base R code (25). Users are required to formally request access to the database.

Baseline Characteristics of Included Patients

The overall mortality rate at hospital discharge was 5.8% (161/2,790). The proportion of men was higher in non-survivors than that in survivors (70 vs. 59%; $p = 0.014$). Patients with pneumonia were more likely to die than other sites of infection. However, non-survivors showed a shorter length of stay in both hospital and ICU, which was attributable to the fact that many severely ill patients chose to withdraw life-support interventions and died shortly after a few days of treatment.

Sample data for a single patient stay in the ICU are shown in **Figure 1**. The patient was transferred to ICU and experienced septic shock. Norepinephrine was used to maintain blood pressure. Organ failures including acute kidney injury, respiratory failure, and circulatory shock occurred sequentially during the disease course. Supportive treatments such as continuous renal replacement therapy (CRRT), mechanical ventilation (MV), and vasopressor were used. However, the clinical conditions deteriorate and suffered from sudden cardiac arrest (**Figure 1**).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://physionet.org/content/icu-infection-zigong-fourth/1.0/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Zigong Fourth People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZZ, LC, and PX conceived the idea. YZ and SY curated data. RC and WH checked the accuracy of the data. FW performed a patient follow-up. All authors contributed to the article and approved the submitted version.

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Prognostic Value of Syndecan-1 in the Prediction of Sepsis-Related Complications and Mortality: A Meta-Analysis

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Aim: Syndecan-1 (SDC-1) has been shown to have a high predictive value for sepsis development, though uncertainty around these results exists. The aim of this meta-analysis was to assess the prognostic ability of SDC-1 in predicting sepsis-related complications and mortality.

Methods: We searched PubMed, EMBASE, Cochrane Library, and Google Scholar databases from January 01, 1990, to March 17, 2021, to identify eligible studies. The search terms used were "SDC-1," "sepsis," "severe sepsis," and "septic shock," and a meta-analysis was performed using the RevMan 5.4 software.

Results: Eleven studies with a total of 2,318 enrolled patients were included. SDC-1 concentrations were significantly higher in the composite poor outcome group [standardized mean difference (SMD) = 0.55; 95% CI: 0.38–0.72; $P < 0.001$] as well as in deceased patients (SMD = 0.53; 95% CI: 0.40–0.67; $P < 0.001$), patients with septic shock (SMD = 0.81; 95% CI: 0.36–1.25; $P < 0.001$), and patients with acute kidney injury (SMD = 0.48; 95% CI: 0.33–0.62; $P < 0.001$). Statistical significance was also found in the subgroup analysis when stratified by different sepsis diagnostic criteria.

Conclusion: Baseline SDC-1 levels may be a useful predictor of sepsis-related complications and mortality.

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INTRODUCTION

Sepsis is a life-threatening condition characterized by a dysregulated response to infection and is associated with organ dysfunction and high mortality rates (1, 2). Early identification of sepsis patients with a high risk of poor outcomes is vital and can reduce mortality and improve prognosis.

Glycocalyx degradation is a critical driver of organ failure in sepsis due to a combination of pathophysiologic insults (3, 4). It is associated with the development of shock (5, 6), acute kidney

injury (AKI) (7), coagulopathy (8), acute respiratory distress syndrome (ARDS)/respiratory failure (9, 10), and mortality (9, 11). Identifying biological markers of glycocalyx degradation may be an essential step in improving outcomes in patients with sepsis.

Syndecan-1 (SDC-1) has been identified as one such biomarker (12, 13), with levels of SDC-1, being elevated in some studies (14, 15). Moreover, multiple studies have shown that SDC-1 levels increased in patients with sepsis, including those with severe sepsis and septic shock. However, only a few studies have demonstrated SDC-1 as a prognostic tool and predictive marker of poor outcomes in patients with sepsis (5, 11, 16, 17). Some previous studies have also included patients with severe sepsis and septic shock. The diagnostic criteria for sepsis have changed three times from 1991 to 2016, which complicates generalization across these studies. Moreover, SDC-1 levels are variable across the longitudinal course of sepsis (11, 18, 19).

The aim of this meta-analysis was to examine the prognostic value of SDC-1 levels upon admission as a predictor of sepsis-related complications and mortality.

MATERIALS AND METHODS

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol was registered with PROSPERO (CRD42021246344).

Search Strategy and Study Selection

A systematic search of the literature across the PubMed, EMBASE, Cochrane Library, and Google Scholar databases from January 01, 1990, to March 17, 2021, was performed using the following keywords: "sepsis," "severe sepsis," "septic shock," and "SDC-1." We excluded review articles, letters, communications, case reports, and articles published in languages other than English. The reference lists of articles were also reviewed to identify additional relevant studies.

Studies containing the following were included: (1) a prospective study method, (2) patient cohorts aged >18 years, (3) an SDC-1 assessment of serum or plasma within 24 h after admission, and (4) clear diagnostic criteria for sepsis. Moreover, the following reports were excluded: (1) duplicated publications, (2) studies with data not reported or data that could not be transformed into a mean with the standard deviation (SD), and (3) studies which included patients without sepsis. Two investigators (TS and YW) independently extracted studies that complied with the criteria.

Data Extraction

A standardized form containing first author, year of publication, admission setting, study design, age, sex, number of participants, serum or plasma concentrations, outcomes, and the standards used to define sepsis was recorded. The mean difference and SD were used to pool data, while other forms of data were transformed and described as the mean ± SD (20, 21). For this meta-analysis, "poor outcome" was a composite measure, incorporating mortality and sepsis-associated complications, including septic shock, AKI, disseminated intravascular coagulation (DIC), and ARDS.

Two authors (YW and XW) performed the data extraction independently, using the Newcastle-Ottawa Scale (NOS) to assess the quality of the observational studies. The NOS assigns studies a score of up to nine points based on subject, comparability, and the outcome of interest assessed, with a score of ≥6 indicating a high-quality study.

Diagnostic Criteria

Sepsis and septic shock definitions were based on three criteria: sepsis 1 (ACCP/SCCM 1991) (22), sepsis 2 (SCCM/ACCP/ATS/SIS 2001) (23), or sepsis 3 (SCCM/ESICM 2016) (24). The diagnosis of AKI was based on either the Acute Kidney Injury Network (AKIN) (25) or Kidney Disease Improving Global Outcomes (KDIGO) (26) criteria. Diagnoses of DIC and ARDS were based on the criteria specified by the International Society of Thrombosis and Hemostasis (27) and Berlin ARDS definition 2012 (28), respectively.

Statistical Analysis

For this meta-analysis, we used the Review Manager 5.4 (Cochrane Collaboration) software to investigate the association between SDC-1 and poor outcome. Heterogeneity between studies was assessed using the χ^2 test and inconsistency index (I^2). An $I^2 > 50\%$ with $P < 0.05$ was considered indicative of significant heterogeneity. In such cases, a random effect model was chosen, where each measure for poor outcome was then sub-analyzed to explore the source of heterogeneity. Otherwise, a fixed effect model was used. We evaluated publication bias by examining funnel plots when the number of studies reporting the primary clinical outcomes was 10 or more. All tests were two-tailed, and $p < 0.05$ was defined as statistically significant.

RESULTS

Results of Literature Search

Our initial search of the databases led to the identification of 628 reports, of which 208 were duplicates and subsequently discarded. The titles and abstracts of the remaining 420 reports were then screened, after which, 380 reports were discarded. The full-text articles for 40 studies were read. In total, 11 studies conducted in Asia, Europe, and North America met our inclusion criteria. The procedures used for study selection are described in **Figure 1**.

Basic Characteristics of the Included Studies

The eligible studies had a total of 2,318 enrolled patients, 1,375 of whom were male (59.3%). The measures of poor outcome examined included mortality in six studies (11, 29–33), septic shock in five studies (11, 33–36), AKI in three studies (29, 30, 37), and DIC in two studies (36, 38). The study characteristics (i.e., country, year, study design, sepsis definition, age, gender, sampling to analysis, and outcome), Sequential Organ Failure Assessment (SOFA) scores of patients in each

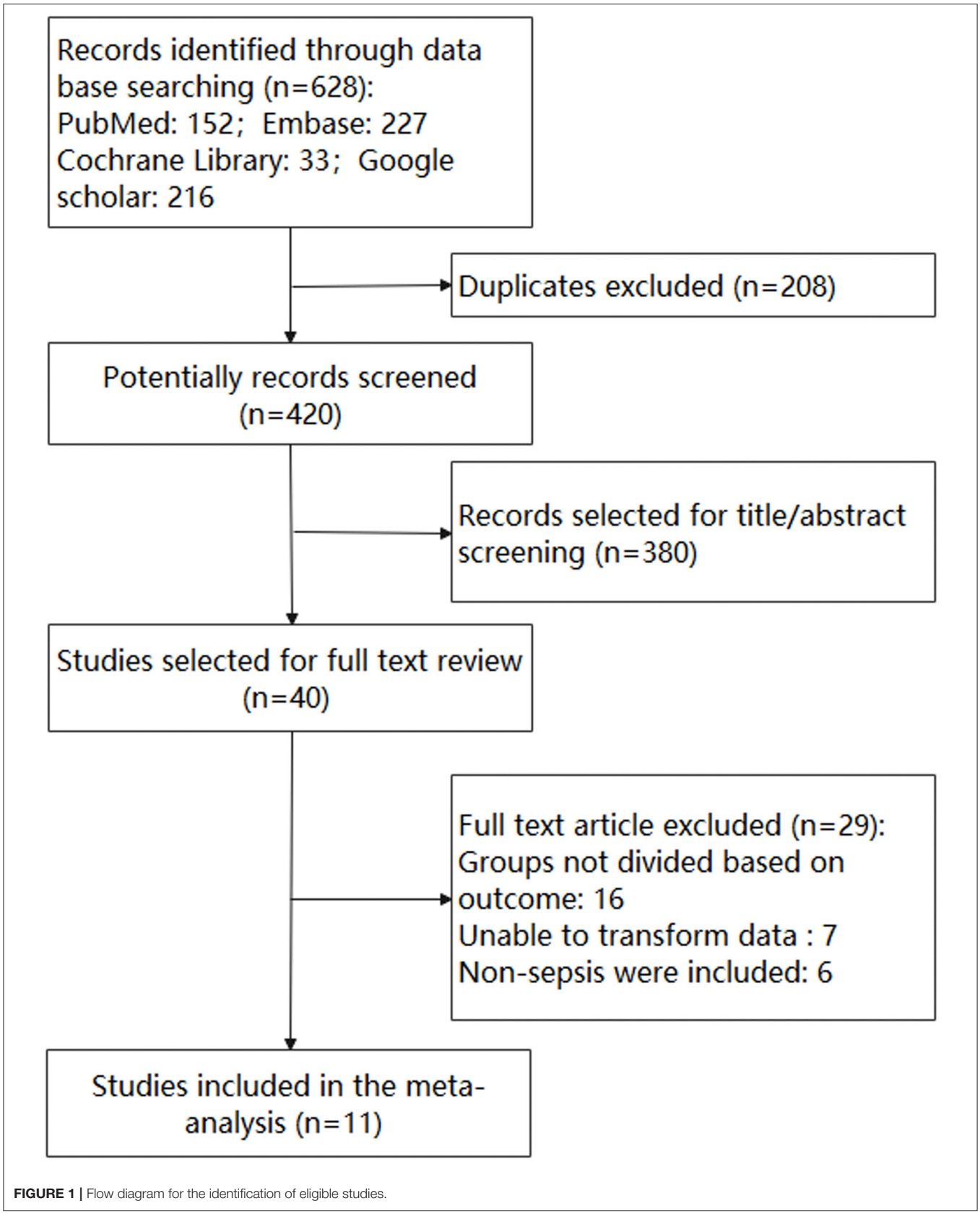

FIGURE 1 | Flow diagram for the identification of eligible studies.

TABLE 1 | Characteristics of included studies.

References	Country	Design	Setting	Sepsis definition	SOFA scores	Patient characteristic	Age (median)	Male (%)	Sample	Assay	Outcome	NOS
Anand et al. (11)	India	PC	ICU	Sepsis-2	6 (4–8)	Sepsis	56	61	Serum	ELISA	Mortality/Septic shock	7
Beurskens et al. (31)	Netherland	PO	ICU	Sepsis-3	8 (7–11)	Sepsis	67	43	Plasma	ELISA	Mortality	6
Huang et al. (36)	China	PO	ICU	Sepsis-3	9.4 ± 3.8	Sepsis	66	82.2	Plasma	ELISA	Septic shock/DIC	6
Ikeda et al. (38)	Japan	PO	ICU	Sepsis-1	9 (5–12)	Sepsis	73	66.7	Serum	ELISA	DIC	7
Inkinen et al. (30)	Finland	PC	ICU	Sepsis-1	8 (6–10)	Sepsis and septic shock	66	64	Plasma	ELISA	Mortality/AKI	7
Johansen et al. (35)	Denmark	PO	ICU	Sepsis-1	Not report	Sepsis	Not report	55.4	Serum	ELISA	Septic shock	7
Johansson et al. (34)	Denmark	PO	ICU	Sepsis-2	5 (5–7)	Severe sepsis	66	59	Serum	ELISA	Septic shock	7
Puskarich et al. (29)	USA	PC	ED	Sepsis-2	7 (4–9)	Severe sepsis	61	53	Plasma	ELISA	Mortality/AKI	7
Saoraya et al. (33)	Thailand	PO	ED	Sepsis-3	4.0 (2.0–6.0)	Sepsis	76	62	Plasma	ELISA	Mortality/Septic shock	7
Sexton et al. (32)	USA	PC	ICU	Sepsis-3	9.12 ± 3.96	Sepsis and septic shouk	52	55	Plasma	ELISA	Mortality	7
Yu et al. (37)	USA	PC	ICU	Sepsis-2	Not report	Severe sepsis	55	51	Plasma	ELISA	AKI	6

PC, prospective cohort; PO, prospective observational; ICU, intensive care unit; ED, emergency department; SOFA, Sequential organ failure assessment score; DIC, disseminated intravascular coagulation; AKI, acute kidney injury; NOS, Newcastle-Ottawa Scale.

study, and NOS scores of the 11 studies (range = 6–7) are shown in **Table 1**.

Meta-Analysis and Subgroup Analysis

SDC-1 levels were significantly higher in the poor outcome group (standardized mean difference [SMD] 0.55; 95% confidence interval [CI] 0.38–0.72; $I^2 = 57\%$; $p < 0.001$), indicating their potential use for early prediction of poor outcome (**Figure 2**).

Results from the subgroup analysis are presented in **Figure 3A**. SDC-1 levels were significantly higher in patients who died (SMD = 0.53; 95% CI: 0.40–0.67; $I^2 = 0\%$; $p < 0.001$), as well as in those who developed septic shock (SMD = 0.81; 95% CI: 0.36–1.25; $I^2 = 79\%$; $p < 0.001$) or AKI (SMD = 0.48; 95% CI: 0.33–0.62; $I^2 = 0\%$; $p < 0.001$). Similar results were found in a subgroup analysis when patients were stratified according to the different diagnostic criteria of sepsis 1, sepsis 2, and sepsis 3, as shown in **Figure 3B** ($p < 0.001$, $p < 0.001$, and $p = 0.01$, respectively). When combining studies which used the same diagnostic criteria, similar results were found.

Among the studies which used the sepsis 1 and 2 diagnostic criteria, five reported SOFA scores (11, 29, 30, 34, 38) ranging from 5 to 9. Another study by Yu et al. (37) only included patients with severe sepsis. According to the sepsis 3 definition (24), patients in these six combined studies, with SOFA scores above 2 or with severe sepsis noted, could be categorized as having sepsis. A meta-analysis including 10 of the studies was also conducted, and a significant difference in SDC-1 levels was noted between patients with poor and good outcomes (SMD = 0.57; 95% CI: 0.45–0.68; $I^2 = 40\%$; $p < 0.001$), as illustrated in **Figure 4**.

Sensitivity Analysis

A leave-one-out meta-analysis was performed to detect the influence of heterogeneity on SMD. Sensitivity analysis revealed that heterogeneity decreased when the studies conducted by Huang et al. (from 55 to 36%) and Johansen et al. (from 55 to 40%) were individually removed. When both were removed, heterogeneity was further reduced (from 55 to 0%), and higher SDC-1 levels were noted in the poor outcome group (SMD = 0.54; 95% CI: 0.42–0.66, $p < 0.001$).

Publication Bias

To evaluate publication bias, the included studies were examined using a funnel plot. A qualitatively symmetrical funnel plot was noted, indicating that no significant publication bias existed in this meta-analysis (**Figure 5**).

DISCUSSION

This is the first meta-analysis to examine the prognostic value of baseline SDC-1 levels to predict sepsis-related complications and mortality. SDC-1 levels were higher in the poor outcome group compared with the good outcome group. In a subgroup analysis,

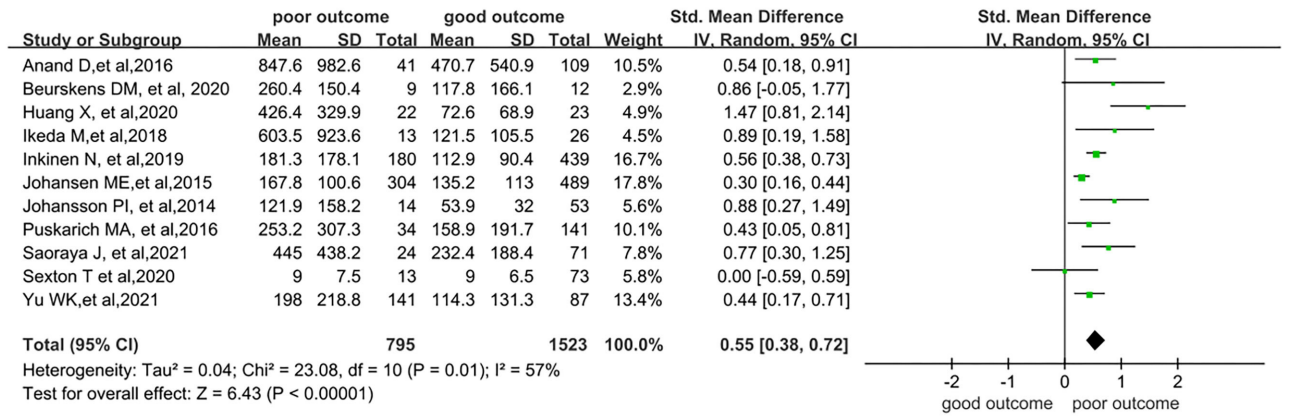

FIGURE 2 | Forest plots of overall poor outcome.

SDC-1 levels were significantly higher in deceased patients as well as in those with septic shock or AKI. These results suggest that sepsis patients with higher baseline SDC-1 levels may be at a higher risk of poor outcomes.

A common factor in organ failure is endothelial dysfunction. Degradation of the endothelial glycocalyx occurs in inflammatory states and quickly alters the physiological function of the endothelium, which is implicated in the pathogenesis of critically ill (39), ARDS (40), coronavirus disease 2019 (COVID-19) (41), and pneumonia patients (42). The Sidestream Dark Field (SDF) imaging of the sublingual area is a direct method to assess the thickness of glycocalyx in sepsis patients, but its application is limited by specialized equipment and software. Donati et al. (43) used SDF imaging and found more severe glycocalyx alterations in sepsis patients than in intensive care unit (ICU) patients without sepsis. Beurskens et al. (31) also found significantly lower endothelial glycocalyx thickness in non-survivors than in survivors with sepsis. The most common method for measuring glycocalyx breakdown products is through plasma/serum measurements. SDC-1, a biomarker of glycocalyx degradation, increases with disease severity and is related to poor prognosis in sepsis patients (10, 16, 17, 44). In our meta-analysis, only one of the included studies did not support the prognostic role of SDC-1 in patients with sepsis (32).

However, SDC-1 levels vary over the longitudinal course and relative progression of sepsis (11, 15). Anand et al. (11) found that SDC-1 levels increased over the first week of ICU admission in non-surviving patients with sepsis, compared with those who survived. In the surviving group, SDC-1 levels tended to decrease after the first week. Fraser et al. (45) found a persistent elevation in SDC-1 levels over the first 3 days of ICU admission in patients with COVID-19. In our meta-analysis, the unified selection criteria included prospective studies where SDC-1 levels were measured within the first 24 h after admission, which allowed us to further confirm the prognostic value of SDC-1 for the outcome prediction in patients with sepsis.

Although, there was significant heterogeneity across the 11 studies included in this review, sensitivity analyses indicated that the pooled results were robust. In sensitivity analysis testing, similar results were found when the two studies by Johansen et al. (35) and Huang et al. (36) were removed. The present meta-analysis suggests that SDC-1 may be a useful biological marker for the prediction of sepsis-related complications and mortality.

In the subgroup analysis, we found considerably higher heterogeneity in the septic shock and sepsis 3 subgroups. In the septic shock group, two studies used sepsis 2 criteria, two used sepsis 3 criteria, and only one used sepsis 1 criteria. Therefore, we speculated that the heterogeneity may have been due to the different diagnostic criteria of sepsis, as the diagnosis of septic shock varied considerably across the three criteria. A subgroup analysis, which included 10 studies that all met the sepsis 3 criteria, was also performed. Significantly higher concentrations of SDC-1 were observed in this subgroup compared with patients with good outcomes.

Despite the results of our meta-analysis, the use of a single biomarker to predict sepsis may not always be reliable. We hope that ongoing randomized trials (NCT 04718623 and NCT 04644302) will include a more in-depth analysis of the predictive markers for patients with sepsis.

This meta-analysis had several limitations. First, SDC-1 levels had a high SD, indicating a high level of variability. SDC-1 levels were reported using medians and interquartile range, which were then used to calculate the means and SDs in this meta-analysis. Second, the sample sizes of the included publications were small. Although we pooled the results of these publications, it may still have been possible to miss the effectiveness of the meta-analysis. Third, the included studies used different definitions of sepsis, which may have affected our results. In particular, the definition of septic shock was different, which could partially explain the substantial heterogeneity noted in the septic shock subgroup. However, subgroup and sensitivity analyses indicated that the

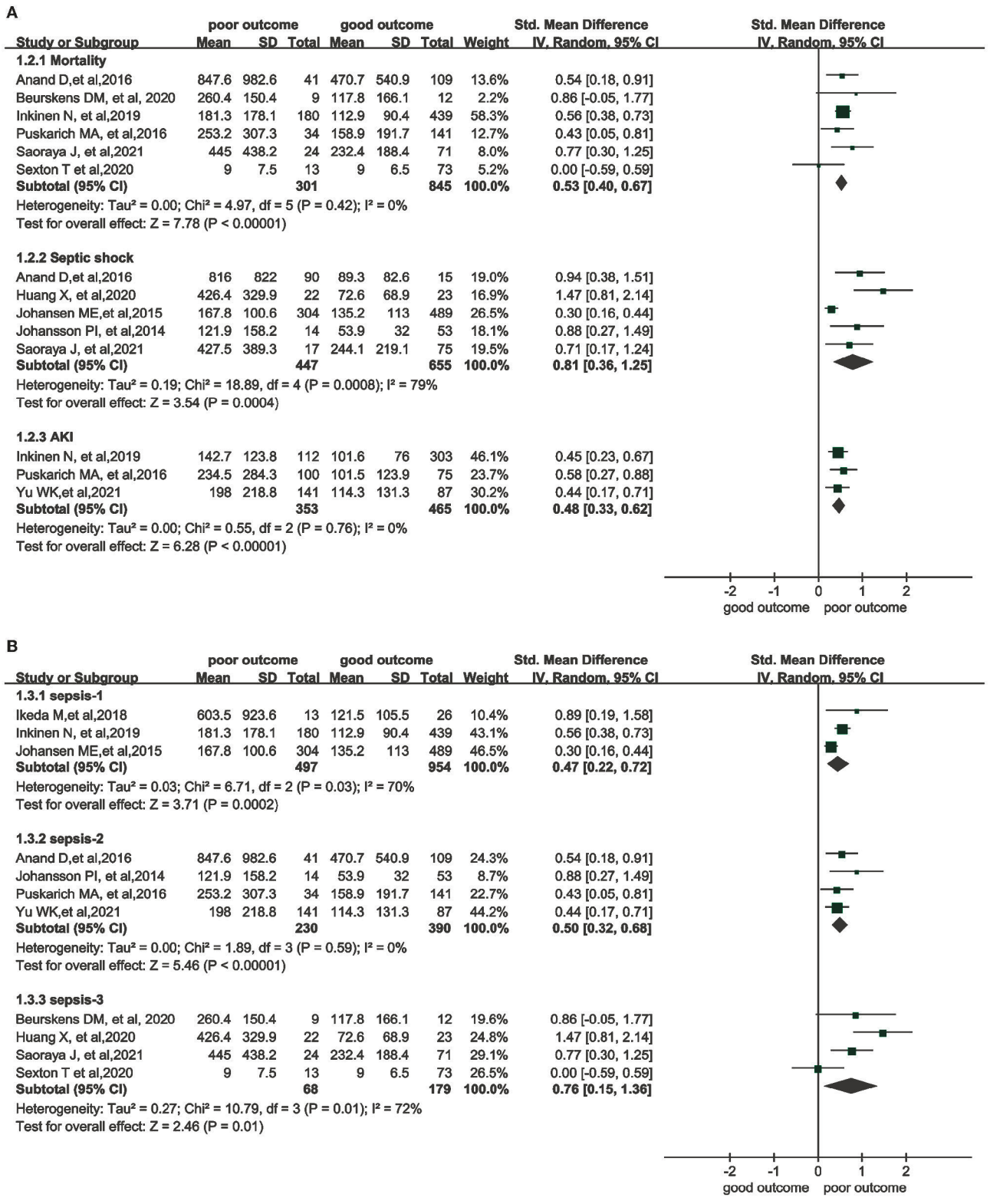

FIGURE 3 | Forest plots of subgroups. **(A)** Subgroup of complications and mortality; **(B)** Subgroup of three diagnostic criterias for sepsis.

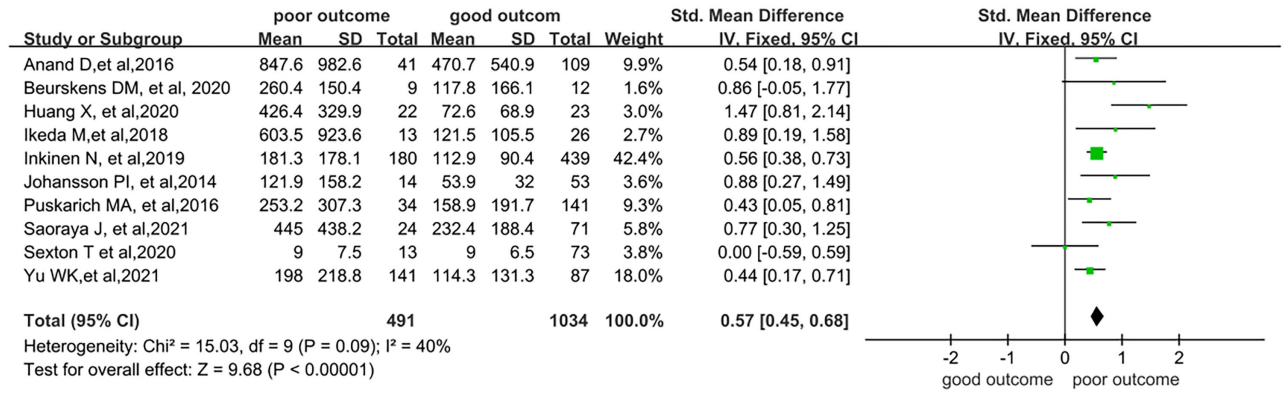

FIGURE 4 | Forest plots of subgroups included 10 studies that meet the sepsis-3 diagnostic criteria.

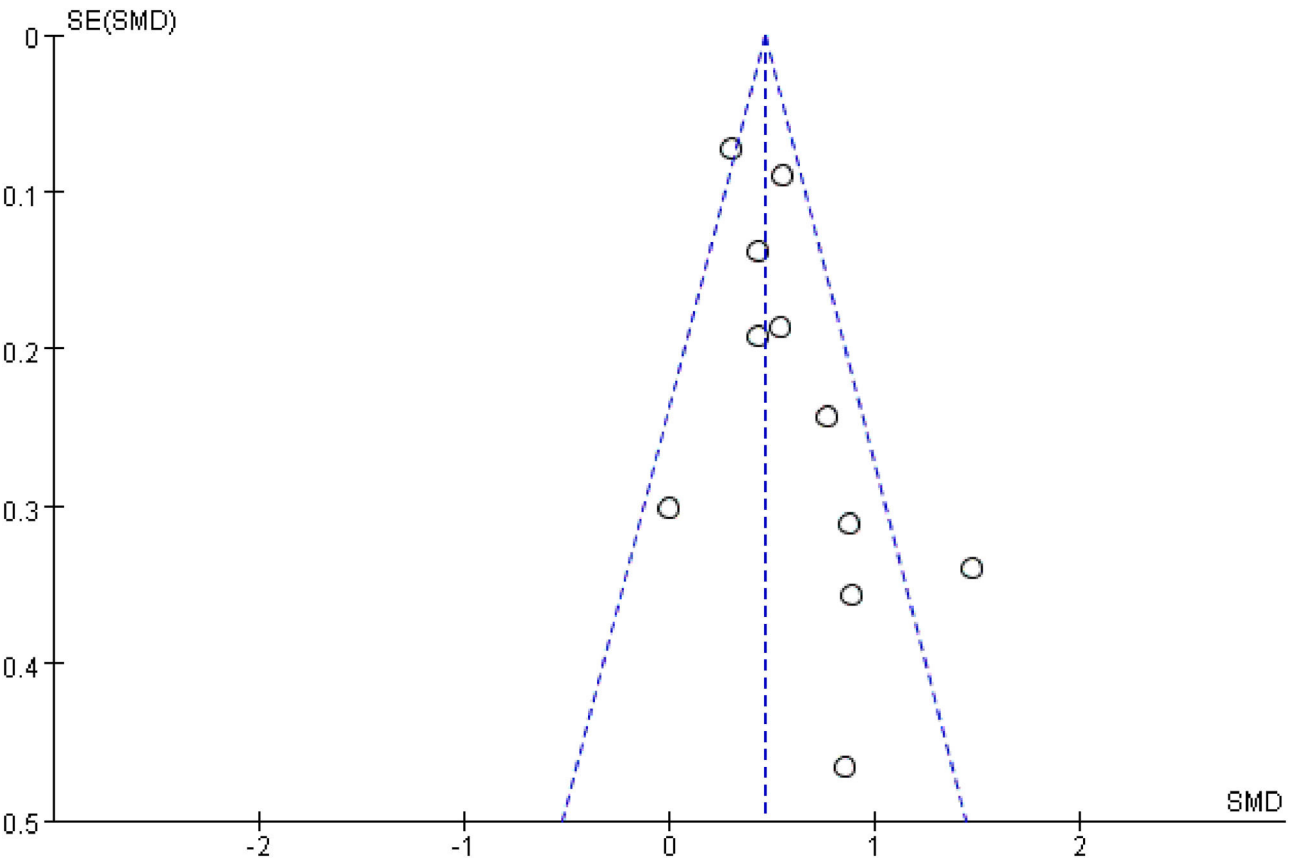

FIGURE 5 | Establish publication bias with funnel plot regarding to all publications.

pooled results were robust. Finally, prospective cohort trials were most qualified for our study objective, as the intervention could not be randomized. Therefore, our meta-analysis of the observational studies, and not of randomized control trials, could only support the potential association between increased SDC-1 and poor outcome in patients with sepsis.

CONCLUSION

This meta-analysis supported the prognostic value of SDC-1 as a predictor of mortality and sepsis-related complications.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

TS and YW extracted studies from the eligible papers. YW and XW performed the data extraction. TZ and YC analyzed the data. TS and QZ wrote the paper. QZ reviewed and revised the paper. All authors contributed to the conception and design of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.870065/full#supplementary-material

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The German Quality Network Sepsis: Evaluation of a Quality Collaborative on Decreasing Sepsis-Related Mortality in a Controlled Interrupted Time Series Analysis

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Background: Sepsis is one of the leading causes of preventable deaths in hospitals. This study presents the evaluation of a quality collaborative, which aimed to decrease sepsis-related hospital mortality.

Methods: The German Quality Network Sepsis (GQNS) offers quality reporting based on claims data, peer reviews, and support for establishing continuous quality management and staff education. This study evaluates the effects of participating in the GQNS during the intervention period (April 2016–June 2018) in comparison to a retrospective baseline (January 2014–March 2016). The primary outcome was all-cause risk-adjusted hospital mortality among cases with sepsis. Sepsis was identified by International Classification of Diseases (ICD) codes in claims data. A controlled time series analysis was conducted to analyze changes from the baseline to the intervention period comparing GQNS hospitals with the population of all German hospitals assessed *via* the national diagnosis-related groups (DRGs)-statistics. Tests were conducted using piecewise hierarchical models. Implementation processes and barriers were assessed by surveys of local leaders of quality improvement teams.

Results: Seventy-four hospitals participated, of which 17 were university hospitals and 18 were tertiary care facilities. Observed mortality was 43.5% during baseline period

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and 42.7% during intervention period. Interrupted time-series analyses did not show effects on course or level of risk-adjusted mortality of cases with sepsis compared to the national DRG-statistics after the beginning of the intervention period ($p = 0.632$ and $p = 0.512$, respectively). There was no significant mortality decrease in the subgroups of patients with septic shock or ventilation >24 h or predefined subgroups of hospitals. A standardized survey among 49 local quality improvement leaders in autumn of 2018 revealed that most hospitals did not succeed in implementing a continuous quality management program or relevant measures to improve early recognition and treatment of sepsis. Barriers perceived most commonly were lack of time (77.6%), staff shortage (59.2%), and lack of participation of relevant departments (38.8%).

Conclusion: As long as hospital-wide sepsis quality improvement efforts will not become a high priority for the hospital leadership by assuring adequate resources and involvement of all pertinent stakeholders, voluntary initiatives to improve the quality of sepsis care will remain prone to failure.

Keywords: sepsis, mortality, quality improvement, risk adjustment, administrative claims, interdisciplinary health team, diagnosis-related groups (DRG)

INTRODUCTION

Sepsis is a life-threatening organ dysfunction resulting from infection and the leading cause of death due to infectious diseases (1). It might also be the leading cause of preventable deaths in hospitals (2). Timely recognition and adequate anti-infective treatment have been shown to decrease mortality, but awareness of sepsis is often low in everyday clinical practice (2–8). A recent meta-analysis showed that performance improvement programs substantially improved implementation of sepsis guidelines including early adequate antimicrobial treatment – and decreased odds of death (9). Such quality initiatives typically use a multifaceted approach by assessing and reporting quality, staff education, and implementing changes in care processes (9). Prospective inclusion of patients with sepsis and documentation of clinical data for quality indicators put a high workload on participating hospitals, which can cause poor reporting or even the drop-out of hospitals from quality improvement projects (10, 11).

Using claims data for performance measurement has the advantage of covering all International Classification of Diseases (ICD) coded cases with data readily available and needing minimal time and costs (12). This approach is extensively used within quality initiatives in the United States of America (USA) (13). It has also achieved the first promising results in Germany, where a large quality initiative combines benchmarks of quality indicators based on administrative data with peer reviews (14). Therefore, the German Quality Network Sepsis (GQNS) was founded as a quality collaborative to support participating hospitals to improve sepsis care by offering quality reports based on claims data, peer reviews, and support to implement a continuous quality management and regular staff education. The participation in the GQNS was voluntary and the full responsibility for implementation of quality improvement measures was on the side of the participating hospitals. This study aims to evaluate the effect of hospitals' participation in the GQNS on mortality among patients with sepsis.

Abbreviations: DRG, diagnosis-related groups; GQNS, German Quality Network Sepsis; ICD, International Classification of Diseases; ICD-10-GM, International Statistical Classification of Diseases and Related Health Problems – German Modification – 10th Revision; RSMR, risk standardized mortality rate; USA, United States of America.

MATERIALS AND METHODS

Context

The GQNS was founded in February 2016. The start-up period of the GQNS was funded by grants from the German Federal Ministry of Education and Research (BMBF) and ran from August 2015 to July 2018. The funded start-up phase and its scientific evaluation used the acronym quality Improvement in infection COntrol and Sepsis management in MOdel regionS (ICOSMOS). The study was approved by the Ethical Review Board of the Jena University Hospital (IRB protocol 4536-08/15). The necessity of informed consent by patients was waived since only pseudonymized claims data were used. Details on the concept and conduction of the GQNS, as well as the planned evaluation, are given in the study protocol (15). Passages cited from the study protocol are not individually marked in the manuscript. The study description follows the Standards for QUality Improvement Reporting Excellence (SQUIRE 2.0) recommendations (16).

Participating Hospitals

Eligible for participation in the GQNS were acute care hospitals with at least one adult intensive care unit. Invitation letters were sent to management boards of hospitals that were participating in former or ongoing sepsis-related quality initiatives or research networks and all German university hospitals; a total of 148 individual hospitals were contacted. In addition, letters were

sent to management boards of five regional and three national hospital groups. Hospitals could join the GQNS at the time of its foundation or any later time.

Project Organization

The GQNS was coordinated by the central study coordinating bureau at the Jena University Hospital. Claims data were collected and processed to generate quality reports by a medical information technology service provider (3M Health Information Systems). Participating hospitals of the GQNS named a local leader of the quality improvement process. The quality improvement leaders were encouraged to establish interdisciplinary and interprofessional quality improvement teams right from the beginning of the participation in the GQNS. The formation of these teams was not mandatory and selection of the members was at the discretion of the quality improvement leader. It was suggested by the study coordinating bureau to include at least intensive care departments, the emergency department, quality management department, and medical and surgical departments responsible for inpatient treatments of adult patients. Major decisions were made in the general assembly of representatives of all participating hospitals. This general assembly was formed by the local quality improvement leaders and met once a year in autumn. A steering committee was elected among the delegates of the general assembly to supervise the work of the coordinating bureau. Meetings of the steering committee and the study coordinating bureau were conducted by phone or web-conference every few months.

Interventions

The core interventions for hospitals in the GQNS are: (a) reporting and publication of quality indicators; (b) case analyses within the participating hospitals; (c) peer reviews for hospitals, which were outliers in the quality reports; and (d) hospital-wide staff education in participating hospitals. Peer review is a process by which health care providers evaluate each other's performance (17). The only mandatory intervention was the reporting, benchmarking, and publication of quality indicators. The study coordination bureau provided information and support regarding the conduction of case analyses and staff education and coordinated peer reviews. The full responsibility for implementation was on the side of the participating hospitals, and the participation in peer reviews was voluntary.

Reporting of Quality Indicators

Data for assessment of quality indicators were provided by diagnosis-related group (DRG) data of each participating hospital, which were sent to the information technology service provider. These data can be exported easily in a standardized format from the hospitals' patient data management system. The service provider supplied the quality reporting quarterly to each hospital beginning in April 2016. Cases with sepsis were identified based on specific codes of the International Statistical Classification of Diseases and Related Health Problems 10th Revision German Modification (ICD-10-GM) for sepsis with organ dysfunction or septic shock according to sepsis-1 definitions (R65.1: sepsis with organ dysfunction, R57.2: septic shock) (18). Although new clinical sepsis definitions ("sepsis-3") were introduced in 2016 (1), the ICD-10-GM-coding of sepsis relied on the old sepsis-1 definitions until the end of 2019 in Germany. Quality reports contained incidence and risk-adjusted mortality for cases with sepsis and the subgroups of patients with septic shock, sepsis, and mechanical ventilation of more than 24 h, admission to the hospital *via* a surgical department, and admission to the hospital *via* a medical department. Hospitals' own results could be compared to other participating hospitals, subgroups of participating hospitals (primary, secondary, tertiary care, and university hospitals), the overall average in the GQNS, as well as to the average among all German hospitals. Also the longitudinal course of quality indicators could be inspected using monthly, quarterly, half-yearly, and yearly periods. Initially, quality reports were presented in tabular form by Microsoft Excel spreadsheets. From July 2017 onward, quality indicators were additionally reported in an online-reporting accessed *via* a web-browser. This online-reporting also contained interactive graphical presentations of quality indicators, e.g., boxplots, and caterpillar plots. Both calculation and presentation of quality indicators were continuously improved.

Mortality was risk-adjusted by a validated complex model developed for the GQNS, which was based on German national DRG-statistics (19). This database contains DRG-data of all German hospitals that are reimbursed *via* DRG. It is provided for scientific analysis in anonymized form by the German Federal Bureau of Statistics (20). Therefore, the same type of data, which are provided by the GQNS-hospitals for quality measurement, are available in the national DRG-statistics and patients with sepsis were identified by the same criteria as given above. The detailed development and validation of the risk-model is described elsewhere (19). Included risk-factors are age, gender, type of admission, clinical characteristics of infection and sepsis, comorbidities, and specific procedures – like treatment of stroke (19). Definitions of variables for risk-adjustment and quality reporting are presented in **Supplementary Data Sheet 1**.

Quality reporting also included case lists presenting predicted and observed mortality for each sepsis case sent to the hospitals, which provided the basis for case analysis and peer reviews. The study coordinating bureau provided hospitals with instructions on how to use the quality reports, and how to conduct case analyses. This was done during annual meetings and by providing educational material on the website of the GQNS.

Publication of Quality Indicators

Hospitals within the GQNS consented to publish their major quality indicators compared to the average of the German national DRG-statistics on their own website. Two indicators were to be published: risk-adjusted mortality of patients with sepsis, and risk-adjusted mortality of patients with sepsis and mechanical ventilation >24 h. To allow hospitals to analyze their data as well as to learn and implement improvements, the first publication of quality indicators was mandatory after 2 years of participation in the GQNS. Therefore, there was one publication of quality indicators at the end of the start-up period of the GQNS in Summer of 2018. All hospitals, which had signed their

contract for participation in the GQNS in 2015 were obliged to publish their quality indicators of the year 2017. This was the case for 11 hospitals.

Case Analyses and Peer Reviews

Based on the provided case lists, expired patients with sepsis with the lowest risk of in-hospital mortality as predicted by the risk-adjustment model were identified and used to analyze and discuss possible problems in the quality of care in interdisciplinary case conferences within the individual participating hospitals (21). The same method was used to select cases for analysis by external peers. An external peer review was suggested by the central study coordinating bureau to hospitals with the highest risk-adjusted mortality among patients with sepsis. Peers were physicians and nurses, who were recruited among the participating hospitals and had a special qualification to conduct peer reviews. A team of at least four peers visited the respective hospital, conducted analyses of up to 10 selected charts of patients with sepsis, and discussed improvement strategies with local clinicians. Contents and results of peer reviews were only reported to the participating hospital and the central study coordinating bureau. Peer reviews were voluntary and hospitals could refuse to take part. Six peer-reviews were conducted from May 2017 to April 2018.

Staff Education

The main focus of staff education was the implementation of strategies for increasing awareness and early recognition of sepsis, as well as the implementation of key elements of the updated Surviving Sepsis Campaign guidelines among all health care workers involved in care for patients with sepsis (22, 23). The study coordination bureau supported the local hospital quality improvement leaders by providing educative material (presentations, pocket cards, posters). Hospitals were also provided with a concept for a screening algorithm for the early detection of sepsis as well as recommendations for its implementation. Educational materials were provided for download *via* the website of the GQNS and concepts were presented at the annual meetings. The local quality improvement teams were responsible for implementing education. In addition, five web-based educational sessions were conducted between March 2017 and February 2018, recordings of these sessions were provided on the website of the GQNS. Due to overall low participation rates and technical problems reported by many participants, no further web based sessions were done. Further details on the interventions are provided in the study protocol (15).

Evaluation of the Effect of Participating in the German Quality Network Sepsis

The effect of participating in the GQNS was evaluated in a controlled interrupted time series analysis (24). The start of the intervention was defined individually for each participating hospital as the month of supply of the first quality report. Thus, for each hospital an individual baseline period and an individual intervention period was defined. This allowed to use all available information of all participating hospitals. The retrospective baseline period began in January 2014 and ended when the hospital received its first quality report. Most hospitals switched to intervention in April 2016; the analyzed intervention period ended, when a hospital stopped its participation in the GQNS or with June 2018 – the time point of the latest delivered DRG-data. Since this analysis might be biased by seasonal variation or history bias, a control condition was included (24). As the control condition, the German national DRG-statistics was used to calculate the monthly risk-standardized mortality rate (RSMR) for all coded sepsis cases in Germany (20), which can be regarded as the population value.

Outcome Measures

The evaluation was based on the data of the quality reports, which were provided to the research team by the medical information technology service provider. Due to data privacy restrictions, no data of individual cases were provided, but all data were aggregated to the hospital level. The primary outcome was the monthly risk-adjusted hospital mortality per hospital of cases with primary or secondary hospital discharge ICD-10-GM code for sepsis with organ dysfunction including septic shock (R65.1, R57.2). Secondary outcomes were the risk-adjusted mortality among patients with septic shock (ICD-10-GM code R57.2) and among cases with sepsis and mechanical ventilation of more than 24 h. Risk-adjusted mortality was calculated as RSMR (see **Supplementary Data Sheet 1** – Definition of variables, and **Supplementary Data Sheet 2** – Calculation of risk-adjusted mortality).

Measures for Intervention Processes and Implementation

To assess fidelity and extent of the local implementation of interventions in the participating hospitals, local quality improvement team leaders were surveyed in the autumns of the years 2016, 2017, and 2018. The survey used a standardized online questionnaire, which contained items on the status of existing quality management structures, extent of usage of quality analysis and implementation of recommended interventions, as well as perceived barriers to change, and rating of the support provided in the GQNS. Items of this questionnaire were designed based on results of qualitative interviews among quality improvement leaders during the MEDUSA study, a cluster-randomized controlled trial on a multifaceted educational intervention to improve acute sepsis care (10, 11).

Statistical Analysis

Retrospective baseline (January 2014–March 2016) and intervention phase (April 2016–June 2018) were descriptively compared regarding patients' demographics, risk factors, the proportion of cases with mechanical ventilation >24 h, hospital length-of-stay, and mortality. The quarterly prevalence and RSMR were calculated and plotted to descriptively compare GQNS and the national DRG-statistics. To test the intervention effect, controlled interrupted time series analyses were calculated for each outcome (24). In this analysis each participating hospital provided its individual time series of monthly RSMRs. To incorporate the control condition, the difference between each monthly RSMR of each hospital and the RSMR obtained from

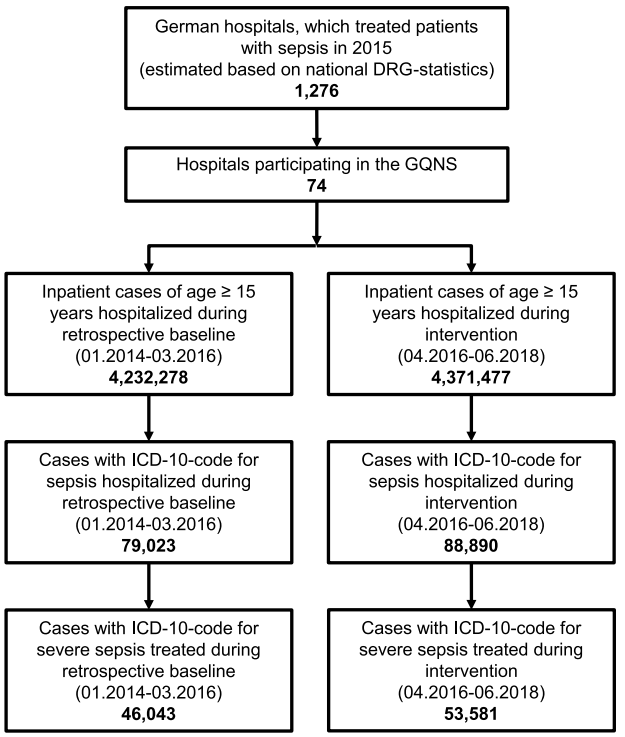

FIGURE 1 | Study flow chart.

the national DRG-statistics for this month was calculated. This defined a new time series for each hospital, representing the difference of its monthly RSMRs to the respective population value. The overall time series analysis incorporating this information from all hospitals was calculated by a piecewise hierarchical model (25). The intervention effect was then tested by the significance of the change in the linear slope as well as the significance of the change in level. Since small sample sizes of sepsis cases per month and hospital might cause bias by the unreliability of the RSMR estimate, the inverse of the noise-variance (see **Supplementary Data Sheet 2**) of the RSMR were used as precision weights in a sensitivity analysis.

Subgroup analyses were conducted among hospitals, which participated through the complete intervention phase, hospitals without complete participation, hospitals with ≤700 beds, and hospitals with >700 beds. Among the hospitals, which participated through the whole intervention period, a subgroup of hospitals was identified, which reported an early implementation of a sepsis-related quality management. This was defined, by the reporting of having implemented a quality improvement team as well as analyses of quality reports in the survey of quality improvement leaders in autumn of 2016.

To analyze the overall success of implementation of interventions as well as barriers and facilitators to change, descriptive statistics were calculated on the items of the last survey of quality improvement leaders – conducted in autumn of 2018. All analyses were conducted using the statistical software R, version 3.6.1 (26).

TABLE 1 | Characteristics of included cases with coded sepsis.

Variable	Retrospective baseline (01.2014–03.2016)	Intervention phase (04.2016–06.2018)
Number of cases with coded sepsis	46.043	53.581
Age (years)	72 (60, 79)	72 (61, 79)
Sex: female	39%	38.7%
Admission: referral by physician or dentist	21.1%	19%
Emergency	63.7%	65.2%
Hospital transfer with pre-treatment >24 h	10.9%	11.3%
Hospital transfer with pre-treatment <24 h or rehabilitation hospital	4.3%	4.5%
Comorbidities		
CCI: cerebrovascular disease	12.8%	13.9%
CCI: dementia	8.5%	8.5%
CCI: mild liver disease	9.7%	10.1%
CCI: moderate or severe liver disease	4.2%	4.1%
CCI: myocardial infarction	10.5%	10.9%
CCI: peptic ulcer disease	4%	4.1%
ECI: alcohol abuse	7.1%	7.1%
ECI: blood loss anemia	0.9%	1%
ECI: cardiac arrhythmias	42.6%	44.7%
ECI: coagulopathy	39.3%	37.4%
ECI: congestive heart failure	34.4%	34.8%
ECI: deficiency anemia	4.4%	4.8%
ECI: depression	6%	5.9%
ECI: drug abuse	1.5%	1.8%
ECI: hypertension, complicated	10.1%	10.7%
ECI: hypertension, uncomplicated	42.2%	42.6%
ECI: hypothyroidism	11.6%	13.2%
ECI: lymphoma	3.5%	3.4%
ECI: metastatic cancer	7.6%	7.7%
ECI: obesity	9.1%	9.7%
ECI: other neurological disorders	15.6%	16.7%
ECI: paralysis	9.2%	9.8%
ECI: peripheral vascular disorders	16.6%	16.5%
ECI: psychoses	1.2%	1.1%
ECI: pulmonary circulation disorders	7.8%	8.1%
ECI: renal failure	30.2%	30.9%
ECI: solid tumor without metastasis	15.2%	14.6%
ECI: valvular disease	13%	14.4%
ECI: weight loss	11.6%	13.5%
Leukemia	3.8%	3.5%
Characteristics of infection and sepsis		
Infection of lower respiratory tract	48.5%	49%
Urinary tract infection	29.2%	30.9%
Abdominal infection	21.8%	20.3%
Foreign body associated infection	12.9%	12.6%
Soft tissue and wound infections	7.3%	8%
Infection of vascular system	5.6%	6%
Infection of central nervous system	1.9%	2.2%
Infection of upper respiratory tract	1.7%	2.9%
Sepsis as primary diagnosis	35.2%	33.4%
Conduction of chemotherapy	6.2%	6.4%

(Continued)

TABLE 1 | (Continued)

Variable	Retrospective baseline (01.2014–03.2016)	Intervention phase (04.2016–06.2018)
Conduction of palliative care	2.1%	2.1%
Hospital length of stay (days)	17 (8, 33)	16 (8, 31)
Hospital mortality	43.5%	42.7%

Descriptive statistics presented as median (first quartile, third quartile) or %. CCI, Charlson comorbidity index; ECI, Elixhauser comorbidity index. Cases with sepsis defined by presence of ICD-10-GM codes R65.1 (sepsis with organ dysfunction) or R57.2 (septic shock). The beginning of the intervention phase is defined uniformly by April 2016 for all hospitals.

Changes in the Evaluation Concept as Compared to the Study Protocol

The strategy of the evaluation was changed in some minor points. First, the primary analysis was not conducted as a difference-in-differences analysis but by a controlled interrupted time-series calculated using piecewise hierarchical models. This allowed to use all available information from all hospitals, regardless from when they joined the intervention, while at the same time controlling for seasonal variation and history bias. Second, the primary analysis was based on all hospitals participating in the GQNS, not only the hospitals participating from the beginning. Third, since the new sepsis-3 definitions do not include a sepsis without organ dysfunction anymore, no analysis was conducted for cases without coding of ICD-10-GM codes R65.1 or R57.2.

RESULTS

Forty-six hospitals received the first quality reports in April 2016, 28 additional hospitals joined later during the intervention phase. The participating 74 hospitals represent 5.7% of 1,276 German hospitals, which treated patients with sepsis (estimated based on national DRG-statistics of 2015). **Figure 1** presents the flow chart of the inclusion of hospitals and cases. Characteristics of participating hospitals are presented in **Supplementary Data Sheet 3 – Supplementary Table 1**.

Characteristics of cases with sepsis are presented in **Table 1**. There were no relevant changes in demographics, comorbidities, or characteristics of the infection and sepsis. Hospital mortality was 43.5% during the retrospective baseline and 42.7% during the intervention period.

Tests of the Effect of Participation in the German Quality Network Sepsis

Figure 2 presents the time-line diagram of the progress of the GQNS. The results of the interrupted time series analysis on the

	2015					2016											2017											2018						
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
Prepartory work and invitation of hospitals	X	X	X	X	X	X																													
Kick-off-Meeting							X																												
Annual meeting with general assembly														X												X									X
Delivery of quality reports (cumulative N of hospitals receiving first report)									46			57			65			65			68			72			73			74			74			74
Addition of web-reporting																								X			X			X			X			X
Peer reviews (N per month)																						2		1			1		1				1			
Web-based educational sessions																				X	X	X		X							X					
Publication of quality indicators (N of hospitals)																																			11	

FIGURE 2 | Time-line diagram on the progress of the GQNS.

TABLE 2 | Results of interrupted time-series analyses on risk-standardized mortality rate difference between GQNS hospitals and the national diagnosis-related groups statistics.

Analysis	Number of hospitals	Slope before intervention (95% CI)	Slope during intervention (95% CI)	*P*-value of test of difference in slopes	Change in level (95% CI)	*P*-value
RSMR-difference for sepsis	74	0.002 (−0.074, 0.078)	0.033 (−0.069, 0.134)	0.632	−0.667 (−2.659, 1.324)	0.512
RSMR-difference for septic shock	74	0.058 (−0.073, 0.188)	0.048 (−0.123, 0.218)	0.928	−0.783 (−4.17, 2.603)	0.65
RSMR-difference for sepsis and mechanical ventilation >24 h	74	0.043 (−0.066, 0.152)	0.112 (−0.032, 0.256)	0.447	−1.827 (−4.669, 1.015)	0.208

Results of piecewise hierarchical models on the difference in the risk-standardized mortality rate (RSMR) between GQNS hospitals and the national German diagnosis-related-groups statistic. Slopes give the linear trajectory of RSMR-difference in % per month across time before and after start of the intervention, change in level gives the change at the time of the beginning of the intervention. Time of beginning of the intervention is defined for each individual hospital as the time of supply of the first quality report.

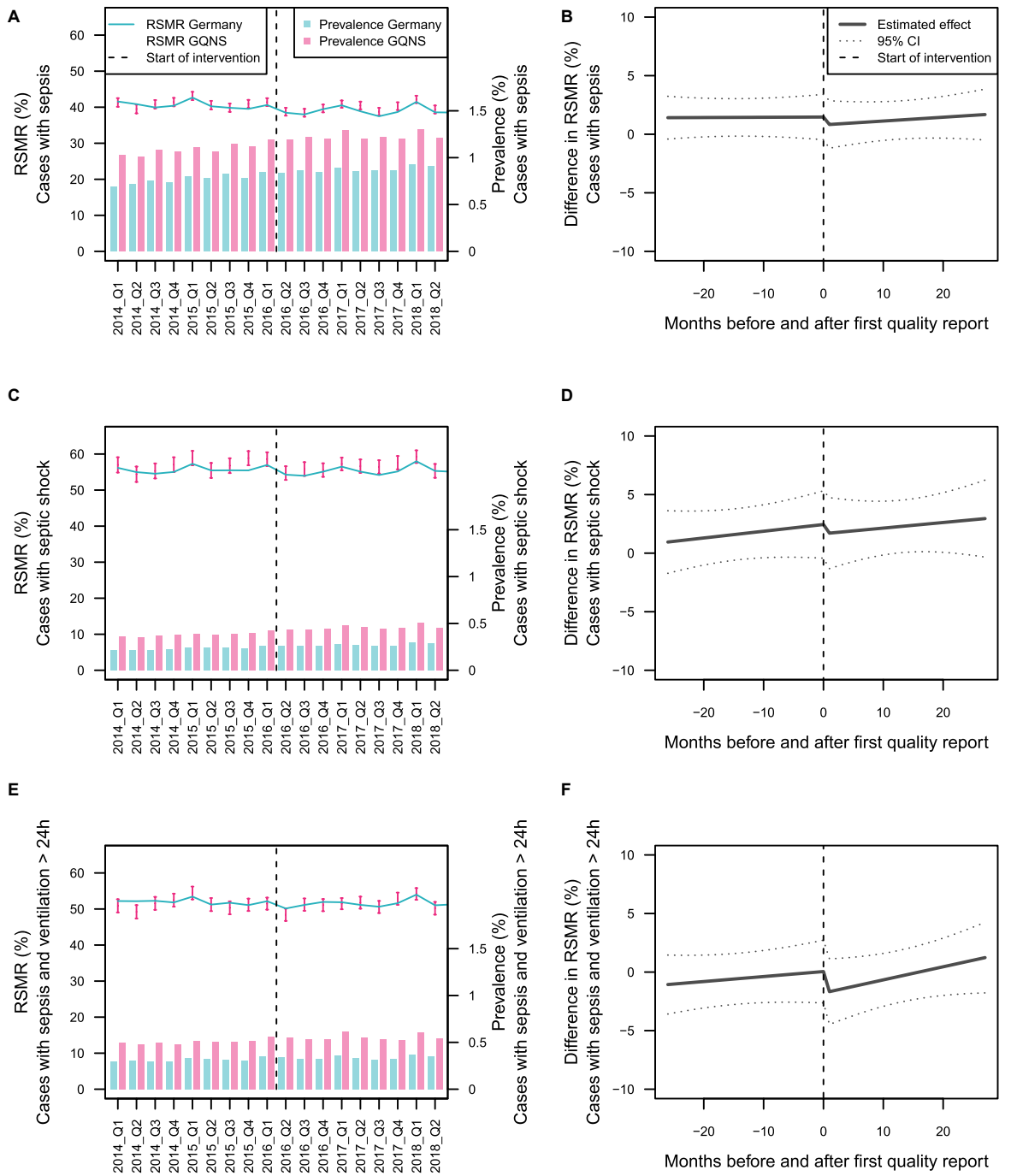

FIGURE 3 | Depiction of the effect of hospitals' participation in the GQNS. Panels **(A,C,E)** present the descriptive changes in prevalence and risk-standardized mortality rate (RSMR) for patients with sepsis, septic shock, and sepsis with mechanical ventilation >24 h. The beginning of the intervention phase is defined uniformly by April 2016 for all hospitals. Panels **(B,D,F)** depict the slopes before and after the beginning of the intervention, as well as the change in level at the beginning of the intervention with 95% prediction limits as estimated from interrupted time series analyses on the monthly RSMR-difference between GQNS hospitals and the national DRG-statistics. The beginning of the intervention phase is defined individually for each hospital by the date the first quality reports were provided to this hospital.

difference between the RSMR of GQNS-hospitals and the RSMR from the national DRG statistics are presented in **Table 2**. There was no change in the trajectory of mortality for cases with sepsis across time before and after the intervention [percent change per month: 0.002 (95% CI: −0.074, 0.078), and 0.033 (−0.069, 0.134), respectively, test of difference: p = 0.632], and no significant

change in level at the beginning of the intervention [percent change: −0.667 (−2.659, 1.324), $p = 0.512$]. This indicates that participation in the GQNS did not affect risk-adjusted mortality compared to the national DRG-statistics. **Figure 3A** presents the descriptive course of prevalence and RSMR for sepsis before and during the intervention period comparing participating hospitals in the GQNS and the national DRG-statistics; **Figure 3B** depicts the slopes and change in level calculated from the time series analysis.

There were also no significant differences in slopes or changes in level in mortality among patients with septic shock or mortality among patients with sepsis and mechanical ventilation >24 h (**Table 2** and **Figures 3C–F**). When precision weights were included to adjust for the unreliability of the RSMR estimated from small sample sizes, there were also no significant effects (data not shown).

When subgroups of hospitals were analyzed, there was a significant change in slopes from baseline to intervention for hospitals, which participated through the whole intervention ($p = 0.042$, **Table 3**). While the RSMR-difference showed a small increase during the baseline, there was no change across time observed anymore during the intervention, but there was also no decrease of mortality. No other subgroup showed any significant differences in slopes or level.

Process Evaluation

Table 4 presents the survey results among local quality improvement leaders, 49 of 69 (71%) invited participants took part in the survey. The results show an overall low degree of implementation of quality management processes: only 22 (44.9%) of hospitals did a complete analysis of provided information on the quality of care by using both the comparison of quality indicators as well as individual case analysis, only eight (16.3%) had an interdisciplinary quality improvement team. Likewise, the implementation degree of measures to improve early recognition, and adequate treatment of sepsis was low: in half of the hospitals, there was no regular staff education on sepsis in the emergency department [$N = 23$ (46.9%)], and on normal wards [$N = 25$ (52.1%)]. Medical emergency teams were implemented in only eight (16.3%) of surveyed hospitals; only three hospitals (6.1%) had screening tools for early detection of sepsis in all relevant departments. Local quality improvement leaders reported high barriers to quality improvement efforts. The GQNS was not seen as an important quality measure for the complete hospital in most hospitals. The most important barriers were lack of time of the quality improvement team [$N = 38$ (77.6%)], general staff shortage [$N = 29$ (59.2%)], and lack of participation of relevant departments [$N = 19$ (38.8%)]. The overall rating of the support provided in the GQNS was good (median grade of 2 for work of the coordination bureau, as well as usefulness and usability of quality reports).

DISCUSSION

The GQNS is a quality collaborative network using claims data and a complex risk adjustment to measure and improve the acute care quality for sepsis patients. Because of this pragmatic approach, 74 hospitals participated in the start-up period of the network. This evaluation study compared the development of risk-adjusted hospital mortality in cases with sepsis between the GQNS and the German national DRG-statistics in a controlled time series analysis. It did not show an effect of participation in the GQNS.

The failure to achieve substantial improvement might be caused by specific flaws in the approach taken by the GQNS. First, the GQNS only measured outcome quality in the form of risk-adjusted sepsis mortality, which alone does not give detailed insights into concrete possibly underlying care deficiencies (27). Former successful quality initiatives on sepsis also used process quality indicators – primarily compliance to sepsis

TABLE 3 | Results of interrupted time-series analysis in subgroups of participating hospitals.

Subgroups	Number of hospitals	Slope before intervention (95% CI)	Slope during intervention (95% CI)	*P*-value of test of difference in slopes	Change in level (95% CI)	*P*-value
Participating through complete intervention period	45	0.133 (0.03, 0.236)	−0.018 (−0.12, 0.085)	0.042	−1.133 (−3.405, 1.138)	0.328
Participating through complete intervention period and early implementation of quality management[a]	8	−0.089 (−0.345, 0.167)	−0.035 (−0.29, 0.22)	0.771	2.841 (−2.784, 8.466)	0.323
Not participating through complete intervention period	29	−0.076 (−0.193, 0.041)	0.165 (−0.085, 0.415)	0.084	−1.67 (−5.525, 2.184)	0.396
Number of beds ≤700	40	0.017 (−0.117, 0.152)	0.02 (−0.153, 0.194)	0.98	−0.997 (−4.468, 2.474)	0.573
Number of beds >700	34	−0.015 (−0.073, 0.042)	0.047 (−0.033, 0.127)	0.21	−0.285 (−1.828, 1.257)	0.717

Results of piecewise hierarchical models on the difference in the risk-standardized mortality rate (RSMR) in patients with sepsis between GQNS hospitals and the national German diagnosis-related-groups statistic considering different subgroups. Slopes give the linear trajectory of RSMR-difference in % per month across time before and after start of the intervention, change in level gives the change at the time of the beginning of the intervention. Time of beginning of the intervention is defined for each individual hospital as the time of supply of the first quality report. [a]Early implementation of quality management was defined based on the survey of local quality improvement leaders of participating hospitals in autumn of 2016, if the implementation of a quality improvement team as well as analyses of quality reports was reported. Survey data were available for 28 of 45 hospitals (62%).

TABLE 4 | Results of survey of the local quality improvement leaders of participating hospitals.

Items of the survey	Descriptive statistics for answers (*N* = 49 participants)
Implementation of quality improvement measures	
Usage of quality reports	
None received yet/unknown	6 (12.2%)
Not used yet	7 (14.3%)
Quality indicators analyzed	14 (28.6%)
Quality indicators and individual cases analyzed	22 (44.9%)
Existence of a quality improvement team	
No	33 (67.3%)
Yes, but not interdisciplinary	8 (16.3%)
Yes, interprofessional and interdisciplinary	8 (16.3%)
Staff education on ICU	
No or unknown	7 (14.3%)
Partly implemented	25 (51%)
Fully implemented	17 (34.7%)
Staff education in emergency department	
No or unknown	23 (46.9%)
Partly implemented	15 (30.6%)
Fully implemented	11 (22.4%)
Staff education on normal wards[a]	
No or unknown	25 (52.1%)
Partly implemented	19 (39.6%)
Fully implemented	4 (8.3%)
Implementation of screening tools	
Not implemented	19 (38.8%)
Implemented on ICU	8 (16.3%)
Implemented in at least one other department	19 (38.8%)
Implemented on ICU, normal wards, and emergency department	3 (6.1%)
Existence of medical emergency team	
Not planned	24 (49%)
Planned	17 (34.7%)
Existing	8 (16.3%)
Barriers to implementation of quality improvement	
Importance of GQNS for the hospital	
No importance	14 (28.6%)
One among many quality improvement measures	17 (34.7%)
Important in some departments	13 (26.5%)
Important for the complete hospital	5 (10.2%)
Lack of time of quality improvement team	38 (77.6%)
General staff shortage	29 (59.2%)
Lacking participation of relevant departments	19 (38.8%)
Tribal thinking of departments	12 (24.5%)
Lacking decision making power of responsible team	10 (20.4%)
Lacking support by management	8 (16.3%)
Lacking awareness of the need for quality improvement	5 (10.2%)
Strict management-hierarchy	4 (8.2%)
Rating of the support by the GQNS	
Grade for the work of the GQNS coordination bureau (1–6)	2 (1, 2)
Grade for usefulness of quality reports (1–6)	2 (1, 2)
Grade for usability of quality reports (1–6)	2 (2, 2)

Descriptive statistics given as N (%) and median (first quartile, third quartile). The survey was conducted among the local quality improvement leaders of participating hospitals in autumn of 2018 after the end of the intervention phase, one person per hospital was surveyed, since some local champions were responsible for more than 1 hospital, 69 participants were invited of which, 49 (71%) took part in the survey.
[a]One participant did not provide information on this item.

management bundles like timeliness of adequate antimicrobial therapy (9, 28–31). Additionally, benchmarking indicators of structural quality – like availability of in-house microbiological or standard operating procedures on antimicrobial treatment – could inform hospitals to implement concrete improvements. Second, the GQNS relied on only using administrative claims data. This approach has high feasibility and low costs, but lacks the information necessary to define process quality indicators. Above that, identification of cases based on ICD-coding in administrative data can be impaired by a misclassification bias (32). Several studies reported low sensitivity for coding of sepsis (33, 34). Misclassification also explains the high observed sepsis-related mortality of more than 40%, since studies have shown that patients with higher risk of death have a higher probability of having an explicit sepsis code in administrative data (34, 35). Also risk factors for mortality – like comorbidities – have been shown to be subject to misclassification (36). The low validity of the data might have impaired the usefulness of the quality reports to identify possible deficiencies of care and opportunities for improvement (12). Automated surveillance may overcome these deficits to track sepsis rates and outcomes based on electronic health records (37), but cannot currently be used among the majority of German hospitals due to the lack of implementation of electronic health records. Third, the only mandatory elements of the intervention were reporting, benchmarking, and publication of quality indicators. Hospitals were advised to form interdisciplinary quality improvement teams to establish a continuous quality improvement based on the analysis of the data provided in the quality reports, case analysis, and peer-reviews. This approach might not have been sufficient to achieve substantial changes, since hospitals have repeatedly been shown to have major deficiencies in organizational and professional capacity to adequately learn and improve based on quality measurement (10, 38, 39), Therefore, implementing a core set of well-defined interlinked improvement measures, like hospital-wide staff education on early recognition and treatment, regular screenings on wards and in emergency departments, and medical emergency teams, in all participating hospitals using a well-structured implementation strategy, could be more successful (9, 28, 29, 40).

The major reason for the failure of the GQNS to achieve a reduction of sepsis-related mortality can be seen in the lack of implementation of measures for quality improvement by the majority of hospitals. Local quality improvement leaders reported high barriers to effective quality management – most importantly, lack of time and resources for quality improvement activities, as well as failure to generate hospital-wide improvement efforts due to general staff shortage and lack of involvement of all relevant departments and stakeholders. Similar reasons had been identified for the failure of the cluster-randomized controlled MEDUSA trial, which comprised 40 German hospitals and aimed to improve sepsis care by the establishment of change teams and prospective documentation and reporting of indicators of process and outcome quality, and staff education (10, 11). Likewise, the only published successful quality initiative on sepsis in Germany, by which an absolute reduction of mortality of 19% was achieved, received financing and full support by the hospital's management board, which facilitated the hospital-wide role out of this program and the involvement of the crucial stakeholders (31).

The failure to replicate such successes in multicenter initiatives like the GQNS and MEDUSA point to the limitations of voluntary quality initiatives, which may often not be able to achieve adequate priority among hospital management boards and department leaderships. Sepsis-specific mandatory quality improvement indicators and tools have been implemented on the national and regional levels in several countries and were associated with decreased sepsis-related mortality (30, 37, 40, 41). Care processes for patients with sepsis are also affected by more general tools for quality assurance and patient safety – such as rapid response systems, nation-wide education of health care workers in early warning scores for deteriorating patients, and the effective use of critical incidence reporting systems. These are mostly standard in other high-income countries like the United Kingdom, Australia or in part the United States, but are poorly adopted in Germany (42–46). German authorities and regulatory bodies in health care should follow these examples, become fully aware of the existing severe deficits in sepsis prevention and care, and take the necessary actions. An essential step would be the inclusion of indicators on the quality of acute sepsis care to the mandated quality assurance system for hospitals in Germany (47). These indicators should include aspects of structural quality – like regular education of all clinical staff on early detection and treatment of sepsis, aspects of process quality – like implementation of a standardized screening for patients at risk (40), and documentation of adequacy of implementation of guideline elements (9, 31), as well as outcome quality – like risk-adjusted mortality and morbidity of survivors (48).

Strengths and Limitations

The evaluation study of GQNS has several strengths. Because of its controlled interrupted time series design, it has higher internal validity compared to most previous evaluation studies on sepsis-related quality initiatives, which only used before-after comparisons (9). In addition, a diverse sample representing the full spectrum of German acute care hospitals was included, which permits generalizing conclusions to the German health care system. The evaluation study also has limitations. It was based on claims data and might therefore be biased by changes in coding practices among participating hospitals across time. Although new clinical sepsis definitions ("sepsis-3") were introduced in 2016 (1), the ICD-coding of sepsis relied on the old sepsis-1 definitions until the end of 2019 in Germany, which might influence the generalization of the results of this study. The national DRG-statistics, which were used as control condition, also included the data of the hospitals participating in the GQNS. This reduced the effect size of possible differences between GQNS and the national statistics, but we believe this bias to be small, since the GQNS-hospitals represent only 6% of all German hospitals. Process evaluation was only based on yearly standardized surveys of local quality improvement leaders and not all hospitals provided this data. A more frequent assessment and report of the implementation progress could have helped to motivate stakeholders of participating hospitals to increase their efforts. The duration of the intervention phase of roughly 2 years might have been too short to result in observable changes (49). We were only able to conduct six peer reviews during the intervention phase of the GQNS, since the number of qualified peers was limited and finding appointments was complicated due to the busy schedules of involved clinicians. To overcome this problem, education of peer reviewers was established in 2020 within the GQNS. The first publication of the main quality indicators occurred in the summer of 2018, at the end of the intervention phase, and only by the 11 hospitals, which were obligated to do so since they had signed their contract for participation in 2015. It is unclear if a broader early implementation of these core elements of the intervention would have resulted in greater success.

CONCLUSION

Participation in this voluntary quality initiative did not result in a reduction of sepsis-related hospital mortality. Major barriers to quality improvement were lack of time and resources for quality improvement teams, general staff shortage, and a failure to involve all relevant stakeholders and departments in the quality improvement process. Voluntary quality initiatives may not be able to achieve adequate priority among pertinent stakeholders among hospital board and department leadership. Therefore, sepsis needs to become part of the mandated external quality assurance for all German hospitals to end preventable suffering from sepsis and reduce the burden for the German health care system.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because by contract with the participating hospitals of the GQNS, the research team is not allowed to publish data, which would make individual hospitals identifiable by third parties. Requests to access the datasets should be directed to DS, Daniel.Schwarzkopf@med.uni-jena.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Internal Review Board of the Jena University Hospital. Written informed consent from the participants or their legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

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AUTHOR CONTRIBUTIONS

KR conceptualized the study design, acquired the funding for the study, and supervised the conduction of the study. DS and HR contributed to the coordination of the GQNS, the conduction of the interventions, and the acquisition of the reported data. DS conceptualized the quality reporting, conducted the analyses, and drafted the manuscript. CF-S, and DT-R contributed to the development of the methods for the quality reporting. AB, CG, MGl, MGr, PM, MP, and TS were involved in the supervision of the conduction of the GQNS as members of the steering-committee. KR, HR, AB, CF-S, DT-R, CG, MF, MGl, MGr, PM, MP, and TS contributed to the interpretation of the data and critically revised the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.882340/full#supplementary-material

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Association of Blood Glucose Level and Glycemic Variability With Mortality in Sepsis Patients During ICU Hospitalization

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Background: There was considerable debate regarding the effect of mean blood glucose (MBG) and glycemic variability (GV) on the mortality of septic patients. This retrospective cohort study aimed to assess the association between MBG and GV with ICU mortality of sepsis patients and to explore the optimal MBG range.

Methods: Sepsis patients were enrolled from the Medical Information Mart for Intensive Care IV database (MIMIC-IV). MBG and glycemic coefficient of variation (Glu_{CV}) were, respectively, calculated to represent the overall glycemic status and GV during ICU stay. The associations between MBG, Glu_{CV}, and ICU mortality of the septic patients were assessed by using multivariate logistic regression in different subgroups and the severity of sepsis. Restricted cubic splines evaluated the optimal MBG target.

Results: A total of 7,104 adult sepsis patients were included. The multivariate logistic regression results showed that increased MBG and Glu_{CV} were significantly correlated with ICU mortality. The adjusted odds ratios were 1.14 (95% CI 1.09–1.20) and 1.05 (95% CI 1.00–1.12). However, there was no association between hyperglycemia and ICU mortality among diabetes, liver disease, immunosuppression, and hypoglycemia patients. And the impact of high Glu_{CV} on ICU mortality was not observed in those with diabetes, immunosuppression, liver disease, and non-septic shock. The ICU mortality risk of severe hyperglycemia (≧200 mg/dl) and high Glu_{CV} (>31.429%), respectively, elevated 2.30, 3.15, 3.06, and 2.37, 2.79, 3.14-folds in mild (SOFA ≦ 3), middle (SOFA 3–7), and severe group (SOFA ≧ 7). The MBG level was associated with the lowest risk of ICU mortality and hypoglycemia between 120 and 140 mg/dl in the subgroup without diabetes. For the diabetic subset, the incidence of hypoglycemia was significantly reduced when the MBG was 140–190 mg/dl, but a glycemic control target effectively reducing ICU mortality was not observed.

Conclusion: MBG and Glu_{CV} during the ICU stay were associated with all-cause ICU mortality in sepsis patients; however, their harms are not apparent in some particular

subgroups. The impact of hyperglycemia and high GV on death increased with the severity of sepsis. The risk of ICU mortality and hypoglycemia in those with no pre-existing diabetes was lower when maintaining the MBG in the range of 120–140 mg/dl.

Keywords: sepsis, glucose metabolism disorders, mortality, restricted cubic splines regression, glycemic control

INTRODUCTION

Sepsis, defined as organ dysfunction caused by a dysregulated host response to infection by the 2021 Surviving Sepsis Campaign (SSC) Guideline, is associated with high mortality and rapidly became a significant global health burden (1, 2). The glycometabolism disorder is highly prevalent in critically ill patients, especially those with sepsis (3). The activation of stress induces this disturbance, typically manifested as hyperglycemia and increased glycemic variability (GV) (4). Specifically, under the attack of infections, the overwhelming release of pro-inflammatory mediums results in excessive hepatic gluconeogenesis and peripheral insulin resistance during sepsis (5). Catecholamines and cortisol, released by the adrenal cortex through the activated hypothalamic-pituitary-adrenocortical axis, also play significant roles (6).

The unified blood glucose (BG) management protocols for sepsis patients has not been established, even though much research has been conducted to clarify the specific mechanisms of the glycometabolism disorder. At present, the controversy on glycemic management in patients with sepsis mainly focuses on two aspects. First, the influence of elevated BG has not been fully elucidated. Previous literature has examined the impact of hyperglycemia on poor prognosis in different critically ill patients, such as those with myocardial infarction (7), acute pancreatitis (8), and stroke (9). However, these connections are not consistent across sepsis patients. Many trials have reported that hyperglycemia is associated with increased short-term mortality of sepsis patients (10–12), but neutral even lower mortality risks have also been found (13–16). These seemingly opposite phenomena suggested complex non-linear relationships between the hyperglycemic effect and the prognosis in sepsis patients (17). Although diabetic condition may be associated with the apparent inconsistencies, it was unreasonable to consider it as a specific interpretation. This was because the influence of hyperglycemia also differs in sepsis patients combined with diabetes (11, 13), and it suggested that other disease states likely also play a role. Second, the interaction between overall BG and GV levels was not clear. Although Magee et al. and Chao et al. have respectively demonstrated that early fluctuation disorder in BG increased 30-day mortality and all-cause hospital mortality in sepsis patients (18, 19), the majority of sepsis patients experienced a relapse of the disease. Thus, the overall GV levels during ICU hospitalization seem more relevant to septic prognosis than early BG fluctuation. Third, the optimal BG target is not yet confirmed. Several multicenter studies have disproved the protective effect of traditional intensive glucose control in sepsis patients, such as VISEP and NICE-SUGAR (20, 21). Furthermore, the 2021 SSC Guidelines recommend initiating insulin therapy when the glucose level ≧180 mg/dl and maintenance ranges from 144 to 180 mg/dl (1). Nevertheless, this recommendation draws on the American Diabetes Association Standards of Medical Care in Diabetes Guideline, specific to the entire critically ill population (22). Few studies have focused on the optimal target of BG control, and thus further investigations are necessary considering the heterogeneity of septic patients.

Therefore, we performed a retrospective cohort study based on an extensive, publicly available database called Medical Information Mart for Intensive Care IV (MIMIC-IV). Our primary aim was to examine the association of overall BG and GV levels during ICU admission with all-cause ICU mortality in sepsis patients. The secondary aim of this study was to investigate the optimal range of BG in patients with sepsis and each subgroup. We hypothesized that the influence of BG and GV in different subgroups of sepsis patients on ICU mortality might differ, and the ideal glucose range might also be different across sepsis subgroups.

METHODS

Study Population and Data Extraction

The Massachusetts Institute of Technology established the MIMIC-IV (1.0 version) database, which contained the medical records of 382,278 in-patients who received care at the Beth Israel Deaconess Medical Center between 2008 and 2019 (23). The latter is one of the preeminent academic medical and referral centers in the Boston area, in which 77 critical care beds are contained. Users can screen demographic characteristics, vital signs, laboratory test results, imaging examinations of each patient by using a unique code given during admission. Lu has completed the Collaborative Institutional Training Initiative program course (Certification number 36763801). Because the MIMIC-IV database is a publicly available anonymized database, approval from the ethical committee was not necessary.

In the present study, we extracted patients' parameters, including (1) demographic features (age, gender), type of care unit, body mass index (BMI); (2) neutrophil to lymphocyte ratio (NLR), white blood cell count (WBC), Sequential Organ Failure Assessment (SOFA) score, Acute Physiology Score III (APS III), Charlson Comorbidity Index within the first 24 h after ICU admission; (3) anamnesis (diabetes, immunosuppression, myocardial infarct, congestive heart failure, peripheral vascular disease, liver disease, renal disease, cerebrovascular disease, and chronic obstructive pulmonary disease), infection site; (4) mean

Abbreviations: SSC, Surviving Sepsis Campaign; GV, glycemic variability; MBG, mean blood glucose; MIMIC-IV, Medical Information Mart for Intensive Care IV; NLR, neutrophil to lymphocyte ratio; WBC, white blood cell count; SOFA, Sequential Organ Failure Assessment; APS III, Acute Physiology Score III; MV,mechanical ventilation; RRT, renal replacement therapy; ICD-9, International Classification of Diseases, Ninth Revision; ICD-10, International Classification of Diseases, Tenth Revision; RCS, Restricted0cubic0splines; ROC, receiver operating characteristic.

BG and glucose variability during ICU stay; (5) the use of mechanical ventilation (MV), renal replacement therapy (RRT), norepinephrine and insulin during ICU stay; (6) incidence of septic shock and hypoglycemia during ICU stay; (7) the length of ICU stay, 7-day mortality, 28-day mortality, ICU mortality of all patients. Immunosuppression was defined as having any of the following major immune diseases: lymphoma, acquired immune deficiency syndrome, solid metastatic tumor, malignant tumor, or autoimmune diseases. All related diseases were identified by the International Classification of Diseases, Ninth Revision (ICD-9), combined with Tenth Revision (ICD-10) diagnosis codes when the patient is discharged.

All adult sepsis patients (≧18 years) were screened for analysis. We excluded patients who stayed <48 h in the ICU to avoid inaccurate valuation of the condition of glycemic fluctuations. Furthermore, patients were also excluded if they had missing daily BG records. In this study, the diagnosis of sepsis was based on the criteria of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), which define sepsis as SOFA ≧2 and the presence of infection or suspected infection (24). Suspected infection refers to antibiotics administered within 3 days or before 24 h of culture collection. It is difficult to implement the procedure which is strictly based on the Sepsis-3 standard to screen septic shock patients in the MIMIC-IV database, and thus we draw on previous experience in this study (25). Septic shock was defined as sepsis with hypotension, and the hypotension was assumed for sepsis patients when any vasopressor was administered during the ICU stay, including norepinephrine, epinephrine, phenylephrine, vasopressin, and dopamine dobutamine or milrinone. For patients with multiple ICU and hospital admissions, we only included data from the first hospital admission and first ICU stay. The flowchart is shown in **Supplementary Figure 1**.

Glucose Measurement and Glycemic Variability Definition

For each included patient, we have calculated the mean BG (MBG) during ICU stay using all biochemical glucose records. MBG were stratified as follows: no hyperglycemia (≦140 mg/dl), mild hyperglycemia (140–200 mg/dl), and severe hyperglycemia (≧200 mg/dl) based on previous work (26). We defined hypoglycemia as at least one glucose record <70 mg/dl during ICU stay. Here, we considered glucose≦140 mg/dl as a reference value to which each category is compared. In this analysis, the overall GV was evaluated by calculating the coefficient of variation (Glu_{CV}), which is the ratio of the standard deviation (Glu_{CD}) to the glycemic average. Due to the lack of universally accepted clinical criteria for grading the Glu_{CV} status of critically ill patients, we grouped Glu_{CV} into three categories according to the percentiles (low: <25th; mild: 25–75th; high: >75th).

Restricted Cubic Splines

Linear regression was often used to identify the relationship between independent and dependent variables in clinical trials, but this linear relationship was not always easy to meet and particularly likely to occur when the independent variable was continuous. We usually transformed continuous variables into categorical variables based on some special cutoff points to explore the unknown non-linear relationship. However, this approach may change the shape of the dose-response relationship and induce inevitable information loss. Restricted cubic splines (RCS) analysis as a smoothness function is well-fit to non-linear relationships and retains independent local structure. Recently, RCS was widely used to assess the dose-response relationship between continuous variables and dependent variables (27, 28). RCS can be seen as a piecewise polynomial, it requires a continuous second-order derivative existing in each segmented spot (29). The main operation of RCS is that the setting of the knots count and position is required before its use and it may have an influence on the overall structure. With the reference from the previous study (28), we used RCS with five knots, corresponding to the 5, 35, 50, 65, and 95th percentiles, to explore the relationship between MBG with all-cause ICU mortality in sepsis patients. The reference was set at 140 mg/dl.

Statistical Analysis

The present retrospective study of the collected observational data set was stratified according to MBG and Glu_{CV}. We performed a normality test (*Agostino tests*), followed by a descriptive analysis of the data. Continuous variables were expressed as mean (standard deviation) while non-parametric variables were expressed as the median (interquartile ranges, IQR) and were compared using the *one-way ANOVA test* or *non-parametric Kruskal-Wallis test*. The categorical variables are expressed as a frequency (percentage) and were compared using the X^2 or *rank-sum tests*. The random forests function handled missing values. However, the variable was deleted when >30% of the values were lacking. Outlier expressions were defined as values that are greater than the 99th or lower than the 1st percentile. Variables with outliers were winsorized using the *winsor2* command in STATA software.

Multivariate logistic regression was performed to determine the connection between MBG, Glu_{CV}, and ICU mortality of sepsis patients separately. MBG was modeled as both continuous and categorical scale; while the MBG category set cutoffs on 140 and 200 mg/dl, and the Glu_{CV} set on the $_{first}$ and third quartile. The potential confounders were adjusted gradually in three models. Initially, we adjusted for age and gender (Model 1). Subsequently, related comorbidities, such as diabetes and immunosuppression, have been adjusted (Model 2). Finally, we adjusted for NLR- related early disease severity scores (APS III, SOFA, and Charlson Comorbidity Index), MBG/Glu_{CV}, occurrence or not of septic shock and hypoglycemia, and related interventions including the use of MV, RRT, and insulin except norepinephrine during ICU stay (Model 3).

In the subgroup analyses, we stratified the study population by age (≧65, <65 years), gender (male, female), diabetes, immunosuppression, liver disease, hypoglycemia, and septic shock. The interaction of the levels of MBG and Glu_{CV} with the above covariates for stratification of ICU mortality was examined by including two-factor interaction terms in the multivariate logistic regression model. Meanwhile, the interactions were visualized by the slopes of the regression lines.

To evaluate the performance of MBG and Glu_{CV} in predicting ICU mortality in sepsis patients, we conducted receiver operating

TABLE 1 | Baseline demographic and clinical characteristics by mean glucose level and glycemic variability in patients with sepsis.

Features	Mean blood glucose level during ICU hospitalization			*p*-value	Glycemic variability during ICU hospitalization			*p*-value
	Glucose <=140	Glucose 140 to <200	Glucose >= 200		GlUcv < 15.174	GLUcv 15.174 to <= 31.429	GlUcv > 31.429	
	N = 4,407	*N* = 2,029	*N* = 668		*N* = 1,776	*N* = 3,552	*N* = 1,776	
Demographic features								
Age (years)	68.0 (54.9, 80.5)	69.8 (58.8, 79.8)	68.8 (59.6, 78.1)	0.001	68.5 (55.5, 80.7)	68.5 (56.4, 80.1)	68.8 (57.4, 79.6)	0.9
Gender				0.289				<0.001
Female	2,010 (45.6%)	920 (45.3%)	283 (42.4%)		720 (40.5%)	1,670 (47.0%)	823 (46.3%)	
Male	2,397 (54.4%)	1,109 (54.7%)	385 (57.6%)		1,056 (59.5%)	1,882 (53.0%)	953 (53.7%)	
Care unit type				<0.001				<0.001
Cardiac vascular intensive care unit	568 (12.9%)	171 (8.43%)	13 (1.95%)		267 (15.0%)	339 (9.54%)	146 (8.22%)	
Coronary care unit	267 (6.06%)	184 (9.07%)	72 (10.8%)		114 (6.42%)	254 (7.15%)	155 (8.73%)	
Medical intensive care unit	1,403 (31.8%)	647 (31.9%)	267 (40.0%)		507 (28.5%)	1,180 (33.2%)	630 (35.5%)	
Medical/surgical intensive care unit	964 (21.9%)	411 (20.3%)	166 (24.9%)		364 (20.5%)	765 (21.5%)	412 (23.2%)	
Neuro intermediate	112 (2.54%)	38 (1.87%)	11 (1.65%)		55 (3.10%)	81 (2.28%)	25 (1.41%)	
Neuro stepdown	60 (1.36%)	7 (0.34%)	5 (0.75%)		23 (1.30%)	43 (1.21%)	6 (0.34%)	
Neuro surgical intensive care unit	70 (1.59%)	42 (2.07%)	11 (1.65%)		41 (2.31%)	64 (1.80%)	18 (1.01%)	
Surgical intensive care unit	559 (12.7%)	316 (15.6%)	78 (11.7%)		228 (12.8%)	482 (13.6%)	243 (13.7%)	
Trauma SICU	404 (9.17%)	213 (10.5%)	45 (6.74%)		177 (9.97%)	344 (9.68%)	141 (7.94%)	
Inflammatory indicators#								
WBC_max (X 10^3/uL)	13.6 (9.50, 19.0)	15.1 (10.7, 20.6)	15.0 (10.9, 20.3)	<0.001	13.4 (9.60, 18.3)	14.2 (9.80, 19.6)	15.1 (10.6, 21.1)	<0.001
NLR	8.73 (5.80, 14.4)	9.46 (6.33, 15.9)	9.71 (6.90, 16.4)	<0.001	8.20 (5.55, 12.9)	9.13 (6.13, 15.3)	9.77 (6.61, 16.7)	<0.001
Severe scoring								
SOFA	3.00 (2.00, 5.00)	3.00 (2.00, 5.00)	3.00 (2.00, 4.00)	0.231	3.00 (2.00, 4.00)	3.00 (2.00, 5.00)	3.00 (2.00, 5.00)	<0.001
APSIII	54.0 (39.0, 73.0)	61.0 (47.0, 82.0)	65.0 (52.0, 83.0)	<0.001	49.0 (36.0, 63.0)	58.0 (43.0, 77.0)	67.0 (50.0, 87.0)	<0.001
Charlson comorbidity index	6.00 (4.00, 8.00)	6.00 (5.00, 8.00)	7.00 (5.00, 9.00)	<0.001	5.00 (4.00, 7.25)	6.00 (4.00, 8.00)	6.00 (5.00, 8.00)	<0.001
Comorbidity								
Diabetes	708 (16.1%)	996 (49.1%)	536 (80.2%)	<0.001	324 (18.2%)	992 (27.9%)	924 (52.0%)	<0.001
Septic shock	1,761 (40.0%)	868 (42.8%)	238 (35.6%)	0.003	624 (35.1%)	1,465 (41.2%)	778 (43.8%)	<0.001
Immunosuppression	839 (19.0%)	373 (18.4%)	124 (18.6%)	0.811	323 (18.2%)	707 (19.9%)	306 (17.2%)	0.046
Myocardial infarct	598 (13.6%)	374 (18.4%)	174 (26.0%)	<0.001	234 (13.2%)	554 (15.6%)	358 (20.2%)	<0.001
Congestive heart failure	1,279 (29.0%)	728 (35.9%)	263 (39.4%)	<0.001	503 (28.3%)	1,139 (32.1%)	628 (35.4%)	<0.001
Peripheral vascular disease	455 (10.3%)	214 (10.5%)	84 (12.6%)	0.211	167 (9.40%)	362 (10.2%)	224 (12.6%)	0.004
Cerebrovascular disease	612 (13.9%)	330 (16.3%)	122 (18.3%)	0.002	299 (16.8%)	517 (14.6%)	248 (14.0%)	0.034
COPD	1,215 (27.6%)	576 (28.4%)	187 (28.0%)	0.79	444 (25.0%)	1,052 (29.6%)	482 (27.1%)	0.001
Renal disease	899 (20.4%)	568 (28.0%)	224 (33.5%)	<0.001	353 (19.9%)	794 (22.4%)	544 (30.6%)	<0.001
Liver disease	808 (18.3%)	398 (19.6%)	119 (17.8%)	0.398	281 (15.8%)	686 (19.3%)	358 (20.2%)	0.001
Infection site								
Skin subcutaneous tissue	334 (7.58%)	173 (8.53%)	63 (9.43%)	0.16	137 (7.71%)	286 (8.05%)	147 (8.28%)	0.823

(Continued)

TABLE 1 | Continued

Features	Mean blood glucose level during ICU hospitalization			*p*-value	Glycemic variability during ICU hospitalization			*p*-value
	Glucose <=140	Glucose 140 to <200	Glucose >= 200		GlUcv < 15.174	GLUcv 15.174 to <= 31.429	GlUcv > 31.429	
	N = 4,407	*N* = 2,029	*N* = 668		*N* = 1,776	*N* = 3,552	*N* = 1,776	
Catheter related	88 (2.00%)	56 (2.76%)	16 (2.40%)	0.154	32 (1.80%)	84 (2.36%)	44 (2.48%)	0.325
Urinary tract	874 (19.8%)	359 (17.7%)	150 (22.5%)	0.016	331 (18.6%)	707 (19.9%)	345 (19.4%)	0.545
Intestinal infection	248 (5.63%)	110 (5.42%)	32 (4.79%)	0.667	75 (4.22%)	214 (6.02%)	101 (5.69%)	0.023
Septicemia	1,584 (35.9%)	762 (37.6%)	286 (42.8%)	0.002	516 (29.1%)	1,370 (38.6%)	746 (42.0%)	<0.001
Pulmonary infection	1,468 (33.3%)	706 (34.8%)	210 (31.4%)	0.239	530 (29.8%)	1,282 (36.1%)	572 (32.2%)	<0.001
Curing								
RRT	419 (9.51%)	271 (13.4%)	79 (11.8%)	<0.001	106 (5.97%)	388 (10.9%)	275 (15.5%)	<0.001
MV	2,408 (54.6%)	1,273 (62.7%)	389 (58.2%)	<0.001	814 (45.8%)	2,144 (60.4%)	1,112 (62.6%)	<0.001
MV duration (h)	6.50 (0.00, 45.0)	19.0 (0.00, 80.0)	14.3 (0.00, 61.0)	<0.001	0.00 (0.00, 22.4)	14.0 (0.00, 66.3)	20.0 (0.00, 75.4)	<0.001
Norepinephrine	1,156 (26.2%)	614 (30.3%)	188 (28.1%)	0.003	343 (19.3%)	1,028 (28.9%)	587 (33.1%)	<0.001
Insulin	1,627 (36.9%)	1,597 (78.7%)	643 (96.3%)	<0.001	667 (37.6%)	1,884 (53.0%)	1,316 (74.1%)	<0.001
Outcomes								
Length of ICU stay	3.92 (2.76, 6.64)	4.52 (2.91, 8.65)	3.89 (2.74, 6.87)	<0.001	3.28 (2.55, 5.05)	4.48 (2.93, 8.01)	4.37 (2.86, 7.93)	<0.001
Mortality_hospital	666 (15.1%)	481 (23.7%)	152 (22.8%)	<0.001	223 (12.6%)	652 (18.4%)	424 (23.9%)	<0.001
Mortality_ICU	407 (9.24%)	330 (16.3%)	104 (15.6%)	<0.001	124 (6.98%)	416 (11.7%)	301 (16.9%)	<0.001
Mortality_ICU_7day	262 (5.95%)	205 (10.1%)	68 (10.2%)	<0.001	105 (5.91%)	242 (6.81%)	188 (10.6%)	<0.001
Mortality_ICU_28day	607 (13.8%)	434 (21.4%)	142 (21.3%)	<0.001	209 (11.8%)	587 (16.5%)	387 (21.8%)	<0.001
Hypoglycemia	759 (17.2%)	198 (9.76%)	56 (8.38%)	<0.001	43 (2.42%)	460 (13.0%)	510 (28.7%)	<0.001

Continuous data are presented as the median (interquartile range) and categorical data as n (%).

WBC, white blood cell; NLR, neutrophil to lymphocyte ratio; SOFA, Sequential Organ Failure Assessment; APS III, Acute Physiology Score III; COPD, chronic obstructive pulmonary disease; RRT, renal replacement therapy; MV, mechanical ventilation.

[#]*Inflammatory indicators use the maximum value in the first 24 h after ICU admission.*

TABLE 2 | Odds ratio for death in ICU according to the mean glucose levels and glycemic variability on a continuous scale or in tertile groups.

	ICU mortality	*N*	Event	Crude OR (95% CI)	P^a	a.OR (95% CI) Model 1	P^b	a.OR (95% CI) Model 2	P^c	a.OR (95% CI) Model 3	P^d
MBG	Glucose (per 20 mg/dl)	7,104	841	**1.12 (1.08, 1.15)**	**<0.001**	**1.12 (1.08, 1.15)**	**<0.001**	**1.17 (1.13, 1.21)**	**<0.001**	**1.14 (1.09, 1.20)**	**<0.001**
	Glucose (mg/dl)										
	Glucose <=140	4,407	407	Ref		Ref		Ref		Ref	
	Glucose >140 and <200	2,029	330	**1.91 (1.63,2.23)**	**<0.001**	**1.88 (1.61, 2.19)**	**<0.001**	**2.16 (1.82, 2.55)**	**<0.001**	**1.97 (1.61, 2.41)**	**<0.001**
	Glucose >=200	668	104	**1.81 (1.43, 2.28)**	**<0.001**	**1.80 (1.42, 2.16)**	**<0.001**	**2.39 (1.83, 3.10)**	**<0.001**	**2.23 (1.64, 3.03)**	**<0.001**
GLUcv	GLUcv (per 10%)	7,104	841	**1.18 (1.13, 1.23)**	**<0.001**	**1.18 (1.13, 1.23)**	**<0.001**	**1.21 (1.16, 1.26)**	**<0.001**	**1.05 (1.00, 1.12)**	**0.05**
	GLUcv %; (quartile: 25%, 75%)										
	GLUcv < 15.174	1,776	124	Ref		Ref		Ref		Ref	
	GLUcv >=15.174 and <= 31.429	3,552	416	**1.77 (1.44, 2.19)**	**<0.001**	**1.76 (1.43, 2.18)**	**<0.001**	**1.76 (1.43, 2.19)**	**<0.001**	1.02 (0.81, 1.29)	0.86
	GLUcv >= 31.429	1,776	301	**2.72 (2.19, 3.40)**	**<0.001**	**2.71 (2.18, 3.39)**	**<0.001**	**2.98 (2.37, 3.76)**	**<0.001**	**1.36 (1.04, 1.77)**	**0.03**

Model 1: adjusted for age and gender.

Model 2: adjusted for age, gender and comorbidity.

Model 3: adjusted for age, gender, comorbidity, SOFA, APS III, Charlson Comorbidity Index, MBG/Glu_{CV}, septic shock or not, mechanical ventilation, renal replacement therapy, insulin infusion, NLR, and hypoglycemic event.

aOR and corresponding 95% CIs are presented in bold when the 95% CI cross 1.

[a]*P for crude analysis.*

[b]*P for analysis based on Model 1.*

[c]*P for analysis based on Model 2.*

[d]*P for analysis based on Model 3.*

characteristic (ROC) curves. We also conducted the dose-response association using the RCS model with five knots located at the 5, 35, 50, 65, and 95th percentiles of the overall distribution for MBG levels based on the multivariate logistic regression model. The exact number and location of knots from the overall population splines were also applied in the splines for each subgroup to allow direct comparison of the overall and stratified analyses. All statistical analyses were performed using STATA 15.1 (College Station, Texas) and R 3.6.2 (Chicago, Illinois) software. The *p*-values with < 0.05 were taken as statistically significant (two-sided).

RESULTS

Characteristics of Included Sepsis Participants

In the MIMIC-IV database, a total of 12,274 patients were diagnosed with sepsis at their first ICU admission according to the definition of sepsis 3.0; ultimately, 7,104 patients were included in the analysis; 2,661 patients lacked the height data. Thus, all pre-defined features were included except the BMI index. During the whole ICU stay, the minimum and maximum values of MBG were 81.33 and 294.78 mg/dl, respectively; in addition, the minimum Glu_{CV} was 4.22 %, and the maximum Glu_{CV} was 84.76%. The incidence of septic shock was 40.36% (2,867/7,104), insulin treatment was 54.43% (3,867/7,104), hypoglycemia was 14.26% (1,013/7,104), diabetes was 31.53% (2,240/7,104), and liver disease was 18.65% (1,325/7,104). Among the included septic patients, 841 (11.84%) died during the ICU stay. The MBG of patients who died was significantly elevated compared with survivors [128 (112–155) vs. 142 (119–173); $p < 0.001$]. The dead group also showed significantly increased Glu_{CV} [21.5 (14.8–30.5) vs. 26.4 (18.4–37.1); $p < 0.001$]. The distribution of MBG, Glu_{CV} within the two cohorts is shown in **Supplementary Figure 2**.

The clinical characteristics based on MBG and Glu_{CV} categories can be found in **Table 1**. An upward trend was observed at higher MBG levels for initial NLR value, APS III scoring, and the prevalence of diabetes, myocardial infarct, congestive heart failure, peripheral vascular disease, cerebrovascular disease, and renal disease. Similarly, as MBG levels increased, elevated risk of urinary tract infection and septicemia was also presented; but higher MBG levels were not positively correlated with poor prognosis in sepsis patients. For different Glu_{CV} categories, the initial inflammatory markers (WBC, NLR) were higher in individuals with higher Glu_{CV}, and the incidence of diabetes, septic shock, myocardial infarct, congestive heart failure, renal disease, and related treatments (RRT, MV, norepinephrine, and insulin infusion) was also elevated. Unlike the MBG levels, there was a positive association between Glu_{CV} and the risk of poor outcomes.

Association Between MBG, Glu_{CV}, and ICU Mortality

On a continuous scale, the results of multivariable logistic regression showed that every 20 mg/dl or 10% rise in MBG and

TABLE 3 | Results of subgroup analyses of mean blood glucose level and ICU mortality according to clinical characteristics.

Subgroup	*N*	Event	a.OR (95%CI)	Subgroup	*N*	Event	a.OR (95%CI)	*P* for interaction
Age < 65				**Age >= 65**				0.98
Glucose <= 140	1,923	157	1.00 (1.00, 1.00)	Glucose <= 140	2,484	250	1.00 (1.00, 1.00)	
140 < Glucose < 200	763	111	1.99 (1.42, 2.81)	140 < Glucose < 200	1,266	219	1.98 (1.54, 2.55)	
Glucose >=200	259	33	2.32 (1.35, 3.93)	Glucose >=200	409	71	2.22 (1.52, 3.24)	
Male				**Female**				0.88
Glucose <= 140	2,397	221	1.00 (1.00, 1.00)	Glucose <= 140	2,010	186	1.00 (1.00, 1.00)	
140 < Glucose < 200	1,109	158	1.83 (1.37, 2.44)	140 < Glucose < 200	920	172	2.16 (1.63, 2.86)	
Glucose >=200	385	65	2.77 (1.83, 4.19)	Glucose >=200	283	39	1.67 (1.04, 2.66)	
Diabetes				**Non-Diabetes**				<0.001
Glucose <= 140	708	66	1.00 (1.00, 1.00)	Glucose <= 140	3,699	341	1.00 (1.00, 1.00)	
140 < Glucose < 200	996	114	1.04 (0.70, 1.55)	140 < Glucose < 200	1,033	216	2.44 (1.94, 3.06)	
Glucose >=200	536	66	1.21 (0.77, 1.93)	Glucose >=200	132	38	3.52 (2.18, 5.60)	
Immunosuppression				**Non-Immunosuppression**				0.83
Glucose <= 140	839	114	1.00 (1.00, 1.00)	Glucose <= 140	3,568	293	1.00 (1.00, 1.00)	
140 < Glucose < 200	373	79	1.73 (1.13, 2.63)	140 < Glucose < 200	1,656	251	2.15 (1.71, 2.71)	
Glucose >=200	124	27	1.83 (0.97, 3.42)	Glucose >=200	544	77	2.64 (1.85, 3.76)	
Liver disease				**Non-Liver disease**				0.002
Glucose <= 140	808	137	1.00 (1.00, 1.00)	Glucose <= 140	3,599	270	1.00 (1.00, 1.00)	
140 < Glucose < 200	398	70	1.15 (0.74, 1.79)	140 < Glucose < 200	1,631	260	2.36 (1.88, 2.96)	
Glucose >=200	119	17	1.15 (0.56, 2.32)	Glucose >=200	549	87	2.85 (2.01, 4.01)	
Hypoglycemia				**Non-Hypoglycemia**				0.001
Glucose <= 140	759	141	1.00 (1.00, 1.00)	Glucose <= 140	3,648	266	1.00 (1.00, 1.00)	
140 < Glucose < 200	198	48	1.33 (0.81, 2.18)	140 < Glucose < 200	1,831	282	2.22 (1.78, 2.77)	
Glucose >=200	56	11	0.91 (0.39, 2.04)	Glucose >=200	612	93	2.78 (1.98, 3.88)	
Septic-shock				**Non-Septic shock**				0.25
Glucose <= 140	1,761	186	1.00 (1.00, 1.00)	Glucose <= 140	2,646	221	1.00 (1.00, 1.00)	
140 < Glucose < 200	868	176	2.31 (1.73, 3.09)	140 < Glucose < 200	1,161	154	1.69 (1.27, 2.23)	
Glucose >=200	238	50	2.79 (1.77, 4.37)	Glucose >=200	430	54	1.84 (1.20, 2.79)	

Adjustment factors are the same as those in Model 3.

Glu_{CV} was, respectively, associated with 1.14-fold (95% CI 1.09–1.20) and 1.05-fold (95% CI 1.00–1.12) increase in the risk of ICU mortality (**Table 2**, Model 3). As described previously, we divided the patients into three tertiles according to their MBG levels. Compared with MBG levels ≦140 mg/dl, septic patients with MBG levels between 140 and 200 mg/dl and ≧200 mg/dl had an increased risk of ICU mortality, the aORs were 1.97 (95% CI 1.61–2.41) and 2.23 (95% CI 1.64–3.03), respectively (**Table 2**, Model 3). Similarly, the 25 and 75th percentiles of Glu_{CV} were used as the cutoff values to subdivide patients with sepsis into three risk categories. Mortality among patients in the lowest category of Glu_{CV} was 6.98%, increasing to 11.7 and 16.9% in the median and highest category (**Table 1**). The patients with Glu_{CV} ≧ 31.429% had a 0.36 (95% CI 0.04–0.77) higher risk of ICU mortality than those with Glu_{CV} < 15.174% (**Table 2**, Model 3).

A subgroup analyses indicated that the effect of hyperglycemia on ICU mortality is more pronounced in non-diabetic, non-immunosuppression, non-liver disease, non-hypoglycemia, and septic shock patients. Interestingly, different levels of hyperglycemia did not seem to have obvious adverse impacts on the risk of ICU mortality in patients with diabetes or liver disease. Additionally, a significant interaction effect was found between diabetes ($p < 0.001$), hypoglycemia ($p = 0.001$), liver disease ($p = 0.002$), and MBG levels (**Table 3**). **Supplementary Figures 3–5** visually depicted these interactions, respectively. We observed that the ICU mortality risk among non-diabetics was consistently higher than among people with diabetes at the same level as hyperglycemia (**Supplementary Figure 3**). Meanwhile, increased MBG had a weak impact on ICU mortality risk for patients who experienced at least one episode of hypoglycemia (**Supplementary Figure 4**). One of the possible reasons is that the influence of hypoglycemia may mask the effect of hyperglycemia on death. And this phenomenon also occurred in the liver disease cohort (**Supplementary Figure 5**). The impact of Glu_{CV} on different subgroups varied greatly. Despite the ICU mortality risk appearing incremental with increasing Glu_{CV}, the difference was only significant in non-elderly, males, non-diabetics, non-immunosuppression, non-hypoglycemia, non-liver disease, septic shock, and patients not treated with insulin (**Table 4**). Furthermore, a significant interaction between age and Glu_{CV} was observed ($p = 0.02$) (**Supplementary Figure 6**).

In this study, we also subdivided the severity of sepsis according to the initial SOFA score. Those with SOFA scores ≦ 3 (25th) and ≧ 7 (75th) were correspondingly assigned to the mild group and severe group, while the 3–7 were defined as the middle

TABLE 4 | Results of subgroup analyses of Glu_{CV} level and ICU mortality according to clinical characteristics.

Subgroup	*N*	Event	a.OR (95%CI)	Subgroup	*N*	Event	a.OR (95%CI)	*P* for interaction
Age < 65				**Age>= 65**				0.02
Glu_{CV} <15.174	741	34	1.00 (1.00, 1.00)	Glu_{CV} <15.174	1,035	90	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	1,480	152	1.40 (0.93, 2.16)	15.174 <= Glu_{CV} <=31.429	2,072	264	0.85 (0.64, 1.13)	
Glu_{CV} >31.429	724	115	2.21 (1.39, 3.57)	Glu_{CV} >31.429	1,052	186	1.06 (0.76, 1.47)	
Male				**Female**				0.95
Glu_{CV} <15.174	1,056	79	1.00 (1.00, 1.00)	Glu_{CV} <15.174	720	45	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	1,882	201	0.92 (0.68, 1.26)	15.174 <= Glu_{CV} <=31.429	1,670	215	1.22 (0.85, 1.78)	
Glu_{CV} >31.429	953	164	1.45 (1.02, 2.06)	Glu_{CV} >31.429	823	137	1.35 (0.89, 2.05)	
Diabetes				**Non-Diabetes**				0.4
Glu_{CV} <15.174	324	23	1.00 (1.00, 1.00)	Glu_{CV} <15.174	1,452	101	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	992	103	0.92 (0.55, 1.58)	15.174 <= Glu_{CV} <=31.429	2,560	313	1.03 (0.80, 1.35)	
Glu_{CV} >31.429	924	120	0.78 (0.46, 1.39)	Glu_{CV} >31.429	852	181	1.75 (1.29, 2.39)	
Immunosuppression				**Non-Immunosuppression**				0.97
Glu_{CV} <15.174	323	32	1.00 (1.00, 1.00)	Glu_{CV} <15.174	1,453	92	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	707	119	1.17 (0.74, 1.90)	15.174 <= Glu_{CV} <=31.429	2,845	297	0.99 (0.76, 1.30)	
Glu_{CV} >31.429	306	69	1.23 (0.70, 2.18)	Glu_{CV} >31.429	1470	232	1.42 (1.05, 1.93)	
Liver disease				**Non-Liver disease**				0.74
Glu_{CV} <15.174	281	25	1.00 (1.00, 1.00)	Glu_{CV} <15.174	1,495	99	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	686	118	1.03 (0.60, 1.80)	15.174 <= Glu_{CV} <=31.429	2,866	298	1.02 (0.79, 1.33)	
Glu_{CV} >31.429	358	81	1.12 (0.61, 2.10)	Glu_{CV} >31.429	1,418	220	1.43 (1.06, 1.93)	
Hypoglycemia				**Non-Hypoglycemia**				0.66
Glu_{CV} <15.174	43	5	1.00 (1.00, 1.00)	Glu_{CV} <15.174	1,733	119	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	460	92	1.29 (0.46, 4.33)	15.174 <= Glu_{CV} <=31.429	3,092	324	0.97 (0.77, 1.25)	
Glu_{CV} >31.429	510	103	1.29 (0.45, 4.37)	Glu_{CV} >31.429	1,266	198	1.42 (1.07, 1.88)	
Septic-shock				**Non-Septic shock**				0.28
Glu_{CV} <15.174	624	45	1.00 (1.00, 1.00)	Glu_{CV} <15.174	1,152	79	1.00 (1.00, 1.00)	
15.174 <= Glu_{CV} <=31.429	1,465	204	1.22 (0.84, 1.79)	15.174 <= Glu_{CV} <=31.429	2,087	212	0.92 (0.68, 1.25)	
Glu_{CV} >31.429	778	163	1.58 (1.05, 2.41)	Glu_{CV} >31.429	998	138	1.24 (0.87, 1.77)	

Adjustment factors are the same as those in Model 3.

group. The results demonstrated that the impact of MBG on death increased with the severity of sepsis; besides, hyperglycemia was independently associated with increased ICU mortality in each group (**Supplementary Figure 7A**). In addition, the same trends were also found for the relationship between septic severity and Glu_{CV} (**Supplementary Figure 7B**).

After adjustment for confounders contained in Model 3, among the subjects with non-hyperglycemia, increased Glu_{CV} did not associate with an increased risk of ICU mortality (mild Glu_{CV}: aOR 0.94, 95% CI 0.71–1.25; high Glu_{CV}: aOR 1.33, 95% CI 0.92–1.93). Among the patients with hyperglycemia, the risk of ICU mortality significantly increases regardless of higher Glu_{CV}. Notably, adjusted odds of death were markedly higher in patients with MBG above 200 mg/dl and lower Glu_{CV} values (aOR 3.51, 95% CI 1.23–8.58). In the patients without pre-existing diabetes, mild and severe hyperglycemia were also associated with increased mortality when in combination with various levels of Glu_{CV}, that is, mild hyperglycemia plus low Glu_{CV} level (aOR 2.44, 95% CI 1.37–4.20), mild hyperglycemia plus mild Glu_{CV} level (aOR 2.29, 95% CI 1.61–3.28), mild hyperglycemia plus high Glu_{CV} level (OR 2.6, 95% CI 1.77–3.83), high hyperglycemia plus low Glu_{CV} level (aOR 6.25, 95% CI 1.06–30.66), high hyperglycemia plus mild Glu_{CV} level (aOR 3.24, 95% CI 1.34–7.51), and high hyperglycemia plus high Glu_{CV} level (aOR 3.46, 95% CI 1.86–6.29). By contrast, in combination with any Glu_{CV} levels, hyperglycemia was not associated with increased mortality in diabetes patients (**Figure 1**).

The area under the curve (AUC) of MBG, Glu_{CV}, and the combination of two indicators for predicting ICU mortality of all sepsis patients were 0.59, 0.61, and, 0.62, respectively. Three indicators significantly improved risk discrimination in non-diabetics with the AUC increasing from 0.54 to 0.64, 0.55 to 0.64, and 0.56 to 0.66 for the MBG, Glu_{CV}, and combination, respectively, compared with those in the people with diabetes. Nevertheless, the overall predictive performance was only moderate (**Supplementary Figure 8**, **Supplementary Table 1**).

Mean Glucose With the Lowest Risk of ICU Mortality

The results of RCS after multivariable adjustment presented a non-linear dose-response relationship between the levels of MBG on a continuous scale and the risk of ICU mortality. The concentration of MBG associated with the lowest risk of ICU mortality was ∼120 mg/dl in the overall population. The

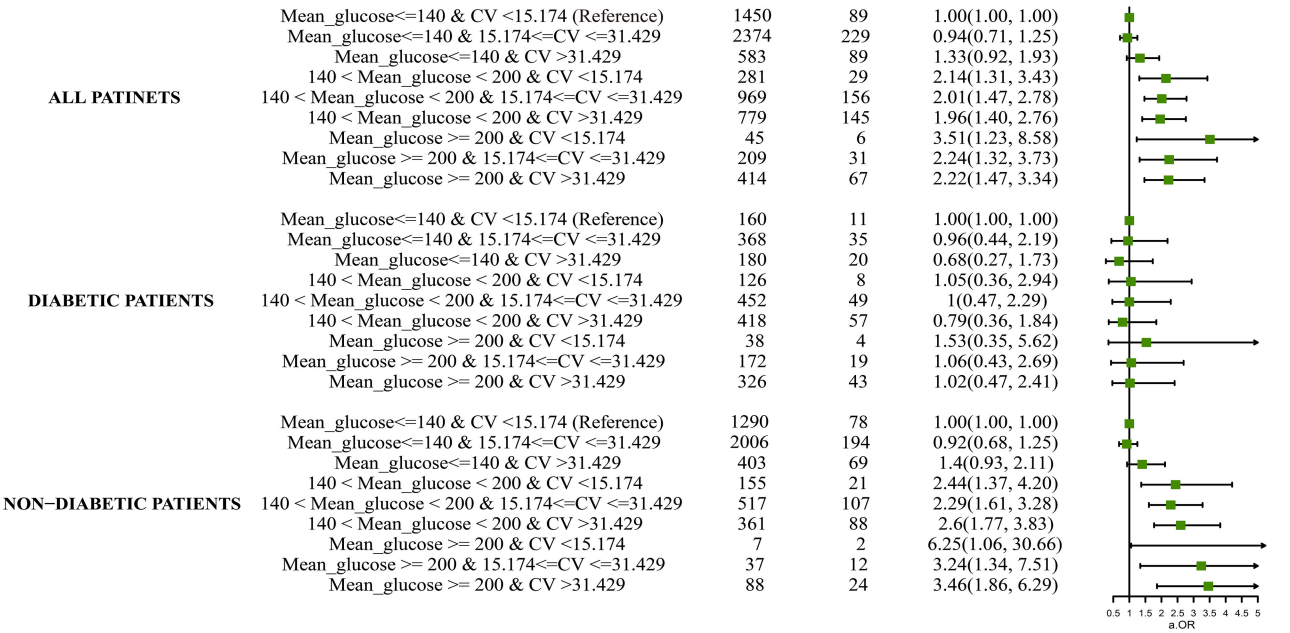

FIGURE 1 | Forest plot depicting ICU mortality risk in septic patients with and without diabetes. Adjustment factors are the same as those in Model 3.

value of aOR has an initial steep increase when MBG is lower than 120 mg/dl or ranges from 120–200 mg/dl, then plateaued. Similarly, the risk reached a minimum when the concentrations of MBG were around 120 mg/dl in non-diabetes patients, and up perpetually with MBG increasing. There was a trend for decreasing the risk of ICU mortality when MBG was between 140 and 190 mg/dl for people with diabetes, but it did not reach statistical significance (**Figure 2**).

Considering the risk of hypoglycemia, we further compared the incidence of hypoglycemia and death in sepsis patients when containing MBG below 120 mg/dl and in the range of 120–140 mg/dl. The results showed that the hazard of hypoglycemia for patients who maintained MBG lower than 120 mg/dl was significantly greater than the rate for those who maintained MBG between 120 and 140 mg/dl (22.8 vs. 9.35%, $p < 0.001$), while there was no significant difference in ICU mortality (8.57 vs. 10.2%, $p = 0.08$). Within non-diabetics, the risk of hypoglycemia was significantly reduced when MBG was between 120 and 140 mg/dl compared to the MBG level below 120 mg/dl (22.8 vs. 9.35%, $p < 0.001$). And there was no difference in ICU mortality (8.5 vs. 10.3%, $p = 0.069$). For diabetics, patients with 140–190 mg/dl of MBG had a lower hypoglycemic event rate than those who maintained MBG below 140 mg/dl (26 vs. 13%, $p < 0.001$). In addition, no statistical difference was observed between the two divided groups in terms of ICU mortality (9.25 vs. 10.2%, $p = 0.589$).

DISCUSSION

This study demonstrated the association between MBG, Glu_{CV} during ICU stay, and the increased ICU mortality of septic patients. For the entire cohort, the MBG levels of 140–200 mg/dl, ≧ 200 mg/dl induced a 1.97- and 2.23-fold higher risk of ICU mortality, respectively; and Glu_{CV} of ≦ 31.429% connected with 1.36-fold higher risk. Nevertheless, we found that the effect of MBG and Glu_{CV} on ICU mortality differed among different subgroups. The unfavorable influence of hyperglycemia was more pronounced in non-diabetic, non-immunosuppression, non-liver disease, and non-hypoglycemia patients. And the impact of high Glu_{CV} was more significant in non-elderly, males, non-diabetic, non-immunosuppression, non-liver disease, non-hypoglycemia, and septic shock patients. Furthermore, the impact of hyperglycemia and high Glu_{CV} on death increased with the severity of sepsis. Our results also indicated that the optimal MBG target of sepsis patients without diabetes during ICU stay was 120–140 mg/dl. In diabetic patients, the incidence of hypoglycemia was significantly reduced when the MBG level was set between 140 and 190 mg/dl. A trend of decreased ICU mortality was observed in this BG range, but statistical differences were not reached.

High GV during ICU stay has a solid and consistent relation with adverse prognosis in critically ill patients (30–32). However, there was no consensus regarding the effect of GV on mortality in septic patients. In this study, we calculated the Glu_{CV} using all available biochemical BG records, reflecting the overall intervention status. The results identified that death presented a higher Glu_{CV} than the surviving patients, and a high Glu_{CV} level (>31.43%) was independently associated with an increased risk of ICU mortality among septic patients. In line with our findings, a recent study demonstrated that the rise of the mean amplitude of glycemic excursions and Glu_{CV} within the 1st day of ICU admission was related to increased risk of 30-day mortality

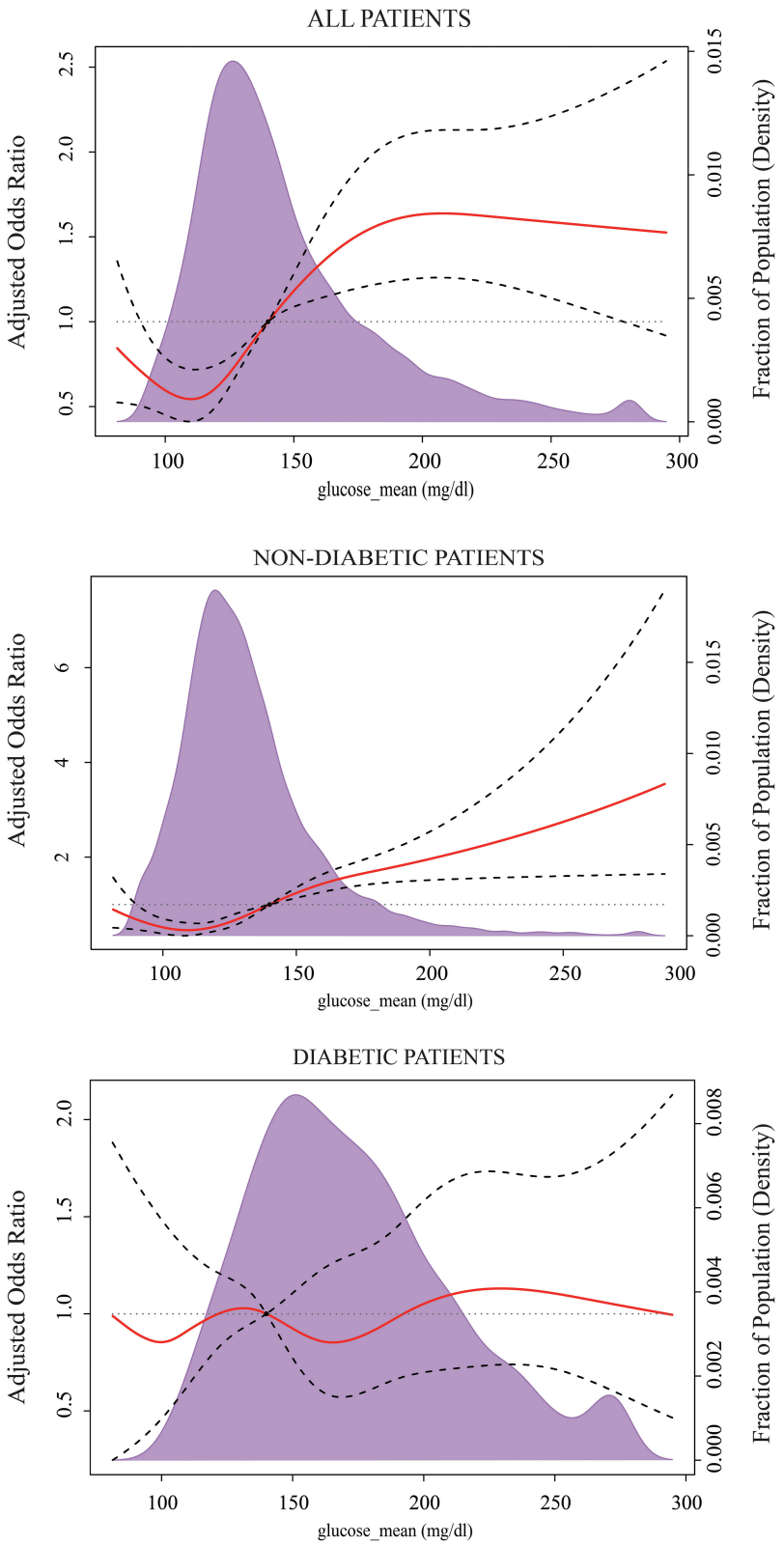

FIGURE 2 | Multivariable-adjusted odds ratios for ICU mortality according to the levels of the mean blood glucose (MBG) on a continuous scale. Solid red lines are multivariable-adjusted odds ratios, with dashed bold lines showing 95% confidence intervals derived from restricted cubic spline regressions with five knots. Reference lines for no association are indicated by the black dashed lines at a hazard ratio of 1.0, and the reference knot set at 140 mg/dl. Purple regions indicate the fraction of the population with different levels of MBG. Adjustment factors are the same as those in Model 3 of **Table 2**.

in septic patients; in contrast, these relations do not exist in those with diabetes (33). In addition, Ali et al. also reported that GV was an important factor connected with hospital mortality using all biochemical and capillary glucose values for the entire hospitalization (19). Unfortunately, they did not further probe whether GV may vary across different populations.

Of course, the divergent results among such trials may not just depend on the presence of diabetes (30–33). Our study also found that the influence of high Glu_{CV} on ICU mortality was attenuated in the elderly, females, or patients with immunosuppression and hypoglycemia. Although the mechanisms underlying these phenomena are unclear, two reasons could explain this discrepancy. First, a higher incidence of cardiovascular disease, diabetes, and the use of related medications increased as the individuals aged, which changed the natural process of GV and obscured their adverse effects. Second, the risk of hypoglycemia induced by increased GV masked the association between GV and mortality of septic patients. Septic patients are especially vulnerable to hypoglycemia, and the occurrence risk is proportional to the viscera injury severity (34). Previous trials have proved the interaction between hypoglycemia and GV in intensive and non-intensive patients (35–37). The present study similarly showed that the probability of hypoglycemic occurrence rises with increases in Glu_{CV}.

The debate surrounding the effect of hyperglycemia on septic patients has been ongoing for more than 10 years. In some studies, hyperglycemia has been argued as an adaptive response under a stress state and plays a protective role in reducing the mortality of septic patients (15, 16). Due to the small number of samples included in these two trials, the stability of this conclusion may be questioned. In contrast, a large multicenter cohort study that contained 7,754 emergency department patients with sepsis demonstrated that high initial BG (>200 mg/dl) was significantly related to increased mortality in non-diabetic patients, but not in those with diabetes (13). In addition, Zohar et al. reported that BG over 200 mg/dl at admission resulted in a 1.48-fold increase in in-hospital mortality, 1.8-fold increase in 30-day mortality, and 1.68-fold increase in 90-day mortality of septic patients (10). However, they claimed that the harm of hyperglycemia was more robust in diabetic patients than in those without diabetes. Although diabetic patients have a greater chance of suffering chronic hyperglycemia, most published papers support that increased BG may not be harmful in septic patients with diabetes (13, 15, 38). Similarly, in this study, we did not observe any relevance between hyperglycemia and the ICU mortality risk of diabetic patients after adjustment in demographic characteristics, other comorbidities, and illness severity. Furthermore, the interaction test also proved that patients without diabetes had a higher risk of mortality in the same MBG range than diabetic patients. Interestingly, our results found that other comorbidities and pathological states, such as immunosuppression, hypoglycemia, liver disease, and septic shock, maybe also affecting the effect of hyperglycemia on the outcomes in septic patients (**Table 2**). However, no existing study targeted this particular population.

Different diseases may require a different optimal range of BG levels to achieve a better prognosis, and it will impact subsequent medical strategy and interventions (39, 40). However, in patients with sepsis, a firm consensus on optimal BG level is not available. The latest 2021 SSC Guideline recommended that BG should be kept in the range of 144–180 mg/dl for sepsis patients (1), and this recommendation was based on the results of a multicenter RCT (NICE-SUGAR) (21). The NICE-SUGAR study randomized 6,104 critically ill patients to either an intensive glycemic control group with BG of 81–108 mg/dl or a conventional glycemic control group in which insulin was administered if the BG level exceeded 180 mg/dl, and then maintained BG in the range of 144–180 mg/dl. The results presented that the patients in the intensive glycemic control group had lower 90-day mortality (27.5 vs. 24.9%, $P = 0.02$). Nevertheless, it is important to note that there was no statistically significant difference in the all-cause mortality between the two groups in the severe sepsis subgroup (OR 1.13, 95% CI 0.89–1.44). Furthermore, the definition of sepsis has undergone a dramatic change in the past 10 years. Thus, these differences limited the application of this recommendation in clinical practice.

The management protocol of BG in septic patients was usually developed according to the local conditions and experiences of the physicians. The optimum glycemic management needs to consider both the survival benefit and the risk of hypoglycemia. This study found that overall, the patients achieved relatively low mortality and hypoglycemic risk when keeping MBG in the range of 120–140 mg/dl; this range was equally applied to those without diabetes. For the diabetic subset, this study did not find an effective MBG interval that could significantly decrease ICU mortality. Nevertheless, we suggest that diabetes patients maintain MBG between 140 and 190 mg/dl to avoid hypoglycemia. A few published papers have explored the optimal level of BG control in septic patients. In 2019, Wang et al. found that the MBG at admission between 145 and 155 mg/dl was associated with the lowest hospital mortality both in the sepsis patients with and without diabetes based on a dose-response meta-analysis (17). The discrepancies between the two studies were on account of different BG measurements. Considering the effect of subsequent interventions, the MBG during the ICU stay was usually lower than MBG at admission.

Strengths and Limitations

The strengths of our study were that it was a large cohort that assessed the relationship between MBG, Glu_{CV}, and ICU mortality in sepsis patients and each subgroup. In addition, we used RCS to explore the optimal MBG range of sepsis patients in ICU stay. Although residual confounding cannot be completely removed, detailed adjustment for potential confounders about patients themselves and subsequent therapies limited the degree of confounding as far as possible.

Despite these strengths, this study has several limitations. First, we were unable to quantify the timing of each BG measurement, such as fasting or non-fasting in this real-world observational study. Thus, each BG record in this study should be regarded as a random BG. Second, the recent BG control of included patients cannot be accurately reflected due to the lack of complete HbA1c records in the MIMIC-IV database. However, chronic hyperglycemia is strongly associated with the risk of

death in critically ill patients (41). Therefore, there could be bias affecting the influence of hyperglycemia in diabetic patients, especially in those with better glycemic control. Third, numerous medications used in the ICU patients and the routes of nutrition are associated with blood glucose metabolism. Nevertheless, this study aimed to determine whether there was a difference in the association of the overall BG and GluCV levels with the prognosis of sepsis patients in the context of the above measures and the reasons for the discrepancy. Furthermore, these related interventions recorded in the MIMIC-IV database were reasonable and recognized. Fourth, this study was a single-center, retrospective cohort study. Our findings need to be validated by an external population.

Clinical Implications and Future Perspectives

The most salient finding of this study is the evidence for differences in the effects of BG and GV in various septic subgroups, and the reason for this discrepancy is not simply due to the diabetes states. Age, gender, immunosuppression, liver disease, septic shock, and the hypoglycemic event also play an essential role in associating overall BG and GV with ICU mortality in sepsis patients. The current investigation findings have important implications for the development of a reasonable medical strategy and individualized treatment. On the other hand, our results suggest that the glycemic management of septic patients during the acute phase should be assessed individually rather than a "one size fits all" approach.

Moreover, this study questions the plausibility of the latest published 2021 SSC Guideline, which recommends a glycemic target range of 140–180 mg/dl for septic or septic shock patients (1). Given the risk of mortality and hypoglycemia, the optimal range of BG should be different between diabetic and non-diabetic patients. The occurrence of hyperglycemia (>140 mg/dl) should be avoided as much as possible for those without diabetes. In contrast, for diabetic patients, the BG should be maintained at a relatively high level to reduce the risk of hypoglycemia.

Although the mechanisms behind these phenomena are currently unknown, this research has provided further explorations some enlightenment. The enlightenment were listed below, as presented in 1.,2.,3.,4:

1. It is necessary to consider BG and GV levels together when implementing glycemic management in septic patients.
2. Future studies should focus on investigating the glycometabolism disorder among specific subgroups rather than all the septic patients.
3. The optimal glycemic target range of septic patients and related subsets is still controversial. Hence, further studies are warranted to resolve it.
4. Despite some new biomarkers and technologies such as capnography and continuous glucose monitoring systems showing a positive effect on clinical glucose management (42, 43), they did not seem to be widely available in sepsis patients. Further studies and consensus are necessary to standardize blood sample collection frequency and time points during the BG monitoring and management of sepsis patients.

CONCLUSION

This study demonstrated that MBG and Glu_{cv} during the ICU stay were associated with all-cause ICU mortality in sepsis patients. However, the harm of hyperglycemia and high GV was not apparent in some particular subgroups, such as those with diabetes, immunosuppression, liver disease, and documented hypoglycemia. Furthermore, the results presented that the impact of hyperglycemia and high GV on death increased with the severity of sepsis based on the initial SOFA scores. We also found that patients with severe hyperglycemia (≥200 mg/dl) and low GV (<15.174%) during ICU hospitalization always had the highest all-cause ICU mortality of any subsets regardless of having diabetes or not, indicating that persistent hyperglycemia states were a significant risk factor for ICU deaths of sepsis patients. Although the AUC of MBG combined with Glu_{cv} was superior to either of them alone for predicting ICU mortality in sepsis patients, the overall predictive performance was moderate. Finally, the results of the RCS analysis showed that the risk of ICU mortality and hypoglycemia of those with no pre-existing diabetes were lower when maintaining the MBG in the range of 120–140 mg/dl, whereas in sepsis patients with diabetes, the incidence of hypoglycemia significantly reduced when the MBG level was set between 140 and 190 mg/dl, but a glycemic control target effectively reducing the ICU mortality was not observed.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZL, MY, and HZ designed the study. XS, MJ, and YZ extracted the data. ZL, YL, ML, and TH conducted data quality management and statistical analysis and drafted the manuscript. JZ and WX participated in the literature search. GT, MY, HZ, and TH critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.857368/full#supplementary-material

Supplementary Figure 1 | The flowchart of this present study.

Supplementary Figure 2 | The distribution of mean blood glucose (MBG) **(A,B)**, glycemic coefficient of variation (Glu_{CV}) **(A,B)** within survivors and deaths during ICU stay. The orange and green solid lines present the density curve.

Supplementary Figure 3 | The interaction between mean blood glucose (MBG) and diabetes. The abscissa and ordinate, respectively, represent the MBG values and predictive risk probability of ICU mortality by multivariable logistic regression analysis. Gray solid dots indicate the distribution of each included patient. Solid blue lines are multivariable regression lines, with gray regions showing 95% confidence intervals. Adjustment factors are the same as those in Model 3 of **Table 2**.

Supplementary Figure 4 | The interaction between mean blood glucose (MBG) and hypoglycemia. The abscissa and ordinate, respectively, represent the MBG values and predictive risk probability of ICU mortality by multivariable logistic regression analysis. Gray solid dots indicate the distribution of each included patient. Solid blue lines are multivariable regression lines, with gray regions showing 95% confidence intervals. Adjustment factors are the same as those in Model 3 of **Table 2**.

Supplementary Figure 5 | The interaction between mean blood glucose (MBG) and liver disease. The abscissa and ordinate respectively represent the MBG values and predictive risk probability of ICU mortality by multivariable logistic regression analysis. Gray solid dots indicate the distribution of each included patient. Solid blue lines are multivariable regression lines, with gray regions showing 95% confidence intervals. Adjustment factors are the same as those in Model 3 of **Table 2**.

Supplementary Figure 6 | The interaction between the glycemic coefficient of variation (Glu_{CV}) and age. The abscissa and ordinate, respectively, represent the Glu_{CV} values and predictive risk probability of ICU mortality by multivariable logistic regression analysis. Gray solid dots indicate the distribution of each included patient. Solid blue lines are multivariable regression lines, with gray regions showing 95% confidence intervals. Adjustment factors are the same as those in Model 3 of **Table 2**.

Supplementary Figure 7 | The associations between MBG and Glu_{CV} with the ICU mortality of sepsis patients in different severity degrees according to the initial SOFA score.

Supplementary Figure 8 | The ROC curve of the mean blood glucose (MBG), glycemic coefficient of variation (Glu_{CV}), and MBG + Glu_{CV} in all septic patients **(A)**, diabetic patients **(B)**, and non-diabetic patients **(C)**.

Supplementary Table 1 | Comparison of performance of MBG, $Glu_{CV,}$ and MBG combined with Glu_{CV} in predicting the ICU mortality of septic patients.

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CASE REPORT
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Case Report: Disseminated Nocardiosis Caused by *Nocardia vulneris* in a Patient With Macroglobulinemia

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This report describes a case of disseminated nocardiosis, caused by *Nocardia vulneris*, in a 61-year-old man with macroglobulinemia and presenting with repeated fever, cough, shortness of breath, and muscle pain. The isolated *Nocardia* strain was resistant to ciprofloxacin, but susceptible to amikacin, gentamicin, tobramycin, linezolid, trimethoprim-sulfamethoxazole, amoxicillin/clavulanic, moxifloxacin, ceftriaxone, cefotaxim, and imipenem. The patient was started on combined meropenem and doxycycline treatment, followed by trimethoprim-sulfamethoxazole, which was subsequently switched to a combination treatment of linezolid, amikacin, and trimethoprim-sulfamethoxazole. The patient recovered, and his condition remained stable. Although infection by *Nocardia vulneris* is rare, and it is easy to miss detection in clinical practice, clinicians should be aware of the possibility of this infection. In addition, the MIC value of the drug sensitivity test should be ascertained when there is a wide choice of medicines. The current case was treated successfully with linezolid, amikacin, and trimethoprim-sulfamethoxazole. In cases of disseminated nocardiosis, the patient should be treated with antimicrobial therapy for at least 12 months. Furthermore, bacteriological examination and antimicrobial susceptibility testing should be performed regularly.

Keywords: disseminated nocardiosis, *Nocardia vulneris*, macroglobulinemia, mass spectrometry, 16S rRNA, linezolid, trimethoprim-sulfamethoxazole, minocycline

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INTRODUCTION

Nocardia species are soil saprophytes which are widespread in soil or water, and always associated with pulmonary infection. They can cause serious human infections, especially in immunocompromised patients (1, 2). With the wide use of steroids, immunosuppressants, broad-spectrum antibiotics, and the development of organ transplantation treatment, nocardiosis has been increasingly reported in recent years worldwide (3, 4). About 1 to 179 new cases of Nocardia infections are reported in China each year since 2009, and *Nocardia farcinica* was the most commonly isolated species (39.9%) (4). However, disseminated infections caused by *Nocardia vulneris* are rare and could present with different clinical symptoms. In this study, we report a case

of disseminated nocardiosis in a macroglobulinemia patient, with the involvement of lung, brain, blood, and skin, caused by the rarely isolated species *Nocardia vulneris*, which was identified by 16S rRNA sequencing analysis.

CASE DESCRIPTION

On 5 December 2018, a 61-year-old man with a 4-year history of macroglobulinemia, undergoing long-term use of prednisone acetate (15 mg/day) and thalidomide (75 mg/night) was sent to our hospital. At the time of presentation, the patient reported a 1-month history of recurrent cough associated with expectoration, repeated fever, and shortness of breath. Within the last week he developed joint pain, muscle pain in the limbs, and a headache. At presentation, a physical examination confirmed that the patient was conscious, and systemic superficial lymph nodes were not affected. Both lungs sounded clear, with no dry or wet rale. Rhythm of the heart was regular, with no murmurs. The abdomen was soft, with no tenderness and rebound tenderness; spine tenderness/pain was observed, without percussion pain; limb muscle tenderness without rebound tenderness was reported and each joint presented as normal. No obvious pitting edema was observed in either of the lower limbs, pathological character was negative. The patient was admitted right in the middle of the fever break, and his admission examination showed a body temperature of 36.3°C, respiration at 20 times/min, pulse 92 beats/min, pulmonary CT: multiple nodular shadows or lamellar shadows in the left lung, new appearance of multiple lymph nodes in bilateral axilla and mediastinum, and partial lymph node swelling, as shown in **Figure 1A**. Laboratory findings were as follows: blood routine examination: WBC, 3.35×10^9/L; neutrophils, 58.8%; hemoglobin, 115 g/L; blood platelet, 193×10^9/L; urine routine: urine protein, 1+; urine sugar, 1+; urine protein, 633.5 mg/L; immunoglobulin IgA, 0.51 g/L; immunoglobulin IgG, 4.54 g/L; immunoglobulin IgM, 6.15 g/L; D-dimer, 1.16 mg/L; C-reactive protein (CRP), 192.78 mg/L; total protein, 47.5 g/L; albumin, 27.5 g/L; and procalcitonin (PCT), 0.54 ng/ml, as shown in **Table 1**. Levofloxacin was administered for 3 days. On December 7, the patient developed fever with a peak value of 39.5°C. On December 8, the patient retained high fever, with a WBC value of 1.09×10^9/L, and the absolute value of neutrophils was 0.41×10^9/L. Thereafter, the patient discontinued treatment with steroids and immunosuppressants, and treated with recombinant human granulocyte stimulating factor (90 ug/day) to increase white blood cells. In addition, meropenem (1 g/day), combined with doxycycline (100 mg/day), was administered. On December 9, two peanut-sized nodules developed, with a moderate hardness in the scalp. The right temporal area was accompanied by an ulcer and obvious tenderness. Multiple reddish plaque and nodules were scattered over the trunk and limbs, with tenderness, and some erythematous pustules were observed, as shown in **Figures 1G,H**. Gram-positive bacilli (suspected *Nocardia*) were found in the blood culture, and further 16S rRNA gene identification confirmed the isolate as *Nocardia vulneris*. Based on the results of drug sensitivity testing, doxycycline was replaced with trimethoprim-sulfamethoxazole (3 g/day). Subsequently, the sputum smear, bronchial washing fluid and scalp all revealed the presence of *Nocardia*. MRI revealed the presence of a brain abscess and multiple nodular shadows as shown in **Figure 1D**, and the patient's fever peaked at 40°C.

On 12 December, trimethoprim-sulfamethoxazole (3 g/day) treatment, in combination with ceftriaxone (2 g/day), was started. However, the patient's symptoms did not abate. On 14 December, treatment was changed to SMZco combined with linezolid. One day later, the patient's temperature remained unchanged, and according to the drug susceptibility analysis, the MIC values of linezolid, amikacin, and trimethoprim-sulfamethoxazole were confirmed and the treatment was changed to the combination of linezolid, amikacin, and trimethoprim-sulfamethoxazole. On 16 December, there was a noticeable drop in the temperature of the patient, and the PCT and CRP significantly decreased. On 24 December, the patient displayed normal body temperature, with no obvious chills, occasional cough and shortness of breath, with production of phlegm. Skin rash and scalp abscess became narrowed, both lungs sounded clear with a little wet rale. Antibiotics were changed to trimethoprim-sulfamethoxazole combined with linezolid, after which the symptoms gradually improved. Multiple microbial cultures of the blood, phlegm, and pus were negative and the lung CT showed absorption of the lesions **Figure 1B,** MRI showed an increased presence of the multiple nodular shadows as shown in **Figure 1E**. On January 28, the patient was discharged from the hospital, with the instruction to continue long-term oral trimethoprim-sulfamethoxazole combined minocycline for 12 months. Follow-up lung CT **Figure 1C** and brain MRI **Figure 1F** showed that the lesions had improved significantly.

DIAGNOSTIC ASSESSMENT

Bacterial Culture and Identification

A set of blood culture was sent for detection on 7 December, and the blood culture was positive after 35 h. Gram-positive long thin filamentous bacterial group was found in the smear, as shown in **Figures 2A,B**. Pin-sized white colonies could be seen on the transferred blood plate after 24 h. Large, dry, white colonies with obvious smell of dust, which did not embed into the agar, was observed after 72 h **Figures 2E,F**. Sputum and pus samples were inoculated into blood plate and cultured at 35°C for 48 h. No growth of *Nocardia* was observed; however, fine colonies were observed after culture for 72 h. Meanwhile, long filamentous Gram-positive bacterial group was found in sputum samples and scalp pus, and the weak acid-fast staining was positive, as shown in **Figures 2C,D**. The colony was identified as *Nocardia brasiliensis* by mass spectrometry system Bruker (Bruker Company, Bruker MALDI Biotyper TOF, America) and VITEK MS (BioMerieux Company, BioMerieux VITEK MS, China), with scores of 1.718, and 2.0, respectively. However, this isolate was identified as *Nocardia vulneris* by 16S rRNA gene sequencing analysis (GenBank NR148265.1). The universal primers used for sequencing were (27F/1492R; 5′-AGAGTTTGATCCTGGCTCAG-3′/5′-CTACGGCTACCTTGTTACGA-3′), with the cycling

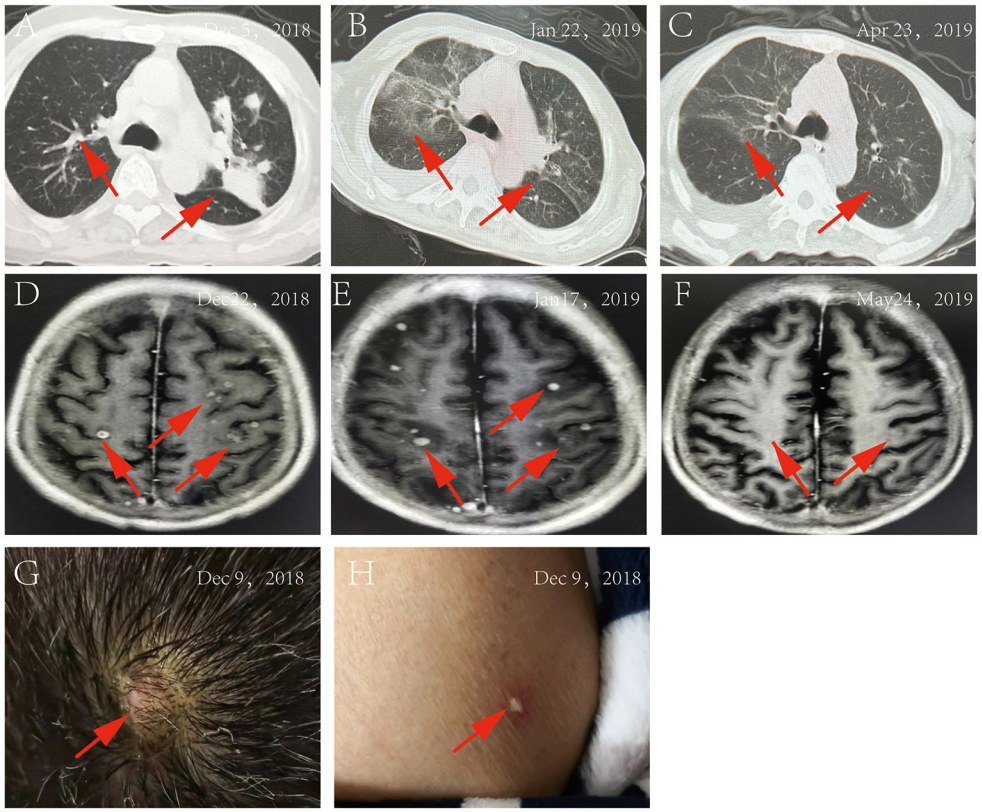

FIGURE 1 | (A) Pulmonary CT on December 5, 2018; **(B)** Pulmonary CT on January 22, 2019; **(C)** Pulmonary CT on April 23, 2019; **(D)** MRI of the brain on December 22, 2018; **(E)** MRI of the brain on January 17, 2019; **(F)** MRI of the brain on May 24, 2019; **(G)** the rash on the patient's head; **(H)** The rash on the patient's body.

TABLE 1 | Clinical information of the patient during hospitalization.

Date	Dec 5, 2018	Jan 22, 2019	April 23, 2019	Reference range
Day after onset	30	48	90	
WBC	3.35 × 10^9/L	3.84 × 10^9/L	2.83 × 10^9/L	3.5 × 10^9/L 9.5 × 10^9/L
Neutrophils	58.8%	67.2%	63.2%	40 70%
Hemoglobin	115 g/L	79 g/L	75 g/L	130 175 g/L
Blood platelet	193 × 10^9/L	93 × 10^9/L	126 × 10^9/L	125 × 10^9/L 350 × 10^9/L
Urine protein	1+	1+	+-	negative
Urine sugar	1+	negative	negative	negative
immunoglobulin IgA	0.51 g/L	/	0.32 g/L	0.7 4.0 g/L
immunoglobulin IgG	4.54 g/L	/	8.37 g/L	7.0 16.0 g/L
immunoglobulin IgM	6.15 g/L	/	10.8 g/L	0.4 2.3 g/L
D-dimer	1.16 mg/L	1.02 mg/L	1.17 mg/L	0 1 mg/L
C-reactive protein	192.78 mg/L	49.43 mg/L	33.21 mg/L	0.06 8.2 mg/L
Total protein	47.5 g/L	56.0 g/L	55.0 g/L	65 85 g/L
Albumin	27.5 g/L	28.2 g/L	31.3 g/L	40 55 g/L
Procalcitonin	0.54 ng/ml	0.32 ng/ml	1.3 ng/ml	<0.5 ng/ml

conditions of initial denaturation 94°C for 5 min, 25 cycles of denaturation 94°C for 30 sec, annealing 57°C for 30 sec, extension 72°C for 90 sec, and a final extension 72°C for 5 min (RuiBo Biotechnology Company, Beijing, China); or used the primer (7F/1540R; 5′-CAGAGTTTGATCCTGGCT-3′/5′-AGGAGGTGATCCAGCCGCA-3′), with the cycling conditions of initial denaturation 94°C for 4 min, 30 cycles of denaturation 94°C for 45 sec, annealing 55°C for 45 sec, extension 72°C

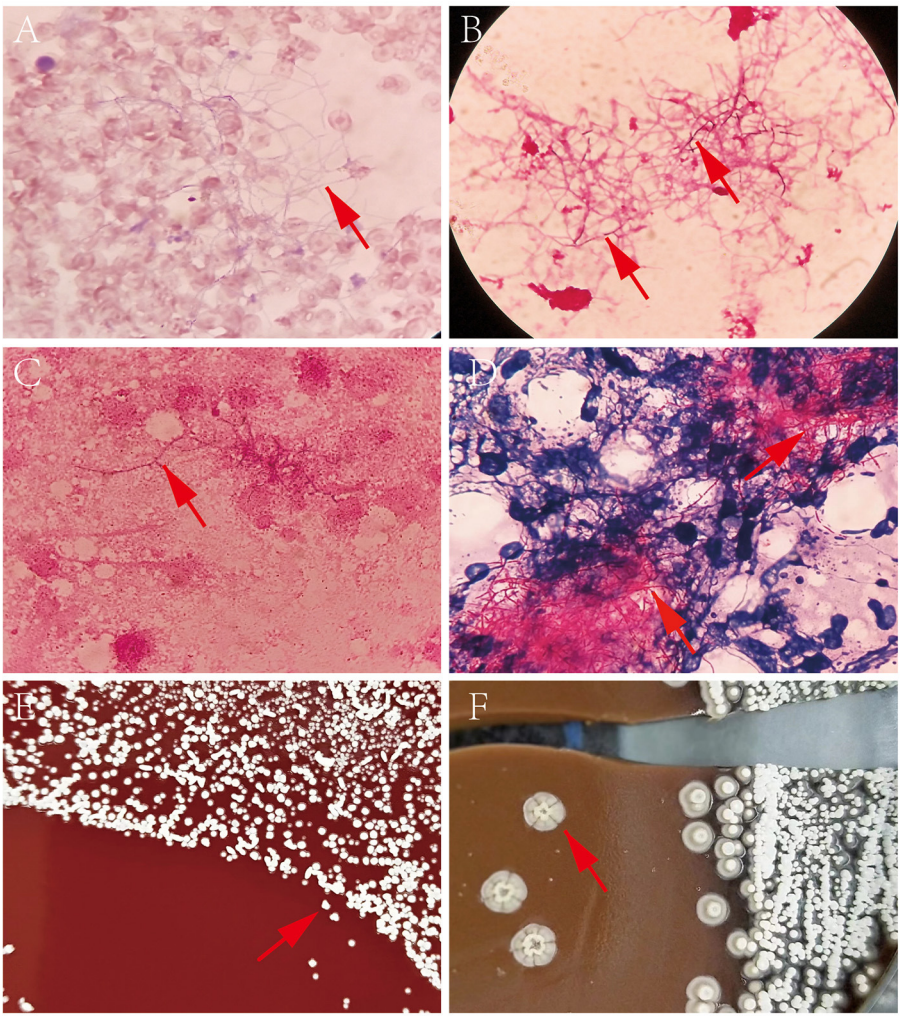

FIGURE 2 | (A) Direct blood culture smear by Wright-giemsa staining (×1,000); **(B)** Direct blood culture smear by Gram staining (×1,000); **(C)** Nocardia vulneris by Gram staining; **(D)** Nocardia vulneris by weak acid fast staining; **(E)** Colonies of Nocardia vulneris cultured for 3 days; **(F)** Colonies of Nocardia vulneris cultured for 15 days.

for 1 min, and a final extension 72°C for 10 min (Sangon Biotechnology Company, Shanghai, China).

Drug Sensitivity Test

The trace broth dilution method was used to determine the sensitivity of the strain to antibiotics, and CLSI M24 was used as the standard for determining drug sensitivity results, as shown in **Table 2**.

DISCUSSION

As a soil saprophyte, *Nocardia* species invades the human body through the respiratory tract, skin, or digestive tract, resulting in the development of nocardiosis. Species of *Nocardia* involved in human pathogenesis include *Nocardia brasiliensis*, *Guinea pig nocardia* and *Picrax nocardia.* Nocardiosis is a life-threatening disease (5) usually found in immunocompromised patients, or populations where it occurs secondary to other diseases. The lung is the most common site of nocardiosis, accounting for about 70–80% (6, 7). Disseminated infections caused by *Nocardia* usually affect immunocompromised patients (8), and typically originate in the lungs, then spread to the brain. However, an individual with normal immune function and disseminated nocardiosis which originated in the lungs and spread to the brain, causing brain abscess, has been reported (9).

Nocardia bacteremia is an extremely severe form of disseminated nocardiosis, the mortality rate may account for approximately 60% (10). However, to the best of our knowledge, *Nocardia vulneris* always manifests as skin and/or subcutaneous tissue infections, and though there are no previous reports on its role in disseminated infections, here, we present a case of disseminated *Nocardia vulneris* infection.

Nocardia do not constitute as a part of the human normal flora; therefore, it can be diagnosed as nocardiosis

TABLE 2 | Susceptibility of *Nocardia vulneris* isolate to different antimicrobials.

Antimicrobials	MIC	Susceptibility
Trimethoprim-sulfamethoxazole	≤0.5/9.5	S
Amoxycillin/clavulanic acid	≤2/1	S
Amikacin	2	S
Gentamicin	≤1	S
Tobramycin	≤1	S
Imipenem	4	S
Linezolid	1	S
Ciprofloxacin	≥8	R
Moxifloxacin	1	S
Ceftriaxone	8	S
Cefotaxim	8	S
Cefepime	16	I
Minocycline	2	I

MIC, minimum inhibitory concentration; S, susceptible; I, intermediate; R, resistant. Susceptibility of the isolate to antimicrobials was defined according to the CLSI M24-A guidelines.

when these organisms are isolated from sputum, alveolar lavage fluid, blood, pus, pleural fluid, subcutaneous tissues, or other samples. In the case reported here, the patient was undergoing immunosuppressant and steroid treatment for macroglobulinemia for 4 years. He was admitted to the hospital suffering from a 1-month history of repeated cough associated with sputum, repeated fever, and shortness of breath. In addition, the patient reported a 1-week history of limb muscle pain, interphalangeal joint pain, and headache. A blood culture was started upon admission, and the aerobic blood culture was positive after 35 h. Gram-positive red filamentous branching bacterial groups were found in the smear. Although visible in the phlegm or alveolar lavage fluid direct smear specimens, stained by wright-giemsa staining and weak acid-fast dye, it is also easily be concealed by other bacteria which grow rapidly, so microscopic examination of the smear is very important in early diagnosis. In addition, *Nocardia* grow slowly, usually taking 72 h to form small colonies, so medical personnel should extend the time of bacteria culture to avoid the problems of misdiagnosis. Of note, small microflora could be seen which came from the positive blood culture, and were transferred to the blood plate for 24 h. This may be the reason that nutrient solution in the blood cultures could neutralize the antibiotics or antibacterial factors which remained in the specimen and made bacteria grow quickly. Because it is time-consuming and difficult for the traditional microbial identification method to identify the species of *Nocardia*, it does not meet the needs of the accurate and rapid clinical identification of pathogenic bacteria. However, mass spectrometry, and other modern molecular biology technologies, especially PCR and gene sequencing, could provide a good foundation for rapid identification of *Nocardia* species. A recent report has shown that 91% of the *Nocardia* species could be accurately identified by the mass spectrometry system VITEK MS (11). In this case, the species identified by mass spectrometry and 16S rRNA gene sequencing was incorrect, possibly because the species *Nocardia vulneris* was not in the library of the mass spectrometry system Bruker (Bruker Company, Bruker MALDI Biotyper TOF, America) and VITEK MS (BioMerieux Company, BioMerieux VITEK MS, China), so the organism was identified as *Nocardia brasiliensis* by Bruker and VITEK MS. This report suggests that 16S rRNA gene sequencing method should be adopted, and the library of the mass spectrometry system should be upgraded frequently or combined with the results of gene sequencing.

Sulfonamides have long been considered the first line of drug treatment in nocardiosis; however, a report regarding the antibiotic susceptibility of *Nocardia* showed that the resistance rate of *Nocardia* against sulfonamides is 57%, and the mortality rate is higher when it is used alone (12). For immunosuppressed patients or patients with disseminated infections, trimethoprim-sulfamethoxazole monotherapy has been changed to amikacin combined with imipenem or linezolid, which is for central nervous system infections or multidrug-resistant bacterial infections (13–15). In this case, as the results of the direct blood culture smear were received that pointed to suspected *Nocardia* infection, the patient was treated by trimethoprim-sulfamethoxazole combined with meropenem for 3 days. Given that the patient still displayed a high fever, the treatment was changed to trimethoprim-sulfamethoxazole combined with ceftriaxone for 2 days, but the patient's symptoms persisted. Following treatment with trimethoprim-sulfamethoxazole combined with linezolid for 1 day, which showed no efficacy, the MIC values of linezolid, amikacin, and trimethoprim-sulfamethoxazole were confirmed and the treatment was changed to the combination of linezolid, amikacin and trimethoprim-sulfamethoxazole due to the severity of the illness. Concomitantly, because the patient had extremely poor renal function, the clinical pharmacists reduced the dosage of amikacin to prevent further renal function damage; the patient's temperature showed a decreasing trend after treatment with trimethoprim-sulfamethoxazole combined with linezolid and amikacin for 1 day. After treatment for 15 days, the patient's infection symptoms had largely resolved. Thereafter, the patient was treated with an oral combination of trimethoprim-sulfamethoxazole and minocycline for 6 months, without any relapse after treatment cessation. The successful treatment of this case fully demonstrates that the MIC value of the drug sensitivity test should be ascertained when there is a wide choice of drugs available. In addition, multidisciplinary cooperation may be warranted and can effectively significantly improve the success rate of treatment. Scott *et al.* reported a case in which retinitis caused by *Nocardia veterana* was successfully treated by a combination of linezolid, amikacin, and meropenem (16). For the cases which are diagnosed, patients should be treated for at least 12 months with antimicrobial therapy. Bacteriological examination and antimicrobial susceptibility testing should be performed regularly. In addition, according to the antimicrobial sensitivity testing, the treatment regimen should be adjusted with the appropriate monitoring, and follow-ups are required to determine whether the patient relapsed after cessation of the treatment.

CONCLUSION

In conclusion, this is the first reported case of disseminated *Nocardia vulneris* infection in a macroglobulinemia patient. Despite the clinical characteristics, diagnostic methods and optimal treatment protocols for *Nocardia vulneris* remain unclear because of its rarity. Our findings provide the information that 16S rRNA gene sequencing method should be adopted, or gene sequencing combined with the results of mass spectrometry, to diagnose *Nocardia vulneris* infection. In addition, we suggest that the MIC value of the drug sensitivity test should be ascertained when there is a wide choice of drugs available, and disseminated cases should be treated with at least 12 months of antimicrobial therapy, and regular bacteriological examination and antimicrobial susceptibility testing.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FQ: data analysis, data interpretation, and writing. ZM: study design, data collection, and data interpretation. RZ: data analysis and data interpretation. H-nH: figures and data collection. YW: pictures. HL: literature search, writing, and funds collection. All authors contributed to the article and approved the submitted version.

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Salivary Alpha Amylase Bronchial Measure for Early Aspiration Pneumonia Diagnosis in Patients Treated With Therapeutic Hypothermia After Out-of-hospital Cardiac Arrest

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Background: Aspiration pneumonia is the most common respiratory complication following out-of-hospital cardiac arrests (OHCA). Alpha-amylase (α-amylase) in pulmonary secretions is a biomarker of interest in detecting inhalation. The main goal of this study is to evaluate the performance of bronchoalveolar levels of α-amylase in early diagnosis of aspiration pneumonia, in patients admitted to intensive care unit (ICU) after OHCA.

Methods: This is a prospective single-center trial, led during 5 years (July 2015 to September 2020). We included patients admitted to ICU after OHCA. A protected specimen bronchial brushing and a mini-bronchoalveolar lavage (mini-BAL) were collected during the first 6 h after admission. Dosage of bronchial α-amylase and standard bacterial analysis were performed. Investigators confirmed pneumonia diagnosis using clinical, radiological, and microbiological criteria. Every patient underwent targeted temperature management.

Results: 88 patients were included. The 34% (30 patients) developed aspiration pneumonia within 5 days following admission. The 55% (17) of pneumonias occurred during the first 48 h. The 57% of the patients received a prophylactic antibiotic treatment on their admission day. ICU mortality was 50%. Median value of bronchial α-amylase did not differ whether patients had aspiration pneumonia (15 [0–94]) or not (3 [0–61], $p = 0,157$). Values were significantly different concerning early-onset pneumonia (within 48 h) [19 (7–297) vs. 3 (0–82), $p = 0,047$]. If one or more microorganisms were detected in the initial mini-BAL, median value of α-amylase was significantly higher [25 (2–230)] than in sterile cultures (2 [0–43], $p = 0,007$). With an 8.5 IU/L cut-point, sensitivity and

specificity of α-amylase value for predicting aspiration pneumonia during the first 2 days were respectively 74 and 62%. True positive and negative rates were respectively 44 and 86%. The area under the ROC curve was 0,654 (CI 95%; 0,524–0,785). Mechanical ventilation duration, length of ICU stay, and mortality were similar in both groups.

Conclusion: In our study, dosage of bronchial α-amylase was not useful in predicting aspiration pneumonia within the first 5 days after ICU admission for OHCA. Performance in predicting early-onset pneumonia was moderate.

Keywords: cardiac arrest, alpha-amylase, aspiration pneumonia, acute lung injury, antibiotics

INTRODUCTION

Out-of-hospital cardiac arrest (OHCA) is a frequent cause of ICU admission. Management of OHCA is clearly defined in specific guidelines (1). The high incidence rate of subsequent infectious complications is described in the literature (2, 3). Aspiration pneumonia is the most common complication, as a result of inhalation mechanisms during the early phase of CA and during cardio-pulmonary resuscitation (CPR) (4). Prophylactic antibiotic treatment is frequently initiated although benefit on mortality or neurological outcome has never been demonstrated (5–7). The recent work from François et al. (7) showed that prophylactic antibiotic treatment with amoxicillin-clavulanate in ICU patients after OHCA resulted in a lower incidence of infectious pneumonias. However, it had no effect on length of ICU stay, ventilator-free days, or mortality. Moreover, antibiotic overuse or misuse is a contributing factor to the spread of multidrug-resistant bacteria (8, 9). This issue should encourage judicious and proper use of antibiotics. The identification of a sensitive and specific biomarker for predicting aspiration pneumonia after OHCA seams necessary.

Alpha-amylase (α-amylase) is the major digestive enzyme in saliva (10). Its detection in bronchial secretions could be a marker of interest in aspiration pneumonia. Data suggests that elevated bronchial α-amylase value is associated with ventilator-associated pneumonia (VAP) (11). Samanta et al. (12) showed that mini bronchoalveolar lavage α-amylase concentrations increase in patients with VAP.

However, there is no data concerning the interest of bronchial α-amylase dosage in predicting the risk of developing aspiration pneumonia post-OHCA resuscitation.

The aim of this study is to determine the interest of bronchial α-amylase dosage in the early diagnosis of aspiration pneumonia, in ICU patients after resuscitation of OHCA.

MATERIALS AND METHODS

Study Design

This is a prospective, observational study, conducted in an adult ICU in the University Hospital of Marseille (APHM, France), during a 5-year period (from July 2015 to September 2020).

Patients

All adult patients admitted to ICU following OHCA (cardiac or respiratory etiology) were included. Targeted temperature management (TTM) was applied during the first 24 h, with continuous sedation and neuromuscular blockade. Exclusion criteria were patients under 18 years old, patients concerned by withdrawal of life-sustaining treatment decisions taken within a few hours after admission, and patients who died during the first 48 h after admission.

Alpha-Amylase and Bronchial Sampling

A respiratory sampling was collected within the first 6 h after admission, using a protected specimen bronchial brushing (Combicath®, Prodimed) following a standardized procedure. This technique consists in inserting the device in the endotracheal tube until feeling it stop, then removing the spacer to allow the catheter to extend. A mini-bronchoalveolar lavage (mini-BAL) is then performed by injecting 40 mL of 0.9% saline solution, immediately aspirated using a sterile syringe. The sample collected was then transferred into 2 distinct sterile tubes, and immediately sent for biochemical and bacteriological analysis.

α-amylase dosage in the bronchial sampling was performed 24/7 in the biochemistry laboratory using a colorimetric enzymatic assay according to the International Federation of Clinical Chemistry (IFCC) recommendations (13). The α-amylase activity was detected without distinction of salivary or pancreatic isoforms. Bacteriological analysis and culture were performed on the same sample.

Diagnosis and Treatment of Aspiration Pneumonia

Aspiration pneumonia was confirmed by the investigators retrospectively. According to the European Society of Intensive Care Medicine (ESCIM) (14) and the Infectious Disease Society of America/American Thoracic Society (IDSA/ATS) guidelines (15), required criteria were hyperleukocytosis > 10,000/μL or leukopenia < 4,500/μL, presence of new or progressive radiological infiltrate or consolidation, associated with microbiologic confirmation in the respiratory sampling. Detection of mixed oral flora in the respiratory fluid was considered as a microbiologic confirmation of aspiration pneumonia in our study. Patients had to meet all 3 types of criteria (clinical, radiological, and microbiologic). Early-onset aspiration pneumonia was defined as pneumonia occurring during the 48 h following admission. Fever or hypothermia were not considered as diagnosis criteria, because of TTM influence

TABLE 1 | Clinical characteristics of patients at Intensive Care Unit (ICU) admission.

	Global population ($n = 88$)	No aspiration pneumonia ($n = 58$)	Aspiration pneumonia ($n = 30$)	p
Age (years)	59 (46–68)	59 (45–68)	59 (47–68)	0.979
Gender: male (*n*, %)	60 (68)	39 (67)	21 (70)	0.792
Weight (Kg)	80 (65–92)	80 (70–92)	76 (65–100)	0.772
No flow duration (minutes)	2 (0–10)	2 (0–10)	5 (0–9)	0.707
Low flow duration (minutes)	15 (10–20)	15 (10–20)	15 (12–21)	0.224
Cardiac arrest etiology				0.731
Cardiac etiology (*n*, %)	54 (61)	34 (59)	20 (61)	
Hypoxia (*n*, %)	23 (26)	17 (29)	6 (20)	
Subarachnoid hemorrhage (*n*, %)	2 (2)	1 (2)	1 (3)	
Hypokaliemia (*n*, %)	1(1)	1 (2)	0 (0)	
Hemorragic shock (*n*, %)	2 (2)	2 (3)	0 (0)	
Electrocution (*n*, %)	1(1)	0 (0)	1 (3)	
No etiology (*n*, %)	5 (6)	3 (5)	2 (7)	
Emergency treatment				0.936
Coronarography (*n*, %)	41 (49)	29 (58)	16 (53)	
ECMO (*n*, %)	2 (2)	2 (3)	0 (0)	
Temporary pacemaker (*n*, %)	1 (1)	1 (2)	0 (0)	
Pericardial drainage (*n*, %)	1 (1)	1 (2)	0 (0)	
Antibiotic therapy at admission (*n*, %)	50 (57)	33 (57)	17 (57)	0.984
SOFA score at H24	10 (8–11)	10 (7–11)	10 (8–12)	0.340
SAPS II score	64 (56–75)	64 (55–71)	69 (58–80)	0.079
SOFA score at H48	8 (6–11)	8 (6–11)	10 (7–12)	0.199
Bronchial amylase level at admission (IU/L)	3 (0–90)	3 (0–61)	15 (0–94)	0.157

No flow represents cardiac arrest delay without cardio pulmonary resuscitation (CPR). Low flow represents CPR duration.
ECMO, Extracorporeal membrane oxygenation; SOFA, Sequential organ failure assessment; SAPS II, Simplfied Acute Physiology Score II.
Values are presented as medians +/– Inter Quartile Range (IQR).

on body temperature. Antibiotic treatment was left to physician discretion based on clinical arguments. No selective digestive decontamination or prophylactic antibiotic was realized. Only curative treatment was initiated by ampicillin-clavulanic acid, gentamicin was added if patient presented septic shock. The duration of treatment was 48 h without microbiological data or 5 days with microbiological data.

Data Collection

Following data was collected during the first 3 days after admission and until day 5: OHCA characteristics (no flow and low flow time, cause), hemodynamic (heart rate, blood pressure, daily urine output, catecholamine use and dose) and respiratory variables (ventilator parameters, ratio of arterial oxygen pressure and fraction of inspired oxygen (P/F) several times a day), and body temperature. Microbiologic results, severity scores SOFA an SAPS2, as well as biochemistry results were collected (white blood cell count, procalcitonin (PCT), arterial blood gas analysis, arterial lactate, serum troponin at admission, H+6, and H+12). We also documented all the procedures underwent by patients: coronary angiography, pericardial drainage, extracorporeal life support, anti-infective treatments. The occurrence of post-cardiac arrest syndrome with myocardial and/or microcirculatory dysfunction was specified. Complications such as multiple organ failure, additional cardiac arrest, hemorrhagic shock, required renal replacement therapy (RRT) or prone position were collected. For each patient, the length of mechanical ventilation and ICU stay, as well as mortality rate were collected.

Statistical Analysis

Qualitative variables, resumed by counts and percentages, are compared using Khi² or Fisher exact test. Quantitative values are presented with medians and interquartile ranges (IQR). They are compared using Mann and Whitney test. Sensitivity, specificity, and predictive values of α-amylase were determined. La ROC (Receiver Operating Characteristic) curve was established to determine the best cut-point value for predicting aspiration pneumonia. We used SPSS software version 20 for statistical analysis. The threshold for statistical significance was defined for $p < 0.05$. The number of needed was calculated on the only value reported a threshold of 125 IU/ml to predict the risk of aspiration pneumonia. If we assume a difference of 20 ± 30 IU/ml, 37 subjects per group would be necessary with a power of 80% and an alpha risk of 5%, i.e., 64 subjects. Hundred patients will be included to overcome any inclusion problem.

Ethical Considerations

This study was accepted by the Committee for the Protection of Persons of Marseille (N°2016A0119744AEC). The

Commission on Data Processing and Freedom was notified (N° CNIL1994062v0). The study was registered in the Clinical Trial database (NCT 03007862). All patients or relatives received an information note, and a written consent was obtained.

RESULTS

Population

From July 2015 to September 2020, 88 patients were included. Clinical and biological data, as well as treatments received are presented in **Table 1**. Cardiac disease (arterial coronary disease, arrhythmia, pulmonary embolism) was the predominant cause of OHCA (61%). Hypoxia was found in 26% of the situations. Median no flow time was 2 mins (IQR 0–10), and median low flow time was 15 mins (IQR 10–20). A coronary angiography was performed immediately after admission in 47% of cases. 57% of patients received prophylactic antibiotic treatment on their admission day. Among them, 50% received amoxicillin/clavulanic acid and 34% received a combination of amoxicillin/clavulanic acid—gentamicin. Median maximal temperature on day 1 was 36°C (IQR 35–37). Post-cardiac arrest syndrome with hemodynamic dysfunction occurred in 25% ($n =$ 22 patients) of cases on day 1, 21% ($n = 9$ patients) on day 3. ICU mortality rate was 50% (**Table 2**).

Aspiration Pneumonia

After clinical, radiological, and bacteriological data analysis, aspiration pneumonia diagnosis within the first 5 days was established for 30 patients (34%). The 17 (56%) pneumonias occurred during the first 48 h.

We found no significant difference concerning α-amylase value in the mini-BAL between the two groups, with and without pneumonia [3 (0–60) vs. 15 (0–130)—$p = 0{,}157$—**Figure 1**].

However, we found a significant difference concerning bronchial α-amylase values between patients who developed early aspiration pneumonia (≤48 h—$n = 17$) and the others [19 (7–297) vs. 3 (0–82)—$p = 0{,}047$—**Figure 2**].

The area under the curve (AUC) was 0.591 (CI 95%: 0.464–0.717) (**Figure 3**). For an 8.1 IU/L cut-point, the sensitivity and the specificity of α-amylase for predicting aspiration pneumonia within the first 5 days were respectively 63% (CI 95%: 44–80) and 62% (CI 95%: 48–74). True and false positive rates (TPR and FPR) were respectively 46% (CI 95%: 31–62) and 77% (CI 95%: 62–87).

Using the same α-amylase cut-point, values of sensitivity/specificity/TPR/FPR for predicting early aspiration pneumonia (48 h—$n = 17$) were respectively 76.5% (CI 95%: 50.1-−93.2), 61% (CI 95%: 48.3–72), 32% (CI 95%: 24–41), and 91.5 % (IC 95%: 81.7–96), with an AUC of 0,653 (CI 95%: 0,524-−0,785) (**Figure 4**).

TABLE 2 | Evolution of patients' clinical characteristics from day 1 to 3.

	Global population ($n = 88$)	No aspiration pneumonia ($n = 58$)	Aspiration pneumonia ($n = 30$)	*p*
Clinical characteristics on day 1				
Body temperature (°C)	36 (35–37)	36 (35–37)	36 (35–37)	0.412
White blood cell count (G/L)	16 (13–21)	16 (12–20)	17 (14–22)	0.500
PaO_2/FiO_2 ratio	213 (130–267)	203 (128–265)	238 (146–321)	0.233
Arterial lactate level (mmol/L)	4 (2–8)	4 (2–9)	4 (2–7)	0.682
PCAS (*n*, %)	22 (25)	9 (16)	13 (43)	0.005
Clinical characteristics on day 2				
Mechanical ventilation (*n*, %)	84 (97)	56 (98)	28 (94)	0.272
Body temperature (°C)	38 (37–38)	38 (37–38)	38 (37–38)	0.683
White blood cell count (G/L)	14 (10–19)	13 (10–19)	15 (13–20)	0.192
PaO2/FiO2 ratio	214 (155–283)	213 (154–278)	215 (153–290)	0.834
Arterial lactate level (mmol/L)	2 (1–3)	2 (1–2)	2 (1–3)	0.313
PCAS (*n*, %)	18 (21)	7 (12)	11 (37)	0.008
Clinical characteristics on day 3				
Mechanical ventilation (*n*, %)	67 (81)	42 (76)	25 (89)	0.158
Body temperature (°C)	38 (37–38)	38 (37–38)	38 (38–38)	0.832
White blood cell count (G/L)	13 (10–17)	13 (10–17)	13 (9–17)	0.952
PaO2/FiO2 ratio	254 (172–298)	260 (189–303)	243 (165–283)	0.414
Arterial actate level (mmol/L)	1 (1–2)	1 (1–2)	1 (1–2)	0.815
PCAS (*n*, %)	9 (11)	2 (4)	*7 (26)*	0.005
PCT (μg/L)	0,7 (0,2–2,9)	1,0 (0,2–3,4)	0,6 (0,2–1,5)	0.063
Mechanical ventilation duration (days)	5 (3–8)	5 (3–8)	6 (4–9)	0.380
ICU hospitalization duration (days)	6 (4–9)	6 (4–9)	6 (4–9)	0.975
ICU mortality (*n*, %)	44 (50)	27 (47)	17 (57)	0.368

Values are presented as medians +/– Inter Quartile Range (IQR).
PCAS, Post Cardiac Arrest Syndrome; PCT, serum procalcitonine.

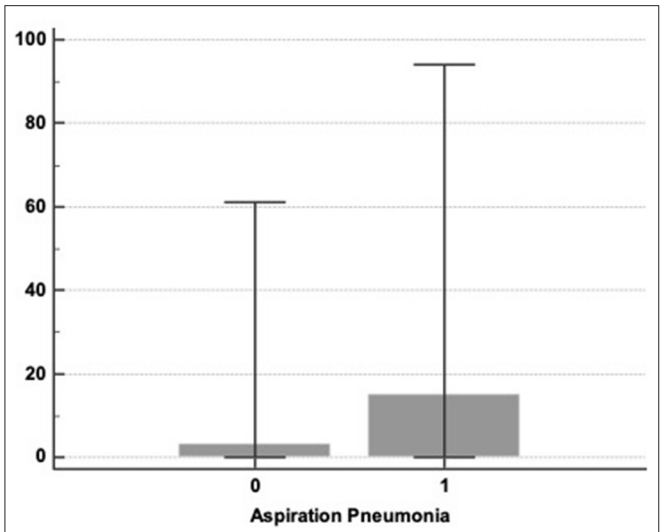

FIGURE 1 | α-amylase levels in mini-BAL at admission and aspiration pneumonia represented by box plot 25–75: 0: no aspiration pneumonia; 1: apiration pneumonia [3 (0–61) vs. 15 (0–94) $p = 0.157$].

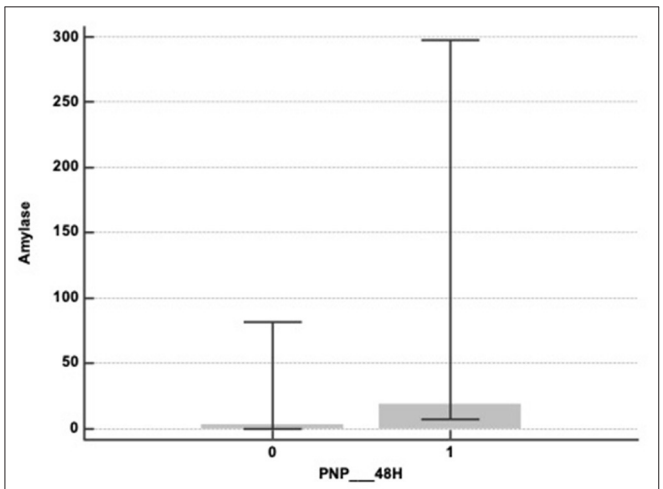

FIGURE 2 | α-amylase levels in mini-BAL at admission and early aspiration pneumonia (within the first 48 h) represented by box plot: 0: no early aspiration pneumonia; 1: early apiration pneumonia [19 (7–297) vs. 3 (0–82)—$p = 0.047$].

Aspiration pneumonia occurrence did not significantly impact median mechanical ventilation duration (5 days, IQR: 3–8), ICU stay (6 days, IQR: 4–9), or ICU mortality (44 patients-−50%).

Microbiologic Data

Table 3 shows the different microorganisms identified. The most common was *Staphylococcus aureus* (29%), followed by *Haemophilus influenza* (13%), and *Streptococcus pneumonia* (10%). Mixed oral flora was found in 26% of our samplings.

If one or more microorganisms were identified in the admission mini-BAL, the median α-amylase value (25 IU/L [IQR: 2–230]) was significantly higher than if a sterile culture was reported (2 IU/L [IQR: 0–43]—$p < 0.01$).

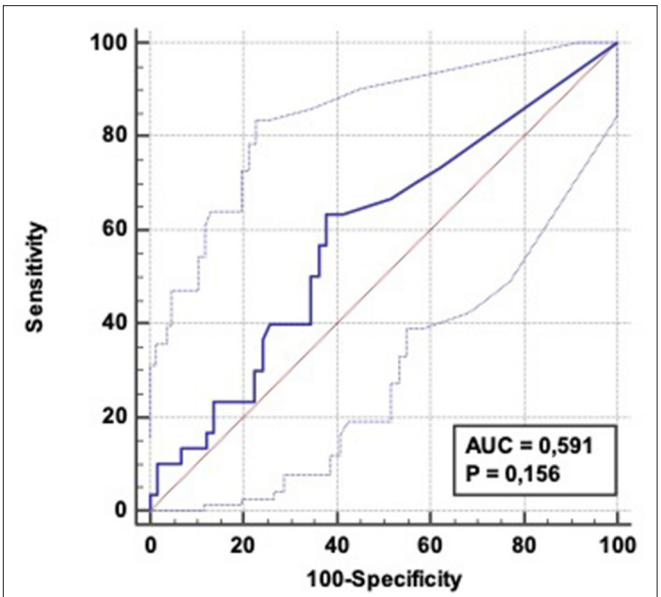

FIGURE 3 | ROC curve: Diagnostic ability of α-amylase level to predict aspiration pneumonia occurrence within 5 days following OHCA admission. AUC = 0.591 (CI 95%: 0.464–0.717) ; Positive LR = 1.67 (CI 95%: 1.1–2.6) ; Negative LR = 0.59 (CI 95 %: 0.4–1.0).

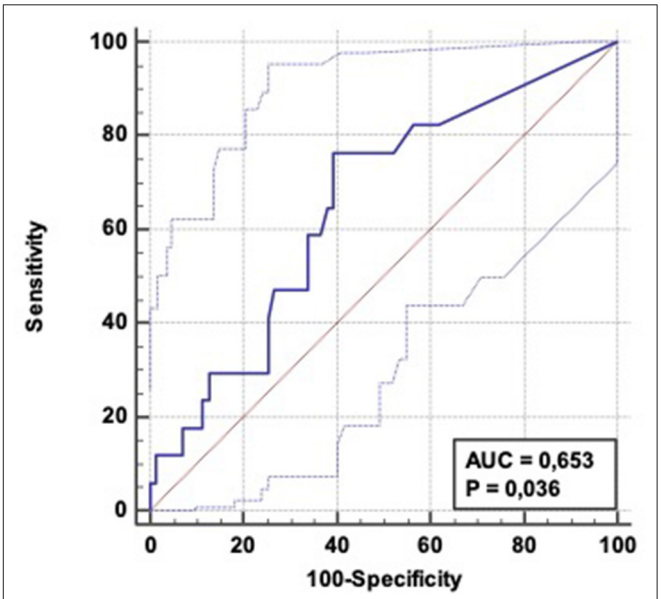

FIGURE 4 | ROC curve: Diagnostic ability of α-amylase level to predict aspiration pneumonia occurrence within 48 h following OHCA admission. AUC = 0.653 (CI 95%: 0.524–0.0.785) ; Positive LR = 1.94 (CI 95%: 1.3–2.9) ; Negative LR = 0.39 (CI 95 %: 0.2–0.9).

DISCUSSION

In this study, with a cut-point of 8.5 IU/L, α-amylase sensitivity and specificity to predict aspiration pneumonia within the first 5 days were, respectively 63 and 62%. TPR and FPR were

TABLE 3 | Microbial identification in ICU admission bronchial sampling.

Bacterial identification	*n (%)**
Gram positive	
Staphylococcus aureus	9 (29)
Streptococcus pneumoniae	4 (9.7)
Other streptococcus	2 (6.5)
Gram negative bacillus	
Haemophilus influenzae	4 (12.9)
Escherischia coli	2 (6.5)
Klebsiella pneumoniae	1 (3.2)
Klebsiella oxytoca	1 (3.2)
Serratia odorifera	1 (3.2)
Serratia liquefaciens	1 (3.2)
Proteus mirabilis	1 (3.2)
Enterobacter cloacae	1 (3.2)
Haemophilus parahemolyticus	1 (3.2)
Moraxella Catarrhalis	1 (3.2)
Other identification	
Mixed oral flora	8 (25.8)

**Multiple germ identification in a same patient led to a total percentage higher than 100.*

respectively 46 and 77%. Rates were more efficient for predicting early aspiration pneumonia, with sensitivity, specificity, TPR, and FPR of respectively 76.5, 61, 32, and 91.5%. Our results suggest that bronchial α-amylase dosage is of moderate interest for predicting aspiration pneumonia after OHCA. However, interesting characteristics can be provided by α-amylase value, as demonstrated by a certain number of findings (11, 12, 14). Our work is the first to study bronchial α-amylase value in this specific context.

General characteristics of patients, causes of OHCA, length of stay, and mortality are in line with current data (5, 7, 16). Global management of such patients, including TTM, follows updated guidelines (1). Microbiologic results were similar to those found in patients admitted to ICU following cardiac arrest (3, 5).

In our study, aspiration pneumonia prevalence was 34%, consistent with recent results. The large variability of criteria used to define infectious pneumonia in the literature is somewhat responsible for a significant heterogeneity of prevalence, ranging from 22 to 61% (3, 7, 16). We decided to use both clinical and radiological criteria, and to consider pneumonia only if bacterial culture was proven within 5 days. Such restrictive conditions may explain the relatively low prevalence found. We did not consider the occurrence of fever or hypothermia because of the confounding factor of TTM, as well as the high incidence of hyperthermia on the following days of OHCA (17, 18). The common initiation of prophylactic antibiotic treatment on admission day (57% in our study) may also explain the lowered prevalence of microbiologic proof of pneumonia.

In the specific context of OHCA, the difference between aspiration pneumonia and ventilator-associated pneumonia (VAP) can lead to a certain confusion. Aspiration pneumonia is defined as the inhalation of either oropharyngeal or gastric contents into the lower respiratory tract. Acid gastric content can cause "chemical" injury, followed by an inflammatory pulmonary response, and eventually an infection (19). When infected secretions are the content of inhalation, they directly result in the infectious process. These two mechanisms are often involved. VAP is defined as pneumonia occurring more than 48 h after intubation and mechanical ventilation. Despite the protective role of the endotracheal tube, micro-aspirations occur and develop VAP (20). Even though these two mechanisms differ and are difficult to distinguish, the underlying physiopathology is similar.

The threshold of 8.5 IU/L identified in our work is lower than values reported in other studies. Samanta et al. (12) worked on bronchial α-amylase values in 151 patients undergoing mechanical ventilation, with suspected VAP. α-amylase concentration was significantly higher in patients with confirmed VAP. It was associated with the presence and number of inhalation risk factors. The 130 IU/L as cut-point had a sensitivity of 84% and a specificity of 67% for predicting VAP occurrence in patients with at least one risk factor of inhalation. The retrospective study of Weiss et al. (11) showed similar results. α-amylase dosage was performed on a blind or endoscopy-guided BAL. Ge-Ping Qu et al. (21) included 147 patients and reported that α-amylase concentration in tracheal samples of intubated patients was a good predictive value for VAP occurrence, with a sensitivity of 80% and a specificity of 79% (AUC 0.813) with a 4,681.5 IU/L threshold. The different sampling methods may explain the large variation of values described.

The population studied in our study may also explain the low median α-amylase value found. Our work focused on patients admitted to ICU after OHCA. In this specific context, inhalation can occur at the time of the cardiac arrest, following the loss of protective reflexes in the airway. Life support techniques (chest compression, mask ventilation) may also favor inhalation, until orotracheal intubation. This period is limited because intubation delay ranges from 10 to 20 mins following OHCA. Other studies focus on ICU patients, undergoing mechanical ventilation since a few days (11, 12, 21). Micro-inhalation events could also explain higher α-amylase values in those patients. Early dosage (within the first 6 h after admission) in our work also accounts for our lower values.

To our knowledge, there is no gold-standard technique to assess aspiration pneumonia. Numerous biomarkers of aspiration have been studied in patients undergoing mechanical ventilation or spontaneously breathing. All of them are of limited use in daily clinical practice. Pepsin is a powerful gastric enzyme. As it attests only gastric content, and poorly reflects oropharyngeal content, its interest remains incomplete (22, 23). Bile acid detection in tracheal samplings has also been studied, in small populations (24). Serum PCT validity in distinguishing bacterial and aspiration pneumonias is not yet proven (25). In the specific context of cardiac arrest, no biomarker seems adequate to predict aspiration pneumonia. α-amylase bears interesting characteristics: quantitative dosage is simple, of rapid response (a few hours), and inexpensive. Along with other clinical, biological, and radiological criteria, α-amylase dosage could help physicians decide when to initiate an antibiotic therapy.

The interest of prophylactic antibiotic therapy in preventing aspiration pneumonia is still debated (5–7, 16). In a controlled, randomized clinical trial, François et al. (7) studied the impact of a prophylactic treatment by amoxicillin-clavulanate during 2 days in patients admitted following OHCA with shockable rhythms. Patients' characteristics, management, and overall incidence of aspiration pneumonias were comparable with our study. Incidence of aspiration pneumonia at day 5 was lower in the treated group compared to the placebo group. Mechanical ventilation duration, ICU length of say, and mortality at day 28 did not differ. In our work, occurrence of aspiration pneumonia did not influence these outcomes either.

Our study has inherent limitations. The monocentric design and the small number of patients are undeniable. The population of interest is specific, and results may not be generalized to all suspected aspiration pneumonias in ICU patients. The lack of a gold-standard technique to assess aspiration pneumonia does not allow the comparison of our results. Despite a standardized sampling protocol, an inter-operator variability remains possible.

CONCLUSION

Bronchial α-amylase value is not an effective biomarker for predicting aspiration pneumonia during the first 5 days following ICU admission after OHCA. The performances of this biomarker were higher but still insufficient for predicting early aspiration pneumonia (inferior to 48H). A systematic prophylactic treatment by amoxicillin-clavulanate during 2 days after ICU admission seems to be the better strategy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Centre de protection des personnes sud méditérannée. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JB and MG were responsible for study concept and design. AM, JB, JC, MG, FB, AL, SH, EC, AB, and JF were responsible for the acquisition, analysis, or interpretation of data. JB, EC, and AM were responsible for drafting the manuscript. MB and AM were responsible for statistical analysis. All authors had full access to all the data in the study, take responsibility for the integrity of the data and the accuracy of the data analysis, interpreted the findings, contributed to writing the manuscript, and approved the final version for publication.

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Incidence, Risk Factors and Outcomes of Sepsis in Critically Ill Post-craniotomy Patients: A Single-Center Prospective Cohort Study

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Background: Data concerning the epidemiology of sepsis in critically ill post-craniotomy patients are scarce. This study aimed to assess the incidence, risk factors, and outcomes of sepsis in this population.

Methods: This was a single-center prospective cohort study. Post-craniotomy patients admitted to the intensive care unit (ICU) were screened daily for the presence of infection and sepsis.

Results: Of the 900 included patients, 300 developed sepsis. The cumulative incidence of sepsis was 33.3% [95% confidence interval (CI), 30.2–36.4%]. Advanced age, male, hypertension, trauma, postoperative intracranial complications, and lower Glasgow Coma Scale (GCS) on the first postoperative day were independent risk factors of sepsis. Septic patients had higher hospital mortality (13.7 vs. 8.3%, $P = 0.012$), longer ICU length of stay (LOS) (14 vs. 4 days, $P < 0.001$), longer hospital LOS (31 vs. 19 days, $P < 0.001$), and higher total medical cost (CNY 138,394 vs. 75,918, $P < 0.001$) than patients without sepsis.

Conclusion: Sepsis is a frequent complication in critically ill post-craniotomy patients. Advanced age, male, hypertension, trauma, postoperative intracranial complications, and lower GCS on the first postoperative day were independent risk factors of sepsis.

Keywords: sepsis, post-craniotomy, incidence, outcome, risk factor

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INTRODUCTION

Post-craniotomy patients are susceptible to central nervous system (CNS) infection, which is associated with the craniotomy procedure, placement of drainage tubes or other intracranial devices (1, 2), and postoperative intracranial complications such as leakage of cerebrospinal fluid (1). Furthermore, paralysis, disturbance of consciousness and dysphagia are common in critically ill neurosurgery patients (3, 4), making these patients vulnerable to extra-CNS infection, particularly pneumonia, urinary tract, and bloodstream infection (5, 6).

Sepsis is a life-threatening condition, which is caused by the dysregulation of the body's inflammatory response to infection and can lead to increased mortality rates and prolonged

hospital stays (7–10). Sepsis is a major challenge for intensive care unit (ICU) clinicians due to its high and increasing incidence as well as clinical complexity. There have been numerous epidemiologic studies of sepsis focused on medical or surgical patients (7–9, 11–18). However, published data on epidemiology, risk factors and outcome parameters of sepsis in critically ill post-craniotomy patients are scare. The study of Pertsch et al., has quantified sepsis after elective neurosurgery (19), but has not reported the incidence of sepsis after emergency procedures. In addition, most of their patients underwent spine procedures, while patients undergoing cranial procedures only accounted for 22.2% of the population. Zhang et al., reported the incidence of sepsis in patients who underwent craniotomy for tumor resection, but not in patients who underwent craniotomy for other reasons (20). Therefore, the results of both studies could not reflect the overall epidemiological characteristics of sepsis after neurosurgery. Given the significant burden of sepsis on patient health and healthcare costs, we conducted this prospective cohort study to assess the incidence, risk factors, and outcomes of sepsis in ICU-admitted post-craniotomy critically ill patients.

METHODS

Study Design

We conducted this study in the ICU ward (70 beds) of a teaching hospital. The study was approved by the institutional review board of the hospital, with a waiver of informed consent, as there was no intervention in this study.

During the study period (from January 1, 2017 to December 31, 2018), all adult (age $\geq$ 18 years) post-craniotomy patients who had stayed in ICU for more than 24 h were eligible for screening. Patients with sepsis before craniotomy surgery were excluded. All of the patients were screened daily for the presence of infection and sepsis. A standard protocol was established to diagnose sepsis according to the definition of sepsis 3.0 (21–23). Patients readmitted to the hospital during the study period would be screened again. For patients with multiple episodes of sepsis during the same hospitalization, only the first episode was counted.

Data Collection

Data were collected using case report forms (CRFs) and were double-entered by two ICU physicians. All recorded data were screened in detail by medical personnel for missing information, logical errors, or insufficient details. Inconsistencies were resolved by an interview with the physicians in charge of collecting the data. Two chief physicians verified the eligibility criteria, characteristics of infection, and sepsis diagnoses.

At study entry, the demographic data, primary diagnosis, chronic comorbidities, Charlson comorbidity index (24) and information about the surgery (operative time, surgical site, indwelling drainage tubes, complications) and Glasgow Coma Scale (GCS) on the first postoperative day were recorded. For patients with infection and sepsis, the infection sites and microbial culture results were collected. The acute physiology and chronic health evaluation (APACHE) II score (25), sequential organ failure assessment (SOFA) score (26) were used to assess the severity of the disease, and the worst parameters within the first 24 h of ICU were selected for calculating the scores. Patients were followed up until discharge or death, whichever came first. Hospital length of stay (LOS), ICU LOS, hospitalization costs, hospital mortality rate, and Glasgow Outcome Scale (GOS) at hospital discharge were calculated.

Diagnostic Criteria

Infections were determined by the attending physicians, and might be diagnosed in the following situations: (1) patients with unquestionable clinical signs of infection (such as fecal peritonitis, necrotizing fasciitis, or wounds with purulent discharge); (2) patients with clinically suspected infections (with symptoms, signs, and anatomical and/or imaging and/or histological evidence of infections) and responding to antibiotic treatments; or (3) positive Gram staining or culture of normally sterile body fluid or tissue (27). CNS infections referred to meningitis, ventriculitis, brain abscess, subdural empyema, and epidural empyema. Meningitis/ventriculitis was defined by organisms present on cerebrospinal fluid (CSF) culture, the presence of clinical signs and symptoms of meningitis or ventriculitis (such as fever, new headache, new meningeal signs, change in mental status, or cranial nerve signs), CSF abnormalities (increased opening pressure, presence of polymorphonuclear pleocytosis, decreased glucose, and increased proteins deemed not to be chemical meningitis), or organisms seen on Gram's stain of CSF (28). Brain abscess, subdural empyema, and epidural empyema were diagnosed by magnetic resonance imaging (MRI) or computed tomography (CT) with contrast and confirmed by positive culture of needle aspiration or open drainage specimens. Infections that occurred 48 h or more after admission and might not have been incubated at the time of admission were defined as hospital-acquired infections (29). Sepsis was defined according to the sepsis-3 criteria (21–23). For infected patients, if the GCS decreased compared with before, neurological examination, cranial imaging examinations (such as CT and MRI), blood gas analysis, blood biochemistry, etc., would be routinely performed to determine the reason for the decline in GCS. In the absence of other causes, the decline in GCS might be considered to be caused by infections.

Statistical Analysis

Statistical analyses were conducted using SPSS software version 19.0 for Windows. Continuous variables were expressed as the mean ($\pm$SD) or median (interquartile range, IQR), and were analyzed using Student's *t*-test, Mann-Whitney *U*-test or one-way ANOVA. Categorical variables are presented as absolute number (%) and were analyzed using chi-square test or Fisher's exact test, as appropriate. According to the presence of infection and sepsis, the patients were divided into non-infection group, non-septic infection group and sepsis group. Multinomial logistic regression was used to evaluate the risk factors for infection and sepsis. Variables with *P*-values lower than 0.2 by univariate analysis were entered into the model. All comparisons were unpaired. Two-tailed $P < 0.05$ were considered statistically significant. UpSet plots were used to depict the distribution of infection sites in

patients with infection and sepsis, and were implemented using the TBtools software (30).

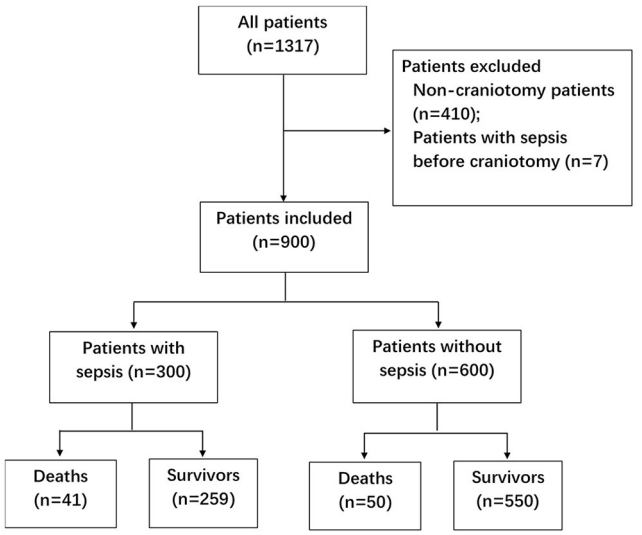

FIGURE 1 | Flow diagram of enrolled patients. ICU, intensive care unit; LOS, length of stay.

RESULTS

Incidence of Sepsis

During the two-year study period, 1,317 patients were screened (**Figure 1**). Seven patients with sepsis before craniotomy were excluded, as well as 410 non-craniotomy cases. Nine hundred patients were included, among whom 55.3% were male (**Table 1**). Most of the patients (78.1%) underwent elective surgery. The most common comorbidities were hypertension, diabetes, and cerebrovascular disease. Nearly 3/4 (n = 668, 74.2%) of the patients were admitted into ICU from the operating theater. The other patients (n = 232, 25.8%) were from general wards, and the most common reasons for their ICU admission were respiratory failure (n = 99, 11.0%) and CNS disorders (n = 71, 4.2%).

A total of 509 patients (56.6%) with infection were identified, of whom 300 patients developed sepsis. The cumulative incidence of sepsis was 33.3% [95% confidence interval (CI), 30.2–36.4%]. Nearly 2/3 of sepsis episodes occurred during the first week after craniotomy (**Figure 2A**), and almost three-quarters of patients were diagnosed with sepsis during the first week of their ICU stay (**Figure 2B**).

The mean age of patients with sepsis was higher than that of patients without sepsis (52.8 vs. 47.9 years, $P < 0.001$). The incidence of sepsis was higher in men than in women (39.0%vs.

TABLE 1 | Demographic characteristics and outcomes of patients.

Variables	Total (n = 900)	Sepsis (n = 300)	Non-sepsis (n = 600)	P-value
Age (years)*	49.5 (14.6)	52.8 (15.0)	47.9 (14.2)	<0.001
Male, *n* (%)	498 (55.3%)	194 (64.7%)	304 (50.7%)	<0.001
Smoking, *n* (%)	151 (16.8%)	58 (19.3%)	93 (15.5%)	0.147
Alcoholism, *n* (%)	91 (10.1%)	37 (12.3%)	54 (9.0%)	0.118
Comorbidities				
Hypertension, *n* (%)	259 (28.8%)	111 (37.0%)	148 (24.7%)	<0.001
Diabetes, *n* (%)	89 (9.9%)	38 (12.7%)	51 (8.5%)	0.048
Cerebrovascular disease, *n* (%)	71 (7.9%)	28 (9.3%)	43 (7.2%)	0.256
Tumor, *n* (%)	42 (4.7%)	12 (4.0%)	30 (5.0%)	0.503
Coronary heart disease, *n* (%)	35 (3.9%)	14 (4.7%)	21 (3.5%)	0.393
Chalson comorbidity index†	0 (0, 0)	0 (0, 1)	0 (0, 0)	0.180
Type of patients				0.002
Elective surgery, *n* (%)	703 (78.1%)	216 (72.0%)	487 (81.2%)	
Emergency surgery, *n* (%)	197 (21.9%)	84 (28.0%)	113 (18.8%)	
Operative time (hours)†	4.3 (3.0, 6.0)	4.0 (2.8, 5.5)	4.6 (3.0, 6.2)	0.002
GCS on postoperative day 1†	10 (7, 11)	8 (5, 10)	10 (7, 14)	<0.001
APACHE II†	16 (11, 20)	18 (14, 23)	14 (10, 18)	<0.001
SOFA of ICU day1†	4 (3, 6)	5 (4,6)	4 (2, 5)	<0.001
GOS at hospital discharge†	4 (3, 5)	3 (3,4)	4 (3, 5)	<0.001
Death, *n* (%)	91 (10.1%)	41 (13.7%)	50 (8.3%)	0.012
ICU LOS, days	6 (3, 13)	14 (8,22)	4 (3, 7)	<0.001
Hospital LOS before ICU admission, days†	4 (2, 11)	10 (4,19)	4 (2, 7)	<0.001
Total hospital LOS, days†	22 (15, 32)	31(21,43)	19 (14, 27)	<0.001
Hospitalization costs (CNY)†	93,179 (62,590, 138,496)	138,394 (101,060, 189,994)	75,918 (56,297, 107,793)	<0.001

**Data were expressed as mean and SD; † Data were expressed as median and quartiles; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; ICU, Intensive care unit; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; LOS, length of stay.*

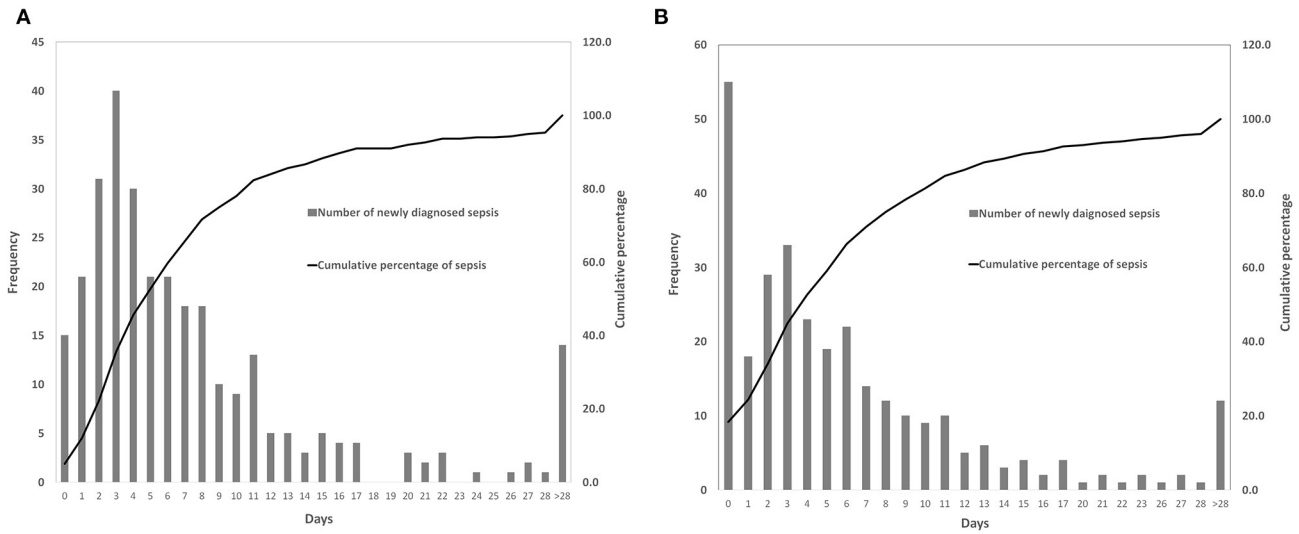

FIGURE 2 | Time of the patients diagnosed with sepsis. **(A)** The occurrence time of sepsis after craniotomy. **(B)** The occurrence time of sepsis after intensive care unit (ICU) admission.

TABLE 2 | Indications for craniotomy, surgery category, surgical site, contamination class of surgical wound, and the corresponding incidence of infection and sepsis.

	Infection (n = 509)	Non-infection (n = 391)	P value	Sepsis (n = 300)	Non-sepsis (n = 600)	P-value
Indications for craniotomy						
Tumor	293 (57.6%)	253 (64.7%)	0.03	161 (53.7%	385 (64.2%)	0.002
Glioma	97 (19.1%)	65 (16.6%)	0.346	56 (18.7%)	106 (17.7%)	0.713
Meningiomas	68 (13.4%)	82 (21.0%)	0.002	32 (10.7%)	118 (19.7%)	0.001
Tumors of the cranial and paraspinal nerves	35 (6.9%)	27 (6.9%)	1.000	13 (4.3%)	49 (8.17%)	0.032
Tumors of the sellar region	30 (5.9%)	26 (6.6%)	0.642	25 (8.3%)	31 (5.2%)	0.064
Mesenchymal, non-meningothelial tumors	25 (4.9%)	15 (3.8%)	0.438	13 (4.3%)	27 (4.5%)	0.909
Embryonal tumors	21 (4.1%)	18 (4.6%)	0.727	10 (3.3%)	29 (4.8%)	0.297
Metastatic tumors	7 (1.4%)	4 (1.0%)	0.765	4 (1.3%)	7 (1.2%)	1
Other tumors*	10 (2.0%)	16 (4.1%)	0.059	8 (2.7%)	18 (3.0%)	0.778
Trauma	80 (15.7%)	22 (5.6%)	<0.001	53 (17.7%)	49 (8.2%)	<0.001
Cerebrovascular disease	116 (22.8%)	98 (25.1%)	0.427	74 (24.7%)	140 (23.3%)	0.658
Aneurysm	52 (10.2%)	38 (9.7%)	0.805	34 (11.3%)	56 (9.3%)	0.346
Vascular malformation	30 (5.9%)	28 (7.2%)	0.443	18 (6.0%)	40 (6.7%)	0.701
Intracranial hemorrhage	24 (4.7%)	18 (4.6%)	0.937	15 (5.0%)	27 (4.5%)	0.737
Occlusive cerebrovascular disease	10 (2.0%)	14 (3.6%)	0.136	7 (2.3%)	17 (2.8%)	0.661
Other indications†	20 (3.9%)	18 (4.6%)	0.618	12 (4.0%)	26 (4.3%)	0.815
Surgery category			0.004			0.002
Elective surgery	380 (74.7%)	323 (82.6%)		216 (72.0%)	487 (81.2%)	
Emergency surgery	129 (25.3%)	68 (17.4%)		84 (28.0%)	113 (18.8%)	
Surgical site			0.186			0.057
Supratentorial	301 (59.1%)	214 (54.7%)		185 (61.7%)	330 (55.0%)	
Infratentorial	208 (40.9%)	177 (45.3%)		115 (38.3%)	270 (45.0%)	
Contamination class			0.257			0.036
Clean	456 (89.6%)	359 (91.8%)		263 (87.7%)	552 (92.0%)	
Clean-contaminant	53 (10.4%)	32 (8.2%)		37 (12.3%)	48 (8.0%)	

**Other tumors included neuronal and mixed neuronal-glial tumor, choroid plexus tumor, lymphomas, tumors of the pineal region, melanocytic tumors and germ cell tumors; †Other indications included dysplasia diseases, functional neurological diseases, hydrocephalus and intracranial infections.*

26.3%, $P < 0.001$). Comorbidities of diabetes (12.7 vs. 8.5%, $P = 0.048$) and hypertension (37.0 vs. 24.7%, $P < 0.001$) were more prevalent in septic patients than in non-septic patients. The septic group had a lower postoperative GCS (8 vs. 10, $P < 0.001$), a higher APACHE II score (18 vs. 14, $P < 0.001$), and a higher SOFA score (18 vs. 14, $P < 0.001$) compared with the non-septic group (**Table 1**).

The incidence of infection and sepsis varied among patients with different craniotomy indications, different surgical categories, different surgical sites, and different contamination

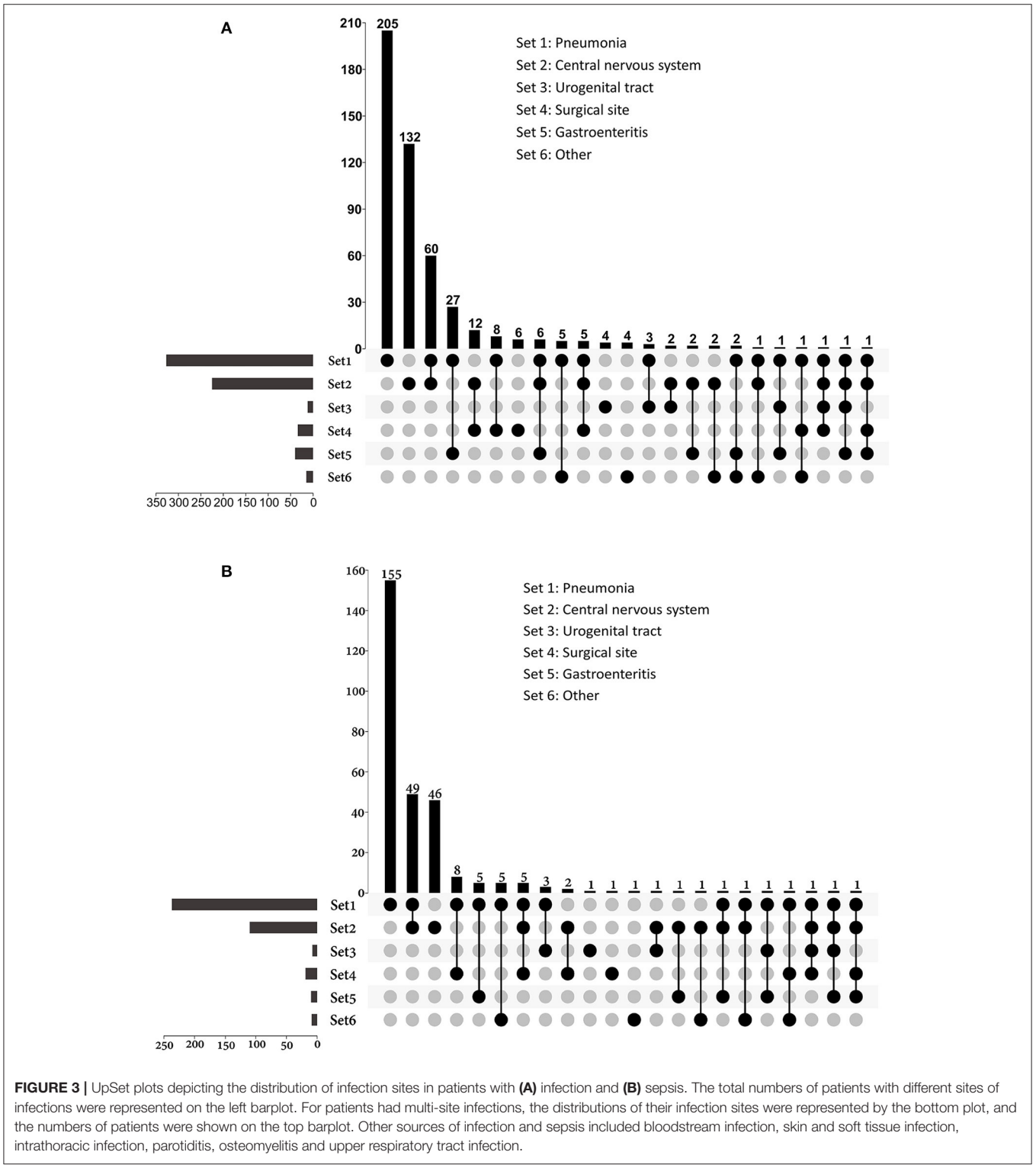

FIGURE 3 | UpSet plots depicting the distribution of infection sites in patients with **(A)** infection and **(B)** sepsis. The total numbers of patients with different sites of infections were represented on the left barplot. For patients had multi-site infections, the distributions of their infection sites were represented by the bottom plot, and the numbers of patients were shown on the top barplot. Other sources of infection and sepsis included bloodstream infection, skin and soft tissue infection, intrathoracic infection, parotiditis, osteomyelitis and upper respiratory tract infection.

classes of surgical wound (**Table 2**). Patients undergoing emergency surgery had a higher incidence of sepsis than those undergoing elective surgery (42.6 vs. 30.7%, $P = 0.002$). Compared with patients with intracranial tumors (29.5%) and cerebrovascular diseases (34.6%), patients with traumatic brain injury (52.0%) were more likely to develop sepsis.

Infection Types, Sources of Infection and Pathogens

Among patients with infections, 96.3% had hospital-acquired infections, and 3.7% had community-acquired infections. The occurrence rate of sepsis in patients with hospital-acquired infections was similar to that in patients with community-acquired infections (56.6% vs. 63.2%, $P = 0.570$).

Of all the infections, lower respiratory tract infections ($n = 336$) and central nervous system infections ($n = 230$) were the most common, and they were also the main causes of sepsis (**Figure 3**). Among patients with sepsis, 81.3% ($n = 244$) had pneumonia; 37.3% ($n = 112$) had central nervous system infections. Lower respiratory tract infection (72.6%) and gastroenteritis (66.7%) were more likely to develop sepsis than surgical site (55.9%), CNS (48.7%), and urogenital tract (25.0%) infections.

A total of 271 cultures were isolated from 230 patients with sepsis, including 161 growing gram-negative bacilli, 86 growing gram-positive cocci, and six growing other pathogens (**Table 3**). *Klebsiella pneumoniae* was the most common isolated pathogen, followed by methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.

Most of the pathogens were cultured from sputum ($n = 229$) and cerebrospinal fluid specimens ($n = 36$). In patients with lower respiratory tract infection, more gram-negative bacilli ($n = 157$) were isolated than gram-positive cocci ($n = 67$), and the most common isolated pathogens included *Klebsiella pneumoniae* ($n = 65$), MRSA ($n = 53$), and *Acinetobacter baumannii* ($n = 45$). The numbers of gram-negative and gram-positive cocci isolated from cerebrospinal fluid were similar, and the most common pathogens were coagulase-negative *Staphylococcus* ($n = 10$) and *Klebsiella pneumoniae* ($n = 9$).

Risk Factors for Sepsis

Multinomial logistic regression analysis found that patients with advanced age, male sex, hypertension, trauma, lower GCS on the first postoperative day and postoperative intracranial complications were at higher risk of sepsis (**Table 4**). Other factors entered into the model but no longer significant after adjustment included smoking, alcoholism, diabetes, categories of surgery (elective or emergency surgery), surgical sites (supratentorial or infratentorial surgery), surgical wound classifications (clean or clean-contaminated) and intracranial tumors (**Supplementary Table 1**). Longer operative time was associated with infection, but not sepsis. Advanced age, male and hypertension were associated with sepsis, but not infection. The chi-square value of the Pearson's Chi-square test was 294.296, and the *P*-value was 0.240, suggesting that the model of logistic regression was fit to the data well.

TABLE 3 | Pathogens isolated from patients with infection and sepsis.

Pathogens	Infection ($n = 509$)	Sepsis($n = 300$)
Gram-negative bacteria	205 (40.3%)	161 (53.7%)
Klebsiella pneumoniae	90 (17.7%)	69 (23.0%)
Acinetobacter baumannii	58 (11.4%)	49 (16.3%)
Pseudomonas aeruginosa	24 (4.7%)	20 (6.7%)
Escherichia coli	12 (2.4%)	10 (3.3%)
Enterobacter aerogenes	9 (1.8%)	7 (2.3%)
Serratia marcescens	8 (1.6%)	5 (1.7%)
Enterobacter cloacae	8 (1.6%)	7 (2.3%)
Gram negative, others*	11 (2.2%)	8 (2.7%)
Gram-positive bacteria	126 (24.8%)	86 (28.7%)
MRSA	83 (16.3%)	59 (19.7%)
MSSA	14 (2.8%)	13 (4.3%)
Staphylococcus epidermidis	11 (2.2%)	5 (1.7%)
Other *Staphylococcus*†	13 (2.6%)	7 (2.3%)
Gram positive, others‡	10 (2.0%)	7 (2.3%)
Other pathogens§	9 (1.8%)	7 (2.3%)

**Other Gram negative bacteria included Burkholderia cepacia, Stenotrophomonas maltophilia, Proteus mirabilis, Citrobacter braakii and Klebsiella oxytoca; †Other Staphylococcus included Staphylococcus hominis, Staphylococcus capitis, Staphylococcus warneri and Staphylococcus saprophyticus; ‡Other Gram-positive bacteria included Clostridium difficile, Enterococcus faecium, Enterococcus faecalis and Streptococcus; §Other pathogens included Candida, Aspergillus and Chlamydia. MRSA, Methicillin-resistant Staphylococcus aureus; MSSA, Methicillin-sensitive Staphylococcus aureus.*

Outcomes

The mortality rate was 13.7% in septic patients and 8.3% in non-septic patients. The fatality rates varied greatly among patients with different surgery types, different surgical sites and different surgical contamination classes (**Table 5**), and were higher in patients undergoing emergency procedures, patients with supratentorial lesions and patients with clean-contaminant surgical wounds. Patients with trauma and intracranial hemorrhage had higher mortality rates than those with other indications of craniotomy. Septic patients had lower GOS at hospital discharge, longer ICU LOS, longer hospital LOS and higher hospitalization costs (**Table 1**).

DISCUSSION

We conducted this prospective observational study to identify the incidence, risk factors, and outcomes of sepsis in post-craniotomy critically ill patients over the course of 2 years. We found that the incidence of sepsis in our patients was 33.3%, and the hospital mortality rate of patients with sepsis was 13.7%. Advanced age, male, hypertension, trauma, postoperative intracranial complications, and lower GCS on the first postoperative day were independent risk factors of sepsis for post-craniotomy patients. Septic patients had higher hospital mortality, lower GOS at hospital discharge, prolonged ICU LOS, prolonged hospital LOS, and higher total hospital costs than patients without sepsis.

TABLE 4 | Risk factors for infection and sepsis in critically ill post-craniotomy patients.

Risk factor*	Infection		Sepsis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	-	-	1.014 (1.002, 1.027)	0.027
Male	-	-	1.739 (1.217, 2.485)	0.002
Hypertension	-	-	1.526 (1.025, 2.274)	0.038
Trauma	2.741 (1.275, 5.892)	0.010	2.294 (1.157,4.548)	0.017
Operative time (hours)	1.12 (1.043, 1.203)	0.002	-	-
GCS on postoperative day 1	0.943 (0.894, 0.995)	0.031	0.819 (0.777, 0.864)	<0.001
Postoperative intracranial complications†	1.785 (1.064, 2.994)	0.028	2.086 (1.307, 3.330)	0.002

**Factors listed are those found statistically significant after Multinomial Logistic Regression analysis. Other factors entered into the model but no longer significant after adjustment included smoking, alcoholism, diabetes, categories of surgery (elective or emergency surgery), surgical sites (supratentorial or infratentorial surgery), surgical wound classifications (clean or clean-contaminated) and intracranial tumors. †Postoperative intracranial complications included intracranial hemorrhage, cerebral infarction, hydrocephalus, cerebrospinal fluid leakage and other intracranial complications. OR, Odds ratio; CI, Confidence interval; GCS, Glasgow Coma Scale.*

TABLE 5 | Mortality rates of patients with different intracranial diseases, surgical categories, surgical sites, and surgical wound classifications.

	All patients		Patients with sepsis		Patients without sepsis		*P*-value
	Number	Mortality	Number	Mortality	Number	Mortality	
Indications for craniotomy							
Tumor							
Glioma	162	6.2%	56	8.9%	106	4.7%	0.316
Meningiomas	150	2.0%	32	3.1%	118	1.7%	0.516
Tumors of the cranial and paraspinal nerves	62	6.5%	13	7.7%	49	6.1%	1.000
Tumors of the sellar region	56	10.7%	25	24%	31	0%	0.005
Mesenchymal, non-meningothelial tumors	40	5.0%	13	7.7%	27	3.7%	1.000
Embryonal tumors	39	7.7%	10	20%	29	3.4%	0.156
Metastatic tumors	11	18.2%	4	25%	7	14.3%	1.000
Other tumors*	26	15.4%	8	25%	18	11.1%	0.563
Trauma	102	26.5%	53	26.4%	49	26.5%	0.989
Cerebrovascular disease							
Aneurysm	90	12.2%	34	8.8%	56	14.3%	0.524
Vascular malformation	58	1.7%	18	5.6%	40	0%	0.31
Intracranial hemorrhage	42	26.2%	15	26.7%	27	25.9%	1.000
Occlusive cerebrovascular disease	24	20.8%	7	0%	17	29.4%	0.272
Other indications‡	38	5.3%	12	0%	26	7.7%	1.000
Surgical category							
Elective surgery	703	6.1%	216	9.7%	487	4.5%	0.008
Emergency surgery	197	24.4%	84	23.8%	113	24.8%	0.875
Surgical site							
Supratentorial	515	14.6%	185	17.3%	330	13.0%	0.188
Infratentorial	385	4.2%	115	7.8%	270	2.6%	0.025
Contamination class							
Clean	815	9.0%	263	11.4%	552	7.8%	0.091
Clean-contaminant	85	21.2%	37	29.7%	48	14.6%	0.090

**Other tumors included neuronal and mixed neuronal-glial tumor, choroid plexus tumor, lymphomas, tumors of the pineal region, melanocytic tumors and germ cell tumors; ‡Other indications included dysplasia diseases, functional neurological diseases, hydrocephalus and intracranial infection.*

Our results suggested that sepsis was common in post-craniotomy patients admitted to the ICU. Compared with previous studies, the incidence of sepsis in our study was relatively low. Previous studies have shown that the incidence of sepsis varied among different populations (7, 8, 14, 31, 32). The disparity in the patient population might be the main reason for the difference in the incidence of sepsis. Differences in the definitions of sepsis could partly explain the variation in

incidence. Most previous studies had defined sepsis as systemic inflammatory response syndrome (SIRS) due to infection (9, 32, 33). SIRS has proven to be extremely sensitive but has poor specificity for sepsis (9, 22). Even in the absence of sepsis, SIRS can also be frequently observed in ICU patients, including patients with acute cerebral injury (34, 35). Previous studies that determined sepsis based on SIRS criteria might have overestimated the incidence of sepsis.

Post-craniotomy critically ill patients were rarely involved in previous literature. Pertsch et al., estimated the epidemiology of sepsis in elective neurosurgery patients using the data of the American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP) (19). In their study, the incidence of sepsis in patients undergoing craniotomy was 1.21%. Zhang et al., also using the ACS NSQIP database, reported a 1.35% incidence of sepsis in patients undergoing craniotomy for tumor resection (20). Compared with the incidence of sepsis in the above two studies, the incidence of sepsis in our study was much higher. Several reasons may explain the high incidence of sepsis in our patients. Although all of the studies included patients undergoing neurosurgery, we only focused on patients admitted into the ICU. Our patients might be more severely ill and more prone to infections and sepsis (10, 36). Furthermore, Pertsch et al., only included elective neurosurgical patients, while both elective and emergency surgery patients were included in our study. It is well-known that the rates of postoperative sepsis were significantly greater for non-elective than for elective procedures in the general surgical and mixed surgical patients (36, 37). Excluding patients undergoing non-elective procedures might be one of the reasons for the lower incidence of sepsis in the study of Pertsch et al.

In our patients, pneumonia was the leading cause of sepsis. Unfortunately, a review of the literature yielded few studies on sepsis that included similar patients and were comparable with ours. Some studies have described the epidemiology of infections in neurological patients (5, 38–40). Consistent with previous reports (5, 39, 40), the lungs were the most frequent focus of infection in our patients. The incidence of pneumonia in the present cohort ($n = 336$, 37.3%) was relatively lower than that in critically ill stroke patients (75.2%) (41), and was very close to that (37.5%) in post-craniotomy patients in the study of Kourbeti et al. (40). Zhang et al. (42) found that the incidence of pneumonia varied among post-craniotomy patients, with the highest in patients with cerebrovascular diseases and the lowest in patients with tumors. Differences in patient populations might the main reason for the difference in the incidence of pneumonia between our study and others.

CNS infections were the second most common cause of sepsis in this study. The incidence of CNS infection in this study was higher than those reported in previous studies (40, 43–45). Those previous studies included patients treated in general wards after craniotomy, while we only included patients admitted into ICU. In the study of Kourbeti et al. (40), a much higher incidence of meningitis was found in patients admitted into the ICU than that in non-ICU admission patients [9.1% (16/176) vs. 0 (0/148), $P < 0.001$]. More serious condition might be the main reason for the high incidence of CNS infection in our patients. However, compared with their patients admitted to the ICU, the incidence of CNS infection in our patients was still significantly higher. Different diagnostic criteria may have resulted in the disparity. In the study of Kourbeti et al. (40), meningitis was diagnosed only if the bacterial culture was positive. The occurrence rate of meningitis might be underestimated as the CSF cultures might be negative in some meningitis patients, especially in those who have hospital-acquired meningitis and have received antibiotic therapy prior to obtaining CSF studies (46).

Nearly two-thirds (65.7%) of patients were diagnosed with sepsis within 1 week of surgery, and 71% of patients developed sepsis within 1 week of ICU admission. In the first week after craniotomy, patients were prone to infections due to cerebral edema, increased intracranial pressure, bed-ridden state, dysphagia, disturbance of consciousness, or requirement of mechanical ventilation (47), indicating the importance of preventing infections, especially nosocomial pneumonia and CNS infections, as they were the leading causes of sepsis. Hand hygiene, head of bed elevation, oropharyngeal decontamination, gastric residual monitoring and contact precautions were implemented in our center to prevent hospital-acquired infections. Further stringent prevention strategies having yet to be implemented, such as selective digestive tract decontamination, subglottic suction and surveillance cultures for multidrug-resistant bacteria colonization, might be needed to reduce the risk of nosocomial infections and sepsis. In addition, removing unnecessary drainage / monitoring intracranial tubes (45), timely administration of prophylactic antibiotics (43, 48, 49), proper skin preparation, and maintenance of sterile conditions might be helpful for the prevention of meningitis and surgical site infections after craniotomy.

Similar to previous studies, we found that male (50), an older age (50, 51), and a lower postoperative GCS (32, 52) were independent risk factors for sepsis. We also found that trauma patients and patients with postoperative intracranial complications were at higher risk for sepsis. Understanding these risk factors associated with sepsis may help physicians in the identification of high-risk patients, and in the prevention, early diagnosis and early treatment of sepsis. Previous studies (19, 20) reported that pre-operative ventilator dependence, functional status, bleeding disorders, dyspnea, severe chronic obstructive pulmonary disease (COPD) and chronic steroid use were independent risk factors of sepsis for craniotomy. However, data on pre-operative bleeding disorders, pre-operative ventilator dependence and chronic steroids use were not collected in our study. Our patients were much younger, and there might be few people with the above comorbidities. Pre-operative functional status was not evaluated in our study as functional status might change significantly before and after surgery in many patients. We collected post-operative GCS, which could reflect the postoperative status of patients, and found GCS was independent risk factor for sepsis. Chronic lung disease was not associated with sepsis in our patients. However, since only four patients had chronic lung diseases, this result might be related to the fact that the sample size was too small to detect differences between groups. Unlike previous studies (19, 20), we found that operative time was associated with infection

but not sepsis. The result might have been influenced by the heterogeneity of patients. In this study, the occurrence rate of sepsis in trauma patients (34.6%) was higher than those in patients with intracranial tumors (29.5%) and cerebrovascular diseases (34.6%), while the operative time of trauma patients [Median 2.7 h, IQR (2.0, 3.5)] was shorter than those of patients with intracranial tumors [Median 5.2 h, IQR (3.9, 6.9)] and cerebrovascular diseases [Median 3.3 h, IQR (2.5, 4.6)].

The mortality rate of sepsis in our patients was lower than those in general ICU wards (15, 33). Our patients were much younger and had fewer comorbidities than those in general ICU wards, which might be the most important reasons for the low mortality rate. In addition, improved therapeutic strategies and compliance with practice guidelines (53–55) might have resulted in decreasing mortality rates of sepsis (16), which may also be one of the reasons for the low mortality rate in this study.

Limitations

Our study has several limitations. First, this is a single-center study. Most of the patients admitted to this center were transferred from other hospitals because of severe cerebral diseases, and the results of our study might not be generalizable to other centers. Second, the data cannot reflect the epidemiology of sepsis in all patients undergoing craniotomy, as we only screened patients admitted to the ICU ward. Septic patients who had been treated in general wards were not included. ICU-unadmitted septic patients might have milder conditions and better prognoses, and were not the population of interest in this study. Furthermore, we used logistic regression analysis to evaluate the risk factors for sepsis. Logistic regression assumes linearity between the predicted (dependent) variable and the predictor (independent) variables. However, this is not always the case in reality (56). Therefore, the results of the regression analysis need to be further verified in future researches.

CONCLUSION

Sepsis is a frequent complication in critically ill post-craniotomy patients. Advanced age, male, hypertension, trauma, postoperative intracranial complications, and lower GCS on the first postoperative day were independent risk factors of sepsis. Early identification of high-risk patients based on risk factors may facilitate early diagnosis and treatment of sepsis and ultimately improve the prognosis of these patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Review Board of Beijing Tiantan Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JZ and LZ designed the study, conducted the statistical analysis, interpreted the results, drafted, and critically revised the manuscript. JZ and X-YL contributed in data collection and analysis. X-YL, G-QC, H-LL, SL, GS, MX, Y-LY, and J-XZ contributed in data analysis, interpretation of data, and drafting the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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Bulk RNA Sequencing With Integrated Single-Cell RNA Sequencing Identifies BCL2A1 as a Potential Diagnostic and Prognostic Biomarker for Sepsis

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Background: Sepsis is one of the leading causes of morbidity and mortality worldwide in the intensive care unit (ICU). The prognosis of the disease strongly depends on rapid diagnosis and appropriate treatment. Thus, some new and accurate sepsis-related biomarkers are pressing needed and their efficiency should be carefully demonstrated.

Methods: Differential expression analysis and weighted gene co-expression network analysis (WGCNA) were applied to detect sepsis and monocyte/macrophage-related genes. Least absolute shrinkage and selection operator (LASSO) and random forest regression analyses were used in combination to screen out prognostic genes. Single-cell RNA sequence profiling was utilized to further verify the expression of these genes on a single cell level. Receiver operating characteristic (ROC) curve and decision curve analysis (DCA) were also applied to verify the diagnostic value of the target biomarkers.

Results: The intersections of the genes detected by differential expression and WGCNA analyses identified 141 overlapping candidate genes that were closely related to sepsis and macrophages. The LASSO and random forest regression analyses further screened out 17 prognostic genes. Single-cell RNA sequencing analysis detected that *FCGR1A* and *BCL2A1* might be potential biomarkers for sepsis diagnosis and the diagnostic efficacy of *BCL2A1* was further validated by ROC curve and DCA.

Conclusions: It was revealed that *BCL2A1* had good diagnostic and prognostic value for sepsis, and that it can be applied as a potential and novel biomarker for the management of the disease.

Keywords: sepsis, diagnosis, biomarker, single-cell, sequencing

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INTRODUCTION

Sepsis, which is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection," is one of the leading causes of morbidity and mortality worldwide in the intensive care unit (ICU) (1). As a disease of heterogenous and imprecise syndromes, it also has multiple phenotypes. Based on some phenotypic identification methods of sepsis, most of the patients are grouped according to the degree of inflammatory response, the stability of hemodynamic, the severity of coagulopathy and by using the clinical or genomic variables (2).

Regardless of the subtypes and phenotypic of the disease, the prognosis of sepsis strongly depends on rapid diagnosis and appropriate treatment (1, 3). Therefore, it is particularly important to find accurate, sensitive, and early biomarkers for diagnosing sepsis. To date, multiple biomarkers have been identified and applied for the diagnosis of the disease, including several classic biomarkers [C-reactive protein (CRP) and procalcitonin (PCT)], and some novel ones (decoy receptor-3 and hepcidin) (4, 5). However, classical biomarkers such as CRP and PCT lack specificity, which makes diagnosing sepsis in its early stages extremely difficult. At the same time, the precise roles of newly identified biomarkers such as decoy receptor-3 and hepcidin in the management of patients with septic shock have not been well defined. Moreover, among the biomarkers that have been studied, only a few have been properly evaluated in large cohort studies (6).

Previous studies have shown that monocytes and macrophages play an important role in the pathogenesis of sepsis (7). Recent studies have also demonstrated that monocytes can facilitate the proliferation and exhaustion of T cells via interleukin-1B (IL-1B) signaling pathways and finally lead to monocyte-dependent suppression of T cell function in sepsis (7). Furthermore, monocytes can be activated through aggregation with platelets and release multiple proinflammatory cytokines [e.g., IL-6, IL-1β, and tumor necrosis factor-α (TNF-α)]. Platelet-monocyte aggregates can also facilitate polarization of CD14+ monocytes toward a proinflammatory M1 phenotype (8). Moreover, macrophage polarization has been found to be closely related to the pathogenesis of sepsis (9). Macrophages can mainly polarize into two distinct phenotypes with opposite influences on immune function: M1-like macrophages with proinflammatory function and M2-like macrophages with anti-inflammatory function. In patients with sepsis, elevated levels of multiple cytokines [TNF-α and interferon-γ (IFN-γ)] and pathogen-related molecular patterns [e.g., lipopolysaccharide (LPS)] can activate inflammatory pathways, especially the nuclear factor-κB (NF-κB) pathway, and eventually trigger macrophage polarization toward the M1 phenotype. A continuous M1-like macrophage polarization can further induce an inflammatory response and cause organ, tissue, and immune cell damage (9).

Considering the key roles of monocytes and macrophages in the pathogenesis of sepsis, our study aims to screen some novel diagnostic and prognostic biomarkers that are related to monocytes and macrophages by using bulk RNA sequencing with integrated single-cell RNA sequencing. We hypothesis that the identified novel sepsis-related biomarkers might provide new ideals and research directions for the diagnosis and treatment of sepsis.

METHODS

Bulk RNA-Sequencing Data Downloading and Processing

The datasets GSE65682, GSE28750, GSE69528, and GSE100159 were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) using the GEOquery R package (version 2.60.0, The R Foundation for Statistical Computing, Vienna, Austria) (10). We designated GSE65682 as the training set for the downstream analysis, and GSE28750, GSE69528, and GSE100159 were designated as the validation sets to confirm the results. The GSE65682 dataset included data on 760 ICU patients and 42 healthy cases. Differentially expressed genes (DEGs) were detected using the "limma" R package which had been recognized as a classic algorithm in bioinformatics analysis (11). The DEGs with an adjusted *P*-value (adj. P. val.) of <0.05 and $|\log FC| \geq 1.5$ were considered statistically significant.

WGCNA Network Construction

Clusters of highly correlated genes were screened and a weighted gene co-expression network was constructed using the WGCNA algorithm which is a widely used approach to identify potential biomarkers of interest (12). The soft-threshold β was set to eight to ensure the network followed a scale-free distribution. Next, the adjacency matrix was transformed into a topological overlap matrix (TOM). Subsequently, hierarchical clustering was applied to generate modules and every module consisted of at least 30 genes (min Module Size = 30). Finally, the module eigengene (ME) was calculated, and cluster analysis was performed on the modules. Modules that were similar were merged into a new module.

Identification of Clinically Significant and Immune Cell Infiltration-Related Module

We performed immune infiltration profiling on the samples of the GSE65682 dataset using the Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) deconvolution algorithm (13). The algorithm can count the immune cell infiltration score and quantify the degree of infiltration of 22 types of immune cells on target samples. Next, gene significance (GS) and module membership (MM) were calculated to evaluate the association between the modules and sample traits (including immune cell infiltration score and clinical data). Finally, clinical significance and immune cell infiltration-related modules were identified, and hub genes of this module were extracted for subsequent analysis.

LASSO and Random Forest Regression

LASSO and random forest regression has been widely applied to screen prognosis-related genes in the previous studies (14, 15). Thus, they were utilized in combination to identify the genes that were correlated with the prognosis of sepsis. The LASSO

regression analysis was conducted using the "glmnet" R package, and random forest regression analysis was performed using the "randomForest" R package.

ROC Curve and DCA Analysis

The receiver operating characteristic (ROC) curve analysis was performed using the "pROC" R package and was visualized using the "ggplot2" R package. The decision curve analysis (DCA) was conducted using the "DecisionCurve" R package.

Single-Cell RNA Sequencing Data

Bacterial sepsis data were downloaded from the SCP548 of the Broad Institute Single Cell Portal (SCP) (https://singlecell.broadinstitute.org/single_cell). The single-cell data includes 19 healthy control samples from Research Blood Components (Watertown, MA, USA) and 46 infected samples from three different medical services (an emergency department, a medical department and an ICU) (16). As our study was focusing on patients with sepsis, we extracted the data from Bac-Sep (defined as having bacteremia and sepsis but not requiring ICU admission, $n = 4$), ICU-Sep (defined as patients with sepsis requiring ICU care, $n = 8$), and healthy controls ($n = 19$) for subsequent analysis. The data of coronavirus disease of 2019 (COVID-19) was also downloaded from the GSE150728 of the GEO database. This data was extracted from seven hospitalized patients due to COVID-19 and six healthy controls.

Single-Cell RNA Sequencing Data Processing and Analysis

The bacterial sepsis datasets were based on the 10x Genomics platform (https://www.10xgenomics.com). We used the Seurat pipeline to analyze the single-cell RNA (scRNA) data. The original data matrix downloaded from SCP was inputted into R (version 4.1.1) and processed with the Seurat R package (version 4.0.4) (17). The "Create Seurat Object" function was utilized to transform the dataset into a "Seurat object."

Quality control was conducted through filtering out cells with <200 genes, >2,500 genes, or >10% mitochondrial genes. A total of 60,543 filtered cells were included in the subsequent analysis. Data normalization was performed using the "LogNormalize" method, and 2,000 highly variable genes (HVGs) were identified using the "vst" method. Subsequently, the "Harmony" R package (version 0.1.0) was utilized to remove the batch effect of the sample identity (18). Cell cycle scores for every cell were calculated using the "CellCycleScoring" function, and the cell cycle effect was removed using the "Scaledata" function. Subsequently, we applied principal component analysis (PCA) to identify significant principal components (PCs) and to choose 30 PCs for t-distributed stochastic neighbor embedding (t-SNE) analysis. Moreover, 15 different clusters were identified using the "FindClusters" function with a parameter resolution of 0.6. Finally, we used a published list of marker genes to annotate the cell type of each cluster. The GSE150728 scRNA sequencing dataset was processed as described above. In all, 72,849 cells were included in the analysis. Cluster analysis was performed through the "FindNeighbors" and "FindClusters" functions at a resolution of 0.8.

Immunity-Related Genes Score

The DEGs of each cluster between the control group and the disease group were screened using the "FindMarkers" function. Then, we used the ImmPort database (https://www.immport.org/shared/home) (19) to screen the DEGs of each cluster and identified immunity-related genes (IRGs). The IRGs were considered as a gene set to calculate the IRG scores in every cell using the AUCell R package (version 1.14.0) (20). The IRGs scores were calculated based on gene set enrichment analysis (GSEA). The cell which expresses more genes within the IRGs revealed a higher area under the curve (AUC) value. The threshold to distinguish gene set active cells was determined using the "AUCell explore Thresholds" function. Finally, we used the "ggplot2" R package (version 3.3.5) to visualize the active clusters by mapping the IRGs score in every cell relative to the t-SNE.

Gene Ontology and/or GSEA

The Metascape website (https://metascape.org/gp/index.html) was used for functional enrichment analysis upon the hub genes of target module in bulk-sequencing profiling. The ClusterProfiler package (21) was utilized for performing Gene Ontology (GO) and GSEA on the marker genes of cell clusters in scRNA-sequencing profiling, where $p < 0.05$ indicated statistically significant enrichment.

RESULTS

Our study integrated four bulk-seq datasets and two scRNA-seq datasets. All the datasets included in our study were shown in detail in **Table 1**. The flowchart of our study was shown in **Figure 1**.

Identification of DEGs in the GSE65682 Dataset

The "limma" R package was used to detect DEGs between the ICU samples and healthy samples of the GSE65682 dataset. The DEGs were screened according to an adj. P. val. of <0.05 and $|\log FC| \geq 1.5$. After screening the dataset, a total of 524 DEGs were obtained, of which 270 genes were upregulated and 254 genes were downregulated (**Supplementary Table 1**). These results were visualized using a volcano map (**Figure 2A**) and a heatmap (**Figure 2B**).

Weighted Co-expression Network Construction and Identification of key Modules

Next, WGCNA analysis was conducted to detect the co-expression genes and modules based on 720 samples from the GSE65682 dataset. To ensure the network followed a scale-free distribution, a soft threshold power of eight was chosen as the most appropriate one for network construction (**Figure 3A**). Hierarchical clustering analysis was then performed to generate modules, and similar modules were merged. The cut height for merging modules was 0.25, which meant that modules whose eigengenes were correlated above 0.75 were merged (**Figure 3B**).

TABLE 1 | Information for selected datasets in this study.

Datasets	Type	Platform	Sample size (Disease/Control)	Cells (Disease/Control)
GSE65682 (Training set)	Microarray	GPL13667	720/42 (ICU/health)	NA
SCP548 (Validation set)	scRNA sequencing	Illumina Novaseq S2 (Homo sapiens)	12/19 (sepsis/health)	13438/47105 (sepsis/health)
GSE150728 (Validation set)	scRNA sequencing	Illumina NovaSeq 6000 (Homo sapiens)	7/6 (COVID-19/health)	45105/27744 (COVID-19/health)
GSE28750 (Validation set)	Microarray	GPL570	10/20 (sepsis/health)	NA
GSE69528 (Validation set)	Microarray	GPL10558	83/55 (sepsis/health)	NA
GSE100159 (Validation set)	Microarray	GPL6884	35/11 (sepsis/health)	NA

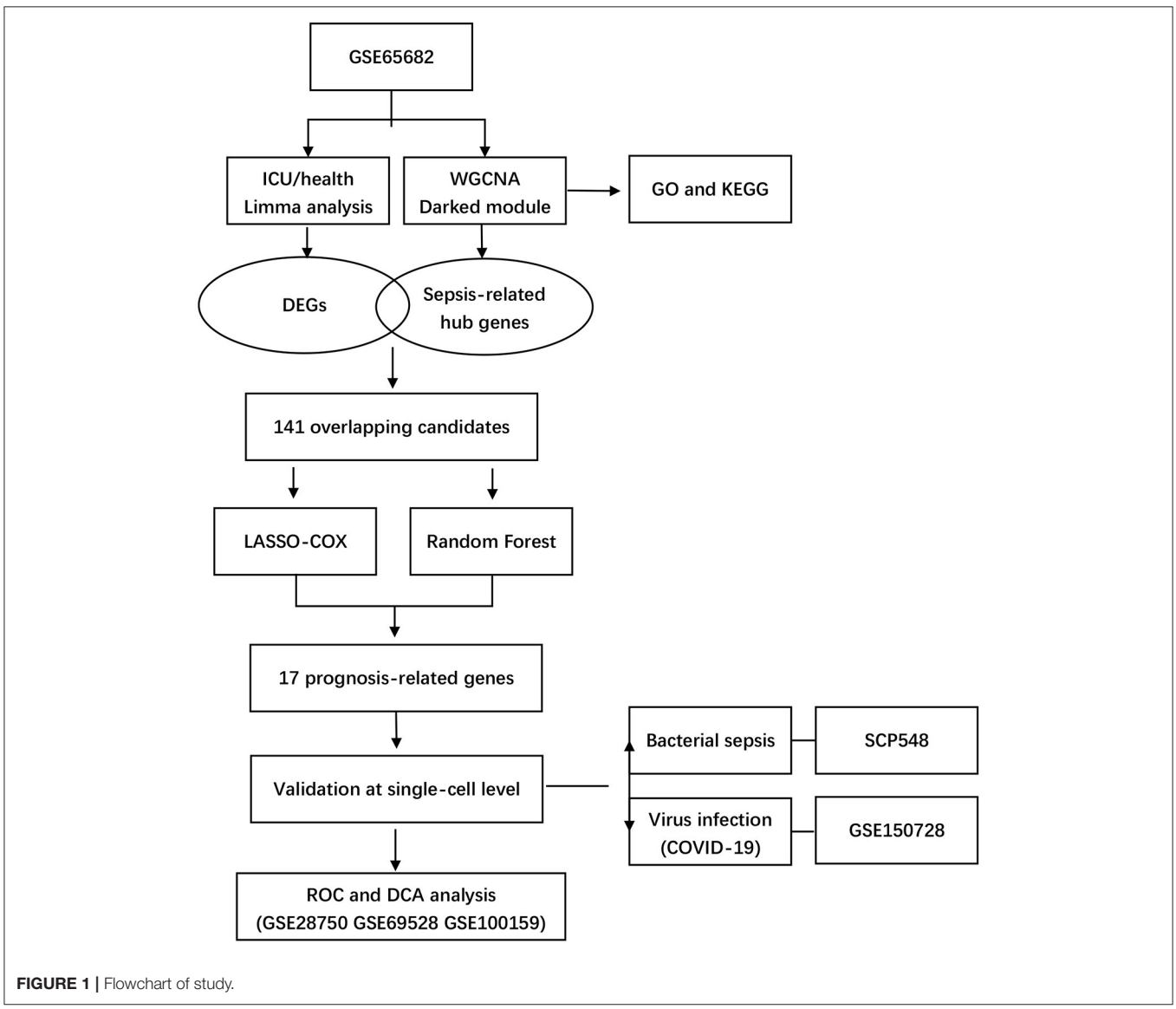

FIGURE 1 | Flowchart of study.

Finally, 22 distinct gene co-expression modules were constructed, and these are shown in different colors in **Figure 3C**.

To identify the clinical significance and immune cell infiltration-related modules, the CIBERSORT deconvolution algorithm was used first to calculate the immune cell scores. Subsequently, GS and MM were calculated to evaluate the correlation between the modules and sample characteristics (including immune cell infiltration score and clinical data) (**Figures 3D,E**). Finally, the Darked module was chosen as the target modules. This module showed the strongest significant

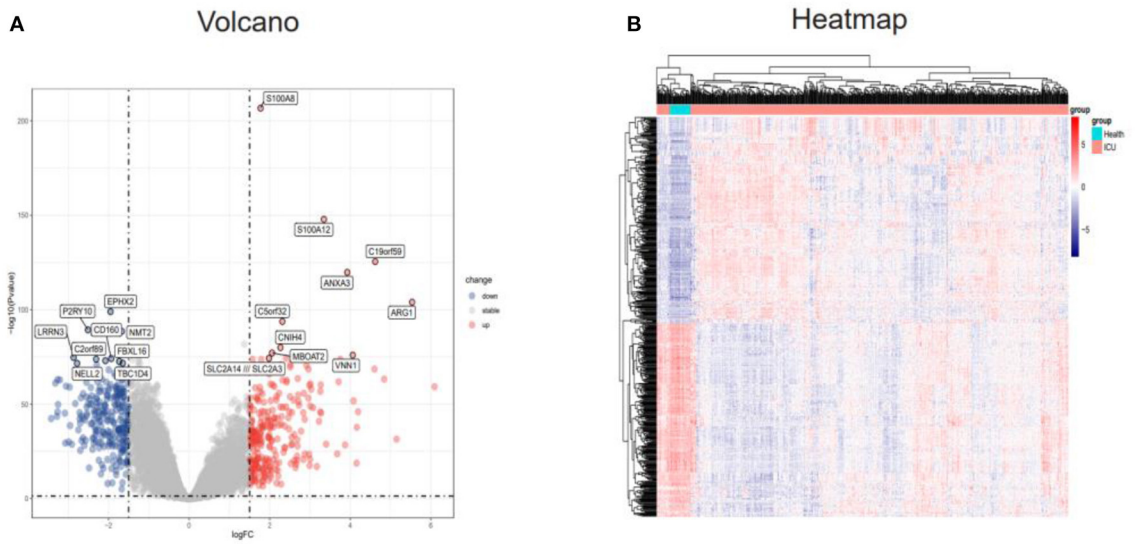

FIGURE 2 | Identification of DEGs in the GSE65682 dataset. **(A)** Volcano plot of the DEGs (|logFC| > 1.5 and adjusted P-value I < 0.05) in GSE65682. Upregulated genes are in red, and downregulated genes are in blue. **(B)** Heatmap of the DEGs in GSE65682. DEGs, differentially expressed genes.

correlation with both admission to ICU ($r = 0.46$, $p = 2e\text{-}43$) and occurrence of abdominal sepsis ($r = 0.57$, $p = 1e\text{-}69$). Furthermore, the Darked module showed a positive association with macrophages M0 ($r = 0.44$, $p = 1e\text{-}39$) (**Figures 3D,E**). To further explore the function of the Darked module, Kyoto Encyclopedia of Genes and Genomes (KEGG) and GO enrichment analyses were performed on the hub genes of this module. It was apparent that the Darked module was mostly enriched in the inflammatory and infection-related pathway (**Figure 3F**). Furthermore, the function of the Darked module was also closely related to monocytes/macrophages, which is consistent with the results of the WGCNA analysis (**Figure 3G**). These results indicated that the patients with sepsis might show predominately a monocyte/macrophage infiltration.

Identification of Prognosis-Related Genes

As the Darked module revealed a tight correlation with the status of sepsis, we extracted the hub genes of the Darked module for the following analysis (**Figure 4A**). The Darked module consisted of 463 genes. The intersection of hub genes among the Darked module and DEGs in the GSE65682 dataset were taken, and 141 genes were obtained, as shown in **Figure 4B**.

Subsequently, LASSO regression analysis in conjunction with random forest regression analysis was used to identify the prognostic genes (**Figures 4C–E**). Taking the intersections of the target genes screened out by the two algorithms, a Venn diagram was constructed (**Figure 4F**). A total of 32 genes in LASSO regression algorithms and 30 genes in random forest algorithms were retained. More importantly, 17 genes were obtained in both LASSO regression analysis and random forest analysis. The list of these 17 genes is shown in **Supplementary Table 2**.

ScRNA Profiling of PBMCs in Bacterial Sepsis

To confirm the result of bulk-RNA sequencing profiling, bacterial scRNA-sequencing data were analyzed. After data processing, 60,543 cells comprising 13,438 cells from patients with sepsis and 47,105 cells from healthy controls were retained. Subsequently, 15 clusters were identified via the t-SNE analysis of unsupervised clustering. These clusters were then annotated into six cell types based on the marker genes reported in the previous study. The six cell types were visualized using t-SNE analysis (**Figure 5A**). The expression of cell type marker genes was shown in a dot plot (**Figure 5B**).

Next, the expression level of the 17 genes, which had been screened out by the bulk RNA-sequencing profiling, was then examined at the single-cell level (**Figure 5C**). Finally, *FCGR1A* and *BCL2A1* were found to be considerably increased in the sepsis group. More importantly, both of them were predominantly expressed in the monocyte/macrophage cluster, which indicated that the two genes might be closely related to the function of this cluster (**Figures 5D,E**).

To further investigate the role played by monocyte/macrophage clusters in the pathology of sepsis, IRG scores were first calculated using the AUCell R package. The dendric and monocyte/macrophage clusters (in yellow color) were found to exhibit higher IRG scores (**Figures 6A,B**), suggesting these clusters were in an active state. In addition, when compared with the healthy control group, the number of monocyte/macrophage cells was considerably elevated in the sepsis group (**Figures 6C,D**). It was indicated that a prominent monocyte/macrophage infiltration could be found in patients with sepsis. We then performed GO and

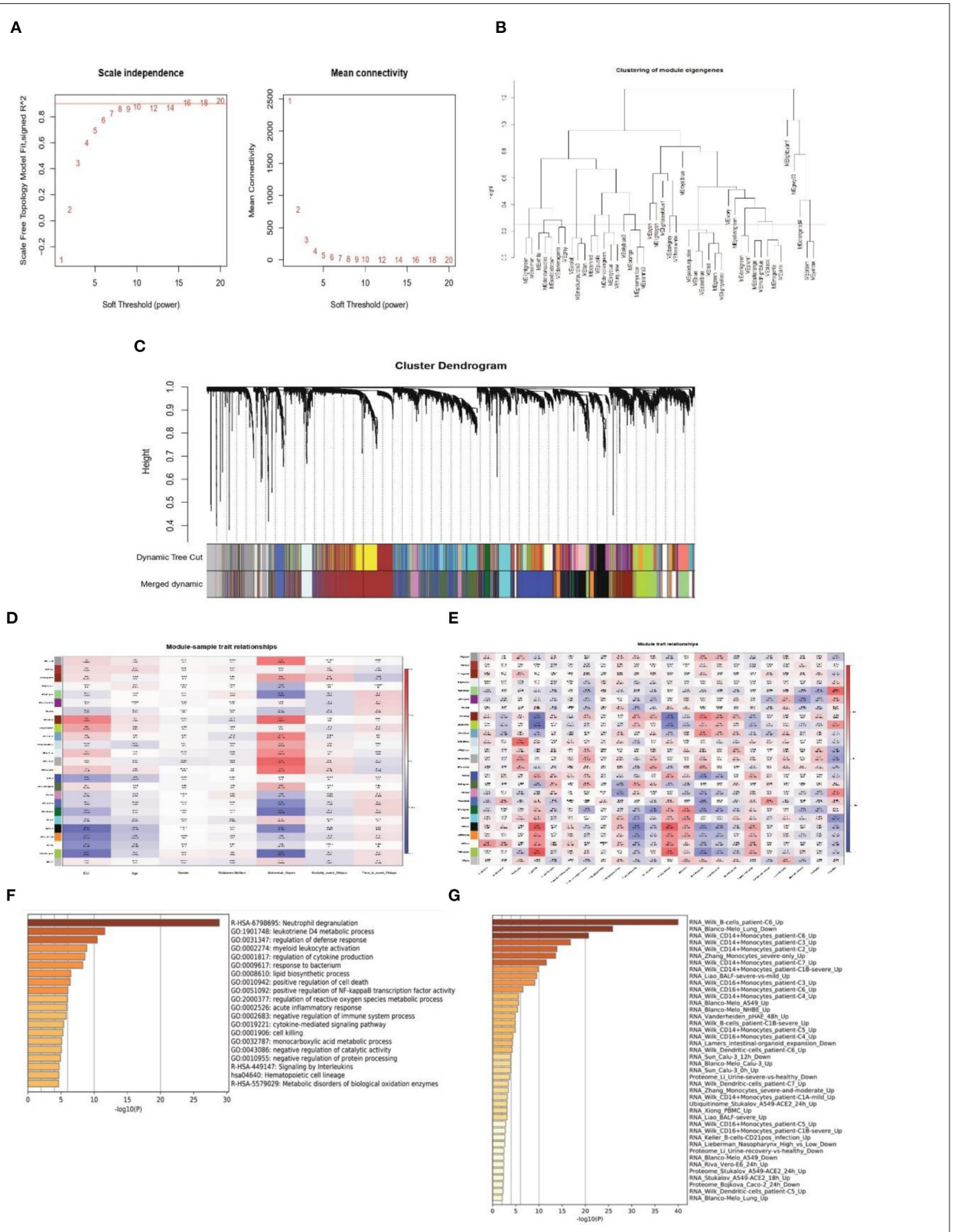

FIGURE 3 | Weighted co-expression network construction and identification of key modules. **(A)** Correlation between the modules in the network topology analysis for various soft-thresholding powers. **(B)** Cut height for merging modules. **(C)** Gene dendrogram and related module colors. **(D)** Correlation between the 22 modules and sample traits. **(E)** Correlation between the 22 modules and immune cell scores. **(F,G)** Functional enrichment analysis of the Darked module genes.

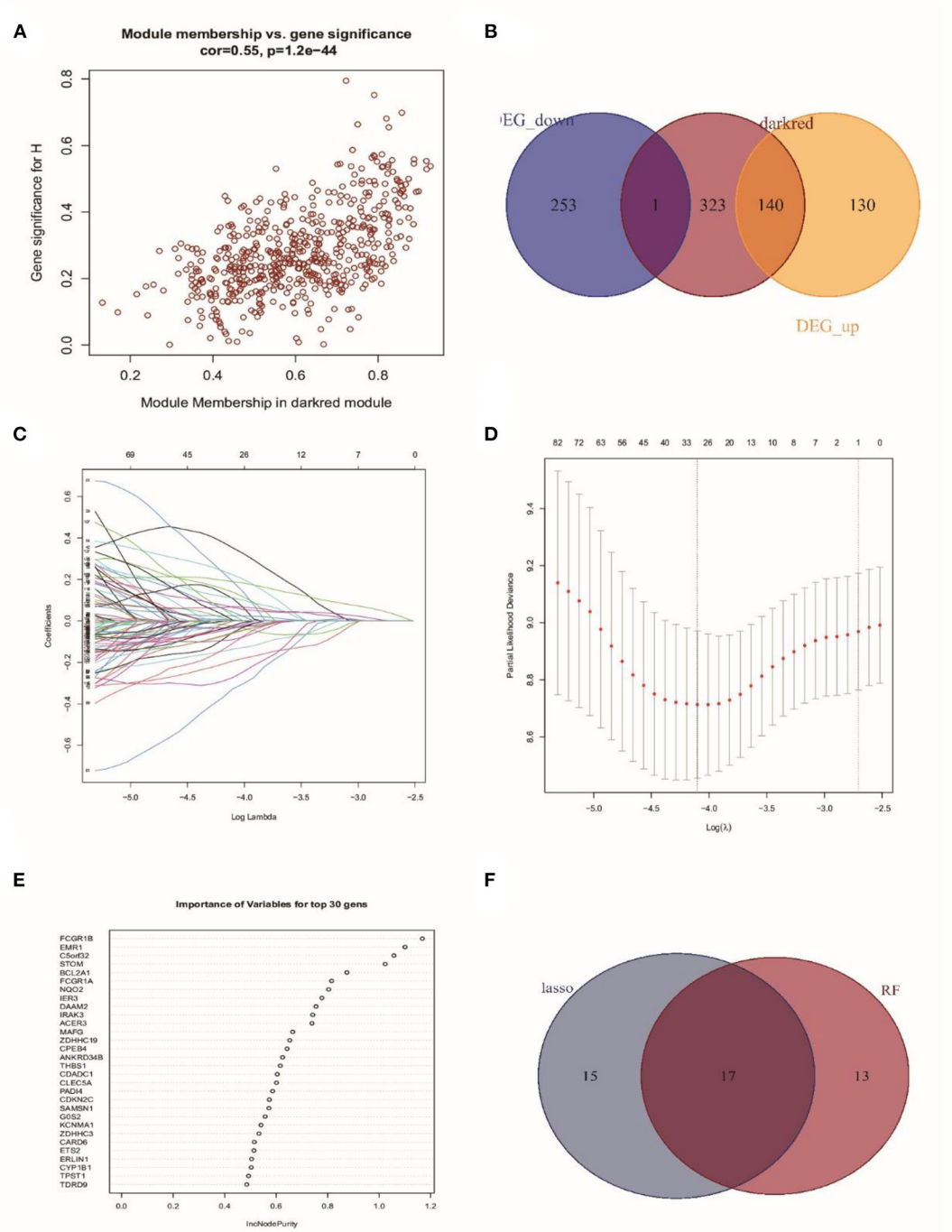

FIGURE 4 | Identification of prognostic genes in patients with sepsis. **(A)** Extraction of the hub genes of the Darked module. **(B)** 141 overlapping candidates in the intersection of the DEGs and the Darked module genes. **(C,D)** LASSO Cox analysis identified 32 prognostic genes. **(E)** The top 30 prognostic genes in the random forest regression analysis. **(F)** Venn diagram showed the genes identified by LASSO Cox and random forest regression analyses. A total of 17 prognostic genes were found in the intersection of the results of from LASSO Cox and random forest regressions.

GSEA on the monocyte/macrophage cluster. The results demonstrated that the cluster was mostly enriched in the proinflammatory and infection-related pathways, especially the NF-κB pathway, which was consistent with the functional enrichment analysis result of the Darked module in the WGCNA analysis (**Figures 6E–G**).

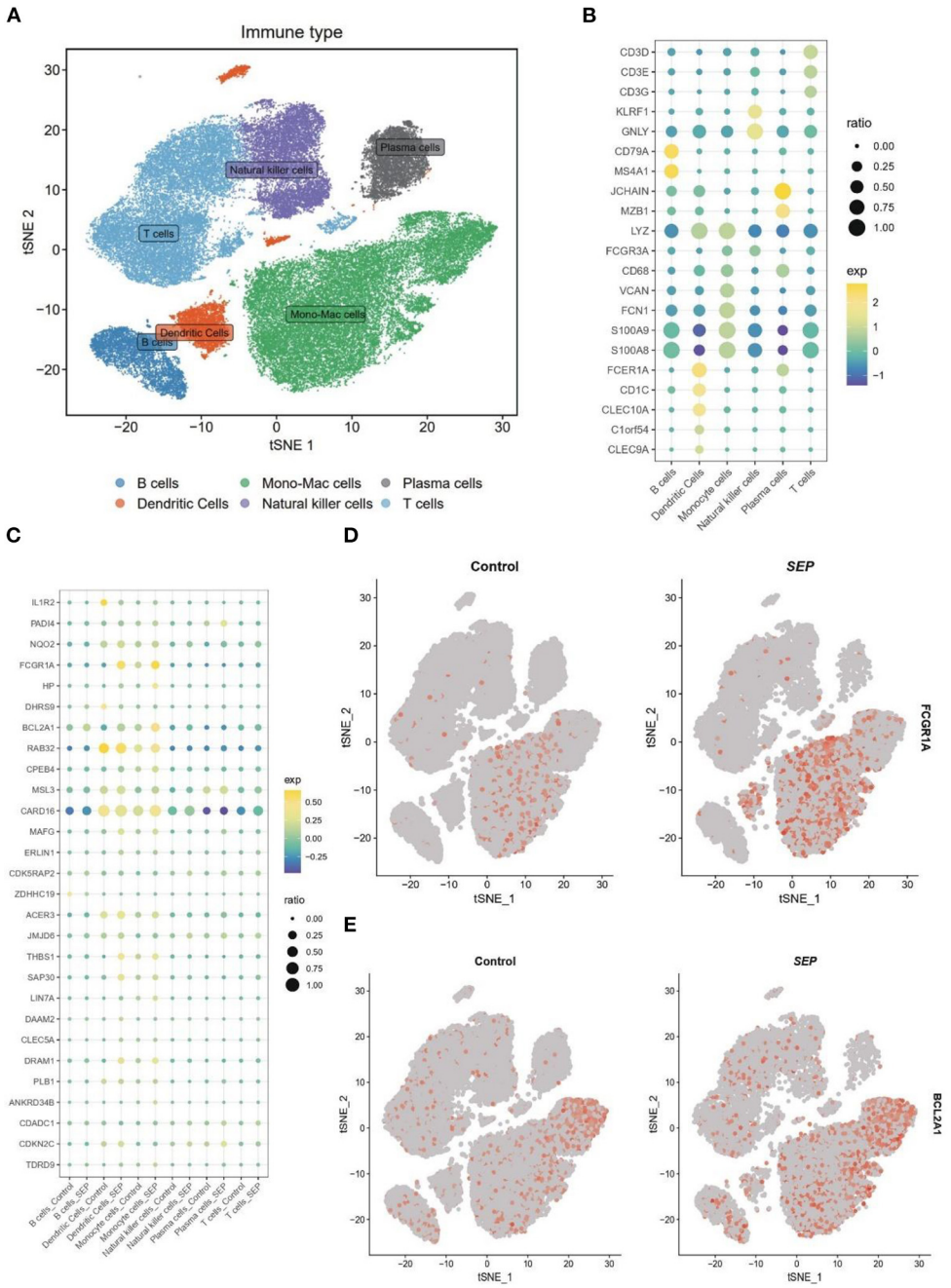

FIGURE 5 | Differential expression of target genes on a single-cell level. **(A)** t-SNE plot of 60,543 cells from SCP548 datasets. Distinct cell types are shown in different colors. **(B)** Dot plot showing the respective marker genes of different cell types. **(C)** A dot plot showing that the expression levels of 17 genes differ between the sepsis and healthy samples. **(D,E)** Expression of *FCGR1A* and *BCL2A1* shown using a t-SNE plot. t-SNE, t-distributed stochastic neighbor embedding.

ScRNA Profiling of Peripheral Blood Mononuclear Cells in COVID-19 Infection

As the above scRNA dataset was focusing on bacterial infection, another virus infection scRNA dataset was also needed to verify our result. Previous studies have revealed that severe COVID-19 infection shares similar clinical symptoms and laboratory characteristics with sepsis (22). The GSE150728 dataset comprising seven in patients with COVID-19 and six healthy volunteers was selected for subsequent analysis. The clusters were annotated into nine types of cells and visualized using t-SNE analysis (**Figure 7A**), and their respective marker genes are shown in a dotplot (**Figure 7B**). The 17 genes were also

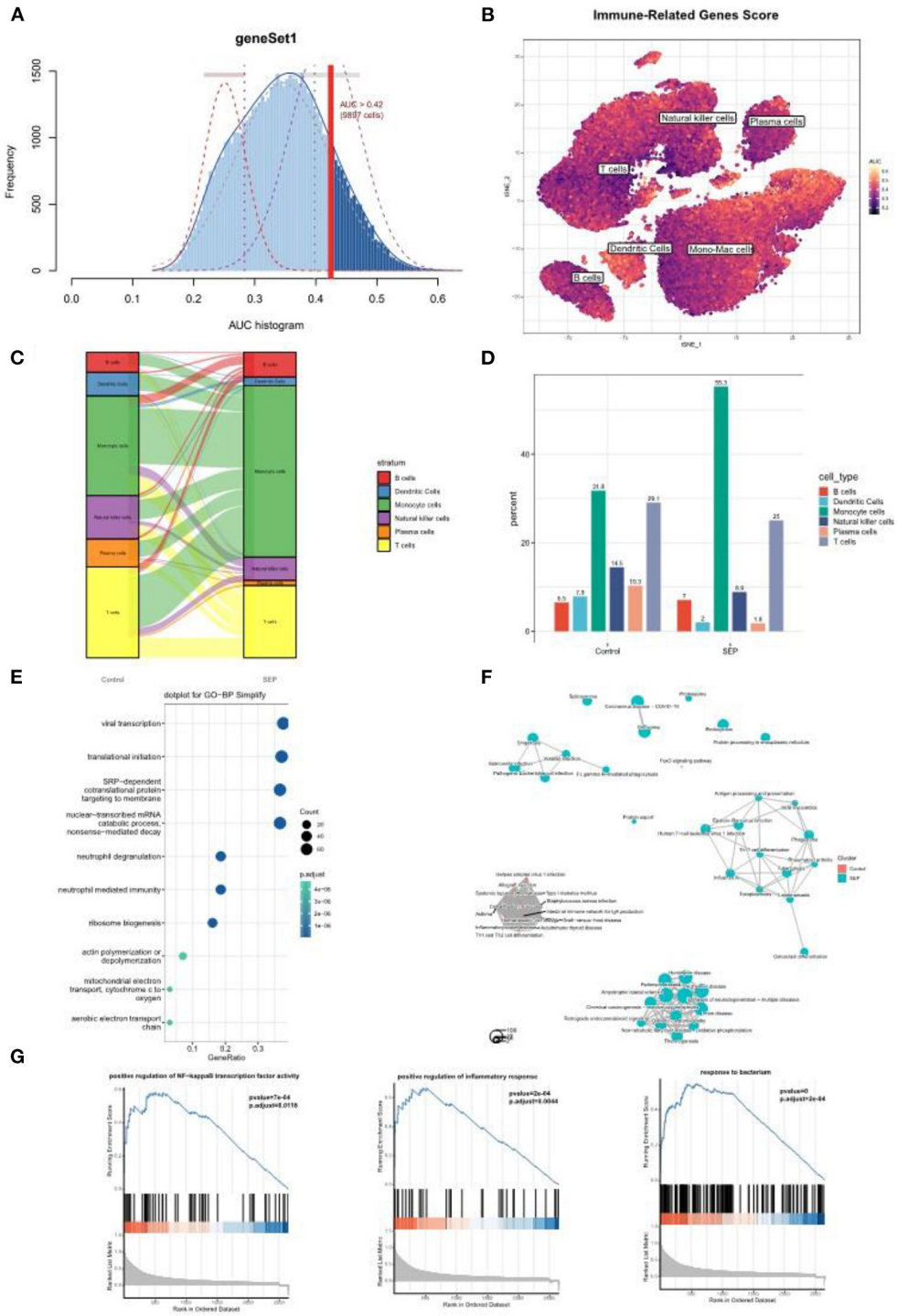

FIGURE 6 | Single-cell analysis revealed a prominent monocyte/macrophage infiltration in sepsis patients. **(A)** The threshold for distinguishing gene set active cells was set at 0.42. **(B)** t-SNE plot of the immune-related genes score (IRGs score) in all of the cell types. The dendric and monocyte/macrophage cells (in yellow color) exhibited a higher IRGs Score. **(C,D)** Sankey diagram and histogram showed the proportion of monocyte/macrophage cells was significantly increased in the patients with sepsis. **(E–G)** GO and/or GSEA showed that the monocyte/macrophage clusters were mostly enriched in the proinflammatory and infection-related pathway. t-SNE, t-distributed stochastic neighbor embedding; GO, Gene Ontology; GSEA, gene set enrichment analysis.

examined at a single-cell level (**Figure 7C**). As expected, *FCGR1A* and *BCL2A1* were significantly upregulated in the disease group and were primarily expressed in the monocyte and/or macrophage cluster and the neutrophil cluster (**Figure 7D**). Moreover, the monocyte/macrophage cluster revealed a high IRG score (**Figures 7E,F**). Taken together, these results were consistent with that of bacterial sepsis in the scRNA dataset.

Comparing the Diagnostic Performance of *BCL2A1* and *FCGR1A* as Biomarkers for Sepsis

The gene *FCGR1A*, which is also called CD64, is a classic sepsis-related biomarker which has been well studied and applied in the diagnosis of sepsis (23). On the other hand, *BCL2A1* has rarely been reported in the pathogenesis of sepsis. To further verify *BCL2A1* as a novel diagnostic biomarker for patients with sepsis, we selected three other sepsis datasets (GSE28750, GSE69528, and GSE100159) to compare the diagnostic accuracy of *BCL2A1* and *FCGR1A* in the disease. The ROC curve analysis revealed that both *BCL2A1* and *FCGR1A* had a high AUC for the diagnosis of sepsis in all three datasets (**Figures 8A–C**). We used DCA to evaluate the clinical utility of *BCL2A1* and *FCGR1A* by qualifying the net benefit at a distinct threshold. As expected, the DCA results showed that *BCL2A1* and *FCGR1A* yielded similar clinical values in the diagnosis of sepsis. In GSE69528 and GSE100159, *BCL2A1* exhibited an even higher clinical value when compared with *FCGR1A* (**Figures 8D–F**).

DISCUSSION

Sepsis, as a prevalent and severe disease, is one of the major causes of death worldwide in the ICU (1). An early and accurate diagnosis of sepsis is crucial, as delays in prescribing appropriate therapy can greatly influence the outcome of this disease (3). Although a variety of treatment and laboratory testing technologies have been gradually applied to clinical practice, the prognosis of sepsis is still not satisfactory (24, 25). In addition, multiple sepsis-related diagnostic biomarkers have been identified, and most of them have not been clearly demonstrated as effective (26). A novel biomarker is still needed in the management of patients with sepsis. Taking the important role of monocytes and macrophages in the pathogenesis of sepsis into account, we attempted to explore sepsis-related biomarkers from the viewpoint of monocyte/macrophages. In this study, we integrated bulk-RNA sequencing data and scRNA data and identified two biomarkers (*FCGR1A* and *BCL2A1*), which were closely related to sepsis and monocyte/macrophage.

The gene *FCGR1A*, also called CD64, is a classic sepsis-related biomarker. Neutrophil CD64 was considered as a marker of neutrophil activation in acute inflammatory reaction. Multiple studies have demonstrated that neutrophil CD64 expression as a candidate biomarker for diagnosing sepsis (27, 28). Our study also found that CD64 was up-regulated in neutrophil cells at the single-cell level. In addition, it also demonstrated that CD64 was significantly elevated in monocyte/macrophage cells, which was consistent with previously published studies (29, 30). However, controversy remains about whether CD64 expression on monocytes can be a diagnostic and prognostic biomarker for sepsis (31). Some researchers found that simultaneously analyzing CD64 expression for both types of cells can improve the accuracy of diagnosis (32). It was also revealed from our study that the level of *FCGR1A* was considerably elevated in sepsis, and this gene was also correlated to the prognosis of sepsis. Nonetheless, further research is still needed to verify the results of our study in clinical practice.

The gene *BCL2A1* is a member of the B-cell lymphoma 2 (BCL2) protein family and is also an important cell death regulator. The gene exerts its antiapoptotic function by sequestering proapoptotic BCL2 proteins (33). It has been well studied in the tumor setting and has been identified as a potential target for cancer therapy (34). However, its role in the pathology of sepsis is still uncertain. Our study showed that *BCL2A1* was significantly upregulated in patients with sepsis at both the tissue- and single-cell levels. The LASSO Cox and random forest regression algorithms demonstrated that *BCL2A1* was closely related to the prognosis of sepsis.

With the development of monitoring techniques and treatment, the mortality rate of sepsis declines to 15–25%, while the in-hospital mortality rate of septic shock is still as high as 30–50% (1). *BCL2A1* was identified as a prognostic biomarker for sepsis patients in our study through LASSO and Random Forest regression analysis. However, due to the lack of demographic and clinical data, some clinical studies are needed to further analysis the efficacy of the novel marker in identification of sepsis and prognostic prediction.

In addition, to further verify *BCL2A1* as a novel biomarker for diagnosing sepsis, we selected three other sepsis datasets to perform ROC curve analysis and DCA. The results showed that *BCL2A1* had a good diagnostic value in all datasets, which indicated that it might be considered as a potential biomarker for sepsis. Most importantly, scRNA profiling showed that this gene was primarily expressed with monocyte/macrophage and neutrophil cells, which indicated that the function of *BCL2A1* was closely related to these two types of cells.

Our study also found that patients with sepsis mainly manifested a monocyte/macrophage cell infiltration. Moreover, the monocyte/macrophage cluster showed a higher IRG score, suggesting that these types of cells were in an active state during the acute stage of sepsis. The GO analysis and GSEA revealed that these types of cells were strongly correlated with inflammation activation, especially the NF-κB pathway. The activation of NF-κB can not only lead to monocyte/macrophage polarizing into an M1-like macrophage (9) but can be an important inducer of *BCL2A1* expression as well (30). This could explain partly why *BCL2A1* was significantly increased in patients with sepsis and was primarily expressed in terms of monocytes/macrophages. The effect of increasing *BCL2A1* expression on monocyte/macrophage cells appears to exert its antiapoptotic function and further exacerbate the imbalance between M1-and M2-like macrophages and eventually worsen the status of sepsis. In addition, over-expression of *BCL2A1* on neutrophils might be correlated to delayed neutrophil apoptosis (35) and can lead to immune dysfunction and persistent

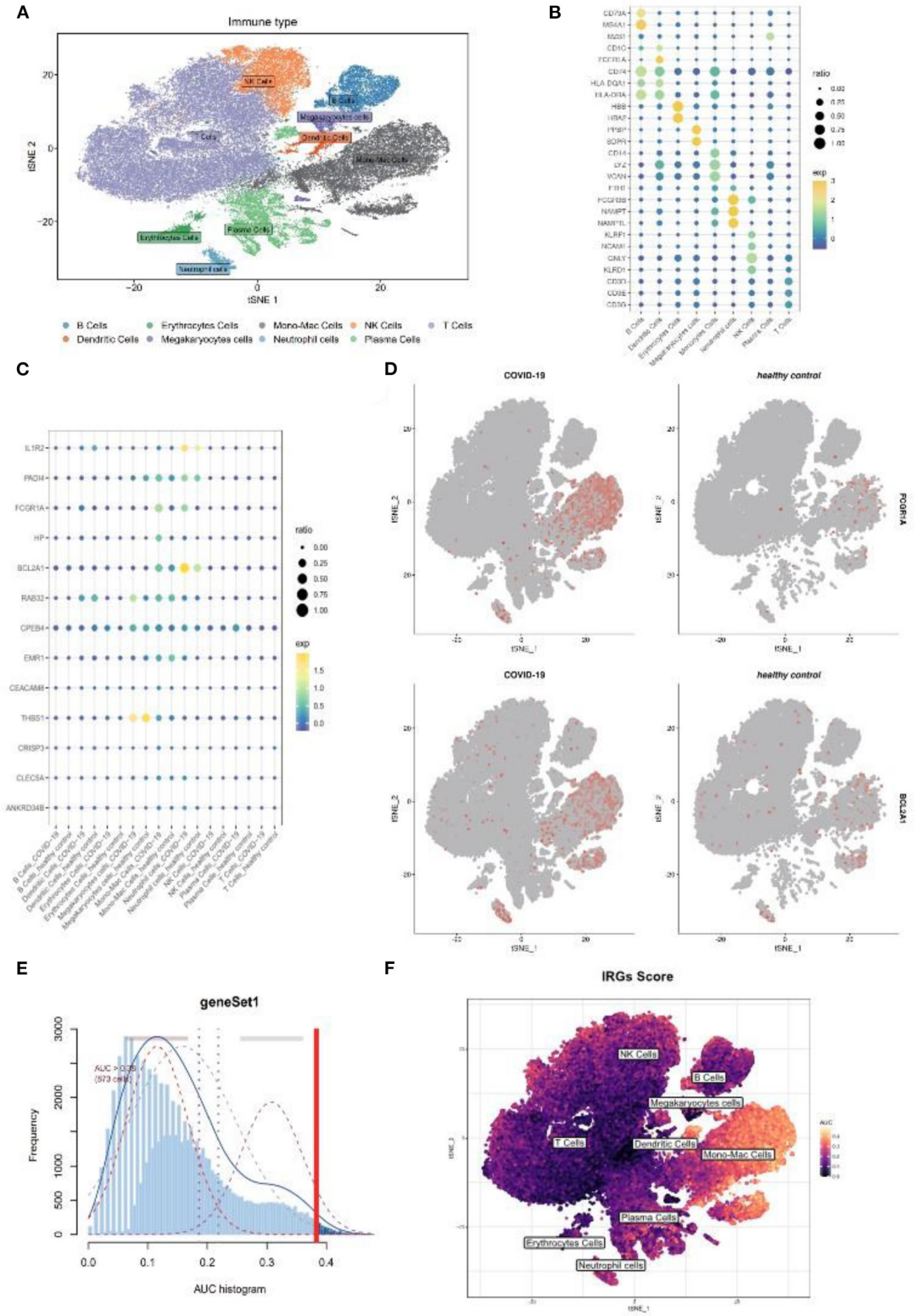

FIGURE 7 | Further validation of above results in a COVID-19 scRNA dataset. **(A)** t-SNE plot visualization of nine clusters. **(B)** The respective marker genes of nine clusters in a dot plot. **(C)** Dot plot shows the expression level of target genes in the COVID-19 and healthy samples. **(D)** Expression of *FCGR1A* and *BCL2A1* using a t-SNE plot. **(E)** A threshold of 0.38 for immunity-related genes (IRGs) score calculation was selected. **(F)** t-SNE plot of IRGs score in nine clusters. t-SNE, T-distributed stochastic neighbor embedding; COVID-19, coronavirus of 2019; scRNA, single-cell RNA.

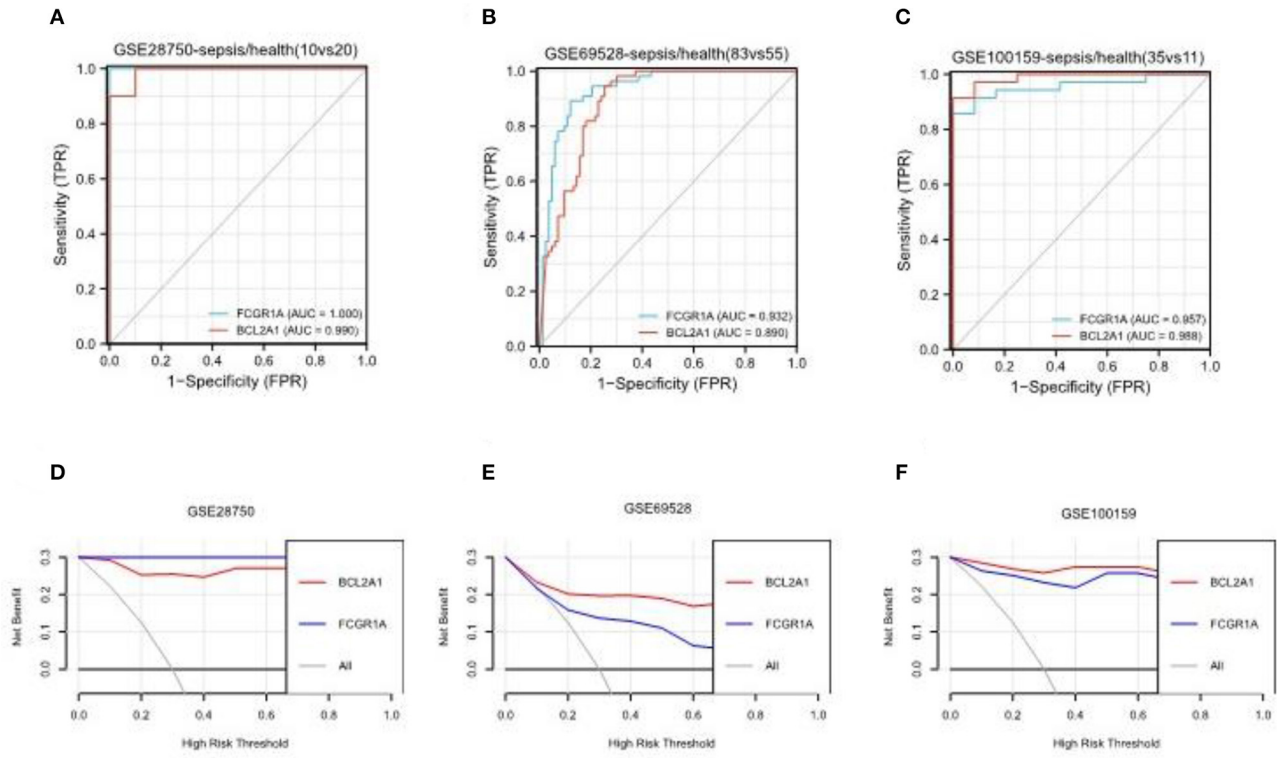

FIGURE 8 | ROC curve analysis and decision curve analysis of *BCL2A1* and *FCGR1A* in three sepsis datasets. **(A–C)** *BCL2A1* revealed a high AUC value in all three datasets, suggesting its diagnostic value in sepsis. **(D–F)** DCA of *BCL2A1* and *FCGR1A* in the three datasets. ROC, receiver operating characteristic; AUC, area under the curve; DCA, decision curve analysis.

inflammation. Thus, further study is also needed to explore the possible mechanisms.

BCL2A1 was identified as a promising and novel biomarker for sepsis diagnosis in our study, limitation still exists. The data of our study was downloaded from public databases, the effectiveness of clinical application is uncertain due to the lack of demographic and clinical information of patients with sepsis. Thus, further clinical studies are needed to verify the efficacy of the novel marker.

In conclusion, our study found that *BCL2A1* revealed good diagnostic and prognostic value for sepsis. The *BCL2A1* gene can be applied as a potential and novel biomarker for the management of sepsis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for this study in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

JL, MZ, and Y-TY: Conception and design. L-JL: Administrative support. CZ, L-XZ, S-YY, and W-YL: Provision of study materials or patients. JL, MZ, and J-QF: Collection and assembly of data. JL, MZ, Y-TY, and S-MH: Data analysis and interpretation. All authors manuscript writing and final approval of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.937303/full#supplementary-material

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Comparison of the Relationship Between SI and RASI Scores With the Outcome of Sepsis Patients

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The aim of this study was to compare the relationship between shock index (SI) and respiratory adjusted shock index (RASI) scores with the final outcome of sepsis patients referred to the emergency department. This was prospective research that examined individuals who had been diagnosed with sepsis, determined by the presence of at least two of the three quick sepsis-related organ failure assessment (qSOFA) criteria and the presence of an infectious disease based on a diagnosis made by a hospital physician of Imam Reza and Ghaemshahr of Mashhad in 2019. Demographic information of patients, SI score, RASI score, and information related to the patient's clinical symptoms were recorded in the checklist. The final outcome of this study was considered mortality. Data analysis was performed using descriptive and inferential tests. In the present study, a total of 178 patients, 46 patients (25.8%) were transferred to the intensive care unit, and 98 patients (55.1%) were admitted to the normal wards. Eighty-five patients (47.75%) died and the mean length of hospital stay of all patients was 11.07 ± 9.23 days. Forty-four patients (24.7%) had referred with a decreased level of consciousness and 44 patients (24.7%) presented with confusion. The rest of the patients reported normal levels of consciousness. Kaplan Mir analysis with log-rank was performed to determine the difference in survival distribution in different SI groups: Survival distribution was not statistically different for the four defined groups (based on statistical quartiles ($P = 0.320$). Receiver operator curves were considered as the date of death in the case of the deceased and the date of discharge from the hospital in the case of the living as censored. The AUC of the RASI scoring system for predicting mortality was 0.614 ($P = 0.009$) while this value was not significant for SI ($P = 0.152$). In logistic regression analysis, it was found that by adjusting for the variables of age, sex, sepsis etiology, blood pressure and heart rate, level of consciousness, and gender, patients with the lower respiratory rate (OR 1.6, z = −0.159 $p = 0.007$), younger age (OR 1.6, z = −0.029 $p = 0.006$) and higher RASI score are more in risk of mortality (OR 1.29, z = 1.209, $p = 0.031$). The results of our

study showed that RASI scoring can be a good criterion for predicting the chance of mortality in patients with sepsis and could be used complementary to previous criteria such as SI. Patients with high RASI scores should be given more attention to reducing the chance of death.

Keywords: SI, RASI, sepsis, emergency, infection

INTRODUCTION

Sepsis is a systemic reaction of the body to invasive microorganisms such as bacteria and fungi and is one of the diseases that patients admitted to different parts of the hospital may be infected with (1). Sepsis is the second greatest cause of mortality among admitted patients with a variety of illnesses, and one of the top 10 causes of mortality in all inpatients (2). Sepsis is more common in the elderly and significantly affects people with cancer and defective immune systems. So that in its most acute form, the infection disrupts several organs of the body and creates critical conditions (4). Rhabdomyolysis has been reported in bacterial, viral, and fungal infections (5). Sepsis-induced hypoxia, bacterial invasion of myocytes, decreased activity of glycolytic, oxidative, lysosomal enzymes, and endotoxin-induced damage all lead to rhabdomyolysis during infections. Legionella is the most common cause of rhabdomyolysis due to sepsis (6). The host's reaction to infection is sepsis. The invading agent and the host body's activated inflammatory mediators impair the body's defensive and regulatory systems, causing the body's homeostasis to be disrupted. The most frequent primary signs of the systemic response, also known as the systemic inflammatory response syndrome, are tachycardia, tachypnea, fever or hypothermia, and immune system activation (leukocytosis or leukopenia) (SIRS) (7). The definition of SIRS based on quick SOFA criteria is that the patient has at least two of the following three criteria: (1). The respiratory rate of higher than 22 times per minute or more, (2). The change in consciousness and (3). Systolic blood pressure 100 mmHg or less. When SIRS is verified or presumed to be caused by bacteria, it is referred to as sepsis. Similarly, if sepsis is affiliated with one or more organ dysfunction signs, such as hypoperfusion, hypotension, metabolic acidosis, acute mental state change, oliguria, or ARDS, it is referred to as severe sepsis and is referred to as septic shock with hypotension which does not adapt to intravenous fluids and interrupts organ dysfunction or contributes to perfusion impairment (7). The consequences of sepsis have greatly improved, probably because of the focus on early diagnosis and the rapid and timely administration of effective antibiotics, and advances such that early detection of the disease is a major challenge (3). In the early stages, the diagnosis of sepsis from non-infectious conditions, especially in critically ill patients is difficult and diagnosis, treatment, and its results are significantly different among patients with sepsis and without sepsis (6). Notwithstanding the advent of new explanations on the origin and pathogenesis of sepsis, as well as the development of extremely powerful antibiotics and antifungal agents, there has been little progress in decisively lowering mortality from this syndrome (3). One of the most essential issues in this respect is the establishment of precise procedures for diagnosing the outcomes in patients with sepsis, particularly critically ill individuals. A basic scoring system for measuring shock and hemodynamics in patients is the SI score (8). Recent studies have shown the importance of tachycardia in predicting cardiac arrest and as an indicator of organ dysfunction; For this reason, a new criterion was defined with the aim of including the RR effect in shock prediction called RASI, which is calculated according to the formula RR / 10 × HR / SBP (9, 10). Jiang et al. (10) evaluated 360 individuals with sepsis in research aiming at employing RASI to detect latent shock and quality of care in sepsis patients. Lactate (OR 1.55, z = 4.38, p0.0001) and RASI (OR 2.27, z = 3.03, p0.002) were shown to indicate the need for more care in regression analysis. For shock detection, the AUCs for RASI, SI, and qSOFA were 0.71, 0.6, and 0.61, respectively. In contrast to SI (0.64) and qSOFA, RASI exhibited a substantial AUC of 0.75 in identifying the degree of care (0.62). They concluded that RASI might be effective as a quick-response method for forecasting critical diseases in sepsis patients (10). In a retrospective study, Caputo et al. (9) examined the RASI criteria for determining the presence of latent shock in trauma patients. A total of 3,093 patients participated in this study. In terms of the SI index, there was no significant difference between discharged and hospitalized patients' rates [0.6 (95% CI, 0.5–0.7) vs. 0.7 (95% CI, 0.5–0.8)]. However, in the study of the RASI index, a significant difference was observed between discharged and hospitalized patients [1.1 (95% CI, 1.04–1.18) vs. 1.46 (95% CI, 1.35–1.55)]. The range under the ROC curve was 0.58 for the SI score and 0.94 for the RASI score. They concluded that the RASI score improves diagnostic accuracy for detecting latent primary shock in trauma patients compared with SI (9). One of the most essential issues in this respect is the establishment of precise techniques for assessing the prognosis of patients with sepsis, particularly critically sick patients, as well as the kind of therapy and prioritization of patient care. As a result, we decided to look into the link between RASI (Respiratory adjusted shock index) and the final outcome of sepsis patients who were brought to the emergency room.

METHODS

This was prospective research conducted on individuals having an initial impression of sepsis (depending on the existence of at least two of the three qSOFA signs and the existence of an infectious condition based on a hospital physician's diagnosis) who were seen in Mashhad city's emergency departments. The

purpose of the study was explained to the patients and their consent or that of their companions was taken to participate in the study. Demographic information of patients including gender, age, medical history, and information related to the patient's clinical signs were collected in a checklist by the resident.

The present study was conducted during three main phases, each of which is referred to below:

1- Data collection and preprocessing according to the parameters required to calculate SI and RASI in patients with sepsis, including heart rate, systolic blood pressure, and respiratory rate. After preparing a comprehensive form regarding the desired parameters, the residents of emergency medicine were asked to complete and submit the relevant information.

2- Performing calculations related to determining the score of SI (HR / SBP) and RASI (RR / 10 × HR / SBP) for each patient according to the mentioned formulas.

3- Evaluating and analyzing the obtained data and comparing SI and RASI scores in determining the final outcome of patients, including 1- Mortality during hospitalization 2- Type of admission of patients, in the emergency department, ward, ICU, or discharge; 3- the duration of hospitalization.

Data analysis was performed using descriptive statistics in SPSS software version 20. The characteristics of the subjects were presented by descriptive statistical methods including central indicators, dispersion, and frequency distribution in the form of appropriate tables and graphs. *T*-test was used to compare quantitative variables in case of normal distribution of data and the Mann-Whitney test was used otherwise. Survival analysis was used to investigate the relationship between the scales and the incidence of mortality. ROC analysis to check the AUC of each score was performed. We used STATA version 17 to perform Delong's test to compare AUC of scores. Logistic regression model was used to adjust for other contextual and clinical variables. Kendall tau Rank Correlation (v1.0.13) was used to show the correlation diagram. In all calculations, a value of 0.05 was considered significant.

RESULT

In the present study, a total of 178 patients with sepsis were studied. Of these, 101 (56.7%) were male and 77 (43.3%) were female. The mean age of these individuals was 68.41 ± 17.35 years. Regarding the source of infection, the final diagnosis was pneumo-sepsis in 154 patients (86.51%). Eighteen patients (10.11%) had urosepsis. Cellulite and catheter infections were seen in two patients (1.12%). Diarrhea was read as a cause of sepsis in one patient (0.6%). One patient had both pneumo-sepsis and urosepsis (**Table 1**).

Forty-four patients (24.7%) were referred with a decreased level of consciousness and 44 patients (24.7%) presented with confusion. The rest of the patients reported normal levels of consciousness.

Finally, 46 patients (25.8%) were transferred to the intensive care unit. Ninety-eight patients (55.1%) were admitted to normal wards. Thirty-four people (19.1%) were treated in the emergency department. Hemodynamic status of patients are shown in **Table 2**.

TABLE 1 | Demographic data and clinical parameters.

		n	%
Sex	Male	101	56.74
	Female	77	43.26
Source of sepsis	Pneumo-sepsis	154	86.52
	Urosepsis	18	10.11
	Cholangin	2	1.12
	Cellulitis	2	1.12
	Catheter related infection	2	1.12
	Gastroenteritis	1	0.56
Level of consciousness	Normal	90	50.56
	Decreased	44	24.72
	Confusion	44	24.72
Admission type	ICU	46	25.84
	Ward	98	55.06
	ED	34	19.1

TABLE 2 | Hemodynamic status of patients.

	Mean	SD	Median	Q1	Q3
RR	30.32	8.59	29.5	24	36
PR	112.78	21.98	110	103	124.25
SBP	122	29.3	122.5	100	144.25

RASI, SI, and BE estimates are calculated and presented in **Figure 1**. The relationship between the main variables of the study with hemodynamic status, age and with each other was measured using the Spearman correlation test and shown in **Figure 2**.

RSAI correlated significant inverse correlation with SI (Spearman rho = −0.555; $P < 0.001$), significant inverse correlation with RR (Spearman rho = −0.486; $P < 0.001$), Significant direct correlation with SBP (Spearman rho = + 0.467; $P < 0.001$), significant direct correlation with age (Spearman rho = + 0.58; $P < 0.001$) and a direct significance correlation with BE (Spearman rho = + 0.711; $P < 0.001$). SI had a direct correlation with RR (Spearman rho = + 0.719; $P < 0.001$), significant direct correlation with PR (Spearman rho = + 0.240; $P = 0.00012$), Significant inverse correlation with SBP (Spearman rho = −0.841; $P < 0.001$), significant inverse correlation with age (Spearman rho = −0.719; $P < 0.001$) and There was a significant inverse correlation with BE (Spearman rho = −0.750; P <0 .001). BE had a significant inverse correlation with RR (Spearman rho = −0.682; $P < 0.001$), weak significant direct correlation with SBP (Spearman rho = + 0.005; $P < 0.001$) and had a

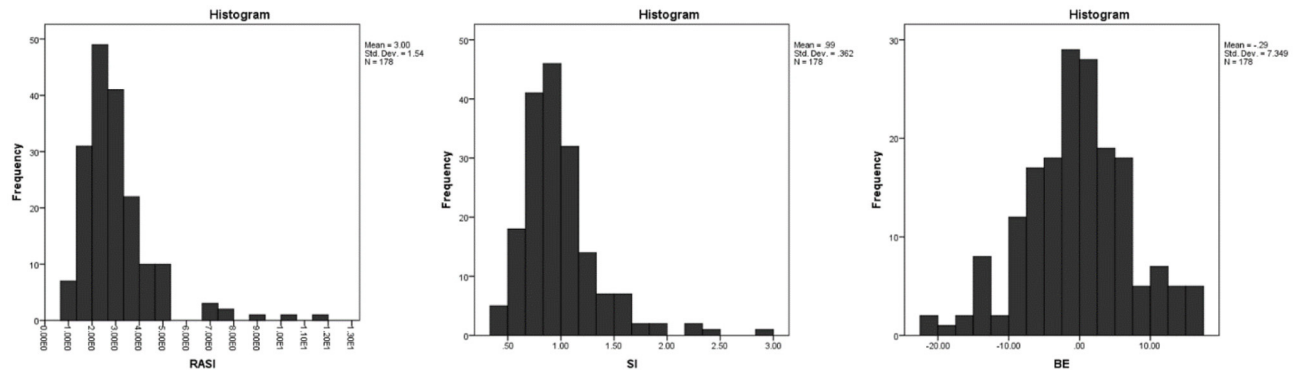

FIGURE 1 | Frequency of different values of BE, RASI and SI.

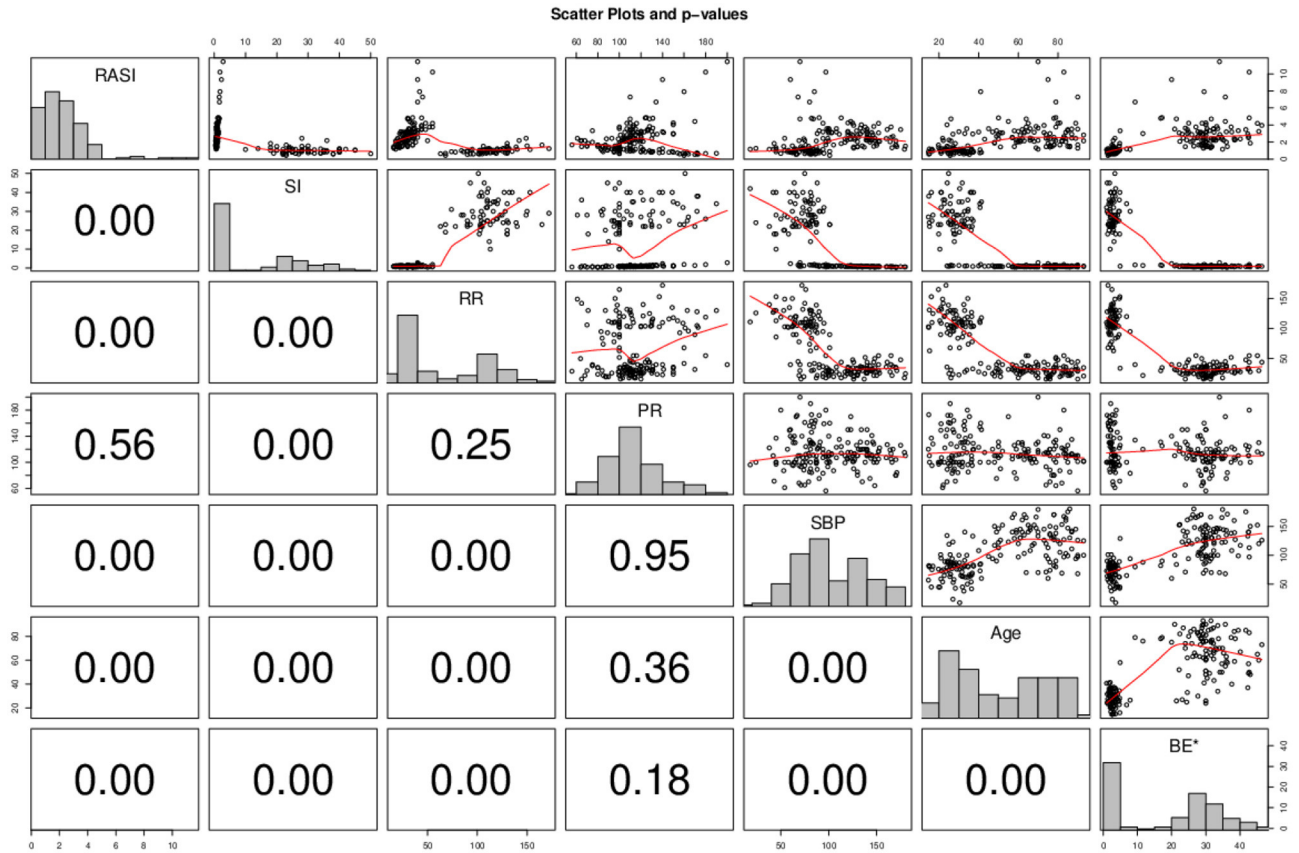

FIGURE 2 | Correlation matrix of study variables. *P*-value is shown numerically on the left side of the matrix. * To display BE numerical values in the graph due to the negative number of data, all values are added with a fixed value. X border and corresponding border in front is belonging to the first variable in each histogram and the Y and the border in front is belonging to the second variable of the correlation test.

significant inverse correlation with age (Spearman rho = −0.631; $P < 0.001$).

Then, the relationship between the study variables and the final outcome of mortality and length of hospital stay was investigated. Of the total population, 85 (47.75%) died. The mean duration of hospitalization was 11.07 ± 9.23 days. The last day of hospitalization was considered the date of death in the case of the deceased and the date of discharge from the hospital in the case of the living was censored.

As shown in **Figure 3**, patients were categorized based on RSAI quartiles into 4 categories of <2.07 (Q1), between 2.07 and 2.71 (Q2), between 2.71 and 3.48 (Q3) and more than

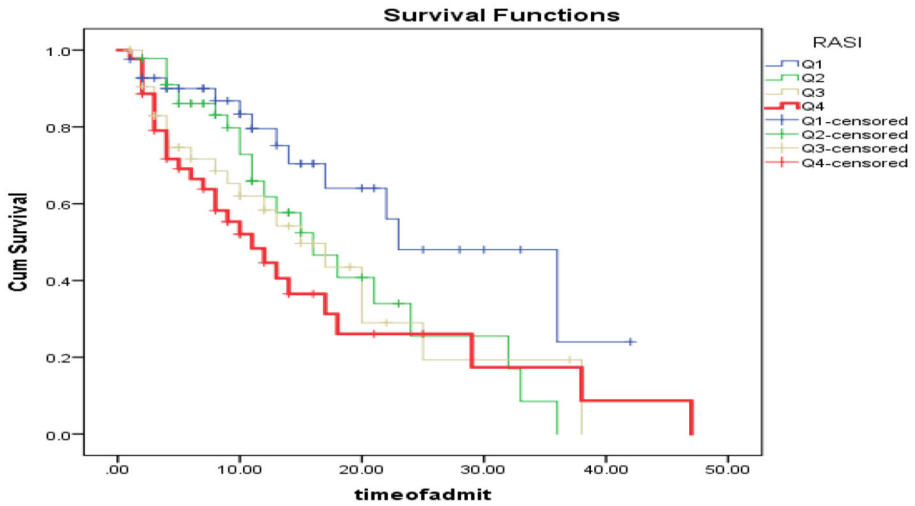

FIGURE 3 | Patient survival analysis based on RASI quarters.

TABLE 3 | Mean survival of live and deceased patients.

		n Total	*n* Death (%)	Mean survival	SE	*P**
RASI	Q1	42	13 (30.95)	25.900	3.050**	0.04
	Q2	46	22 (47.83)	18.380	2.170	
	Q3	44	22 (50)	17.160	2.580	
	Q4	44	28 (63.64)	15.980	2.800	
SE	Q1	41	19 (46.34)	20.680	2.660	0.57
	Q2	44	16 (36.36)	22.210	2.570	
	Q3	46	25 (54.35)	17.630	2.500	
	Q4	45	25 (55.56)	17.660	3.340	
BE	Q1	44	31 (70.45)	16.350	2.560	0.55
	Q2	44	22 (50)	18.800	2.330	
	Q3	45	17 (37.78)	20.760	2.760	
	Q4	43	15 (34.88)	21.240	2.900	

** One-way ANOVA. ** post-hoc Tukey test showing the significant difference with Q4 group.*

3.48 (Q4). Kaplan Mir analysis with log-rank was performed to determine the difference in survival distribution in different RSAI groups: The survival distribution was statistically significantly different for the four defined groups, $\chi 2$ (3) = 9.76, $P < 0.0005$. As shown in **Table 3**, One-way ANOVA of mean survival of patients was compared based on the quartiles of RASI, SE, and BE. It was found that the mean survival of patients having RASI within Q1 ranges was significantly higher than those within Q4 range ($P = 0.014$); while other groups had no significant difference in case of RASI ($P > 0.05$). Also, these comparisons were not statistically significant for SE and BE ($P > 0.05$).

As shown in **Figure 4**, patients were categorized based on SI quartile into 4 categories <0.76 (Q1), between 0.76 and 0.9 (Q2), between 0.9 and 1.14 (Q3) and more than 1.14 (Q4). Kaplan Mir analysis with log-rank was performed to determine the difference in survival distribution in different SI groups: Survival distribution was not statistically different for the four defined groups, $\chi 2$ (3) = 4.31, $p = 0.320$.

As shown in **Figure 5**, patients were categorized based on the BE quartile into 4 categories <5- (Q1), between −5 and −0.6 (Q2), between −0.6 and 4.45 (Q3) and more than 4.45 (Q4). Kaplan Mir analysis with log-rank was performed to determine the difference in survival distribution in different SI groups: Survival distribution was not statistically different for the four defined groups, $\chi 2$ (3) = 3.87, $p = 0.275$.

According to **Figure 6**, in the study of RASI and SI scoring systems, Receiver operator curves analysis showed that the AUC of RASI scoring system for predicting mortality was 0.614, 95% CI (0.531–0.697, $P = 0.009$) and this value was equal to 0.354, 95% CI (0.277–0.441, $P = 0.001$) for BE; while this value was not significant for SI with AUC of 0.562, 95% CI (0.477–0.647, $P = 0.152$); while Delong's test showed no

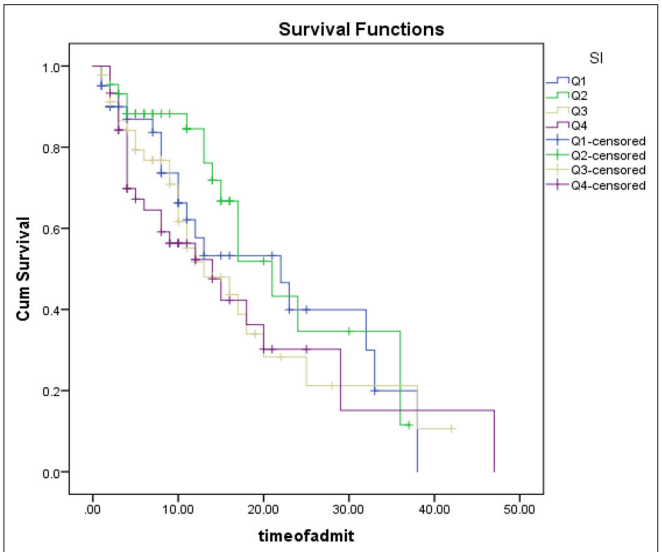

FIGURE 4 | Patient survival analysis Based on SI quarters.

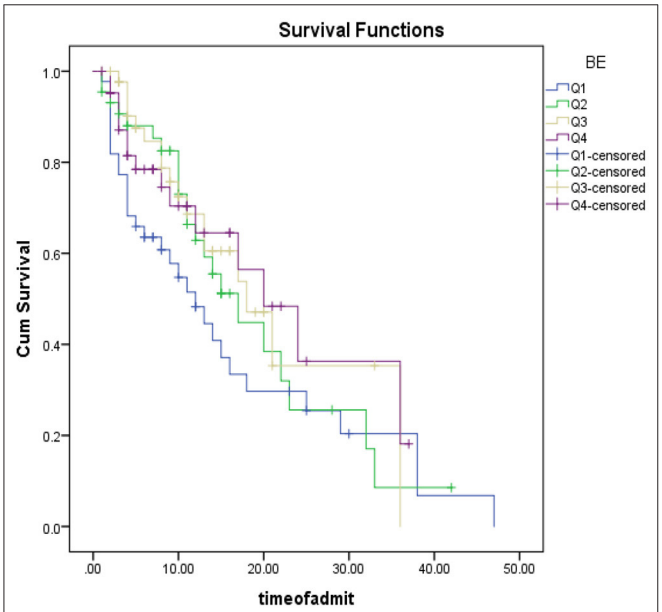

FIGURE 5 | Patient survival analysis based on BE quartiles.

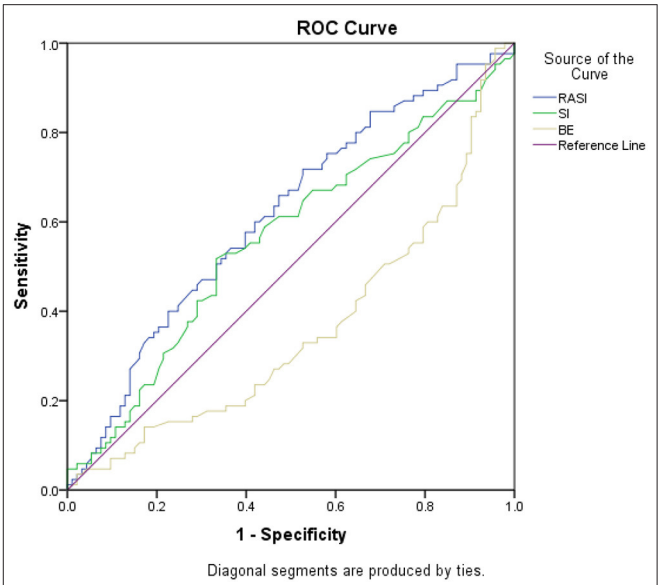

FIGURE 6 | ROC analysis for mortality.

TABLE 4 | Relationship between demographic variables and mortality.

		HR	95% CI		*P*
			Lower	Upper	
Sex, male		0.774	0.476	1.259	0.302
Age		1.012	1.029	0.996	0.137
RASI		0.895	1.985	0.403	0.785
SI		1.841	82.176	0.041	0.753
BE		1.041	1.005	0.969	0.801
RR		1.027	1.11	0.95	0.497
PR		1.003	1.028	0.977	0.847
SBP		0.996	1.017	0.975	0.69
Level of consciousness	Normal	0	0	0	Ref
	Decreased	1.47	2.642	0.818	0.197
	Confusion	1.036	2.146	0.5	0.925
Admission type	ICU	0	0	0	Ref
	Ward	1.631	3.313	0.803	0.176
	ED	6.723	2.638	17.131	0.002

significant difference in AUC of RASI and SI ($chi^2(1) = 2.09$, $P = 0.1480$). RASI scoring system with 97% sensitivity and 96% specificity predicted mortality with a 1.285 cut-off. The BE scoring system, with a sensitivity of 98.8% and a specificity of 97.8%, predicted mortality with a cut-off of 16.9. Cox analysis showed that none of the variables were associated with mortality ($P > 0.05$); Except for hospitalization in the intensive care unit, which was significantly identified as a risk factor for death with a risk ratio of 6.723 ($p = 0.002$) (**Table 4**).

DISCUSSION

Sequential Organ Failure Assessment (SOFA) score is being used as a prognostic factor in sepsis, but it requires multiple laboratory indices that would not be rapidly available in the setting of an emergency department (11). This was the reason that many later studies tried to develop easier to use prognostic factors like the quick SOFA as we discussed. This study also aimed at evaluating one of these newly developed scoring systems, RASI.

In the present study, 56.7% of subjects with sepsis were male and 43.3% were female. As reviewed in the study by Angele et al., Numerous experimental and clinical studies have shown

gender differences in infectious diseases and sepsis. Females are less likely to develop sepsis and infection, while the male gender may be a risk factor for disease due to decreased cellular immune response and cardiovascular function (12). This was somewhat true in our study as well, and the number of men with sepsis was higher.

86.51% of the patients we studied had pneumo-sepsis. This has been seen in several studies and the most common site of infection leading to sepsis is the lung (64% of cases) (13).

The Systemic Inflammatory Response Scale (SIRS) has conventionally been utilized to monitor for sepsis in emergency department patients (14), but the requirement of using other indexes in this field was felt due to the new diagnostic criteria outlined in the third session of the International Consensus Definitions Task (15). The main purpose of our study was to investigate the relationship between RASI and SI systems and paraclinical data related to BE in predicting the course of sepsis. SI is a criterion that has been used before in predicting the clinical course of treatment of different patients in traumatic injuries (16), gynecological diseases (17), cardiovascular diseases (18), and sepsis.

SI was compared to the SIRS-2 and modified SIRS criteria (SIRS omitting white blood cell count) in a retrospective analysis of 2,524 adult individuals. The 28-day mortality prognosis for SI, SIRS, and modified SIRS was low in their research (19). In our study, the SI criterion was not able to predict mortality in sepsis patients and our study is consistent with this study.

In another study of 295 patients with severe sepsis, SI did not predict the need for vasopressor use or mortality (20). However, this issue was also seen in our study, the need to use vasopressor is a good variable for further studies, which unfortunately was not considered in our study.

But fewer studies have been done on RASI. Our study showed that RASI scores are significantly able to predict mortality in people with sepsis. The study by Jiang et al. showed that the use of RASI in the emergency department was able to predict the incidence of sepsis in patients with suspected sepsis (10). In their study, respiration rate was integrated into SI to increase the RASI predictive ability to identify patients with sepsis, which was ultimately more sensitive to lactate alone as well as to other screening tools. They showed that RASI was significantly able to predict discharge or hospitalization status. However, in their study, the final outcome of treatment was not followed up. However, our study examined mortality in these patients. It can be said that one of the advantages of our study in choosing the final outcome was the absence of confounding factors related to the physician's decision to admit or discharge the patient.

However, it seems that no other study has been done on the use of this index in sepsis patients in order to compare the results of the present study with it. But it is noteworthy that in our study RSAI had a significant inverse correlation with RR (Spearman rho $= -0.486$; $P < 0.001$). The median number of respiration per minute in our patients was 30.32 (24-09.5) which could also be interpreted according to the RASI calculation formula [HR / SBP * (RR / 10)].

In our study, no significant relationship was found between BE and clinical outcome, but other studies found acidosis assessed by BE and/or pH to be promising for predicting risk in septic patients. In the study by Wernly et al. (21), in contrast to our study, BE was an independent predictor of mortality. However, they used BE values along with the pH index in their analyzes, which may be the reason why their study differs from ours. But in another study by Gattinoni et al., "Alactic BE," meaning the total concentration of lactate and negative BE, was not useful in predicting mortality in patients with sepsis (22). Which is somewhat consistent with our study. But according to the research, lactate metabolism is complex, and lactate levels may be close to "normal" even in patients at risk of death and adverse outcomes, and much more research is needed to make that decision.

STUDY LIMITATIONS

One limitation was that the sample size of the subjects in our study was low. However, other limitations in not considering other useful parameters such as lactate and pH made it difficult to interpret our results for the BE index; So considering the BE and pH, assessing the base acid balance and buffer capacity may help us and increase our ability to predict mortality risk. Unfortunately, we did not collect all data needed for the calculation of SOFA score. A comparison of SOFA and RASI could be assessed in further studies. One main limitation of this study was the matter of linearity in statistical analyses. While logistic regression does not require a linear relationship between the dependent and independent variables; we assumed linearity for adjusting potential confounding factors in the regression model. This Intention-to-treat approach might give different results getting adjusted for different variables. But an alternative approach to machine learning, entitled ensemble modeling, is proposed by Zhang et al. that could be used in further studies (23).

CONCLUSION

The results of our study showed that RASI scoring alone could be a good criterion for predicting the chance of mortality in patients with sepsis, while is not superior to previous criteria of SI and could be used complementary. Patients with high RASI scores should be given more attention to reducing the chance of death.

SUGGESTIONS

This study was performed in only one center with a small number of patients. It is suggested that further studies with larger sample sizes be performed to confirm the findings of the present study. Also, in advance, other factors such as lactate levels and pH are also assessed in the study. Checking RASI during hospitalization and its relationship with other indicators can also be helpful.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mashhad University of Medical Sciences, Mashhad, Iran. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

FM and MF designed the study. Data was collected by FB, SA, and MF. MF, FB, SA, and AMH collected the data. MF and AMH analyzed the data. The draft of the manuscript was provided by FM, FB, SA, AMH, and MF. AMH, ZB, RF, and HZM edited the manuscript in revisions. All authors confirmed the final format. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ORIGINAL RESEARCH
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The Diagnostic Value of Mitochondrial Mass of Peripheral T Lymphocytes in Early Sepsis

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Background: Studies have shown that lymphocyte dysfunction can occur during the early stages of sepsis and that cell dysfunction is associated with mitochondrial dysfunction. Therefore, quantifying the mitochondrial function of lymphocytes in patients with sepsis could be valuable for the early diagnosis of sepsis.

Methods: Seventy-nine patients hospitalized from September 2020 to September 2021 with Sepsis-3 were retrospectively analyzed and subsequently compared with those without sepsis.

Results: Univariate analysis showed statistical differences between the data of the two groups regarding age, neutrophil/lymphocyte, procalcitonin (PCT), C-reactive protein, total bilirubin, serum creatinine, type B natriuretic peptide, albumin, prothrombin time, activated partial thromboplastin time, lactic acid, single-cell mitochondrial mass (SCMM)-CD3, SCMM-CD4, SCMM-CD8, and Acute Physiology and Chronic Health Evaluation II score ($P < 0.05$). Multivariate logistic regression analysis performed on the indicators mentioned above demonstrated a statistical difference in PCT, lactic acid, SCMM-CD4, and SCMM-CD8 levels between the two groups ($P < 0.05$). The receiver operating characteristic curves of five models were subsequently compared [area under the curve: 0.740 (PCT) vs. 0.933 (SCMM-CD4) vs. 0.881 (SCMM-CD8) vs. 0.961 (PCT + SCMM-CD4) vs. 0.915 (PCT+SCMM-CD8), $P < 0.001$].

Conclusion: SCMM-CD4 was shown to be a better diagnostic biomarker of early sepsis when compared with the traditional biomarker, PCT. Furthermore, the value of the combination of PCT and SCMM-CD4 in the diagnosis of early sepsis was better than that of SCMM-CD4 alone.

Keywords: mitochondrial mass, T lymphocytes, sepsis, receiver operating characteristic curve (ROC), mitochondrial function

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INTRODUCTION

Sepsis is a systemic inflammatory response syndrome caused by a severe infection. Previous research has shown that the pathophysiological process of sepsis is essentially a process of immune system disorders. Despite the hyperimmune responses witnessed during the initial stage of sepsis, the state progresses to immunosuppression (1), resulting in secondary or recurrent infections.

In the long run, patients' conditions will deteriorate due to repetitive infection, ultimately developing multiple organ dysfunction syndromes (2). Studies have established that immune paralysis caused by sepsis is associated with a decrease in the number of immune cells and immune system disorders (3). In contrast, cell dysfunction is associated with mitochondrial function damage (4). The human body relies on mitochondria for metabolism and energy conversion, indicating that once mitochondrial function becomes impaired, it seriously affects cell operation and eventually leads to tissue and organ damage. Recently, a new immunofluorescence technology that reflects mitochondrial function has emerged, detecting mitochondrial quality and expressing it using single-cell mitochondrial mass (SCMM) (5). Therefore, this study discusses the differences in SCMM of T lymphocytes between patients with and without sepsis and evaluates the value of SCMM of T lymphocytes in the diagnosis of sepsis.

MATERIALS AND METHODS

Study Population

Seventy-nine patients who were hospitalized in the emergency intensive care unit of Zhejiang Provincial People's Hospital from September 2020 to September 2021, diagnosed with Sepsis-3 and infection, using a quick sequential organ failure assessment score of $\geq$2 (6), were included in this study. The exclusion criteria were as follows: (i) patients aged <18 years; (ii) patients with a history of malignant tumor; (iii) patients who underwent organ transplantation or were long-term users of drugs that affect immune function (such as adrenocortical hormone); (iv) patients with renal failure requiring kidney replacement therapy; and (v) patients whose hospital stay was less than 48 h. Blood, urine, and sputum cultures were performed on all patients, coupled with ultrasound and computed tomography to determine the location of infection. Evidence of infection refers to the presence of focal infections or results of bacteriological examination. Seventy-five patients without sepsis, hospitalized during the same period as those with sepsis, were selected for comparison. The Ethics Committee of the Zhejiang Provincial People's Hospital (the People's Hospital of Hangzhou Medical College) approved the research protocol of this study. Informed consent was obtained from patients or their families for all treatments and indicators obtained.

Flow Cytometry

Data on indicators, such as population characteristics, infection markers, organ function, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and flow cytometry data of patients with and without sepsis were collected, and univariate and multivariate analyses were performed. Peripheral venous blood was collected 48 h after the diagnosis was established, into collection tubes coated with EDTA-k anticoagulant and examined using flow cytometry. Among the indicators assessed were percentage and absolute counts of lymphocytes, T cells, and their subsets of helper T cells (Th cells: $CD3^+$ $CD4^+$ $CD8^-$), killer T cells (TS cells: $CD3^+$ $CD4^-$ $CD8^+$), and subsets of mitochondrial mass (MM) and SCMM. MM was measured using the medium fluorescence index of mitochondria (5, 7). SCMM of T lymphocytes was obtained by calculating the absolute count of MM and cells (**Figure 1**). Flow cytometry was performed using NovoCyte (Agilent Technologies, US), and flow cytometry antibodies CD3/CD8/CD4/CD45 and Mito dye were produced by UBBIO LTD (Zhejiang, China).

Statistical Analysis

The data were processed using Statistical Package for Social Sciences v. 25.0. Normal distribution statistics are expressed as mean $\pm$ SD, and univariate comparisons between sepsis and non-sepsis groups were performed using the independent samples *t*-test. Measurements of skewed distribution are expressed as medians and quartiles, and the rank-sum test was used for univariate comparison between groups. Count data of the two groups were compared using the χ^2 test. Multivariate logistic regression analysis was performed to control for confounding bias and explore the risk factors affecting the prognosis of sepsis. The receiver operating characteristic (ROC) curves were compared using MedClac. $P < 0.05$ was considered statistically significant for all of the indicators, as mentioned earlier.

RESULTS

Univariate Analysis of Clinical Data Between the Two Groups

This study included 79 and 75 patients in the sepsis and non-sepsis groups, respectively. There were 52 and 40 males in the sepsis (65.8%) and non-sepsis (53.3%) groups, respectively. Univariate analysis was performed for sex, age, body temperature, neutrophil/lymphocyte (N/L), procalcitonin (PCT), white blood cell, C-reactive protein (CRP), total bilirubin (TB), glutamic-pyruvic transaminase, serum creatinine (Scr), type B natriuretic peptide (BNP), mean artery pressure, oxygenation index, pH, albumin (Alb), prothrombin time (PT), activated partial thromboplastin time (APTT), lactic acid (Lac), SCMM-CD3, SCMM-CD4, SCMM-CD8, duration of hospital stay, APACHE II score, number of underlying medical conditions, and emergency surgery between the two groups. The results showed that there were significant differences between the two groups in terms of age, N/L, PCT, CRP, TB, Scr, BNP, Alb, PT, APTT, Lac, SCMM-CD3, SCMM-CD4, SCMM-CD8, and APACHE II score (**Table 1**).

Multivariate Logistic Regression Analysis of the Significant Indices

Following univariate analysis of age, N/L, PCT, CRP, TB, Scr, BNP, Alb, PT, APTT, Lac, SCMM-CD4, SCMM-CD8, and APACHE II score in both groups, multivariate logistic regression analysis was performed on indicators that showed statistical differences to eliminate the interference of confounding factors. Statistical differences were observed in the PCT, Lac, SCMM-CD4, and SCMM-CD8 between the two groups (**Table 2**).

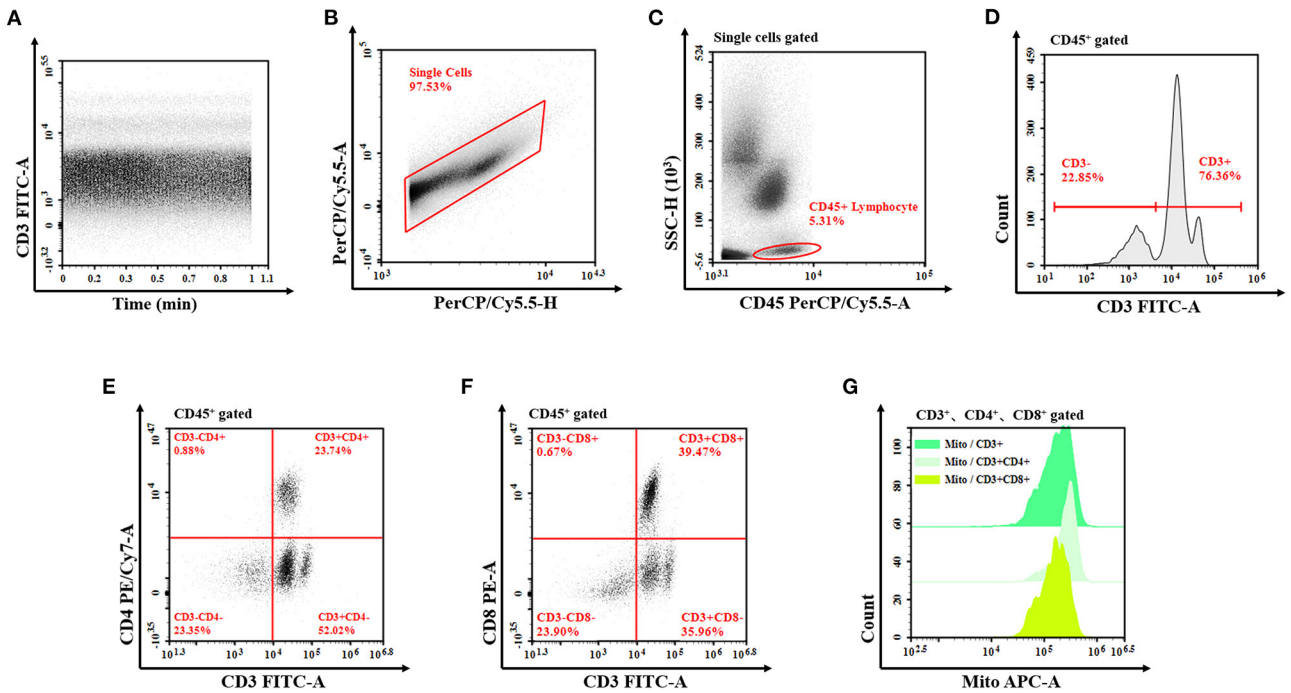

FIGURE 1 | Flow cytometry of T lymphocyte mitochondrial mass. **(A)** The analysis is started by examining the event count over time in order to exclude irregularities from the analysis. **(B)** Exclude the doublets from further analysis by investigating the PerCP/Cy5.5 signal for both the peak and the TOF characteristics compared to the integral attributes. **(C)** The remaining events are analyzed for their CD45 vs. SSC characteristics to define the lymphocyte after single-cell signal acquisition. Draw a gate around the cell population (all lymphocyte cells gate). **(D–F)** In the next step, the T cells (CD3+ T cells, CD3+CD4+ T cells, and CD3+CD8+ T cells) are determined due to the expression of CD3, CD4, and CD8, then set the corresponding gate, respectively. **(G)** Show these fluorescence characteristics of mitochondria by the half-offset histogram of the APC channel. The mitochondrial mass is detected by the MFI of APC. TOF, time of flight; APC, allophycocyanin; MFI, median fluorescence index.

Predictive Efficacy of PCT, SCMM-CD4, and SCMM-CD8

The area under the ROC curve (AUC) was used to evaluate the predictive efficacy of PCT, SCMM-CD4, and SCMM-CD8 levels in sepsis. The results showed that when the PCT cut-off value was 3.66 ng/ml, the AUC to distinguish patients with sepsis from those without sepsis was 0.740 (sensitivity 54.4%, specificity 89.3%, and Youden index 0.437). When the SCMM-CD4 cut-off value was 229.92, the AUC for distinguishing patients with sepsis from those without sepsis was 0.933, with a sensitivity of 72.2%, specificity of 98.7%, and Youden index of 0.722. When the SCMM-CD8 cut-off value was 188.44, the AUC for distinguishing patients with sepsis from those without sepsis was 0.881, with a sensitivity of 79.7%, specificity of 81.3%, and Youden index of 0.611. Furthermore, we combined SCMM-CD4 and SCMM-CD8 with PCT, respectively, to see whether the prediction model could be more optimized or not. The results demonstrated that when the PCT+SCMM-CD4 cut-off value was 0.71, the AUC for distinguishing patients with sepsis from those without sepsis was 0.961, with a sensitivity of 79.7%, specificity of 99.3%, and Youden index of 0.798. When the PCT+SCMM-CD8 cut-off value was 0.40, the AUC for distinguishing patients with sepsis from those without sepsis was 0.915, with a sensitivity of 84.8%, specificity of 82.7%, and Youden index of 0.675. The ROC curves of the five models, which were compared, differed significantly except for the SCMM-CD4 vs. PCT+SCMM-CD8 model (**Figure 2** and **Tables 3**, **4**).

DISCUSSION

The results of the present study revealed statistical differences in age, N/L, PCT, CRP, TB, Scr, BNP, Alb, PT, APTT, Lac, SCMM-CD3, SCMM-CD4, SCMM-CD8, and APACHE II score between the two groups (**Table 1**). However, since the present study adopted a case-control approach with an uneven distribution, multivariate logistic regression analysis was performed for statistically different indicators to eliminate the interference of confounding factors. Because SCMM-CD3 had the problem of multicollinearity in multivariate logistic regression, we excluded this indicator in multivariate logistic regression analysis. The results showed that only PCT, Lac, SCMM-CD4, and SCMM-CD8 levels had statistically significant differences (**Table 2**). Because Lac is not a specific indicator of sepsis, we did not determine its predictive value for early sepsis. After drawing ROC curves for PCT, SCMM-CD4, and SCMM-CD8, the AUC of SCMM-CD4 was the best of the three indicators, suggesting that SCMM-CD4 had a higher value in the early prediction of sepsis.

Studies have shown that sepsis causes a decline in lymphocyte counts and function (8). Although some scholars believe that immunosuppression caused by sepsis occurs during the later

TABLE 1 | Univariate analysis of the variables of the groups with and without sepsis.

Variable	Sepsis ($n = 79$)	No sepsis ($n = 75$)	χ^2/T/U test	
			χ^2/T/Z	*P*-value
Sex				
Male *N* (%)	52 (65.80)	40 (53.30)	2.50	0.114
Female *N* (%)	27 (34.20)	35 (46.70)		
Age (years)	69.91 ± 14.83	64.24 ± 16.18	2.27	0.025*
Temp (°C)	37.40 ± 0.94	37.32 ± 0.84	0.55	0.585
N/L (%)	15.70 (11.00, 28.20)	8.30 (5.30, 17.80)	−4.56	<0.001***
PCT (ng/mL)	4.60 (0.53, 18.00)	0.54 (0.11, 1.80)	−5.14	<0.001***
WBC (10^9/L)	10.20 (7.14, 16.29)	10.57 (7.10, 14.44)	−0.33	0.744
CRP (mg/L)	96.80 (47.50, 214.50)	54.20 (19.50, 105.20)	−3.78	<0.001***
TB (μmol/L)	17.10 (12.20, 37.00)	13.80 (9.10, 20.80)	−3.59	<0.001***
GPT (U/L)	26.00 (16.00, 61.00)	30.00 (13.00, 52.00)	−0.31	0.759
Scr (μmol/L)	104.40 (71.10, 175.00)	73.10 (62.80, 104.80)	−3.08	0.002**
BNP (pg/ml)	368.60 (143.50, 1371.60)	126.75 (65.63, 309.88)	−4.55	<0.001***
MAP (mmHg)	84.09 ± 15.86	86.65 ± 14.04	−1.06	0.293
OI (mmHg)	278.04 ± 121.13	311.31 ± 129.97	−1.64	0.102
PH	7.40 ± 0.78	7.42 ± 0.06	−1.64	0.104
Alb (g/L)	28.90 ± 4.27	31.27 ± 4.12	−3.51	0.001**
PT (s)	14.20 (12.90, 17.90)	13.10 (12.30, 14.10)	−3.22	0.001**
APTT (s)	32.60 (27.40, 39.00)	27.70 (25.60, 30.80)	−3.46	0.001**
Lac (mmol/L)	2.30 (1.50, 3.60)	1.50 (1.00, 2.30)	−4.27	<0.001***
SCMM-CD3	156.00 (94.07, 266.61)	35.19 (17.74, 68.92)	−9.20	<0.001***
SCMM-CD4	343.73 (179.44, 576.94)	68.23 (35.77, 130.21)	−9.27	<0.001***
SCMM-CD8	424.28 (204.38, 673.91)	97.73 (38.84, 175.92)	−8.15	<0.001***
Hospital day	7 (5, 14)	10 (6, 21)	−1.93	0.054
APACHE II score	21.99 ± 7.45	18.45 ± 7.03	3.02	0.003**
Number of basic diseases	2 (1, 3)	1 (0, 2)	−1.78	0.075
Emergency surgery (%)	19 (24.1%)	20 (26.7%)	0.14	0.709

*Data are shown as the mean ± SD and M (IQR). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.*
TEMP, temperature; N/L, neutrophil/lymphocyte; PCT, procalcitonin; WBC, white blood cell; CRP, C-reactive protein; TB, total bilirubin; GPT, glutamic-pyruvic transaminase; Scr, serum creatinine; BNP, type B natriuretic peptide; MAP, mean arterial pressure; OI, oxygenation index; Alb, albumin; PT, prothrombin time; APTT, activated partial thromboplastin time; Lac, lactic acid; SCMM, single-cell mitochondrial mass; SD, standard deviation; M, median; IQR, interquartile range.

stages of sepsis (2), research has shown that the number and function of lymphocytes can decrease 48 h after the diagnosis of sepsis. For example, Inoue et al. showed that 48 h after the diagnosis of sepsis, the CD28 lymphocyte subsets of CD4 lymphocytes decreased. At the same time, the percentage of immunosuppressive PD-1 $^+$ T cells and regulatory T cells increases among geriatric patients with sepsis (9). Cabrera-Perez et al. (10) established that on the second day after mice experienced sepsis impairment, the total number of CD4 lymphocytes in the spleen, inguinal lymph nodes, and blood decreased significantly, along with a decrease in interleukin-17. Therefore, the study population selected for the present study included patients with sepsis hospitalized for more than 48 h. Lymphocytes can play an anti-inflammatory role and repress inflammation by secreting cytokines. In sepsis, changes in the mRNA levels of T-bet, GATA3, and ROR-γT result in the differentiation of Th1, Th2, and Th17 lymphocyte subsets and subsequent changes in secreted inflammatory factors (11–13). CD4 + cells in patients with sepsis have an increased expression of inhibitory receptors, including PD-1, 2B4, BTLA, and TRAIL, which could lead to a weakened immune response (14–17).

In addition to the mechanisms mentioned above, lymphocyte function is also closely related to mitochondrial function. One of the mechanisms of mitochondrial function damage is damage to mitochondrial protein turnover and regeneration (18). Since reactive oxygen species (ROS) are constantly produced during the oxidative phosphorylation of mitochondria, organelles are vulnerable to DNA mutations or protein misfolding (19). Therefore, a quality control system is required to ensure protein functionality. A recent study found that most mitochondrial protein turnover (∼70%) occurs through a variety of non-autophagic degradation processes, such as mitochondrial proteases, the ubiquitin-proteasome system (UPS), and mitochondrial-derived vesicles (MDV). Mild mitochondrial damage is addressed by activating specific proteases in each mitochondrial compartment to degrade misfolded or oxidized

TABLE 2 | Multivariate logistic regression of the significant variables in the groups with and without sepsis.

Variable	B	S.E.	Wald	OR (95% CI)	*P*-value
Age	0.000	0.024	0.000	1.000 (0.954–1.048)	0.995
N/L	−0.005	0.045	0.015	0.995 (0.910–1.087)	0.903
PCT	0.144	0.068	4.505	1.155 (1.011–1.319)	0.034*
CRP	0.005	0.005	1.080	1.005 (0.995–1.015)	0.299
TB	−0.013	0.024	0.281	0.987 (0.941–1.036)	0.596
Scr	−0.003	0.005	0.289	0.997 (0.988–1.007)	0.591
BNP	0.000	0.000	0.210	1.000 (0.999–1.001)	0.647
Alb	−0.039	0.082	0.229	0.962 (0.819–1.129)	0.633
PT	−0.102	0.091	1.251	0.903 (0.755–1.080)	0.263
APTT	0.013	0.036	0.136	1.013 (0.944–1.088)	0.712
Lac	0.498	0.228	4.789	1.646 (1.053–2.571)	0.029*
SCMM-CD4	0.026	0.007	15.485	1.026 (1.013–1.040)	<0.001***
SCMM-CD8	0.009	0.004	5.366	1.009 (1.001–1.017)	0.021*
Hospital day	0.003	0.017	0.025	1.003 (0.971–1.036)	0.874
APACHE II score	−0.022	0.064	0.115	0.978 (0.863–1.110)	0.734

B, Co-efficient for the constant in the null model; S.E., standard error around the Co-efficient.
For the constant; Wald, Wald chi-square test value; OR, odds ratio; CI, confidence interval.
**P < 0.05; ***P < 0.001; N/L, neutrophil/lymphocyte; PCT, procalcitonin; CRP, C-reactive protein; TB, total bilirubin; Scr, serum creatinine; BNP, type B natriuretic peptide; Alb, albumin; PT, prothrombin time; APTT, activated partial thromboplastin time; Lac, lactic acid; SCMM, single-cell mitochondrial mass.*

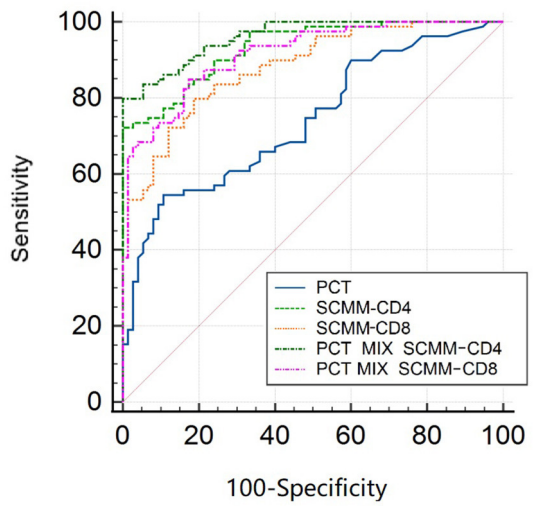

FIGURE 2 | ROC curve of sepsis detected by SCMM-CD4, SCMM-CD8, and their combination with PCT. ROC, receiver operating characteristic; SCMM, single-cell mitochondrial mass; PCT, procalcitonin.

TABLE 3 | Diagnostic values of SCMM-CD4, SCMM-CD8, and their combination with PCT in sepsis.

Variable	AUC	S.E.	Sig.	95% CI
PCT	0.740	0.040	<0.001***	0.662–0.817
SCMM-CD4	0.933	0.018	<0.001***	0.897–0.969
SCMM-CD8	0.881	0.026	<0.001***	0.819–0.927
PCT+SCMM-CD4	0.961	0.013	<0.001***	0.936–0.986
PCT+SCMM-CD8	0.915	0.022	<0.001***	0.859–0.954

*AUC, area under the curve; S.E., standard error around the Co-efficient for the constant; CI, confidence interval. ***P < 0.001; PCT, procalcitonin; SCMM, single-cell mitochondrial mass.*

proteins (20). The deubiquitinase USP30 (21), E3 ubiquitin ligase Parkin (22), mitochondrial ubiquitin ligases MARCHV/MITOL (23), MAPL/MULAN (24), and RNF185 (25) are located in the outer mitochondrial membrane to mediate protein polyubiquitination. These UPS components can remove damaged proteins and regulate mitochondrial morphology and renewal. Mitochondria also undergo dynamic remodeling through repeated fusion and division of the mitochondrial membrane, which can redistribute energy in the mitochondrial potential, metabolites, proteins, and mitochondrial DNA (26, 27) to avoid the accumulation of dysfunctional mitochondria and to maintain their overall function (28). MDV transfer misfolded or oxidized proteins and lipids in the mitochondria to lysosomes for degradation (29). These findings indicate that mitochondria must undergo dynamic renewal to maintain normal cellular function. Diseases lead to mitochondrial dysfunction, preventing the removal of aging mitochondria and further affecting cell function. Therefore, finding a convenient and rapid detection index for the quality of cell mitochondria is of great significance for evaluating the role of immune cells in sepsis, and the emergence of SCMM of T lymphocytes meets this clinical demand.

A fluorescent probe, MitoTracker, is used to detect MM by fluorescently labeling the mitochondria of lymphocytes to reflect the quality of the mitochondria (7). A similar research

TABLE 4 | Comparison between the ROC curves of five models.

Variable	AUC difference	S.E.	Z	95% CI	Sig.
PCT vs. SCMM-CD4	0.193	0.045	4.29	0.105–0.281	<0.001***
PCT vs. SCMM-CD8	0.141	0.048	2.96	0.048–0.023	0.003**
PCT vs. PCT+SCMM-CD4	0.021	0.039	5.71	0.145–0.297	<0.001***
PCT vs. PCT+SCMM-CD8	0.175	0.038	4.63	0.101–0.249	<0.001***
SCMM-CD4 vs. SCMM-CD8	0.052	0.024	2.13	0.004–0.100	0.032*
SCMM-CD4 vs. PCT+SCMM-CD4	0.028	0.013	2.16	0.003–0.054	0.030*
SCMM-CD4 vs. PCT+SCMM-CD8	0.018	0.025	0.711	−0.031–0.067	0.478
SCMM-CD8 vs. PCT+SCMM-CD4	0.080	0.026	3.050	0.029–0.132	0.002**
SCMM-CD8 vs. PCT+SCMM-CD8	0.034	0.017	1.995	0.001–0.068	0.046*
PCT+SCMM-CD4 vs. PCT+SCMM-CD8	0.046	0.021	2.231	0.006–0.087	0.026*

*AUC, area under the curve; S.E., standard error around the Co-efficient for the constant; CI, confidence interval. *P < 0.05; **P < 0.01; ***P < 0.001; PCT, procalcitonin; SCMM, single-cell mitochondrial mass.*

study, conducted by Doherty et al. (30) established that detection of ROS markers (HE and DHR) and reactive nitrogen species markers (DCF-DA and DAF-FM) using MitoTracker, was significantly correlated with the production of ROS and nitrosative stress in the cytoplasm and mitochondria. Yu et al. (5) found that HIV infection leads to an increase in MM in $CD4^+$T and $CD8^+$T cells, resulting in the accumulation of ROS in $CD4^+$T cells, affecting their function. SCMM uses Mito tracker's fluorescent probe to detect the mitochondrial fluorescence intensity of lymphocyte subsets by flow cytometry and then divides it by the count of corresponding lymphocyte subsets to obtain the SCMM of each lymphocyte subset. Compared to MM, SCMM can more sensitively reflect the function of cell mitochondria, which is an innovation in the detection of cell mitochondrial function. The higher SCMM in peripheral blood lymphocytes reflects abnormal mitochondrial metabolism, which is positively correlated with the degree of mitochondrial damage. The present study showed that there was a statistical difference in SCMM-CD4 and SCMM-CD8 between the groups with and without sepsis, which also confirms the argument that sepsis affects the function of lymphocytes by damaging lymphocyte mitochondria. Since this analytical method was more sensitive to CD4 lymphocytes than CD8 ones (7), the predictive value of SCMM-CD8 was not as high as that of SCMM-CD4, which was also consistent with the AUC results of our study. As CD3 includes CD4 and CD8, there was a collinearity problem; therefore, it was not used as a parameter of multivariate logistic regression. However, this research was only able to reflect the overall situation of mitochondrial quality through SCMM of T lymphocytes without clarifying which kind of mitochondrial regeneration mechanism was damaged.

After drawing ROC curves for PCT, SCMM-CD4, SCMM-CD8, PCT+SCMM-CD4, and PCT+SCMM-CD8, the present study found that the AUC of SCMM-CD4 or SCMM-CD8 was greater than that of PCT. PCT is a widely used classic indicator in clinical practice and is mainly used to reflect the severity of gram-negative infections (31). It is often used as a reference for the diagnostic efficacy of new biomarkers of sepsis (32). The present study found that when comparing PCT with SCMM-CD4 or SCMM-CD8 cut-off values, the sensitivity and specificity of SCMM-CD4 or SCMM-CD8 were higher than those of PCT, indicating that the diagnostic value of SCMM-CD4 or SCMM-CD8 in the early prediction of sepsis is higher than that of PCT. Moreover, SCMM-CD4 was better than SCMM-CD8. We further studied the predictive value of SCMM-CD4 or SCMM-CD8 combined with PCT for early sepsis, and the results showed that SCMM-CD4 combined with PCT had the best predictive effect (**Figure 2** and **Tables 3**, **4**). Some studies have shown that mitochondrial function is related to patient prognosis. A meta-analysis by Wang et al. (33) showed that mitochondrial metabolic indices could predict the mortality of patients with sepsis. Maestraggi et al. (34) established that abnormal mitochondrial function of skeletal muscle and lymphocytes during septic shock could trigger intensive care unit-acquired weakness, infectious, and immune paralysis. Therefore, based on the mechanism of mitochondrial damage in sepsis, selecting an appropriate target intervention may be an effective measure to reverse the process of sepsis and improve its prognosis in the future (35). Currently, 5-hydroxydecanoate can block the particle K_{ATP} channel, thereby preventing an increase in line permeability and ATP outflow (36). Mitochondria-targeted Co-enzyme Q10 can target and aggregate into mitochondria to improve the electron transport chain function (37). Animal experiments have confirmed that recombinant human mitochondrial transcription factor A stimulates mitochondrial regeneration, which can significantly increase the expression of mitochondrial DNA and improve mitochondrial function (38). The present study has several limitations, such as the limited sample size, the effect of blood collection time, disease onset, and other factors. Therefore, bias may have existed in the research results. In addition, the present study neither clarified whether SCMM-CD4 could be used as an indicator to judge the prognosis of sepsis, nor did it examine downstream indicators reflecting abnormal mitochondrial protein turnover and regeneration. These issues need to be studied further by collecting more sample data.

The present study established that differences exist in lymphocyte mitochondrial functions between patients with sepsis and those without sepsis. SCMM-CD4 and SCMM-CD8, which reflect lymphocyte mitochondrial function, have better predictive value for early sepsis than PCT, a classical sepsis biomarker. SCMM-CD4 combined with PCT has the highest predictive value. Although the detection method of SCMM in T lymphocytes cannot clarify the specific mechanism of mitochondrial regeneration disorder leading to its functional damage, it can still reflect the overall level of mitochondrial function in patients. Because SCMM of T lymphocytes is superior to PCT in the diagnosis of early sepsis, it will assist in the early identification and treatment of the pathogen and improve the prognosis of patients. Furthermore, practitioners can administer targeted drugs to improve mitochondrial damage based on the levels of SCMM-CD4 in patients. Multidimensional treatment can be provided to patients with severe sepsis to improve their prognosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Zhejiang Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QL, L-XP, and W-WC: study conception and design. LC, JF, C-XX, J-YL, and L-XP: material preparation, data collection, and analysis. L-XP: wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: First Case of Endophthalmitis Caused by an Emerging Pathogen: *Nocardia huaxiensis*

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Nocardia endophthalmitis is a relatively uncommon form of endophthalmitis seen in clinical patients. In general, *Nocardia* endophthalmitis tends to carry a poor prognosis. Here, we report a 3-year-old child who was admitted to the hospital due to a rupture of the left eye. The suturing and anterior chamber formation were performed immediately. Approximately, 16 days after the operation, massive whitish plump and tufted exudates gathered in the pupil area and at the bottom of the anterior chamber, and the child was diagnosed with endophthalmitis. The infection was initially considered to be caused by fungal pathogens for that the hyphae and spores were observed in the smear. However, the isolate obtained after 4 days of culturation was identified as actinomycetes using MALDI–TOF. We further classified it as *Nocardia huaxiensis* by next-generation sequencing (NGS) based on the MinION platform. Amikacin and sulfamethoxazole tablets were used to control the infection and the ocular inflammation subsided gradually. Intraocular lens (IOL) implantation is planned to be performed at an appropriate future time to improve his vision. *Nocardia* endophthalmitis is rare and usually caused by ocular trauma or surgery. In conclusion, *Nocardia huaxiensis* should be considered as an emerging pathogen and deserves more attention.

Keywords: endophthalmitis, nocardiosis, antibiotic susceptibility, Nanopore sequencing, pathogen identification

INTRODUCTION

Nocardia species are a kind of aerobic, gram-positive, and weakly acid-fast bacteria that are commonly found in soil, water, and plants (1). In the past few years, an increasing number of species have been recognized as human pathogens and they are frequently associated with pulmonary infections, mycetoma, and disseminated nocardiosis (2, 3). They can also cause ocular morbidities such as keratitis, scleritis, and endophthalmitis (2). *Nocardia* had been reported to be associated with all the types of endophthalmitis, including post-operative, post-traumatic, and endogenous endophthalmitis, with generally poor-visual outcomes (4).

In recent years, the taxonomy of *Nocardia* has become more complex due to the description of new species that have made some clusters bigger or have even led to the creation of new ones (5). Species-level identification of *Nocardia* relies heavily on biochemical

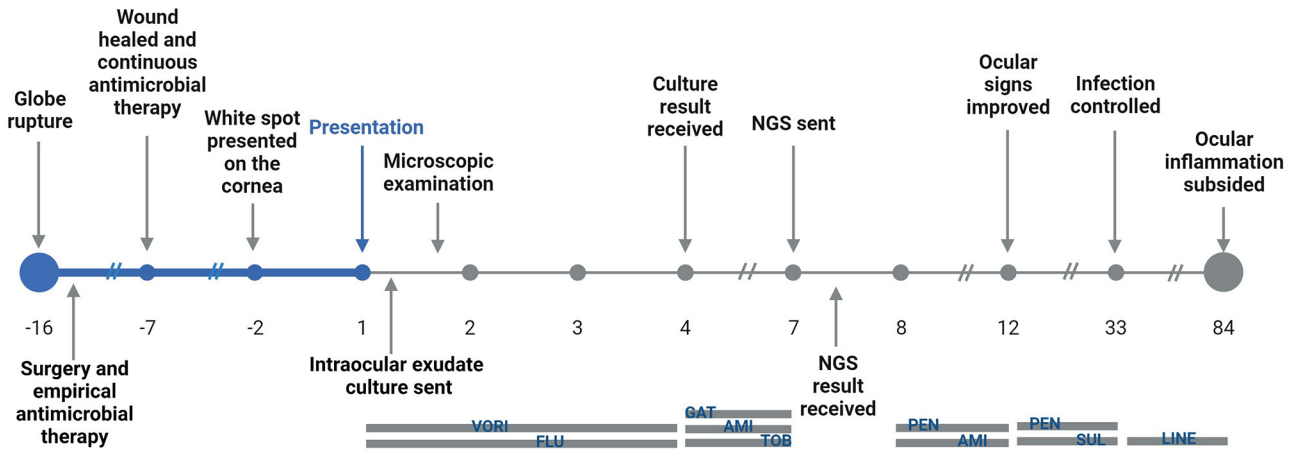

FIGURE 1 | Timeline of the illness progress, pathogen identification, and treatment for the patient. VORI, voriconazole; FLU, fluconazole; GAT, gatifloxacin; AMI, amikacin; TOB, tobramycin; PEN, penicillin; SUL, sulfamethoxazole; LINE, linezolid.

tests and cellular fatty acid analysis, which are cumbersome, ime-consuming, and often not definitive (6). In total, 16S rRNA gene sequencing was considered to be the "gold standard" for *Nocardia* species identification (7). Multi-locus sequence typing (MLST) using concatenated sequences of 5 housekeeping genes (*gyrB, 16S, secA, hsp65*, and *rpoB*) was also used to provide higher accuracy and discriminatory power in species identification of the *Nocardia* genus (6, 8). However, some reports have confirmed that the 16S rRNA sequence or the MLST method cannot provide enough genetic information to distinguish between closely-related *Nocardia* species (6, 9). A recent study showed that the *dapb1* gene, which encodes dipeptidyl aminopeptidase BI, was far superior to commonly-used markers for *Nocardia* and yielded a topology almost identical to that of whole genome-based phylogeny (9).

Nocardia huaxiensis (*N.huaxiensis*) was first isolated from skin biopsy specimens of a patient and identified as a novel *Nocardia* species in 2021 (10). There was no other reported case of human infection caused by this species. Next-generation sequencing (NGS) has the potential to determine pathogen species more specifically and accurately (11). In this report, we present a case of bacterial endophthalmitis caused by *N. huaxiensis* which was mistaken for fungal endophthalmitis before the correct diagnosis was made with NGS testing based on MinION platform. We also performed gram-staining and weak acid-fast staining (modified Kinyoun's method) of this strain and its susceptibility to antibiotics was tested using a broth dilution method according to Clinical and Laboratory Standards Institute (CLSI) standard M24-A2 guidelines. These results show that *N. huaxiensis* should be considered as an emerging pathogen and deserves more attention.

CASE PRESENTATION

A 3-year-old was admitted to the Emergency Department of Beijing Children's Hospital, Capital Medical University with photophobia and a white spot on the cornea of the left eye that had been presented for 2 days. The timeline is shown in **Figure 1** and the complete case progress record is reported later in detail.

Approximately, 16 days before admission, the child fell on the garden steps, resulting in a globe rupture of the left eye with lacerations of the cornea and the sclera. The iris incarcerated in the wound. Corneal and scleral laceration debridement, suturing, and anterior chamber formation were performed immediately. To prevent post-traumatic endophthalmitis, empirical antimicrobial therapy was performed during the pre- and post-operative period with intravenous cefuroxime for 5 days and anti-inflammatory dexamethasone for 3 days post-operatively. In addition, levofloxacin antibiotic eye drops, tobramycin dexamethasone eye ointment, and prednisone acetate eye drops were administered for further anti-inflammatory and anti-infection purposes. In total, 7 days before admission, the corneal wound healed, and the suture remained tight. The anterior chamber was clear with medium depth, and the lens was also clear. Topical antibiotics were continued, and amblyopia treatment was initiated.

On admission, the patient was observed with mixed hyperemia and mild edema of the cornea as well as a shallow anterior chamber in the left eye. A massive white exudate gathered in the pupillary area and at the bottom of the anterior chamber. The iris was partially visible and the lens was completely invisible. Red light reflection was not observed (**Figure 2A**). The vital signs were stable. A systemic work-up revealed no obvious abnormalities in the respiratory system, digestive system, or nervous system. The indexes of liver and kidney function and chest X-ray were normal. B-mode ocular ultrasound showed an abnormal echo in the left eyeball and slight vitreous opacity (**Figure 2B**), suggestive of endophthalmitis caused by microbial infection. Emergency surgery was performed immediately, including anterior chamber irrigation, extracapsular cataract extraction (ECCE), and intravitreal drug injection in the left eye. During

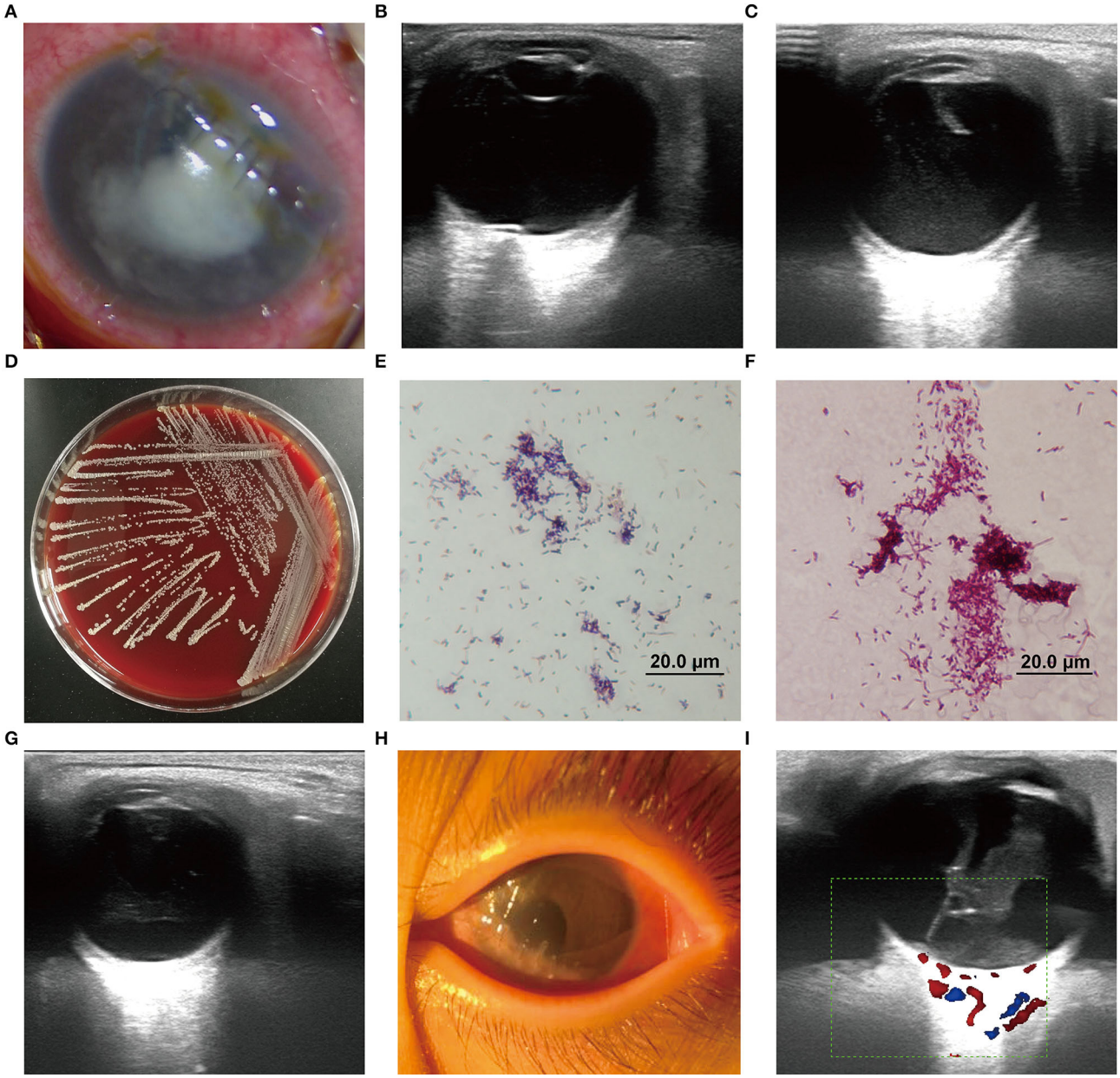

FIGURE 2 | (A) Left eye with a massive white exudate gathered in the pupil area. **(B,C,G,H)** B-mode ultrasound of the left eye in different treatment stages. **(D)** The culture colonies of strain BCHNH01^T on blood agar plate after 48 h culture. **(E,F)** The gram-staining and weak acid-fast staining (modified the Kinyoun's method) of strain BCHNH01^T. **(I)** Vitreoretinal traction and suspicious retinal detachment were observed in the left eye.

the surgery, the intraocular exudate was rinsed and sent for smear examination and culture (using sabouraud medium and columbia blood agar media, under aerobic conditions at 25 and 35°C, respectively). The lens cortex was sucked out, because opacity of the capsular membrane was observed. The optic disc appeared to be red, and the fundus was sightless. Intravitreal injection of ceftazidime (1 mg, 0.1 ml), vancomycin (1 mg, 0.1 ml), and also subconjunctival injection of tobramycin (20,000 U, 0.5 ml) were conducted. Adjunctive antimicrobial therapy was started after surgery with levofloxacin eye drops and gatifloxacin eye gel.

Microscopic examination revealed hyphae and spores, thus, the endophthalmitis was originally considered to be caused by fungal agents. Based on this finding, antimicrobial treatment was modified to 135 mg intravenous infusion of voriconazole combined with voriconazole eye drops every hour and subconjunctival injection of fluconazole. Intravitreal injection of voriconazole (0.1 mg, 0.1 ml) was given to the patient

on the following day after surgery. On hospital day 3, ocular B-mode ultrasound showed an abnormal echo in the left eyeball and vitreous opacity (**Figure 2C**). Meanwhile, corneal edema, shallow anterior chamber, and aqueous flare were still present, indicating that the condition had not improved. On hospital day 4, the medias at 35°C showed that some yellow, dry, and chalky colonies were present after 48 h of culturation (**Figure 2D**) and were identified as actinomycetes by MALDI–TOF mass spectrometry. Following the recommendation of the Department of Infectious Diseases, administration with gatifloxacin eye gel, amikacin eye drops, and 5 consecutive days of subconjunctival injection of tobramycin were initiated to enhance anti-infection effects. Vitrectomy and intravitreal injection [ceftazidime (1 mg, 0.05 ml) and amikacin (0.4 mg, 0.1 ml)] were performed on hospital day 6.

On hospital day 7, in order to determine the pathogen species of the colonies accurately, the isolate was further subjected to whole-genome sequencing (WGS) on a MinION platform with Rapid Barcoding Kit (SQK-RBK004, Oxford Nanopore Technologies, ONT), as described in **Supplementary Methods**. Within 2 h, the MinION-based NGS assay determined that the species belonged to *Nocardia* spp. Subsequently, gram stain and weak acid-fast stain were performed and both were positive in the isolates (**Figures 2E,F**). Thus, we adjusted the treatment strategy again. Systemic use of penicillin and amikacin drugs and compound sulfamethoxazole tablets (0.48 g oral) were given as anti-infection agents. Amikacin eye drops, sulfacetamide sodium and penicillin eye drops, gatifloxacin eye gel, and conjunctival injection of penicillin were given per day.

On hospital day 12, the ocular congestion and the corneal edema had subsided, and the anterior chamber was gradually cleared. However, the ocular B-ultrasound indicated that the vitreous opacity in the left eye had not significantly changed (**Figure 2G**). In this period, to find out if the severe endophthalmitis aroused the intracranial infection, we took cranial MRI for him and no obvious abnormality was observed in bilateral brain parenchyma. Penicillin and sulfamethoxazole oral compound were continuously used for anti-infection. Amikacin was replaced with ceftriaxone sodium from experience due to the high risk of hearing loss in children with its long-term use. Through the continuous application of the above anti-infection treatment for 3 weeks, the infection was effectively controlled: eye congestion was reduced; the cornea became transparent; the anterior chamber was quiet with no cell or flare being noted; and the purulent secretion disappeared (**Figure 2H**). Thereafter, the doses were tapered as the infection was brought under control. Treatment with systemic intravenous cephalosporins and penicillin antibiotics was stopped, and linezolid was given orally. On hospital day 34, vitreoretinal traction and suspicious retinal detachment were observed (**Figure 2I**). Vitrectomy was recommended to relieve vitreoretinal traction. To further treat the vitreoretinal traction, the child underwent vitrectomy on the left eye.

On hospital day 84, the ocular inflammation subsided and the ocular structure was stable. No systemic complications were observed. The vital indexes were always steady during the treatment and no medication side effect appeared. Intraocular lens (IOL) implantation was planned to be performed at an appropriate time to improve vision.

DISCUSSION

Clinically, nocardiosis is a rare and potentially life-threatening gram-positive bacterial infection. The route of infection is usually *via* inhalation or direct inoculation (12). Commonly reported human infections with *Nocardia* are pulmonary nocardiosis, cutaneous nocardiosis, and brain abscesses (13, 14), while there are also some reports about endophthalmitis caused by *Nocardia* (4). *Nocardia* endophthalmitis is considered of a relatively rare but potentially devastating ocular condition (15). *N. huaxiensis* is a novel species identified in 2021, and here, we present the first description of endophthalmitis caused by this species.

Nocardiosis typically affects immunocompromised patients while this report describes an immunocompetent child who suffered ocular trauma by the steps in their garden yard. Our first potential speculation was that the infectious agent penetrated the eye during the primary injury and was possibly encapsulated around the injury to the anterior lens capsule. Our second potential speculation was the treatment time of glucocorticoids was too long. In addition to broad-spectrum antibiotics, the patient was also treated with glucocorticoids during the post-traumatic period, similarly to that reported by Compte et al. (16). *Nocardia* endophthalmitis is rare and these two reasons may work together to result in eye infection. Ocular exposure to soil or plant matter was a common historical point in case of *Nocardia* infection in eyes (17, 18) and our report also indicates that *Nocardia* infection should be considered in patients with such plant-inflicted trauma.

Clinical diagnosis of *Nocardia* is very difficult and complicated, as there are no signs, symptoms, or radiological findings that are pathognomonic for *Nocardia* infection (12). As such, clinical *Nocardia* endophthalmitis misdiagnosed as fungal endophthalmitis have been reported (19). Recently, MALDI–TOF has been shown to provide accurate identification of *Nocardia* species (8). However, while some species are easily identified, the identification of uncommon species remains a challenge (8). In this report, we mistook this case as a fungal infection due to the hyphae and spores observed in the smear. The colonies were observed after 4 days of culture and identified as actinomycetes *via* MALDI–TOF, while we could not identify the bacteria on species level. Nanopore sequencing is an emerging NGS technique that can generate sequencing reads in real time (20). During the sequencing of the pure culture isolate, the first reads file was outputted in 10 min after run started. Following analysis of the sequences determined it as the *Nocardia* genus initially, indicating that 10-mininute sequencing time was sufficient to enable pathogen identification by MinION. We reclassified this case as *Nocardia* Endophthalmitis and the amikacin treatment was used to cure the patient, which indicated that Nanopore sequencing can provide valuable information during the identification of novel *Nocardia* infections.

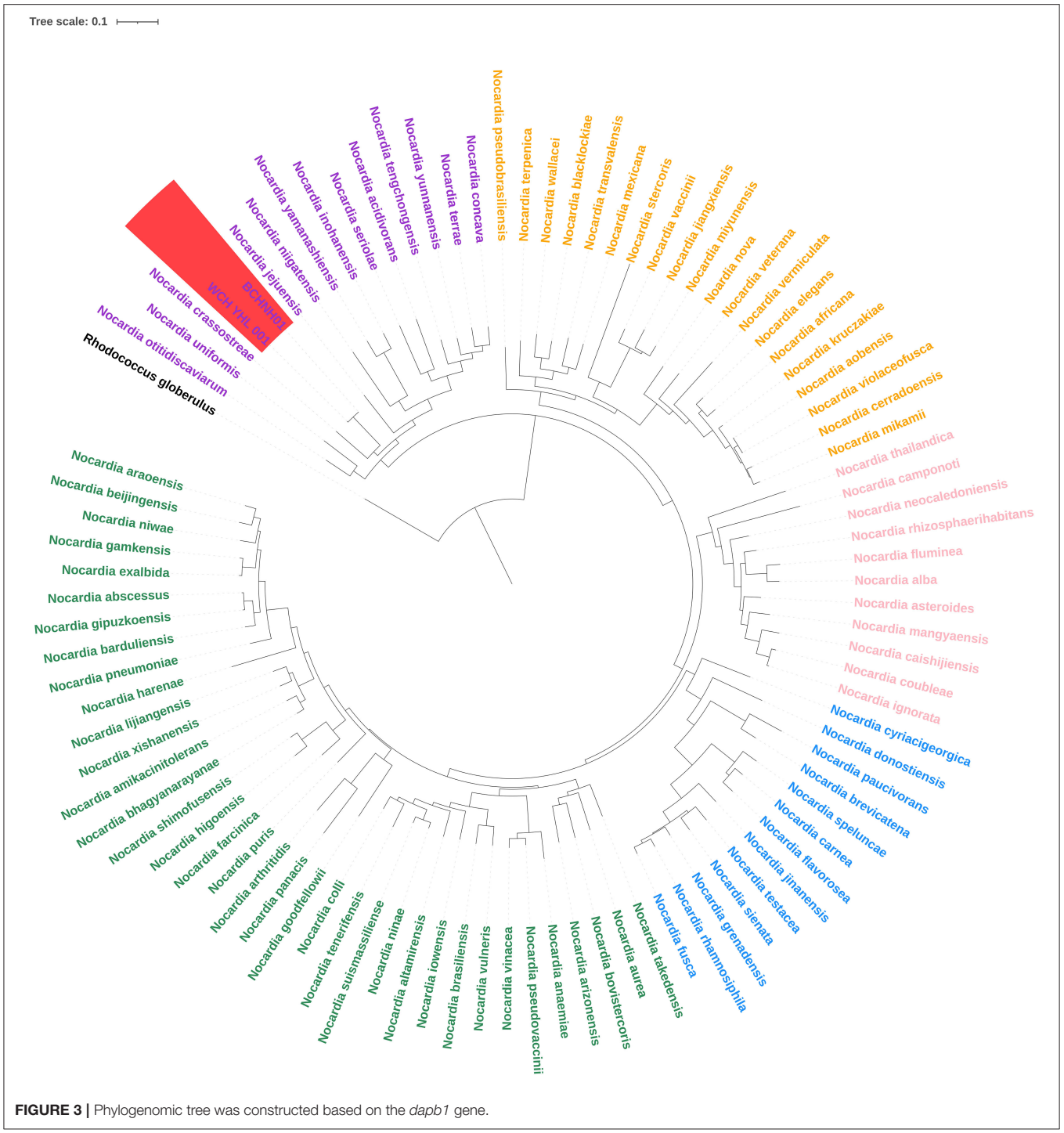

FIGURE 3 | Phylogenomic tree was constructed based on the *dapb1* gene.

The whole genome of this isolate was assembled using Nanopore sequencing and the Illumina Noveseq platform. The genome size of this strain is 8.3 M nucleotides with a G+C content of 67%. We extracted the whole 16S rRNA sequence from the assemble genome and fount it showed the highest similarities (99.93%) with the 16S rRNA sequence of the strain *Nocardia huaxiensis* WCH-YHL-001^{T}. We further compared the Average Nucleotide Identity (ANI) of the two strains and found that the ANI value was determined of 99.47%, indicating that the strain belong to a novel *Nocardia* species, *N. huaxiensis*, whose phylogenetic analysis was performed based on the 16S rRNA sequence (10). However, some reports have confirmed that the 16S rRNA sequence cannot provide enough genetic information to distinguish between closely-related species (8).

TABLE 1 | The antibiotic susceptibility of *Nocardia huaxiensis*.

Antibiotics	Minimum inhibitory concentration (MIC, μg/mL)	Interpretation
Amikacin	2	S
Amoxicillin-Clavulanic acid	≥64/32	R
Ceftriaxone	≥128	R
Ciprofloxacin	0.5	S
Clarithromycin	4	I
Imipenem	≥32	R
Linezolid	2	S
Minocycline	1	S
Moxifloxacin	1	S
Trimethoprim-Sulfamethoxazole	1/19	S
Tobramycin	4	S
Cefepime	≥64	R
Cefotaxime	≥128	R
Doxycycline	0.5	S

S, Susceptible; R, Resistant; I, Intermediate.

The *dapb1* gene of *Nocardia* has been recently demonstrated to yield a topology almost identical to the genome-based phylogeny (9). The whole-genome sequences of 95 *Nocardia* strains were downloaded from the National Center for Biotechnology Information (NCBI) and the sequences of the *dapb1* gene were extracted. Multiple sequence alignment was performed with ClustalW2 and a phylogenetic tree was constructed using the maximum likelihood method with 1,000 bootstrap repeats with IQ–TREE. *Rhodococcus globerulus* NBRC 14531^T served as an outgroup. The phylogenomic tree revealed five main phylogroups, consistent with the previous report (9), and the strains BCHNH01^T and WCH-YHL-001^T formed independent branches with robust bootstrap support, indicating that they indeed belong to a new species (**Figure 3**).

Because of the variety of antibiotic susceptibility depending on *Nocardia* species, it is important to identify *Nocardia* at the species level and to investigate its antibiotic susceptibility (21). Amikacin at a concentration of 2–2.5% is considered to be the best choice for the treatment of *Nocardia* endophthalmitis, as amikacin demonstrates the lowest minimum inhibitory concentrations for *Nocardia* isolates (4). However, some cases of amikacin-resistant *Nocardia* have been described (22, 23), so it is important to test the antibiotic susceptibility of the *N. huaxiensis*. We performed the antimicrobial susceptibility of this strain by using a broth dilution method according to the CLSI standard M24-A2 guidelines. The strain was resistant to amoxicillin-clavulanic acid, ceftriaxone, imipenem, cefepime, and cefotaxime (**Table 1**). Interestingly, these drugs collectively belong to β-lactams which indicates that β-lactam antibiotics are not suitable to cure infection caused by this species.

A limitation of this case study is that we did not identify *N. huaxiensis* from the intraocular exudate directly. Although we identified the cultured isolate correctly with Nanopore sequencing, we wasted nearly 1 week while waiting for the isolate to grow. Nanopore sequencing has the advantage of rapid library preparation and real-time data acquisition and it has been used to identify viral and bacterial pathogens from clinical samples directly (24). Whether the Nanopore sequencing can be used in the rapid species-level identification of the *Nocardia* genus from the clinical samples deserves more investigation. Besides, we did not test the antimicrobial susceptibility of this strain in a timely manner, which led to the improper use of some antibiotics such as ceftriaxone.

This case study demonstrates that it is of great importance to explore new pathogen identification strategy to improve the prognosis of the patients even though the treatment process is tortuous. Accurate identification of *Nocardia* species, complete local debridement, and appropriate antibiotic therapy are important in the treatment of *Nocardia* infections. At present, the child is still being treated with local eye drops combined with systemic oral drugs to control infection. However, the effects of long-term treatment with anti-infection agents still need to be observed moving forward.

In conclusion, here, we described a case of bacteria endophthalmitis caused by *N. huaxiensis*, which was mistakenly diagnosed as fungal endophthalmitis. *Nocardia* endophthalmitis is often difficult to diagnose, mimicking chronic inflammation, or fungal infection. Ophthalmologists should be aware of infections caused by *Nocardia* and suspect *Nocardia* endophthalmitis after plant-inflicted trauma. Earlier and accurate identification of *Nocardia* species can improve patient outcomes. We have initially revealed the application prospects of Nanopore sequencing in the identification of *Nocardia* infection, especially for novel and uncommon species. The antibiotic susceptibility of *N. huaxiensis* was also tested according to CLSI standard M24-A2 guidelines and β-lactam antibiotics were found to be unsuitable to cure the infection caused by this species. *N. huaxiensis* is an emerging pathogen and we hope that our results can provide some instructions for future treatment.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Beijing Children's Hospital Affiliated with Capital Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal

guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

CL analyzed and interpreted patient data. LZ performed the Nanopore sequencing and antimicrobial susceptibility test experiment. LLiu performed microscopic examinations of this strain and performed the surgery described in the case report with YC, TL, and TC. YW analyzed data and improved the manuscript in details. YJ and GL performed the literature review and wrote the manuscript. LLi conceived the idea of describing the case report and reviewed, and made significant revisions to this manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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Expert consensus statement on therapeutic drug monitoring and individualization of linezolid

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Linezolid is an oxazolidinone antibacterial drug, and its therapeutic drug monitoring and individualized treatment have been challenged since its approval. With the in-depth clinical research of linezolid, we have changed our attitude toward its therapeutic drug monitoring and our view of individualized treatment. On the basis of summarizing the existing clinical studies, and based on the practical experience of each expert in their respective professional fields, we have formed this expert consensus. Our team of specialists is a multidisciplinary team that includes pharmacotherapists, clinical pharmacology specialists, critical care medicine specialists, respiratory specialists, infectious disease specialists, emergency medicine specialists and more. We are committed to the safe and effective use of linezolid in patients in need, and the promotion of its therapeutic drug monitoring.

KEYWORDS

linezolid, therapeutic drug monitoring, individualization, expert consensus, pharmacotherapy

Introduction

Linezolid is a synthetic antibacterial drug of the oxazolidinone class. It was approved by the U.S. Food and Drug Administration (FDA) in 2000 and entered the Chinese market in 2007 for clinical use (1, 2). Linezolid inhibits protein synthesis by binding at the P site of the ribosomal 50S subunit. In terms of pharmacokinetics (PK), it has good penetration, and its accumulation in tissue—including bone, lung, vegetation, hematoma, and cerebrospinal fluid (CSF)—allows for its use in treating surgical infection (3–7). However, differences in linezolid exposure between individuals are related to differences in efficacy and adverse reactions (8–12).

Currently there is no guideline or consensus for linezolid therapeutic drug monitoring (TDM) and individualized treatment. Therefore, the Division of Therapeutic Drug Monitoring of Zhejiang Pharmacological Society and the Infectious Diseases Pharmacist Group of the Society of Hospital Pharmacy of Zhejiang Pharmaceutical Association recruited experts in related fields to discuss issues related to linezolid TDM and individualized treatment. The expert group considered 18 clinical practice issues and conducted three rounds of consultation with external experts; the end result is this consensus statement.

Methods

The expert consensus was drawn up by 36 pharmacotherapy experts from the Division of Therapeutic Drug Monitoring of Zhejiang Pharmacological Society and the Infectious Diseases Pharmacist Group of the Society of Hospital Pharmacy of Zhejiang Pharmaceutical Association. The group's agenda was predefined. The expert group first defined the clinical questions to be addressed and then designated experts to each question. The recommendations for the clinical practice questions are in a question-and-answer format and reasons are provided. Three rounds of expert meetings were conducted to provide trustworthy recommendations.

Eighteen external clinicians and experts in clinical pharmacotherapeutics were invited to vote on the issues. Scoring was based on theoretical basis, scientificity, innovation, feasibility, and expert weighting of the consensus items. According to the modified Delphi method (13), the voting results of experts are summarized, and the average score is calculated to propose the corresponding recommendation strength. The final score was on a scale from 1 to 10: 1–4 is not recommended, 5–7 is weakly recommended, and 8–10 is recommended.

Expert panel recommendations

Question 1: Does linezolid require TDM? What is the target range for linezolid TDM?

Expert panel recommendations

(1) We recommend TDM for linezolid. (Score: 8.90) (2) We recommend maintaining a linezolid trough concentration of 2–8 mg/L. (Score: 8.60)

Reason

A decade-long retrospective study by Pea et al. included 1,049 patients treated with linezolid 600 mg q12h and collected 2,484 trough concentration points. They set the trough concentration range to 2–7 mg/L. Only 50.8% of patients

were in this range, and the incidence of overexposure (33%) was significantly higher than that of underexposure (16.2%). Overexposure to linezolid is significantly correlated with an estimated Creatinine clearance (CrCL) was estimated by means of the Cockcroft and Gault formula ($CrCL_{C-G}$) $\leq$40 mL/min, and underexposure is significantly correlated with an estimated $CrCL_{C-G}$ > 100 mL/min. This suggests that TDM could be used to optimize linezolid exposure in most patients (14). A monocentric, prospective, open-label, interventional study found that proactive TDM of linezolid may be beneficial for preventing or recovering from dose-dependent thrombocytopenia (15). A prospective observational study enrolled 84 patients who received the standard linezolid regimen (600 mg q12h) and collected 153 trough concentration points. Only 57.52% of patients had trough concentrations within the effective range (2–8 mg/L), 31.37% had underexposed trough levels, and 11.11% had overexposed trough levels (16). A retrospective, single-center, observational trial from 2008 to 2013 included 70 patients treated with linezolid 600 mg q12h. A linezolid trough concentration of 2–6.3 mg/L achieved the best therapeutic effect while minimizing adverse reactions (17). Another retrospective study included 108 patients treated with linezolid 600 mg q12h. Multivariate analyses showed that the clinical failure rate of the TDM group was significantly lower than that of the non-TDM group. In addition, 90.5% of patients with renal impairment in the TDM group needed a dose adjustment to achieve the target trough concentration (18). A prospective study by Cattaneo et al. (19) suggested a causal relationship between the blood concentration of linezolid and the risk for drug-related hematological toxicity. In addition, case reports of two critically ill patients with normal renal function suggested that overexposure to linezolid in plasma causes toxicity and that subtherapeutic exposure leads to treatment failure. TDM would allow early detection and correction of bias to achieve the target linezolid concentration (20).

In short, both prospective and retrospective trials and case reports suggest that TDM of linezolid is necessary. Although this is contrary to the description in the package insert of linezolid, it is a confirmed, evidence-based recommendation. We recommend that in patients treated with linezolid, in particular critically ill patients, TDM is required to achieve optimal PK.

Question 2: What detection method is recommended for TDM of linezolid?

Expert panel recommendations

We recommended quantification of linezolid in human plasma or serum by high performance liquid chromatography-Ultraviolet detector (HPLC-UV) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. (Score: 8.80)

Reason

HPLC-based methods coupled with UV or MS are the major analytical techniques for determining linezolid in human plasma or serum, and MS is more suitable for clinical application because of its high selectivity and sensitivity (21). LC-MS/MS methods that enable simultaneous quantification of plasma concentrations of linezolid and other antibiotics are available (22–27) and may improve detection efficiency for TDM. Other methods for determining linezolid in human plasma are immunoassay (28), direct analysis in real time mass spectrometry (DART-MS) (29), and square wave voltammetry (30), but these lack comparative verification with HPLC for large-scale clinical sample detection.

In summary, based on the stability of the quantitative method and the availability of TDM equipment, we recommend HPLC-UV or LC-MS/MS methods for quantitative determination of linezolid in plasma.

Question 3: How should laboratories conducting quantitative determination of linezolid perform quality control?

Expert panel recommendations

Quality control procedures should be implemented using drug *in vivo* analysis techniques, quality control standards, and clinical intervention programs. (Score: 9.15)

Reason

The quality control of linezolid by *in vivo* drug analysis techniques should include investigations of specificity, sensitivity, accuracy, reproducibility, and stability (22–27). Quality control standards should include indicators for quantitative determination methods within and between laboratories, qualifications and certification of testing personnel, Standard Operating Procedure (SOPs) for testing procedures, clinical pathways and independent external quality assessments (EQAs) (31). The laboratory should have a dedicated quality control manager. The laboratory should participate in the quality evaluation activities of the TDM professional organization or government-authorized quality management agency and have sufficient capacity. For quantitative detection of linezolid, relevant technical guidance documents, quality control plans, and clinical intervention plans should be formulated in advance. These quality control documents should be released after review by the appropriate authorities before quantitative testing of linezolid (31).

Question 4: How should linezolid TDM results be reported?

Expert panel recommendations

We recommend that the TDM report provide test results, explanations thereof, and treatment suggestions. The report should be individualized for the patient in question. (Score: 9.15)

Reason

There is no best practice for TDM reports, but we base these recommendations on *Antimicrobial Therapeutic Drug Monitoring in Critically Ill Adult Patients: A Position Paper*, *The Expert Consensus on the Standards of Therapeutic Drug Monitoring (2019 Edition)*, and clinical practice experience. A complete linezolid TDM report should include basic information, test results, and treatment suggestions and explanations (31–33). Basic information includes information on the applicant, patient, and sample. Patient information should include the type of patient, identification, sex, age, weight, serum creatinine level, and estimated creatinine clearance rate (CLCr) on the day of sampling or the past 3 days. The minimum inhibitory concentration (MIC) value of pathogens is crucial, and should include confirmed or suspected pathogens and local drug resistance information for subsequent pharmacokinetic/pharmacodynamic (PK/PD) calculations. The PK of linezolid is affected by, for example, age, sex, weight, renal function, and rifampin contamination. Therefore, these factors should be included in the TDM report. Sample information should include the type of sample, collection time, and linezolid regimen (dose and time of administration of the last two doses). Only the qualified samples make the interpretation of significance. Any errors in the timing of specimen collection, collection of blood in arteries or veins or central venous catheter (CVCs), blood collection tubes, etc., can mislead the results.

Test results should include the concentration, quantitative unit, therapeutic range, and over/under limit mark. The interpretation of the test results should include the purpose of monitoring, an analysis of the results, and a conclusion. The conclusion should integrate the regimen, renal function, contaminant medication, and clinical response. Treatment suggestions should be based on the test results, purpose of monitoring, and patient information. If the linezolid dosage needs to be adjusted, we recommend pharmacometrics-based dose calculation. Pharmacometrics enables individualized administration of linezolid (8, 10). In addition, individualized monitoring and follow-up are needed based on characteristics of the patient and disease.

The TDM report should be issued within 24 h to provide time to adjust treatments. The report should contain the signatures of the test operator and the reviewer of the report.

Question 5: For which patients should linezolid TDM be considered?

Expert panel recommendations

We recommend TDM for critically ill patients, children, patients with renal insufficiency/augment or liver cirrhosis, elderly, obese and patients taking co-medications known to interact with linezolid. (Score: 9.50).

Reason

Increased endothelial permeability, renal dysfunction, and hypoalbuminemia are common in critically ill patients. Endothelial dysfunction may increase the distribution volume of hydrophilic antimicrobials. Hypoalbuminemia may increase the free (or unbound) fraction of antimicrobials, potentially resulting in CL and increasing the drug distribution (34, 35). ARC (CrCL > 130 mL/min) is increasingly noted in critically ill subpopulations (incidence: 14–80%). It could enhance renal elimination of antimicrobials and is associated with suboptimal plasma concentrations (36–38). Many critically ill patients have renal impairment or acute kidney injury. All these factors will significantly influence antimicrobial PK in critically ill patients. Therefore, TDM should be routinely performed when linezolid is used to treat critically ill patients. Maintaining a linezolid C_{min} of 2–7 mg/L is recommended to optimize efficacy and minimize hematological toxicity (33). A retrospective, monocenter, observational study by Dong et al. included 70 critically ill patients treated with linezolid 600 mg q12h. Patients were divided into two groups according to whether they developed thrombocytopenia after treatment with linezolid. Logistic analyses showed that C_{min} was significantly related to linezolid-associated thrombocytopenia (17). Two other studies (39, 40) showed that linezolid overexposure in patients with renal insufficiency is related to thrombocytopenia and that linezolid blood concentration monitoring can avoid platelet toxicity caused by overexposure. A retrospective case-control 1:1 study by Luque et al. included 52 patients with and without cirrhosis who received linezolid 600 mg intravenously every 12 h. Patients with liver cirrhosis had higher median linezolid trough plasma concentrations than those without cirrhosis (20.6 mg/L vs. 2.7 mg/L, $P < 0.001$) and a significantly increased incidence of overexposure (76.9% vs. 26.9%, $P < 0.001$). Therefore, liver cirrhosis may influence linezolid PK. TDM of linezolid would be valuable in these patients (41).

A single-center retrospective study by Cojutti et al. included 112 overweight and obese patients on linezolid with a median body mass index (BMI) of 35.4 kg/m^2 and a median weight of 105.8 kg. A total of 52.9% patients who received the standard dosage of 600 mg q12h did not achieve the target steady-state trough linezolid concentration (2–7 mg/L). Indeed, the trough linezolid concentration was <2 mg/L and >7 mg/L in 35.6% and 17.3% of patients, respectively (42). Therefore, the standard

dose of linezolid may not be applicable to obese and overweight patients. TDM is needed in such patients to optimize treatment. Simon et al. (43) suggested that as body weight increases the linezolid concentration in plasma and subcutaneous tissue decreases. The current dosing regimen does not achieve a sufficient concentration to kill bacteria with MIC $\geq$ 2 mg/L, in particular as an empirical antibacterial for severely obese patients. Blackman et al. showed that for 140 kg non-cirrhotic patients, bacteria had MICs of 0.5, 1, 2, and 4 mg/L, and the standard linezolid dose of probability of target attainment (PTA) was 100, 98.8, 34.1, and 0%. For non-cirrhotic patients of body weight $\geq$ 140 kg and MIC $\geq$ 2 mg/L, the standard linezolid dose did not reach the target of $\geq$90%. The standard linezolid dose may not be suitable for all patients (44).

A retrospective study of 23 patients was conducted by Cojutti et al. Standard dosages were suboptimal in 50.0 and 44.4% of patients in group 1 and group 2, respectively. Among those who underwent multiple instances of TDM, the dosage was increased in 33.3% of cases in both groups and decreased in 6.6 and 9.5% of cases in group 1 and group 2, respectively. Monte Carlo simulations showed PTA $\geq$ 90% with the current dosing regimens in both groups for pathogens with MIC $\leq$ 1 mg/L. Therefore, the standard dose of linezolid may not be applicable to pediatric patients. Such patients require TDM to optimize treatment (9). Rao et al. suggested that an AUC:MIC ratio of 80–100 is an appropriate efficacy threshold for children, who tend to clear linezolid significantly faster than adults. However, the threshold for toxicity is less well defined in pediatric patients than in adults (45).

Linezolid is a potential substrate of P-glycoprotein, which interacts with numerous drugs. The putative mechanism underlying such interactions is modulation of P-glycoprotein activity. Clarithromycin, proton pump inhibitors (such as omeprazole and pantoprazole), amiodarone, amlodipine, and calcium channel blockers inhibit P-glycoprotein and therefore increase linezolid concentrations. Rifampin, phenobarbital, and levothyroxine are inducers of P-glycoprotein that increase clearance of linezolid and decrease linezolid plasma concentrations (9, 45–47). Therefore, TDM is required when linezolid is combined with the aforementioned drugs.

Question 6: How should the linezolid dosage be adjusted for patients with renal insufficiency?

Expert panel recommendations

(1) For patients with renal insufficiency not on hemodialysis (HD), we suggest that linezolid can be reduced to a regimen of 300 mg q12h. (Score: 7.90) (2) Insufficient linezolid doses in patients with renal insufficiency on dialysis must be considered, and the dose should be increased based on TDM if necessary. (Score: 8.70) (3) Irrespective of whether patients with renal insufficiency are on dialysis, we recommend adjusting the dose of linezolid based on TDM. (Score: 8.15)

Reason

Pea et al. (14) suggested that overexposure to linezolid is significantly associated with renal insufficiency (Ccr $\leq$ 40 mL/min). A prospective observational study by Fang et al. (16) showed that renal insufficiency (Ccr $\leq$ 40 mL/min) was significantly associated with a linezolid trough concentration $>$ 8 mg/L. A Spanish retrospective study showed that the risk of overexposure for estimated glomerular filtration rate (eGFR) $<$ 40 mL/min is 4.27-fold that of eGFR $>$ 80 mL/min (48). Souza et al. (40) reported that the median linezolid concentration in patients with renal insufficiency was 1.46-fold that of patients with normal function. In summary, renal insufficiency is closely related to linezolid overexposure.

Crass et al. constructed a population pharmacokinetic (PPK) model based on 603 patients on linezolid. For patients with eGFR $<$ 60 mL/min, the risk of overexposure (C_{min} $>$ 8 mg/L) to linezolid at the conventional dosage of 600 mg q12h was $>$50%. Linezolid 300 mg q12h can balance safety and effectiveness and seems to be feasible (39). The PPK/pharmacodynamic model of Sasaki et al. revealed that for Ccr $\leq$ 30 mL/min, 300 mg q12h linezolid is $>$90% likely to achieve the pharmacodynamic target (AUC/MIC $>$ 100). For patients with Ccr $\leq$ 30 mL/min, the author recommends 300 mg q12h (49). A study in Japan showed that among patients with renal insufficiency, the clinical failure rate was significantly lower among linezolid-treated patients in the TDM group than in the non-TDM group. In addition, up to 90.5% of patients in the TDM group required dose adjustment. Patients with renal insufficiency can reach the goal of C_{min} $\geq$ 2.0 mg/L with a 300 mg q12h regimen (18). Kawasuji et al. (50) reported that the initial dose should be reduced to reduce the risk for linezolid-induced thrombocytopenia (LIT) in patients on HD.

In addition, the recommendation in the linezolid instructions that "dose does not need to be adjusted for patients with renal insufficiency" is mainly based on an early PK study. The study included only healthy volunteers (n = 6), individuals with moderately impaired renal function (n = 6), individuals with severely impaired renal function (n = 6), and patients on HD (n = 6). The subjects were given a single oral dose of linezolid 600 mg, and there were no significant differences in linezolid PK parameters (51). We have reason to believe that the recommendations in the instructions do not reflect the clinical situation. In a meta-analysis of the

relationship between renal function and LIT, renal function was closely related to LIT, and poor renal function was related to a higher risk for LIT. Subgroup analyses showed that in patients on the conventional linezolid regimen (600 mg q12h), the risk for LIT was higher in patients with renal insufficiency than in those with normal renal function (unadjusted OR: 2.59, 95% CI: 1.64–4.10, $I^2 = 60.8\%$ vs. adjusted OR: 2.69, 95% CI: 1.83–3.95, $I^2 = 0\%$). Therefore, the dosage recommended in the instructions is unsuitable (52).

A prospective, single-dose pharmacokinetic study included 15 critically ill patients with oliguria and acute renal insufficiency, eight of whom received intermittent HD lasting 3 to 4 h and five of whom received sustained low-efficiency dialysis (SLED) for 8 h. The patients received linezolid 600 mg intravenously within 60 min before dialysis. The average clearance rates of HD and SLED were 32.3 and 33.9%, respectively. After HD and SLED, the serum linezolid concentration in three patients was <4 mg/L (lower than the target MIC). To maintain efficacy, it is recommended that linezolid be used at the end of dialysis (53). A prospective, multi-dose pharmacokinetic study included five critically ill patients with renal failure on intermittent hemodialysis (IHD) who received standard doses of linezolid intravenously. IHD not only reduced the serum linezolid concentration but also delayed a steady-state linezolid concentration. It is recommended that overweight patients and early dialysis patients on linezolid be given one third the full dose or a loading dose of 1,200 mg after the end of dialysis (54). In critically ill patients with continuous veno-venous hemodialysis (CVVHD) and continuous veno-venous hemodiafiltration (CVVHDF) on renal replacement therapy, the standard linezolid dosage (600 mg q12h) is given intravenously, the therapeutic concentration is insufficient, and the target cannot be achieved for pathogens with MIC $\geq$ 2 mg/L (55, 56). A prospective, single-center observational study in China included 40 patients with sepsis who received continuous renal replacement therapy (CRRT). The standard intravenous dose for patients on renal replacement therapy yielded a steady-state concentration effective against pathogens with an MIC of 2 mg/L. The dose group reached the PK/PD target, whereas the high-volume hemofiltration (HVHF) group did not. For MIC > 2 mg/L, neither group achieved the PK/PD target. It is recommended that patients with sepsis receive different doses of CRRT, which should be adjusted according to the TDM results (57). In patients with severe sepsis who receive CRRT, underexposure to linezolid is common; for MIC > 4 mg/L in particular, the conventional dose does not result in optimal exposure and so must be increased (58, 59).

We recommend that patients with renal insufficiency on RRT be monitored for insufficient linezolid exposure. The typical dosage (600 mg q12h) may not be efficacious. Therefore, we recommend monitoring the serum concentration of linezolid in such patients.

Question 7: Does the linezolid dose need to be adjusted for patients with hepatic insufficiency?

Expert panel recommendations

We do not recommend adjusting the linezolid dose for patients with mild to moderate hepatic insufficiency (Child-Pugh grade A or B). (Score: 8.65)

Question 8: How should the linezolid dose be adjusted for patients with hepatic insufficiency?

Expert panel recommendations

We recommend reducing the dose of linezolid in Child-Pugh grade C patients with severe hepatic insufficiency. (Score: 8.40)

Reason

The updated instructions for linezolid tablets (released June 22, 2020) and the updated drug instructions for linezolid glucose injection (September 1, 2020) indicate that there is no need to adjust the dose for patients with mild to moderate hepatic insufficiency. A single-center, retrospective, observational case-control 1:1 study by Luque et al. (41) included 52 patients receiving linezolid 600 mg q12h, of whom 26 were patients with liver cirrhosis (1, 13, and 12 cases of Child-Pugh grade A, B, and C, respectively). The median linezolid trough concentration ($C_{min,ss}$) of patients with liver cirrhosis was 20.6 (17.4) mg/L, which was higher than the control group (2.7 [11.3] mg/L, $P < 0.001$). The median peak concentration ($C_{max,ss}$) was 34.1 (22.7) mg/L, which was higher than the control group [16.5 (11.6) mg/L, $P = 0.001$]. The steady-state C_{min} values of linezolid are similar in patients with different degrees of liver cirrhosis [for Child-Pugh grades A, B, and C: 25.9, 16.2 (15.5), and 22.7 (25.5) mg/L, respectively, $P = 0.547$]. A prospective, open-label, uncontrolled study by Sasaki et al. examined the PK of linezolid in Japanese patients, including four with severe liver cirrhosis (Child-Pugh grade C). The clearance rate at a dosage of 600 mg q12h decreased significantly (~50% decrease) and showed higher $C_{min,ss}$ values of 32.5, 36.4, 40.8, and 45.4 mg/L, respectively, which suggests changes in liver cirrhosis. Therefore, the PK of linezolid is different in patients with liver cirrhosis with different grade (49).

Hepatic insufficiency reduces linezolid clearance in critically ill patients (60). The effects of severe liver dysfunction (Child-Pugh grade C) on the PK of linezolid are unclear. A prospective study by Zhang et al. included 163 concentration samples from 45 patients with liver insufficiency. Their PPK model showed

that PTA and CLcr were positively correlated with the linezolid clearance rate (CL), which confirms that liver insufficiency is an important factor in linezolid PK and dose optimization. Monte Carlo simulation was used to optimize the linezolid dose for patients with liver insufficiency (61). Simulation based on the final model showed that when the MIC was 2 μg/mL, for patients with a PTA of 20–40% or CLcr < 10 mL/min, the 300 mg q12h dosage was safe and effective based on probabilities of AUC_{0-24}: MIC ratio ≥ 80 of 92.9% and 96.9% and probabilities of a $C_{min,ss}$ of 2–8 μg/mL of 99.3% and 99.4%. When the MIC was 2 μg/mL, for patients with PTA ≤ 20% a 400 mg q24h dosage was sufficient to achieve the therapeutic target (the probability of AUC_{0-24}:MIC ratio ≥ 80 was 97.9%). When the MIC was 4 μg/mL, for patients with PTA ≤ 20%, at a 300 mg q12h dosage the probability of AUC_{0-24}:MIC ratio ≥ 80 was 91.9%, that of $C_{min,ss}$ > 8 μg/mL was 0.2%, and that of a $C_{min,ss}$ of 2–8 μg/mL was 99.8%. A prospective, open-label, uncontrolled study by Sasaki et al. included four patients with severe liver cirrhosis (Child-Pugh grade C). Based on a model simulation, for patients with insufficient renal function (CLcr ≤ 30 mL/min) or severe liver cirrhosis (Child-Pugh grade C), it is recommended that the dosage be reduced to 600 mg/day (49). Wicha et al. used the maximal liver function capacity (LiMAx test) tool for linezolid dose adjustment in patients with hepatic insufficiency. They suggested that TDM should be performed for LiMAx < 100 μg/kg/h to reduce the risk of excessively high target plasma concentrations (62, 63).

Question 9: Does the linezolid dose need to be adjusted for patients on extracorporeal membrane oxygenation (ECMO)?

Expert panel recommendations

We recommend optimization of the linezolid dosage for patients on ECMO. (Score: 8.75)

Question 10: How should linezolid administration be optimized for patients on ECMO?

Expert panel recommendations

We recommend that for patients on ECMO the following dosing schedule be used:

- MIC ≤ 1 mg/L: 600 mg q12h.
- MIC = 2 mg/L: 600 mg q8h.
- MIC > 2 mg/L: might require more than 4-fold the conventional dosage to achieve the PK/PD target. The safety of this regime is unknown, so we recommend a switch to other sensitive antibiotics.

Whichever dosage is used, it should be adjusted based on the results of TDM. (Score: 8.40).

Reason

To date, two PK studies of linezolid in patients on ECMO have been published. The case series reported by De Rosa et al. (64) in 2013 comprised three patients on 600 mg q12h linezolid, who had AUC_{0-24h} values of 212.58, 165.65, and 100.59 mg·h/L, respectively. For pathogens with an MIC of 1, the AUC/MIC target was reached; for pathogens with an MIC of 2 mg/L, one patient failed to achieve the target; and for pathogens with an MIC of 4 mg/L, the AUC/MIC target was not reached. Therefore, individualized regimens should be used for patients on ECMO. The PK of a patient infected after right lung transplantation reported by Nikolos et al. (65) differed significantly from that reported by De Rosa et al. After reaching steady state, the peak and trough linezolid concentrations were 1.7 and 0.4 mg/L, and the AUC_{0-24h} was estimated at 21.6 mg/L. This is lower than reported by De Rosa et al. (64). Kühn et al. (66) evaluated 112 and 186 blood samples from patients with and without ECMO, respectively; 34.8% of patients on ECMO were underexposed to linezolid. In addition, the multivariate linear GEE method was used to evaluate the influence of clinical factors on blood antibiotic concentrations. The results showed that linezolid overexposure may have been related to ECMO duration ($P < 0.05$). Therefore, patients on ECMO receiving linezolid should be monitored for underexposure during early use and for overexposure during long-term use.

The four patients differed significantly, so TDM is needed for patients on ECMO. We believe that for bacteria with MIC ≤ 1 mg/L, the optimal PK/PD index can be achieved using the doses on the drug label. For bacteria with an MIC of 2 mg/L, 600 mg q8h linezolid is appropriate. In the case of MIC > 2 mg/L, achieving the PK/PD target is difficult, so we recommend switching to another sensitive antibiotic. The MIC_{90} of linezolid for *Staphylococcus aureus* in China (67) is 2 mg/L, so the empirical linezolid regimen is 600 mg q8h.

Few studies have evaluated linezolid in ECMO patients, and those that have are small samples or case reports. Therefore, it is necessary to adjust the linezolid dose in patients on ECMO and perform TDM.

TABLE 1 Algorithm for determining the optimal linezolid dosage for overweight and obese patients.

$CrCL_{(CKD-EPI)}$ ($mL/min/1.73m^2$)	Coagulase-Negative Staphylococci (CoNS)	S. aureus (MRSA or MSSA)	Enterococcus spp. (VSE or VRE)
0–29	450mg q12h	600mg q12h	600mg q12h
30–59	450mg q12h	600mg q12h	600mg q12h
60–129	450mg q12h	600mg q12h	450mg q8h
130–200	600mg q12h	450mg q8h	450mg q8h

Question 11: Do pediatric patients require linezolid dose optimization?

Expert panel recommendations

We recommend optimizing the linezolid dose for pediatric patients. (Score:9.60)

Question 12: How should the dose of linezolid be adjusted for pediatric patients?

Expert panel recommendations

We recommend linezolid dose adjustment for pediatric patients based on TDM. (Score: 9.25)

Reason

Li et al. (68) conducted a prospective PK study on 112 pediatric patients ages 0–12 years and established a PPK model. If bacterial MIC $\geq$ 2 mg/L, the conventional dosage of 10 mg/kg q8h will lead to a high risk of underdosing in children, and the dosage should be increased to 15 or 20 mg/kg q8h. A single-center, prospective, open-label PPK study on critically ill children in China by Yang et al. showed that for MIC $\leq$ 1 mg/L, a dosage of 10 mg/kg q8h yields PTA > 96%. For MIC > 1 mg/L, the PTA was <70%. For an MIC of 2 mg/L and a dosage of 15 mg/kg q6h, the PTA increased from 63.6 to 94.6% (10). A retrospective study by Wang et al. (69) found that the rate of linezolid treatment for thrombocytopenia in pediatric ICU patients was 25.0% and was related to linezolid overexposure. Linezolid has a better effect in children with tuberculosis, but some children are overexposed to linezolid and the treatment is discontinued because of adverse events. This suggests the importance of linezolid dose optimization for children with tuberculosis (70–72). Cojutti et al. (9) reported that children need a higher linezolid dose for pathogens with MIC > 1 mg/L and that TDM should be encouraged to optimize linezolid exposure. Linezolid TDM is useful for pediatric patients with sparse clinical data or PK changes. Adaptive feedback control and model-informed precision dosing use Bayesian algorithms and PK models to predict linezolid exposure. Linezolid TDM should be included in the dosage optimization workflow (45).

In summary, we support dose optimization in pediatric patients based on TDM.

Question 13: Do obese patients need linezolid dose optimization?

Expert panel recommendations

Standard linezolid doses are not adequate for obese patients, and so dose adjustment is recommended. (Score: 9.30)

Question 14: How should a dose optimization strategy be implemented for obese patients?

Expert panel recommendations

Adjustments to the linezolid dosage should be based on CrCL (CKD-EPI) estimates, and escalation to 600 mg q8h is not recommended because of an unacceptably high risk for thrombocytopenia (Table 1). (Score: 8.95)

Reason

In a controlled clinical study, concentrations of linezolid in plasma and subcutaneous tissue decreased with increasing body weight, and the recommended dosage did not yield concentrations sufficient to kill bacteria with MIC $\geq$ 2 mg/L in obese patients (43). A phase I comparative clinical trial evaluated the PK and PD of a single intravenous fixed dose of linezolid compared to a weight-adjusted dose. A weight-adjusted dose of linezolid 10 mg/kg might be more appropriate than a fixed dose for obese patients (73). Body weight has a marked effect on linezolid clearance, and the PTA decreases with increasing weight. Moreover, standard linezolid dosing in obese patients with pneumonia caused by MRSA (MICs 1–4 mg/L) leads to an unacceptably low (near zero to 60%) PTA for patients <65 years old (74). Furthermore, a prospective study showed that standard linezolid doses might not be adequate for critically ill obese patients with severe skin and soft tissue infection (44).

A retrospective study assessed the PPK and PD of linezolid in overweight and obese hospitalized patients (42). Totals of 352 trough (minimum) and 293 peak (maximum) linezolid concentrations from 112 patients were analyzed. Patients were on linezolid because of sepsis, nosocomial pneumonia, bone and joint infection, skin and soft tissue infection, and central nervous system (CNS) infection. Only the estimated creatinine clearance (using the Chronic Kidney Diseases Epidemiology formula, CrCL [CKD-EPI]) covariate improved the model fit. Dosage reduction to 450 mg q12h might be optimal for patients with coagulase-negative staphylococcal infection and CrCL (CKD-EPI) $<$ 30 mL/min/1.73 m^2. Dosage escalation to 450 mg q8h may be optimal for patients with CrCL (CKD-EPI) $\geq$ 60 mL/min/1.73 m^2. However, dosage escalation to 600 mg q8h is not recommended because of an unacceptably high risk for thrombocytopenia.

Question 15: How should the linezolid dose be individualized for patients with tuberculosis?

Expert panel recommendations

We recommend 1-month treatment with linezolid 600 mg twice daily. For long-term treatment (if tolerated until the end of treatment), a maximum dosage of 600 mg once daily is recommended. Irrespective of the dosage, we recommend TDM of the AUC and determination of the MIC of *Mycobacterium tuberculosis* strains. The recommended target is AUC/MIC $>$ 100. (Score: 9.05)

Reason

Although linezolid was recommended in the revised version of *World Health Organization Recommendations on the Treatment of Drug-Resistant Tuberculosis, 2020 Update*, the optimal dosage for tuberculosis needs to be determined (75). In the Nix-TB open-label clinical study, most participants required a reduction in dose or interruption of linezolid. Thirty-seven (34%) participants completed 26 weeks of linezolid without interruption, although they may have had a dose reduction, and 16 (15%) completed 26 weeks at a 1,200 mg total daily dose of linezolid with no interruptions or dose reductions (76). Although one study recommended a linezolid dosage for tuberculosis of 1,200 mg/day (75), others suggest that $<$600 mg/day is effective and has a treatment success rate similar to that of $>$600 mg/day (77). Indeed, 300 mg/day may have greater clinical efficacy with fewer adverse reactions. The optimal dosage is unclear (78–80). According to clinical studies, linezolid is appropriate for *M. tuberculosis* infection and can maintain a PK/PD target of AUC/MIC $>$ 100 (81–86). Provision by the tuberculosis laboratory of the MIC of *M. tuberculosis* allows calculation of the AUC/MIC, potentially benefitting treatment. If the tuberculosis laboratory cannot provide the MIC of *M. tuberculosis*, it should estimate the AUC/MIC based on the local drug resistance profile of *M. tuberculosis* (87–91).

The problems with using linezolid for *M. tuberculosis* infection deserve further study. We suggest that the dosage be limited to 600 mg/day. Because of the lack of randomized controlled trials, we are unable to provide specific recommendations. However, we can reach a consensus on the TDM of linezolid for tuberculosis patients, and the target can also be determined at AUC/MIC $>$ 100.

Question 16: How should the linezolid dose be adjusted in patients with central nervous system (CNS) infection?

Expert panel recommendations

For CNS infection, we recommend the linezolid dosage as reported in the package insert (i.e., a linezolid dosage of 600 mg q12h in adults and children $>$12 years with normal renal and hepatic function). The recommended linezolid dosage is 10 mg/kg q8h in children up to 11 years of age and 10 mg/kg q12h in preterm (gestational age: 34 weeks) and 7-day-old infants. (Score: 8.65)

Reason

Beer et al. studied the PK profile of linezolid in CSF in five adult patients with staphylococcal ventriculitis. The patients received linezolid 600 mg q12h. The mean area under the concentration-time curve in CFS was 63 $\pm$ 18.9 mg·h/L with a CSF:plasma ratio of 0.8 $\pm$ 0.3. The proportion of time above the MIC in CSF was 99.8% and 57.2% for pathogens with MICs of 2 and 4 mg/L, respectively (92).

Yogev et al. (93) performed two studies in hydrocephalic children and adolescents to assess linezolid penetration of CSF. Patients 12 months to 24 years of age in study 1 and neonates $<$ 12 years of age in study 2 were administered intravenous linezolid 10 mg/kg q12h for 3 days (study 1) or q8h for 2 days (study 2). PK indices were determined for plasma and ventricular fluid (VF) after the first and last doses. In study 1, after the last dose, the mean C_{max} and C_{min} values for VF were 7.54 μg/mL (range: 2.26–12.6 μg/mL) and 1.26 μg/mL (range: 0.19–2.58 μg/mL), respectively. The VF:plasma ratio based on the last dose AUC_{0-12} was 0.98 μg·h/mL (range: 0.64–1.22 μg·h/mL). In study 2, after the last dose, the mean VF C_{max} and C_{min} values were 5.84 μg/mL (range: 1.82–9.34 μg/mL) and 1.94 μg/mL (range: 0.34–4.62 μg/mL), respectively. The VF:plasma ratio based on last dose AUC_{0-8} was 0.95 μg·h/mL (range: 0.62–1.31 μg·h/mL). Therefore, systemic clearance of linezolid decreases with age up to 12 years. However, some case reports suggest that the standard dose of linezolid has a

better drug distribution in the brain and is more effective against common CNS pathogens (94–100).

In short, use of linezolid for intracranial infection is beyond the approved indications in the drug insert, and there are no RCTs on linezolid for CNS infection. Further PK/PD studies of linezolid in the CNS are needed.

Question 17: How should adverse reactions to linezolid be monitored?

Expert panel recommendations

(1) We recommend that during linezolid treatment, platelets be monitored to detect hematological toxicity. (Score: 9.35) (2) We recommend pharmaceutical care for patients who are elderly, have renal insufficiency, have baseline thrombocytopenia, and have low body weight in whom long-term continuous (>1 month) use of linezolid achieves a trough concentration > 8 mg/L. (Score: 9.40)

Question 18: How should linezolid-associated hyperlactacidemia be prevented and treated?

Expert panel recommendations

(1) We recommend monitoring blood linezolid and lactic acid levels while using linezolid. (2) Linezolid should not be used in combination with drugs that affect mitochondrial function. (3) Patients with liver or renal dysfunction, or those treated with linezolid for >1 month, should be monitored for hyperlactacidemia. (4) If hyperlacticacidosis occurs, we recommend stopping linezolid and correcting the acidosis as soon as possible. (Score: 9.35)

Reason

A retrospective study by Guo et al. included 5,336 patients on linezolid, of whom 266 (4.99%) had drug-induced thrombocytopenia. The incidence of thrombocytopenia increased significantly with age. Age was a significant risk factor for linezolid-related thrombocytopenia (OR: 4.887, 95% CI: 3.958–6.035, $P = 0.000$). Thrombocytopenia occurred within 7 days of medication in 45.26% of patients, and the decrease was greater than in other groups (101). Takahashi et al. performed regression tree analyses of 74 patients; the cutoff value for intervention was a reduction in platelet count to <2.3% that at baseline or a trough linezolid concentration ≥ 13.5 mg/L 96 h after initial administration. These cutoff values will appear before the onset of thrombocytopenia, and proactive monitoring can avoid LIT (102). A retrospective study by Morata et al. included 104 patients treated with linezolid; 34.6% had C_{min} > 8 mg/L, and more patients had GFR < 40 mL/L by Modification of Diet in Renal Disease (MDRD). The only factor independently related to C_{min} > 8 mg/L was renal function. The C_{min} of patients with eGFR < 40 mL/min was significantly higher than that of patients with eGFR > 80 mL/min (OR: 4.273), whereas the C_{min} of patients with an eGFR of 40–80 mL/min tended to be higher (OR: 2.109) (48). The hematological toxicity of linezolid in elderly patients with renal insufficiency may be related to drug overexposure (14, 16, 39, 40, 48–50).

An observational study by Niwa et al. (12) showed that a body weight < 55 kg (OR: 33.2, 95% CI: 2.16–510.1, $P = 0.012$) and a baseline platelet count < 200 × 10^3/mm^3 (OR: 24.9, 95% CI: 1.53–404.7, $P = 0.024$) are risk factors for LIT. In subsequent intervention studies, the daily dosage of linezolid was set to 1,200 mg/kg for patients with one or no risk factors. The incidence of thrombocytopenia in the intervention study group was significantly prolonged without a reduction in clinical efficacy (103).

Lactic acidosis is a rare but serious side effect of linezolid. From August 2011 to August 2016, 63 cases (27 deaths) and 243 cases (37 deaths) of linezolid-related lactic acidosis were reported by the U.S. FDA and the European Drug Reaction Report system, respectively (104–106). Based on these data, approximately 50 cases of linezolid-related lactic acidosis occur annually, with a mortality rate of 15% (37/243) to 43% (27/63) (104). Three retrospective studies reported an incidence of linezolid-associated hyperlactacidemia of 2–3% (107–109). A prospective study found that the incidence of lactic acidosis was 33% (5/15) after linezolid treatment for >1 month (110). Therefore, the incidence of linezolid-associated lactic acidosis may increase with treatment for >1 month. Case reports suggest that lactic acidosis occurs 1–16 weeks after linezolid administration (median: 5.5 weeks) (111).

The mechanism of linezolid-associated hyperlactacidemia is unclear, but linezolid has an antibacterial effect by binding to the bacterial ribosome subunit. Bacterial ribosomes have a structure similar to that of mitochondria. Linezolid interacts with mitochondrial ribosomes and affects mitochondrial translation activity, leading to hyperlactacidemia (104, 105, 107, 109, 111). The linezolid concentration required for binding to human mitochondrial ribosomes exceeds that required for therapeutic efficacy (111). Genetic polymorphisms of human mitochondrial 16S rRNA (such as A2706G and G3010A) may increase the risk of toxicity (104). During linezolid therapy, the risk for hyperlactacidemia is elevated by the use of other drugs that interfere with mitochondrial function (e.g., propofol, antiretrovirals, omeprazole, amiodarone, and amlodipine) (104).

Linezolid is metabolized mainly in the liver. Clearance of linezolid is reduced in patients with severe cirrhosis (Child-Pugh grade C) (48, 112). More than 60% of lactic acid is converted into pyruvate in the liver for gluconeogenesis (104). The serum linezolid concentration in patients with liver dysfunction is 4- to 6-fold that of patients with normal liver function (113).

TABLE 2 Overview of expert consensus and summary of recommendations.

Numbers	Questions	Recommendations
1	Does linezolid require TDM? What is the target range for linezolid TDM?	(1) We recommend TDM for linezolid. (Score: 8.90) (2) We recommend maintaining a linezolid trough concentration of 2–8 mg/L. (Score: 8.60)
2	What detection method is recommended for TDM of linezolid?	We recommended quantification of linezolid in human plasma or serum by HPLC-UV or LC-MS/MS methods. (Score: 8.80)
3	How should laboratories conducting quantitative determination of linezolid perform quality control?	Quality control procedures should be implemented using drug *in vivo* analysis techniques, quality control standards, and clinical intervention programs. (Score: 9.15)
4	How should linezolid TDM results be reported?	We recommend that the TDM report provide test results, explanations thereof, and treatment suggestions. The report should be individualized for the patient in question. (Score: 9.15)
5	For which patients should linezolid TDM be considered?	We recommend TDM for critically ill patients, children, patients with renal insufficiency/augment or liver cirrhosis, elderly, obese and patients taking co-medications known to interact with linezolid. (Score: 9.50)
6	How should the linezolid dosage be adjusted for patients with renal insufficiency?	(1) For patients with renal insufficiency not on hemodialysis (HD), we suggest that linezolid can be reduced to a regimen of 300 mg q12h. (Score: 7.90) (2) Insufficient linezolid doses in patients with renal insufficiency on dialysis must be considered, and the dose should be increased based on TDM if necessary. (Score: 8.70) (3) Irrespective of whether patients with renal insufficiency are on dialysis, we recommend adjusting the dose of linezolid based on TDM. (Score: 8.15)
7	Does the linezolid dose need to be adjusted for patients with hepatic insufficiency?	We do not recommend adjusting the linezolid dose for patients with mild to moderate hepatic insufficiency (Child-Pugh grade A or B). (Score: 8.65)
8	How should the linezolid dose be adjusted for patients with hepatic insufficiency?	We recommend reducing the dose of linezolid in Child-Pugh grade C patients with severe hepatic insufficiency. (Score: 8.40)
9	Does the linezolid dose need to be adjusted for patients on ECMO?	We recommend optimization of the linezolid dosage for patients on ECMO. (Score: 8.75)
10	How should linezolid administration be optimized for patients on ECMO?	(1) We recommend that for patients on ECMO the following dosing schedule be used: (2) MIC $\leq$ 1 mg/L: 600 mg q12h (3) MIC = 2 mg/L: 600 mg q8h (4) MIC > 2 mg/L: might require more than 4-fold the conventional dosage to achieve the PK/PD target. The safety of this regime is unknown, so we recommend a switch to other sensitive antibiotics. (5) Whichever dosage is used, it should be adjusted based on the results of TDM. (Score: 8.40)
11	Do pediatric patients require linezolid dose optimization?	We recommend optimizing the linezolid dose for pediatric patients. (Score:9.60)
12	How should the dose of linezolid be adjusted for pediatric patients?	We recommend linezolid dose adjustment for pediatric patients based on TDM. (Score: 9.25)
13	Do obese patients need linezolid dose optimization?	Standard linezolid doses are not adequate for obese patients, and so dose adjustment is recommended. (Score: 9.30)
14	How should a dose optimization strategy be implemented for obese patients?	Adjustments to the linezolid dosage should be based on CrCL (CKD-EPI) estimates, and escalation to 600 mg q8h is not recommended because of an unacceptably high risk for thrombocytopenia (Table 1). (Score: 8.95)
15	How should the linezolid dose be individualized for patients with tuberculosis?	We recommend 1-month treatment with linezolid 600 mg twice daily. For long-term treatment (if tolerated until the end of treatment), a maximum dosage of 600 mg once daily is recommended. Irrespective of the dosage, we recommend TDM of the AUC and determination of the MIC of Mycobacterium tuberculosis strains. The recommended target is AUC/MIC > 100. (Score: 9.05)
16	How should the linezolid dose be adjusted in patients with CNS infection?	For CNS infection, we recommend the linezolid dosage in the instructions (i.e., a linezolid dosage of 600 mg q12h in adults and children > 12 years with normal renal and hepatic function). The recommended linezolid dosage is 10 mg/kg q8h in children up to 11 years of age and 10 mg/kg q12h in preterm (gestational age: 34 weeks) and 7-day-old infants. (Score: 8.65)

(Continued)

TABLE 2 Continued

Numbers	Questions	Recommendations
17	How should adverse reactions to linezolid be monitored?	(1) We recommend that during linezolid treatment, platelets be monitored to detect hematological toxicity. (Score: 9.35) (2) We recommend pharmaceutical care for patients who are elderly, have renal insufficiency, have baseline thrombocytopenia, and have low body weight in whom long-term continuous (>1 month) use of linezolid achieves a trough concentration > 8 mg/L. (Score: 9.40)
18	How should linezolid-associated hyperlactacidemia be prevented and treated?	We recommend monitoring blood linezolid and lactic acid levels while using linezolid. Linezolid should not be used in combination with drugs that affect mitochondrial function. Patients with liver or renal dysfunction, or those treated with linezolid for >1 month, should be monitored for hyperlactacidemia. If hyperlacticacidosis occurs, we recommend stopping linezolid and correcting the acidosis as soon as possible. (Score: 9.35)

The incidence of lactic acidosis is higher in patients with liver function because of the excessive blood concentration of linezolid and lactic acid accumulation (104, 114).

Approximately 30% of linezolid is eliminated by the kidneys, but PK studies have shown that linezolid accumulation is not common in patients with renal dysfunction. Therefore, renal failure is not a major risk factor for linezolid-induced acidosis (51, 109). The kidneys metabolize about 30% of total lactic acid. Although severe renal dysfunction is unrelated to the incidence of linezolid acidosis, it does increase its severity and mortality (109). Discontinuation of linezolid is the most effective treatment for linezolid-associated hyperlactacidemia, and most patients recover in 1–15 days (104, 109, 115). There are no large, randomized cohort studies of RRT for linezolid acidosis. Clinical experience confirms that RRT is beneficial for patients with lactic acid excretion and can be used to correct acidosis (104, 109). When the enzyme formed by vitamin B1 and pyrophosphate is deficient, oxidative metabolism of sugars is blocked, and pyruvate and lactic acid accumulate (114, 116). Vitamin B1 should be supplied to patients with vitamin B1 deficiency on linezolid therapy (117).

Summary

Overview of expert consensus and summary of recommendations in Table 2. There is doubt about the clinical use of linezolid. We hope that this expert consensus will promote individualized, safe, and rational use of linezolid in clinical practice. Because knowledge always lags evidence, this expert consensus has limitations, which we aim to overcome in subsequent editions.

Author contributions

BL, HD, and SJ initiated and organized the writing of expert consensus. BL organization was coordinated and revised the manuscript. HD and SJ reviewed and revised the manuscript. All authors contributed to different sections and manuscript was submitted after the approval.

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Hospital), Min Zhou (Ruijin Hospital, Shanghai Jiao Tong University School of Medicine), Changsheng Xu (Zhongda Hospital, School of Medicine, Southeast University), Yong Li (Suzhou Municipal Hospital), and Chuan Shen (Third Affiliated Hospital of Hebei Medical University), as a physician expert, voted on the expert consensus opinion.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author disclaimer

This expert consensus recommendation is based on a review of a series of clinical studies on linezolid. However, the recommendations may have certain limitations, and clinicians should carry out clinical practice in combination with local resources and laws and regulations on the extended application of drugs. The recommendations made in this expert consensus cannot completely replace the judgment of physicians, and this recommendation needs to be adopted in combination with specific clinical practice.

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Calibration and validation of the Pneumonia Shock Score in critically ill patients with SARS-CoV-2 infection, a multicenter prospective cohort study

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Background: Prognostic tools developed to stratify critically ill patients in Intensive Care Units (ICUs), are critical to predict those with higher risk of mortality in the first hours of admission. This study aims to evaluate the performance of the pShock score in critically ill patients admitted to the ICU with SARS-CoV-2 infection.

Methods: Prospective observational analytical cohort study conducted between January 2020 and March 2021 in four general ICUs in Salvador, Brazil. Descriptive statistics were used to characterize the cohort and a logistic regression, followed by cross-validation, were performed to calibrate the score. A ROC curve analysis was used to assess accuracy of the models analyzed.

Results: Six hundred five adult ICU patients were included in the study. The median age was 63 (IQR: 49–74) years with a mortality rate of 33.2% (201 patients). The calibrated pShock-CoV score performed well in prediction of ICU mortality (AUC of 0.80 [95% Confidence Interval (CI): 0.77–0.83; p-value < 0.0001]).

Conclusions: The pShock-CoV score demonstrated robust discriminatory capacity and may assist in targeting scarce ICU resources during the COVID-19 pandemic to those critically ill patients most likely to benefit.

KEYWORDS

critical care, prognosis, COVID-19, mortality, risk factors

Introduction

The COVID-19 outbreak created a worldwide emergency in the face of rapid dissemination throughout the world (1). To date, the pandemic has more than 240 million cases worldwide and over 4.9 million deaths spread over 220 countries (2). While most infected individuals develop mild forms of the disease, those who develop life threatening infections requiring intensive care units (ICU) care may succumb to their infection with mortality rates up to 49% (3, 4). Scarcity of healthcare resources has profoundly impacted low-middle-income countries, with significant strain on pre-existing limited ICU capacity (2, 5). In Brazil, significant viral transmissibility, associated with excess mortality rates in the elderly and those with a high burden of disease, rapidly overwhelmed health services in the country (3, 6). Existing prognostic tools to triage resources to those most likely to benefit from critical care, such as SAPS3, SOFA and APACHE IV, lack sufficient accuracy in those hospitalized with COVID-19 (7–9). Despite several novel prognostic models emerged during the pandemic, many have been found to have a high risk of bias, and not sufficient attempt has been made to develop a simple routinely applicable scoring system to early predict higher risk of mortality for patients admitted in ICUs (7). Recently, our group developed and externally validated a prognostic score for mortality risk stratification of patients admitted to the ICU with pneumonia, the Pneumonia Shock Score (pShock) (10). This tool demonstrated excellent discriminate function, outperforming other prognostic scores evaluated in our derivation and external validation cohorts. Given the severity of pneumonia in those with COVID-19, this study seeks to calibrate and evaluate the performance of the pShock score in critically ill patients admitted to the ICU with SARS-CoV-2 infection.

Materials and methods

Study design

This was a prospective observational analytical cohort study conducted between January 2020 and March 2021 in four general ICUs in Salvador, Bahia, Brazil. All patients older than 18 years of age with confirmed SARS-CoV-2 infection by reverse transcriptase polymerase chain reaction analysis were included. The primary outcome assessed was ICU mortality. During the study period the assistance provided at all centers was in accordance with the guidelines and protocols for COVID-19 management. Clinical and laboratory data were prospectively collected in the medical records and registered in an encrypted database stored on the RedCap system (11). Study variables included age, weight, height, sex, length of ICU and hospital stay, and physiological and laboratory data within the first 6 h of admission. Complications including need for mechanical ventilation, vasopressors, and other supportive therapy in the ICU were noted. In addition, the score derivation dataset was used to compare the performance of the original score against a calibration of the pShock score (pShock-CoV).

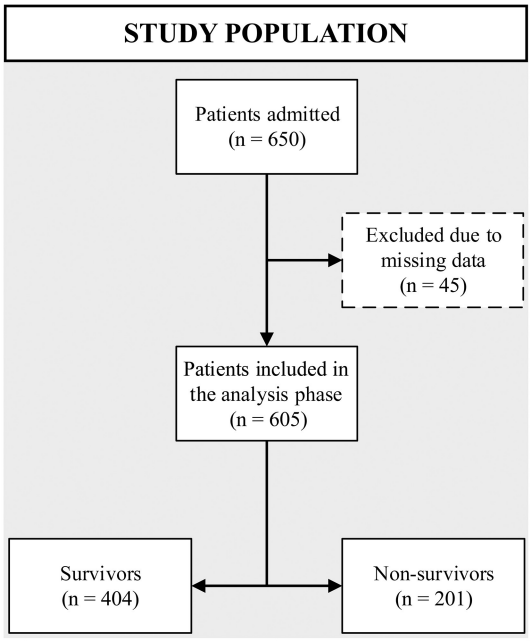


FIGURE 1
Flow chart of study enrollment and analyzed population.

Statistical analysis

Categorical variables were expressed as frequency and percentages, and continuous variables were expressed as medians with inter-quartile ranges (IQR). The proportion of categorical variables between groups were compared using Fisher's exact test. The median of continuous variables was compared using Mann-Whitney *U* test when analyzing the outcome groups. All tests were two-tailed and considered statistically significant for $p \leq 0.05$. Variables that demonstrated possible statistical associations in univariate analysis ($p \leq 0.05$) were transformed from continuous variables into categorical variables whose cutoff values were based on the Youden Index J on AUROC analysis. Additionally, a stepwise multivariate logistic regression was used to identify characteristics independently associated with ICU mortality. Data were categorized, then a ROC curve analysis was performed to assess accuracy and discrimination of the scores. Hosmer-Lemeshow tests for goodness of fit was used to assess the calibration of the model by comparing

TABLE 1 General population description and comparison between survivors and non survivors.

Characteristics	General ($n = 605$)	Survivors ($n = 404$)	Non-survivors ($n = 201$)	p-value
Age, years	63 [49–74]	57 [44–70]	70 [62–80]	**<0.001**
Male sex	366 (60)	247 (61)	119 (59)	0.647
Heart rate, beats/min	90 [79–101]	90 [79,5–100]	89 [77–103]	0.883
Respiratory rate, breaths/min	22 (20-27)	22 (20-26)	23 (20-28)	**0.045**
Systolic blood pressure, mmHg	127 [110–148]	128 [110–146]	124 [108–152]	0.601
Hematocrit, %	37,4 [32,9–40,9]	37,9 [34,3–41,3]	35,3 [30,8–40,4]	**<0.001**
Leukocytes, $\times 10^9$/L	9,89 [6,93–14,59]	[9,22 6,8,9,10,11,12,13,3]	11,9 [7,24–16,2]	**0.001**
Urea, mg/dL	42,6 [29–71]	35,8 [27–56,1]	55 [39–101]	**<0.001**
Sodium, mmol/L	138 [135–141]	138 [135–141]	138 [134–142]	0.661
FiO_2, %	44 [32–100]	40 [28–100]	80 [33–100]	**<0.001**
Glasgow coma scale	15 (13-15)	15 (14,15)	14 (9-15)	**<0.001**
Use of vasopressors	101 (16.7)	35 (8.7)	66 (32.8)	**<0.001**
Mechanical ventilation	271 (45)	100 (25)	171 (85)	**<0.001**

Data are represented as median with interquartile range [25–75th percentile] or frequency (percentage). Clinical groups were compared using the Mann-Whitney U-test for quantitative variables and the Pearson's qui-square test or Fisher exact test for categorical variables.
FiO2, fraction of inspired oxygen.
Bold values which were statistically significant (P-value < 0.05).

both the observed and expected mortality. The study was conducted accordingly with the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) guidelines (12). Extending our analyses, an internal validation using the K (10) Fold Cross Validation was performed (13). Resampling was used to evaluate the models on the data sample, using a parameter called "k" that refers to the number of groups the data sample was split into. One proportion of the data was used to discover the classification and the rest to validate and measure the prediction power of a limited data set. Probability of ICU survival during distinct timepoints since admission, was calculated by Kaplan-Meier analysis. Data analysis was carried out using GraphPad Prism version 6.01, SPSS, version 25.0 software and R statistical software.

Ethics approval and consent to participate

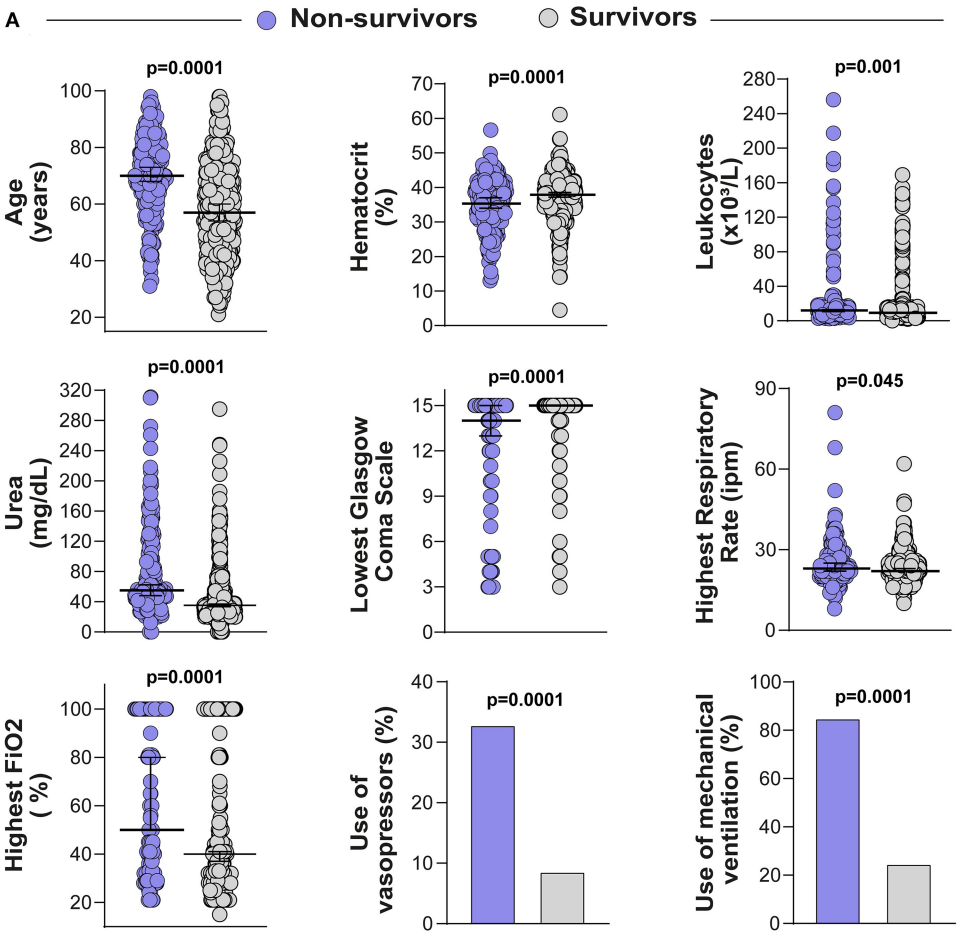
The study was approved directly by the National Committee of Ethics in Research (CONEP) from Brazil in accordance with local guidelines during COVID-19 pandemic (14), Certificate of Presentation of Ethical Appreciation (CAAE) Number: 30660720.0.0000.0008, and by the Ethical Committee of the Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ) under CAAE number 39059320.8.1001.0040. The need for informed consent was waived in both committees and the anonymity of the study subjects was preserved.

Results

During the study period, 650 patients were admitted to the four study ICUs, of whom 605 met inclusion criteria (Figure 1). The median age was 63 (IQR: 49–74) years with a mortality rate of 33.2% (201 patients). Overall, non-survivors were significantly older when compared with survivors [70 (IQR: 62–80) years vs. 57 (IQR: 44–70) years; $p \leq 0.001$]. No mortality differences were observed according to gender, nor objective clinical parameters such as heart rate, lowest systolic blood pressure, and sodium levels (Table 1). Importantly, the following factors were distinct in non-survivors compared to survivors: increased respiratory rate, elevated leukocyte count and urea, increased FiO2 within the first 6 h of admission, need for mechanical ventilation and vasopressors, and a lower Glasgow Coma Scale score and hematocrit in non survivors (Figure 2A). Description of prognostic scores analyzed are detailed in Table 2. Regarding each center characteristics, no significant discrepancies were observed concerning age distribution and vasopressors use meanwhile gender and vital signs exhibited some differences between cohorts.

pShock score development and calibration of the pShock-CoV

The original pShock score was developed in a derivation cohort of critically ill patients admitted with pneumonia in the ICU, with an external validation cohort derived from the Community-Acquired Pneumonia Organization (CAPO).



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Parameter	Model	Odds Ratio (95%CI)	p-value
Age ≥ 65, years	Unadjusted	4.04 (2.82 - 5.80)	<0.001
	Adjusted	3.07 (2.04 - 4.62)	<0.001
Hematocrit ≤ 35, %	Unadjusted	2.24 (1.58 - 3.18)	<0.001
	Adjusted	1.95 (1.30 - 2.95)	0.001
WBC count ≥ 12, 10^9/L	Unadjusted	2.40 (1.69 - 3.41)	<0.001
	Adjusted	1.75 (1.17 - 2.63)	0.007
FiO2 ≥ 50, %	Unadjusted	2.41 (1.70 - 3.40)	<0.001
	Adjusted	2.23 (1.48 - 3.36)	<0.001
Urea ≥ 40, mg/dL	Unadjusted	4.06 (2.78 - 5.91)	<0.001
	Adjusted	2.51 (1.65 - 3.83)	<0.001
Vassopressors use	Unadjusted	5.15 (3.27 - 8.12)	<0.001
	Adjusted	3.71 (2.23 - 6.15)	<0.001

FIGURE 2
General study population description and Calibration of pShock-CoV score. **(A)** Scatter plots depicting the distribution of age, hematocrit, leukocytes, urea, lowest Glasgow coma score, highest respiratory rate and highest FiO2 in non-survivors and survivors. Lines represent median and interquartile range values. The Mann-Whitney *U* test was employed to compare the values detected between the study groups. Use of vasopressors and use of mechanical ventilation variables are shown as frequency (%) and compared using the Fisher's exact test. **(B)** Adjusted and unadjusted binary regression model for ICU mortality. Multivariable regression adjusted for differences in baseline characteristics (variables of $p \leq 0.05$ identified in univariable analysis).

TABLE 2 Prognostic scores in cohort stratified by mortality.

Characteristics	General ($n = 605$)	Survivors ($n = 404$)	Non-survivors ($n = 201$)	p-value
CURB-65				**<0.001**
0	86 (14.2)	81 (20)	5 (2.5)	
1	161 (26.6)	138 (34.2)	23 (11.4)	
2	196 (32.4)	112 (27.7)	84 (41.8)	
3	131 (21.7)	61 (15.1)	70 (34.8)	
4	30 (5)	12 (3)	18 (9)	
5	1 (0.2)	0 (0)	1 (0.5)	
qSOFA				**<0.001**
0	129 (21.3)	113 (28)	16 (8)	
1	305 (50.4)	199 (49.3)	106 (52.7)	
2	147 (24.3)	81 (20)	66 (32.8)	
3	24 (4)	11 (2.7)	13 (6.5)	
pShock-CoV score				**<0.001**
0	78 (12.9)	77 (19.1)	1 (0.5)	
1	87 (14.4)	79 (19.6)	8 (4)	
2	93 (15.4)	77 (19.1)	16 (8)	
3	109 (18)	71 (17.6)	38 (18.9)	
4	104 (17.2)	55 (13.6)	49 (24.4)	
5	70 (11.6)	27 (6.7)	43 (21.4)	
6	37 (6.1)	15 (3.7)	22 (10.9)	
7	19 (3.1)	3 (0.7)	16 (8)	
8	8 (1.3)	0 (0)	8 (4)	

Data are represented as frequency (percentage). Clinical groups were compared using the Pearson's qui-square test or Fisher exact test.
CURB-65, confusion, urea, respiratory rate, blood pressure, age; qSOFA, quick Sequential Organ Failure Assessment.
Bold values which were statistically significant (P-value < 0.05).

The primary outcome evaluated was ICU mortality, and independent risk factors identified by a binary logistic regression were included in the composite score. Of note results were remarkable by a good prediction performance of the pShock score, with an AUC of 0.80 [95% Confidence Interval (CI): 0.73–0.86; p-value<0.0001] and better discriminate function than other models analyzed (SAPS 3, qSOFA, CURB-65, and CRB-65) (10). Further, in this study, pShock variables and clinically important parameters routinely available in the first hours of admission were assessed over multiple analyses and a stepwise multivariate logistic regression model yielded 6 variables associated with ICU mortality: age $\geq$ 65 years, hematocrit $\leq$ 35%, white blood cell count $\geq 12 \times 10^9$/L, FiO2 $\geq$ 50%, urea $\geq$ 40 mg/dL and use of vasopressors (Figure 2B). The calibrated pShock-CoV score system was determined based on variability in the odds ratio for a confidence interval (CI) of 95%. Similar to the original derivation cohort for pShock, age and vasopressor use were weighted 2 points while other variables were given 1 point in the score calculation, with total score values ranging from a minimum of 0 to a maximum of 8. Notably, goodness of fit test exhibited good calibration of the model (Hosmer-Lemeshow statistics, p = 0.65).

pShock-CoV score discrimination and validation performance

In COVID-19 infected patients admitted to the ICU, the pShock-CoV score demonstrated robust performance accuracy with an AUC of 0.80 [95% Confidence Interval (CI): 0.77–0.83; p-value < 0.0001] for mortality prediction without a notable loss in discriminative capacity compared with the derivation cohort for the original pShock score (p-value = 0.9410, Figure 3A). The pShock-CoV score demonstrated superior discriminate function compared with CURB-65 (p = 0.0003) and qSOFA ($p < 0.0001$) (Figure 3B). Internal validation conducted by K (10) Fold Cross Validation analysis confirmed consistent discriminative capacity of the score compared with the original sample, with an AUC of 0.78 [95% Confidence Interval (CI): 0.71–0.83; p-value < 0.0001] (Supplementary Figure 1). Score performance was consistent in 30-day mortality similar to overall ICU mortality (p-value = 0.9759, Figure 4A), and with the others scores analyzed (Figure 4B). Temporal analysis from admission demonstrated decreased survival probability in those with higher scores of pShock-CoV over time (Supplementary Figure 2).

Discussion

The persistence of high ICU mortality rates associated with COVID-19 infection may reflect delayed early recognition of those at highest risk of death resulting in missed opportunities to targeted interventions over the first hours of ICU admission. While ICU specific severity scores have been refined and new scores designed, a robust systematic model to predict mortality risk in a complex and diverse ICU population is lacking. Though vaccines and improved support measures have led to decreased morbidity and mortality, uncertainties remain in how best to stratify who is most likely to survive and target limited ICU resources to these patients (15). While recent studies have sought to develop prognostic tools to predict in-hospital COVID-19 mortality, these tools were not designed to evaluate risk for ICU mortality (16, 17). Other COVID-specific scores focused on triage evaluation to predict ICU admissions, which may inaccurately determine risk of deterioration and mortality in patients already admitted in these units (7, 8, 18, 19). Existing disease severity models including SOFA and SAPS3 lack adequate discriminant function, hindering accurate screening of critically ill patients in areas with supply shortages (20, 21). Furthermore, conclusions from

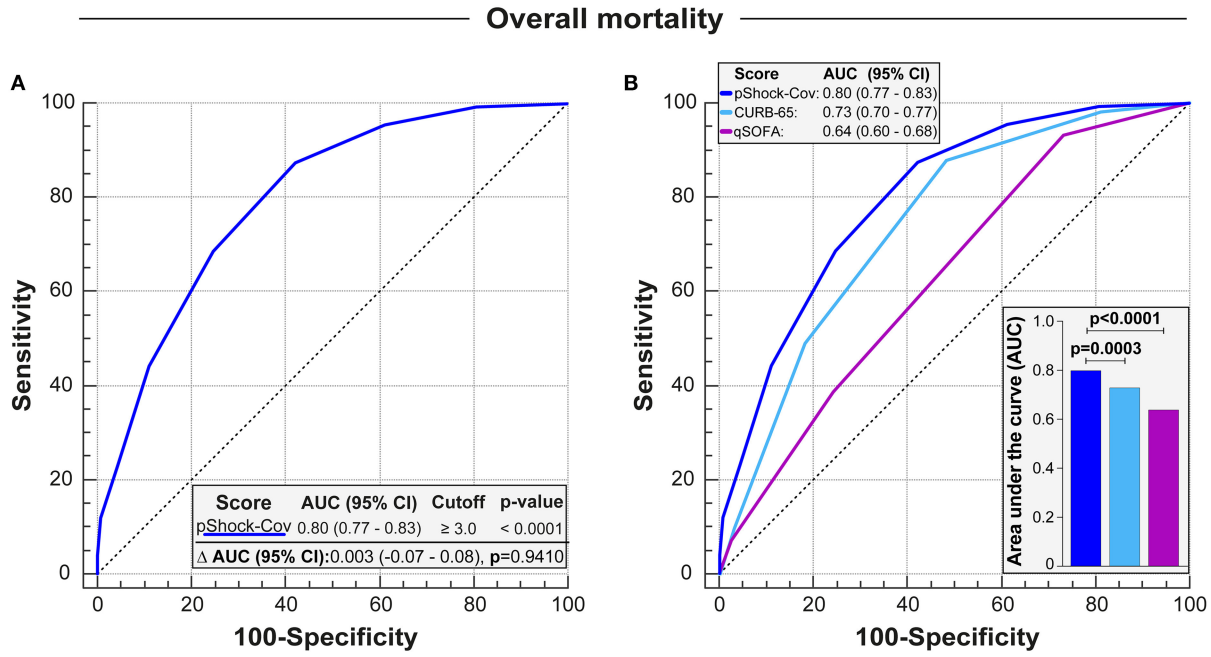

FIGURE 3
Discrimination of pShock-CoV in critically ill patients with SARS-CoV-2 infection and comparison with other severity models. **(A)** Receiver operating characteristic (ROC) curve analysis of pShock-CoV for prediction of ICU mortality in the ICU original sample and comparison of area under the ROC curve (Δ AUC) with pShock in the derivation cohort. **(B)** Overlap between ROC curves showing pShock-CoV performance and comparing with CURB-65 and qSOFA in COVID patients. Differences between AUC-ROCS were accessed by the DeLong test.

clinical trial of novel therapeutics may be confounded as these severity scores are inaccurate in identification of the most critically ill subset of hospitalized patients with COVID-19 infection. Alternatively, to other models, pShock-CoV is a simple straightforward tool that doesn't uses radiographic images or complex variables to be obtained in the first hours of admission. In addition, some of the selected parameters are compatible with earlier described prognostic factors for COVID-19 patients, aiding the applicability of the model in routine clinical practice. While the pShock-CoV Score demonstrated significant discriminatory capacity and sustained performance in ICU and 30-day mortality including cross validation, certain study limitations must be acknowledged. First, the modest number of individuals included in the analysis may have underestimated the performance of the score in a larger ICU cohort of individuals with COVID-19 infection. Secondly, interventions including steroids, remdesivir and possibly a more experienced COVID treatment team could have impacted the performance of the score. Analysis over various time points through the pandemic demonstrated stable score performance reflecting ongoing excess mortality in those admitted to the ICU with COVID-19 independent of new treatment approaches (Supplementary Figure 3).

Conclusions

Our calibrated pShock-CoV score is a robust bedside tool that may better define severity of disease at time of trial enrollment and ensure that results reflect the studied interventions rather than unbalanced study groups.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by National Committee of Ethics in Research (CONEP) (CAAE Number: 30660720.0.0000.0008), and by the Ethical Committee of the Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ) (CAAE Number: 39059320.8.1001.0040). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

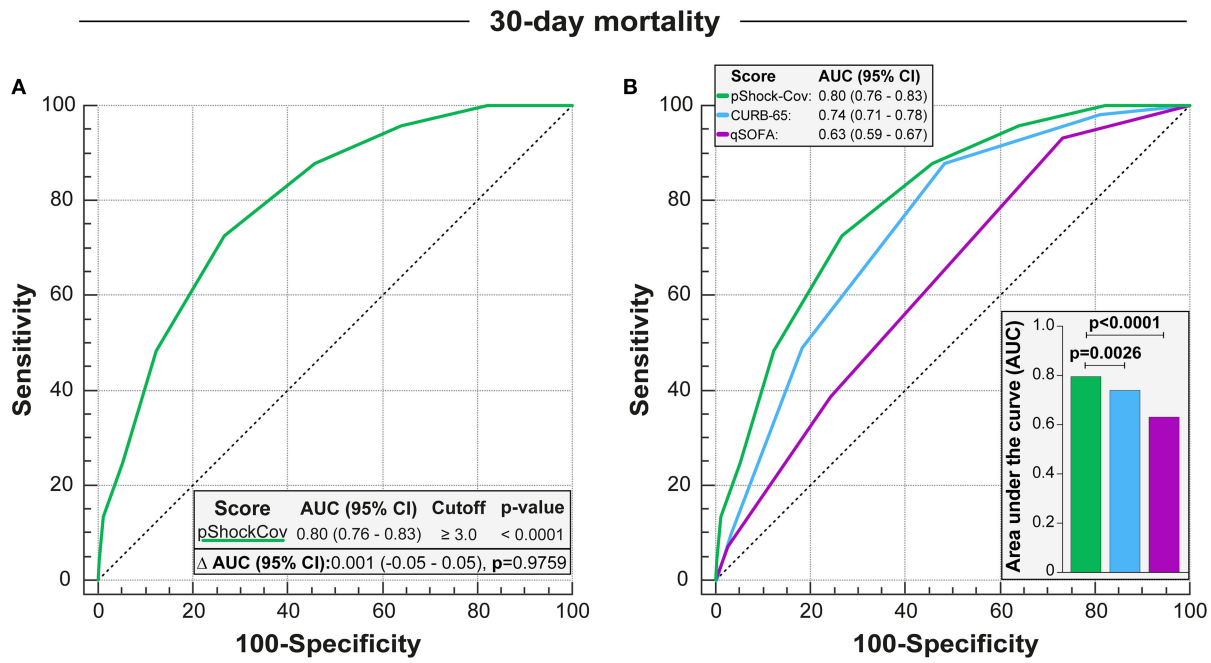

FIGURE 4
Discrimation of pShock-CoV over prediction of 30-day mortality for patients with COVID in ICU and comparison with other severity models. **(A)** Perfomance of the pShock-CoV score in predicting 30-day mortality in the intensive care unit, and comparison with discrimination capacity for overall mortality. **(B)** Comparison pShock-CoV with CURB-65 and qSOFA for prediction of ICU 30-day mortality in COVID patients. Differences between AUC-ROCS were accessed by the DeLong test.

Author contributions

Conceptualization, design of study, and manuscript draft: TC, IF, RM, KA, and BA. Investigation and visualization: TC, MP, RO, GT, AM, TA, JC, and NF. Data acquisition: MP, RO, GT, AM, TA, JC, and NF. Data analysis and interpretation: TC, IF, RM, MA, KA, and BA. Supervision and critical revision: TC, IF, RM, JC, MA, KA, and BA. Editing and final approval of the manuscript: TC, RM, MA, KA, and BA. All authors read and approved the final manuscript.

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Conflict of interest

Authors TC, IF, RM, MA, KA, and BA were employed by fellows from Multinational Organization Network Sponsoring Translational and Epidemiological Research Initiative.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.958291/full#supplementary-material

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Barriers to hand hygiene compliance in intensive care units during the COVID-19 pandemic: A qualitative study

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Background: The practice of hand washing is an effective way to prevent contamination and disease transmission. Following the COVID-19 pandemic, hand washing has become increasingly important. Therefore, this qualitative study aimed to understand barriers to hand hygiene compliance among healthcare workers during the COVID-19 pandemic.

Materials and methods: Twenty-five healthcare workers from intensive care units were sampled using purposive sampling in a qualitative content analysis study. Data were collected through a semi-structured interview and field notes. Based on the Lundman and Graneheim approach, the data were analyzed. COREQ checklist was used to report the research.

Results: According to the findings, there are three main categories of barriers to hand hygiene practice: barriers related to individuals (including two subcategories of lack of knowledge of healthcare workers and healthcare workers' improper attitude), barriers related to management (including two subcategories of wrong behavioral patterns and unsuitable training and planning), and barriers related to organizations (including four subcategories of heavy workloads, improperly designed wards, a lack of equipment, and lack of quality equipment).

Conclusions: This research indicates that hand washing practice increased during the COVID-19 pandemic. Nevertheless, some barriers persist, resulting in a decline in hand washing compliance among health care workers. This finding can help managers and policymakers remove barriers to hand washing compliance and improve healthcare workers' adherence to hand washing.

KEYWORDS

hand hygiene, intensive care units, qualitative study, healthcare workers, barriers, COVID-19

Introduction

COVID-19 is a highly contagious disease that has spread rapidly throughout the world, beginning in Wuhan, China, at the end of 2019. It has affected more than 210 countries in its first wave (1). The path of transmission of COVID-19 facilitates its spread among people and renders all individuals susceptible to the disease (2). As a global public health concern, COVID-19 has been declared an emergency by the WHO (3). Extensive measures were recommended to reduce the spread of infection, including keeping a safe distance, covering the mouth and nose with a tissue when coughing and sneezing, washing hands frequently, and wearing masks (4). In order to prevent nosocomial infections, the WHO and the Centers for Disease Control and Prevention advise hospitals to follow infection control standards and precautions, such as hand washing and wearing personal protective equipment (4).

Hospital-acquired infections (HAIs), also known as nosocomial infections, presented a severe challenge to healthcare professionals worldwide during the COVID-19 pandemic (5). Nosocomial infections are associated with higher clinical costs, drug use, and hospital stays (6). Infection control (IC) practices can prevent and control hospital-acquired infections (7). Due to a lack of self-protection and containment measures, HAIs are increasing (5).

There are two types of IC: expanded precautions and standard precautions. All patients are provided with standard precautions, including hand hygiene (HH), handling bodily fluids, and preventing injury from sharp objects. Alternatively, expanded precautions are applied according to the mode of disease transmission, such as contact, droplet, and airborne transmission (8). Practicing hand hygiene in healthcare settings is crucial to preventing nosocomial infections (7).

Healthcare workers frequently contact patients, making it easy for microorganisms to be transmitted through their hands (9). In intensive care units (ICUs), the problem is more critical due to a higher infection rate than in other wards, as well as high-risk patients due to multiple injuries, low awareness, and weak prevention mechanisms (10). Hand hygiene compliance has improved patient health and safety and decreased complications, hospital stays, and death risks. Despite hand hygiene techniques being simple, individuals find them challenging to follow, and numerous studies have shown that healthcare workers have low acceptance and poor adherence to these practices (11).

In hospitals, hand hygiene is affected by some factors (12). A study found that nurses tend to adhere to hand hygiene less frequently due to high workloads (13). Researchers found that inadequate training in infectious disease control and prevention was one of the reasons for the rise in COVID-19 cases in hospitals (14). A study conducted in a hospital during the COVID-19 revealed that hand hygiene differed according to criteria and moment. Motivation, adequate human resources, supervision, and training are necessary to improve hand hygiene (15). Based on the results of a systematic review, there is a lower compliance rate with hand hygiene in intensive care units compared to other wards. Physicians have a lower compliance rate than nurses. Moreover, before contacting patients, healthcare workers exhibit a lower level of compliance than after contacting patients (16). Furthermore, another study found that hand hygiene is more commonly practiced during night shifts than morning shifts and patient protection than self-protection (10).

While this may be true, the pandemic allowed to change behavior through reactive stimuli such as fear and knowledge (increased infection rates), which led to greater compliance with hand hygiene practices (17); little information is available regarding hand hygiene compliance rates during the COVID-19 pandemic (17). Consequently, most studies have only been quantitative and have examined compliance with hand hygiene practices (9, 10). The behavior associated with HH was also complex and challenging to comprehend, clarify, or alter (18). It is important to use qualitative research methods to investigate individuals' beliefs, attitudes, experiences, and intentions (19). Therefore, this finding contributes to establishing a habit of hand hygiene rather than merely reacting to an incident.

As monitoring and controlling nosocomial infections is one of the essential measures in any hospital, understanding the barriers to hand hygiene can help improve hand washing compliance among healthcare workers. So, this approach was used to explore barriers to hand hygiene compliance in intensive care units during the COVID-19 pandemic as perceived by healthcare workers.

Materials and methods

Study design

This qualitative study applied the conventional content analysis method with a descriptive-explorative approach (20). A qualitative study is a critical tool for studying emotions, perceptions, and knowledge about the complexities of human reactions, which cannot be obtained *via* quantitative research. Content analysis is a systematic coding and categorizing method used to understand, analyze, and conceptualize the underlying concepts of qualitative data (21).

Sample and setting

Twenty-five healthcare workers in the intensive care units of a teaching hospital participated in the study. The healthcare workers included anesthesiologists ($n = 5$), nurses ($n = 18$), and physiotherapists ($n = 2$). Shahid Bahonar Teaching Hospital is the largest trauma center in southeast Iran, with 350 beds and four intensive care units. Purposive

sampling was used to select participants with maximum variation in age, gender, work experience, and education level. The method consisted of interviewing participants suitable for the study, including health care workers who served in intensive care units during the outbreak of the COVID-19 pandemic and were willing and able to share their experiences. These criteria were determined by asking health care workers. The inclusion criteria required healthcare workers with at least 6 months' experience in intensive care units and fluency in Persian. The study excluded participants with a history of mental illness. The sample size of a qualitative study depends on the saturation of the data (21), which determines whether sufficient data are present to form a comprehensive understanding (22). After interviewing 22 participants, the present study reached saturation, however, three additional interviews were conducted to confirm data saturation.

Data collection procedure

Data were collected from late April to late May 2020 through semi-structured individual interviews with open-ended questions. Interviews were conducted by PM in each case. In the beginning, some prepared questions were asked to familiarize the researcher and create a friendly atmosphere with the participant. As part of the interview process, an interview guide was used. Afterwards, the interviews were focused on the study's purpose. Interviews varied from 45–55 min; during each interview, the researcher encouraged healthcare workers to participate in conversation and interaction and share their experiences. Some questions are as follows: "What facilities are available for hand hygiene practices in your ward? According to your beliefs, what are the five moments when hand hygiene should be practiced?" Please talk about your experience regarding barriers to hand hygiene during care provision?" As part of this study, field notes were used to collect data, and in all visits, the conditions of the ward and field observations were noted.

Ethical considerations

This qualitative study is approved by the Ethics Committee of Kerman University of Medical Sciences with the code [No: IR.KMU.REC.1398.581]. After selection of the participants, the study objectives were explained to the participants and informed and written consent was obtained for audio recording at the beginning of the interview. Participants were ensured about the confidentiality of the data and the right to enter and withdraw from the study. Each participant was identified with a number. Interviews were conducted in person at a specified time and place in the health center.

Data analysis

A content analysis methodology was employed in the analysis of the data following Graneheim and Lundman's five steps (23). In the first stage, each interview was transcribed immediately. The full texts of the interviews were read several times to immerse the researchers in the data and obtain a general perception of the content. Each interview text enters into MAXQDA software version 10 to manage the data. In the second step, the full texts of the interviews were read to determine the meaning units relevant to the aim of the study. In the third stage, meaning units were condensed and labeled with relevant codes. The initial codes were divided into subcategories based on similarities and differences in the fourth stage. One category contained similar manifest codes. Finally, the latent content in the data was extracted. During the data collection and analysis process, the researcher recorded any sparks related to the data and used them for subsequent interviews. PM and MJ analyzed the interviews. All extracted categories and themes were checked and approved by the authors. MA, MD, MA, PM, and ZE contributed to the composition, review, and correction of the final written report. Table 1 illustrates an example of the analysis process.

The Guba and Lincoln's criteria, including credibility, dependability, transferability, and confirmability, were used to determine trustworthiness (24). Credibility of data was achieved in various ways; the researchers were engaged in the field and at the site for long periods of time. Peer review was conducted by assessing background information, data collection methods, process, data management, transcripts, data analysis, procedure, and research findings. Several data collection methods were used to obtain supporting evidence, including semi-structured individual interviews and observations in the field. Moreover, participants were asked to participate in a member check during which they reviewed a short report of the analyzed and interpreted data. We did this in order to verify that the results were representative of their experiences and attitudes. By outlining all steps of the data collection process, context, the analysis, and direct quotes from participants, the transferability of the findings was ensured. Furthermore, maximum variability of sampling was considered.

Findings

This study involved 25 healthcare workers working in ICUs with a mean age of 36.44 years and a mean work experience of 11.32 years. The healthcare workers included anesthesiologists ($n = 5$), nurses ($n = 18$), and physiotherapists ($n = 2$). Participants included two men and 23 women. Four of the twenty-nine potential participants declined to participate in the interview due to inadequate preparation and limitations imposed by the COVID-19 pandemic, resulting in a response

TABLE 1 An example of qualitative content analysis process.

Category	Subcategory	Code	Meaning unit
Organizational barriers	Heavy workload	Emergency care of an ICU patient Fatigue following intensive care of critically ill patients High workload of staff Impossibility of keeping away from critically ill patients Simultaneous care of two patients Fatigue following night shifts High number of patients	Many times, I did not have enough time to wash or disinfect my hands due to the high workload in the intensive care unit and the emergency of some procedure. When I have to take care of several patients at the same time especially in the night shift, I am less concerned about hand hygiene practice due to fatigu.

TABLE 2 Main category, categories, and subcategories of barriers to hand hygiene practice in ICU healthcare workers.

Main category	Categories	Subcategories
Barriers to hand hygiene practice	Individual barriers	Lack of knowledge of healthcare workers
		Healthcare workers' improper attitude
	Management barriers	Wrong behavioral patterns
		Unsuitable planning and training
	Organizational barriers	Heavy workload
		Improperly designed wards
		The lack of equipment
		Lack of quality equipment

rate of 86.2% (25/29). Based on the analysis of the data, eight subcategories, three categories, and a main category of "barriers to hand hygiene practice" have been identified. A summary of the main categories, categories, and subcategories is presented in Table 2.

Main category: Barriers to hand hygiene practice

Based on the analysis of healthcare workers' experiences, three subcategories were identified, including barriers associated with individuals, management and organizations, which we will address in the following paragraphs.

Category 1: Individual barriers

According to the participants, improper attitudes of healthcare workers and inadequate knowledge of healthcare workers were two barriers related to knowledge and attitude. So, sufficient knowledge of healthcare workers about nosocomial infections and direct and indirect transmission of infectious agents played an essential role in obeying hand hygiene. In addition, by strengthening a positive attitude toward hand hygiene practice and convincing individuals that their behaviors will significantly impact the behavior of other colleagues, individuals' positive attitudes can be led to more adherence to hand hygiene compliance by healthcare workers.

Subcategory A. lack of knowledge of healthcare workers

In most cases, the participants' experiences indicate that healthcare workers are unaware of the consequences of poor hand hygiene practices, including antibiotic resistance, hospital stay length, nosocomial infections, and even mortality. The absence of awareness of staff, particularly novices, contributed to non-compliance with hand hygiene. Due to the absence of apparent contamination of the hands during the care provision or the use of substitute gloves for hand hygiene, participants felt that hand hygiene was not necessary, which led to less hand hygiene compliance.

> *"Sometimes we do not take hand hygiene seriously because we do not know enough about the complications of poor hand hygiene practice." (P1-A nurse).*
> *"Despite knowing the five moments when hand hygiene should be practiced, I did not fully practice it because I didn't know its importance." (P5-A nurse).*

Subcategory B. healthcare workers' improper attitude

Most participants' experiences revealed that healthcare workers' negative beliefs and attitudes toward hand hygiene practice played an essential role in non-compliance with hand hygiene because of despondency, lower productivity, lower enthusiasm, and low confidence. On the other hand, one aspect of healthcare workers' attitudes is their impact on other colleagues on the ward. Thus, a negative attitude toward hand hygiene could significantly impact the behavior of other healthcare workers, resulting in less compliance with hand hygiene practices.

> *"Often, we do not practice hand hygiene because we do not believe in the importance of hand hygiene and do not get used to it. We would practice hand hygiene more if they reported monthly statistics of nosocomial infections and their complications." (P2-A nurse).*
> *"My hand washing habits became less frequent after the COVID-19 pandemic since I didn't care about it." (P9-An anesthesiologist).*

Category 2: Management barriers

In most cases, the participants addressed the wrong behavioral patterns of supervisors and improper planning and management training. Wrong behavioral patterns hamper the pace of recovery and growth in one place. It is necessary to recognize toxic behavioral patterns before deciding on ways to halt and change them. In social settings, it is crucial that people observe the principles, rules, and guidelines that govern those settings and engage in positive patterns and norms. In addition, managers can promote hand hygiene practices through proper planning, training, and monitoring.

Subcategory A. wrong behavioral patterns

Most participants' experiences demonstrated that healthcare workers mimicked the wrong behavioral patterns of managers as head nurses or in charge and doctors as superiors or heads of departments. Consequently, the lack of adherence to hand hygiene compliance by colleagues, managers, and doctors affected other healthcare workers' performance.

> *"Doctors, residents, and head nurses, who can be good role models, do not often pay enough attention to hand washing practice. Therefore, we underestimate the importance of hand hygiene practice and do not comply with it properly." (p6-A nurse).*
> *"As I often see my colleagues or doctors not complying, I don't do it because they don't." (p4-A nurse).*

Subcategory B. unsuitable planning and training

In most cases, the participants' experiences revealed that inadequate training led to healthcare workers not having a clear picture of what is expected of them at work. Therefore, they will have difficulty performing tasks, including hand hygiene practice. In addition, managers' ineffective planning, poor monitoring, not providing positive feedback, and insufficient support have affected healthcare workers' adherence to hand hygiene. As well, managers did not pay enough attention to the problems and barriers to hand hygiene practices that contributed to poor hand hygiene. Participants considered insufficient management control over the evening and night shifts and inappropriate microbial culture to be administrative barriers to hand hygiene practice.

> *"I easily neglect my hand hygiene because there is no positive culture for hand hygiene and managers do not pay attention to culture building regarding it." (p14-An anesthesiologist).*
> *"There is less supervision during evening and night shifts, so hygiene protocols such as hand washing are less likely to be observed." (p22-A nurse).*

Category 3: Organizational barriers

A number of participants have addressed heavy workloads, poor design of hospital wards, inadequate equipment, and low-quality equipment in this regard. Hand hygiene practices are hindered by the high workload of ICUs. Additionally, changes to the physical design of hospital wards may promote proper hand hygiene. Ample and qualified equipment on the wards, especially in ICUs, will increase hand hygiene practice and reduce poor hand hygiene complications.

Subcategory A. heavy workload

Based on the experiences of several participants, the high workload in ICU is an essential barrier to hand hygiene. The reason is stress, a lack of peace of mind, and the rush to finish the tasks assigned. They mentioned that during the handling of many patients, caring for two patients simultaneously, hand hygiene practice is inevitably forgotten. They also noted that the impossibility of keeping away from critically ill patients was another barrier to hand hygiene practice. Moreover, hand hygiene practice was also impossible for emergency patients who need intensive care in critical situations. In addition, fatigue due to overwork in the ICU and night shifts prevented them from practicing proper hand hygiene.

> *"When I have to take care of several patients simultaneously, especially on the night shift, I am less concerned about hand hygiene practice due to fatigue." (p14-A nurse).*
> *"There are so many patients that I become tired and don't follow handwashing protocols any longer." (p8-A nurse).*

Subcategory B. improperly designed wards

Based on the experiences of several participants, working in non-standard environments was one of the factors that prevented them from practicing hand hygiene effectively. In some situations, for instance, the lack of accessible handwashing sinks and the distance between the sinks and the patients' beds make hand hygiene practice difficult due to inconvenience and dissatisfaction. Many participants indicated that the health system infrastructure was inefficient with regard to hand hygiene practices. As a result, handwashing sinks and reducing the distance between sinks and patients' beds will facilitate access and promote better hand hygiene practices.

> *"Many times, there was no sink when I wanted to wash my hands."* (p16-A physiotherapist).
> *"Patient's bed is far from the toilet."* (p3-A nurse).

Subcategory C. the lack of equipment

In light of the experiences of many participants, the absence of sinks and smart faucets for hand washing was one of the barriers to hand hygiene. Many participants noted that they were unable to dry their hands due to the lack of tissue paper and a hand dryer, causing poor hand hygiene. Other barriers to hand hygiene practices included a lack of detergents or personal protective equipment, a lack of skin moisturizers after hand washing, and insufficient funds to purchase hand washing equipment.

> *"Often, I have neglected hand hygiene due to the lack of tissue papers for drying my hands."* (p9-A nurse).
> *"A few gloves, disposable towels, and disinfectant solution are available."* (p15-A nurse).

Subcategory D. lack of quality equipment

In most cases, the participants' experiences revealed that poor quality equipment reduces hand hygiene practices. A study noted that the poor quality of soap and disinfectants for hand hygiene resulted in skin dryness and itching. This led to inadequate hand washing by healthcare workers.

> *"I have not done hand rub for a month because of the poor quality of the disinfectants and the allergy I felt after using them." (p23-A nurse).*
> *"I washed my hands less often due to extreme dryness and sensitivity caused by hand sanitizers." (p12-A nurse).*

Discussion

In the context of the COVID-19 pandemic, this qualitative study explored nurses' experiences with hand washing compliance barriers in ICUs. This study showed that despite increased compliance with hand hygiene practices due to the COVID-19 pandemic, healthcare workers in intensive care units face several obstacles to hand hygiene practices. Based on the analysis of healthcare workers' experiences, it was discovered that the main categories of "barriers to hand washing practices" consisted of three categories: individual, manager, and organizational barriers.

The lack of awareness of healthcare workers led to poor hand washing practices. Some healthcare workers were unaware of the importance of hand washing and its role in decreasing nosocomial infections and its costs and problems. Also, some healthcare workers did not believe in hand hygiene and were inattentive to it. In contrast, in the present study, (25) lack of obvious contamination on the hands, substitute of gloves for hand hygiene, as barriers to hand washing practice (25). Some studies have shown that healthcare workers have sufficient knowledge about hand washing practices and believe that unclean hands are an essential route of cross-infection in hospitals (26, 27). In addition, (28) believed that non-compliance with hand hygiene was not necessarily related to the knowledge of healthcare workers. Also, the staff was aware of the importance of hand washing, but they did not practice it (28). Despite the COVID-19 pandemic increasing healthcare workers' adherence to hand hygiene practices, compliance has declined over time due to poor understanding of procedures and a lack of educational interventions to recognize hand hygiene opportunities. Additionally, this may result from an inadequate appraisal of the essential issues of hand washing compliance, resulting in inadequate awareness among healthcare workers and poor hand washing practices. A lack of academic training is another factor that contributes to a reduction in the level of knowledge of health care workers, especially novices. Consequently, training healthcare workers on proper hand washing methods with reminder posters can significantly improve their awareness of and knowledge of hand hygiene (29).

The wrong attitude of healthcare workers toward hand washing practice led to less adherence to hand hygiene compliance. Some studies have shown that the positive attitude of healthcare workers has been associated with an increase in hand washing practice (30, 31). Although the COVID-19 pandemic prompted personnel to increase their hygienic practices. After a COVID-19 disease has subsided, healthcare workers may be less inclined to wash their hands due to a lack of positive attitudes toward hand washing, behavioral beliefs such as feeling happy after hand washing, and a lack of evaluation of behavioral outcomes, such as the value of self-care and family

care. Consequently, designing educational programs will play an essential role in increasing healthcare workers' attention and positive attitude regarding hand washing practices and standardizing health behaviors to increase hand hygiene (32).

The wrong behavioral patterns prevented hand washing practice. Similar to the present study, Studies addressed that the behavior of physicians, especially chief physicians, played an essential role in their compliance with hand hygiene standards by other people (31, 33, 34) also reported that role modeling plays a vital role in following hand hygiene standards (34). This issue emphasizes the crucial role of senior hospital staff in promoting hand washing practices and improving patient safety, in addition to the driving role of the COVID-19 disease. Because senior hospital staff plays a lasting and permanent role, the support and involvement of senior hospital staff, including physicians, may assist in removing barriers to hand washing practice. The fundamental concepts of behavioral patterns of hand hygiene must, however, also be emphasized in order to change individual attitudes toward hand hygiene (35).

Barriers related to improper management and planning were identified as essential barriers to hand hygiene practice (36) believed that hospital authorities were responsible for ensuring proper hand washing practices and should have more control over barriers to remove them (29). Therefore, training sessions, positive feedback, managerial support, and a proper environment were recommended to promote hand washing (25). The COVID-19 disease demonstrates the need to change management strategies for improving infection control (IC) practices, including adherence to hand hygiene by healthcare workers.

Participants in the present study considered heavy workload, fatigue, emergency patients who need intensive care in critical situations, and many patients as the main reasons for non-compliance with hand hygiene (37) believed that high workload and high patient-to-nurse ratio caused hand washing compliance to be forgotten or even impossible (37). Also, numerous studies have identified workload as one of the barriers to healthcare workers' compliance with hand hygiene (25, 38). In addition, several studies have shown that healthcare workers believed that they did not have enough time to perform hand washing in emergencies (27, 36, 39) showed that healthcare workers were less concerned about hand washing practice at the end of their shift work due to fatigue, and the longer the rest period between shift work, the higher hand hygiene was practiced (39). As a result, although the healthcare workers are familiar with the correct hand washing technique, they will not be able to practice hand hygiene due to the heavy workload that multiplied during the COVID-19 pandemic. Moreover, a management system capable of handling emergencies, adjusting high workloads, and increasing hand hygiene is necessary.

Improper design of the physical space of the ward was mentioned as some of the obstacles to hand washing practice (25) considered high workload, limited hospital space, and unavailability of sinks as barriers to hand washing practice (25), which were consistent with our study. As a result of a lack of space, the COVID-19 ICUs are located in departments without facilities for washing hands, resulting in poor handwashing practices.

Lack of equipment, on the one hand, and poor-quality equipment, on the other hand, were Also mentioned as significant reasons for hand hygiene non-compliance (40) demonstrated that the staff had sufficient knowledge and readiness to comply with hand hygiene. Unfortunately, the lack of appropriate equipment and facilities prevented them from following recommended hand hygiene protocols (40). To remove barriers and increase compliance with hand hygiene protocols, appropriate cleaning materials such as soap, paper towels, tissue papers, smart faucets, and the availability of hand washing sinks were also considered (41, 42) believed that the main reasons for not practicing hand hygiene were the lack of good hygiene products, insufficient tissue papers, lack of hand dryers, and repeated washings causing skin damage (42). At the beginning of the COVID-19 outbreak, hospitals faced a severe shortage of disinfectants due to the lack of prior forecasting, which adversely affected the observance of hand hygiene. The hospitals purchased low-quality disinfectants because of the dire need for disinfectants, which decreased staff adherence to hand washing, especially after the COVID-19 pandemic subsided. As a result of providing adequate and high-quality equipment, skin damage can be reduced, hand hygiene can be ensured, and cross-infection can be prevented. Therefore, it appears that management's attention promotes handwashing.

Limitation and strengths of the study

The small sample size and the fact that the study took place in a trauma center were among the limitations of the present study. Therefore, generalizations should be made with caution. A small number of participants was present in some groups, which may make it difficult to differentiate between professional groups. Also, The COVID-19 pandemic constraint caused some potential participants to decline to participate in the interview. Notwithstanding these limitations, we gained insight into the conclusion that despite scientific evidence that improved hand hygiene had resulted in a reduction in hospital infections during the COVID-19 pandemic and that pandemic conditions induced personnel to wash their hands more frequently, modifying individual, managerial, and organizational barriers is the feasible solution that can be useful for future research, education, and practice.

Conclusion

Several factors contribute to non-compliance with hand hygiene practices, including lack of knowledge, incorrect behavior patterns, insufficient training, heavy workloads, poorly designed wards, and low-quality equipment. Providing color-coded reminder boards, ensuring easy access to washrooms, monitoring disinfection solutions, and providing training and scientific information regarding the importance of improving hand hygiene can remove hand washing barriers. Standard precautions during COVID-19 reduced HAIs, but employee adherence to hand hygiene declined as the disease subsided; therefore, the hospitals must pay attention to barriers preventing employees from complying.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

PM, MD, MeA, and MaA supervised this study and designed the study. PM and MD prepared the interview guide and analyzed the interviews. MJ conducted the interviews. PM, MD, and ZE cooperated in composing, reviewing, and correcting the written version. The research team read and confirmed the final version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A simple nomogram for predicting infectious diseases in adult kidney transplantation recipients

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Objective: To investigate the risk factors of infectious diseases in adult kidney transplantation recipients and to establish a simple and novel nomogram to guide the prophylactic antimicrobial therapy.

Methods: Patients who received kidney transplantation between January 2018 and October 2021 were included in the study and were divided into a training and a testing set at a 1:1 ratio. Risk factors correlated to infectious diseases were selected using a Least Absolute Shrinkage and Selection Operator (LASSO) regression model. The prediction model was built by incorporating the variables selected by the LASSO model into a logistic regression equation. Calibration curves and receiver operating characteristic (ROC) curves were also applied to assess the model calibration and discrimination. A nomogram consisting of the selected factors was established to provide individualized risks of developing infections. Decision curve analysis (DCA) was adopted to estimate the net benefit and reduction in interventions for a range of clinically reasonable risk thresholds.

Results: In all, 863 adult kidney recipients were included in the study, and 407 (47.16%) of them developed infectious diseases during the 3-year follow–up period. A total of 8 variables were selected using LASSO regression and were retained for subsequent model construction and infection prediction. The area under the curve (AUC) was 0.83 and 0.81 in the training and testing sets, with high F scores of 0.76 and 0.77, sensitivity of 0.76 and 0.81, and specificity of 0.88 and 0.74, respectively. A novel nomogram was developed based on 8 selected predictors (requirement for albumin infusion, requirement for red blood cell infusion, triglyceride, uric acid, creatinine, globulin, neutrophil percentage, and white blood cells). The net benefit indicated that the nomogram would reduce unnecessary interventions at a wide range of threshold probabilities in both sets.

Conclusions: Adult kidney transplantation recipients are high-risk hosts for infectious diseases. The novel nomogram consisting of 8 factors reveals

good predictive performance and may promote the reasonable antimicrobial prescription. More external validations are required to confirm its effectiveness for further clinical application.

KEYWORDS

kidney transplantation, solid organ transplantation, nomogram, prediction model, infectious disease

Introduction

Along with the ongoing advances of modern science, an increasing number of solid organ transplantations (SOTs) have been performed in the last decade, and the life quality of those with chronic organ failure has been greatly improved (1). However, due to the application of glucocorticoids and immunosuppressive agents, secondary infection in transplant recipients is an unavoidable problem and has become the major cause of mortality (2). Therefore, great efforts have been made to manage the infectious diseases appropriately in these immunocompromised hosts (ICHs). These strategies include establishment of transplant infectious diseases (TID) subspecialty, development of relevant practice guidelines, and setting up epidemiological database (3, 4).

Antimicrobial prophylaxis was once considered an effective strategy to prevent the infectious complications in SOT recipients. However, the complexity of the immune function and the atypical clinical manifestations make it difficult to achieve precise treatment (5). Remaining questions include when prophylactic antimicrobial treatment should be started, and who can benefit the most from such therapy are still in

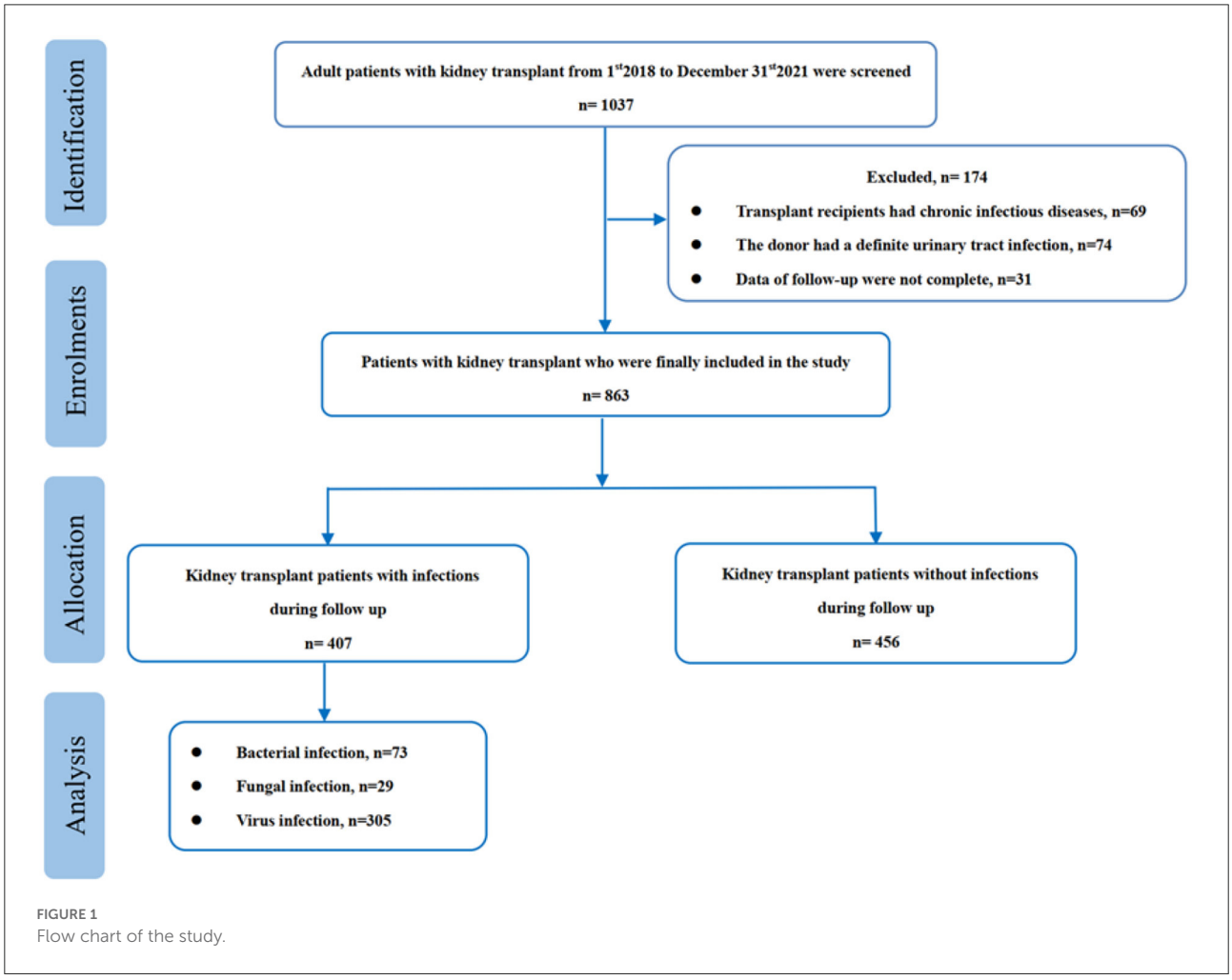

FIGURE 1
Flow chart of the study.

TABLE 1 Demographics and clinical characteristics of patients with or without infectious disease.

Variables	Total ($n = 863$)	Without infection (n = 456)	With infection (n = 407)	*P*-value
Gender, *n* (%)				1.000
Female	344 (39.9)	182 (39.9)	162 (39.8)	
Male	519 (60.1)	274 (60.1)	245 (60.2)	
Age, median (IQR), years	43 (35, 52)	42 (35, 52)	43 (35, 52)	0.736
Blood type, *n* (%)				0.468
O type	301 (34.9)	155 (34.0)	146 (35.9)	
A type	255 (29.5)	128 (28.1)	127 (31.2)	
B type	208 (24.1)	116 (25.4)	92 (22.6)	
AB type	99 (11.5)	57 (12.5)	42 (10.3)	
Dialysis before operation, *n* (%)				0.315
No	45 (5.2)	20 (4.4)	25 (6.1)	
Yes	818 (94.8)	436 (95.6)	382 (93.9)	
Diabetes, *n* (%)				0.457
No	846 (98.1)	445 (97.6)	401 (98.5)	
Yes	17 (1.9)	11 (2.4)	6 (1.5)	
Hypertension, *n* (%)				0.590
No	720 (83.4)	377 (82.7)	343 (84.3)	
Yes	143 (16.6)	79 (17.3)	64 (15.7)	
Delayed graft function, *n* (%)				<0.001
No	664 (76.9)	377 (82.7)	287 (70.5)	
Yes	199 (23.1)	79 (17.3)	120 (29.5)	
Glucocorticoid, *n* (%)				0.384
Methylprednisolone	773 (89.6)	403 (88.4%)	370 (90.9%)	
Metacortandracin	78 (9.0)	47 (10.3%)	31 (7.62%)	
Prednisolone	12 (1.4)	6 (1.32%)	6 (1.47%)	
Tacrolimus, *n* (%)				0.329
No	124 (14.4)	60 (13.2)	64 (15.7)	
Yes	739 (85.6)	396 (86.88)	343 (84.3)	
Immunity induction, *n* (%)				0.577
Thymoglobulin	820 (95.0)	431 (94.5)	389 (95.6)	
Basiliximab	43 (5.0)	25 (5.5)	18 (4.4)	
WBC, median (IQR), 10^9/L	7.1 (5.6, 9.3)	7.8 (6.1,10.3)	6.7 (5.0, 8.2)	<0.001
Neutrophil percentage, median (IQR), %	80.3 (74.0, 85.8)	82.2 (77.3, 86.9)	78.0 (70.0, 82.7)	<0.001
Platelet, median (IQR), 10^9/L	215 (168, 264)	216 (173, 268)	215 (164, 254)	0.058
Globulin, median (IQR), g/L	22.7 (20.2, 25.4)	23.5 (21.3, 26.9)	22.5 (19.1, 23.2)	<0.001
Albumin infusion:				<0.001
No	261 (30.2)	201 (44.1)	60 (14.7)	
Yes	602 (69.8)	255 (55.9)	347 (85.3)	
RBC infusion:				<0.001
No	361 (41.8)	250 (54.8)	111 (27.3)	
Yes	502 (58.2)	206 (45.2)	296 (72.7)	
Prealbumin, median (IQR), mg/L	291 (71.1)	283 (66.2)	299 (75.2)	0.001
Alanine transaminase, median (IQR), U/L	16.0 (11.0, 25.5)	17.0 (11.0, 29.0)	16.0 (11.0, 21.0)	0.005
Aspartate aminotransferase, median (IQR), U/L	15.0 (12.0, 19.0)	15.0 (12.0;19.0)	15.0 (13.0, 19.0)	0.307
Direct bilirubin, median (IQR), umol/L	2.6 (2.0, 3.5)	2.6 (2.1, 3.8)	2.6 (2.0, 3.1)	0.004
Total bilirubin, median (IQR), umol/L	7.7 (6.3, 10.0)	7.7 (6.4, 10.6)	7.7 (6.2, 9.3)	0.090
Urea, median (IQR), mmol/L	9.6 (7.6, 13.5)	9.5 (7.2, 12.7)	9.6 (7.9, 14.2)	0.003
Creatinine, median (IQR), umol/L	117 (93, 162)	107 (82, 136)	128 (108, 217)	<0.001
Uric acid, median (IQR), umol/L	304 (252, 370)	282 (230, 344)	321 (288, 394)	<0.001
Triglyceride, median (IQR), mmol/L	1.8 (1.6, 2.1)	1.8 (1.5, 2.2)	1.8 (1.7, 2.1)	0.474
Cholesterol, median (IQR), mmol/L	4.5 (4.1, 4.9)	4.5 (3.8, 4.6)	4.5 (4.5, 5.2)	<0.001
Blood glucose, median (IQR), mmol/L	4.9 (4.5, 5.4)	4.9 (4.3, 5.2)	4.9 (4.9, 5.6)	<0.001

debate. Moreover, inappropriate use of antibiotics to avoid life-threatening infections is not uncommon, and it may lead to undesirable consequences, including antibiotic resistance and adverse drug effects (ADEs) (6).

Some prediction models or algorithms based on artificial intelligence and machine learning have been established to assist decision-making and assess the prognosis of infectious diseases (7, 8). However, few simple and reliable prediction model about the infections of SOT recipients is available (9, 10).

More than 1,000 kidney transplant operations have been performed in Renji hospital in the last 3 years and nearly half of them had infectious diseases during the follow-up period. The clinical characteristics, laboratory test results and state of illness are properly documented in our hospital information system (HIS). We also find that variables of the recipients with or without infectious diseases are quite different. Therefore, we hypothesized that the risk factors for infections can be identified from the clinical information of these kidney transplantation

TABLE 2 Demographics and clinical characteristics of patients in testing and training groups.

Variables	Total ($n = 863$)	Testing group ($n = 431$)	Training group ($n = 432$)	*P*-value
Gender, *n* (%)				0.461
Female	344 (39.9)	166 (38.5)	178 (41.2)	
Male	519 (60.1)	265 (61.5)	254 (58.8)	
Age, median (IQR), years	43 (35, 52)	42 (35, 52)	43 (35, 52)	0.615
Infection, *n* (%)				0.261
No	456 (52.8)	219 (50.8)	237 (54.9)	
Yes	407 (47.2)	212 (49.2)	195 (45.1)	
Infection types, *n* (%)				0.337
Non-infection	456 (52.8)	219 (50.8)	237 (54.9)	
Bacterial infection	73 (8.5)	34 (7.9)	39 (9.0)	
Fungal infection	29 (3.4)	13 (3.0)	16 (3.7)	
Virus infection	305 (35.3)	165 (38.3)	140 (32.4)	
Blood type, *n* (%)				0.126
O type	301 (34.9)	151 (35)	150 (34.7)	
A type	255 (29.5)	136 (31.6)	119 (27.5)	
B type	208 (24.1)	90 (20.9)	118 (27.3)	
AB type	99 (11.5)	54 (12.5)	45 (10.4)	
Dialysis before operation, *n* (%)				0.766
No	45 (5.2)	21 (4.9)	24 (5.6)	
Yes	818 (94.8)	410 (95.1)	408 (94.4)	
Diabetes, *n* (%)				0.621
No	846 (98.0)	421 (97.7)	425 (98.4)	
Yes	17 (2.0)	10 (2.3)	7 (1.6)	
Hypertension, *n* (%)				0.572
No	720 (83.4)	356 (82.6)	364 (84.3)	
Yes	143 (16.6)	75 (17.4)	68 (15.7)	
Outcomes, *n* (%)				0.551
Survival	852 (98.7)	427 (99.1)	425 (98.4)	
Non-survival	11 (1.3)	4 (0.9)	7 (1.6)	
Delayed graft function, *n* (%)				0.323
No	664 (76.9)	325 (75.4)	339 (78.5)	
Yes	199 (23.1)	106 (24.6)	93 (21.5)	
Glucocorticoid, *n* (%)				0.485
Methylprednisolone	773 (89.6)	383 (88.9)	390 (90.3)	
Metacortandracin	78 (9)	40 (9.3)	38 (8.8)	
Prednisolone	12 (1.4)	8 (1.9)	4 (0.9)	
Anti-proliferation, *n* (%)				0.281
Mycophenolate mofetil	740 (85.7)	373 (86.5)	367 (85.0)	
Mycophenol sodium	123 (14.3)	58 (13.5)	65 (15.0)	
Tacrolimus, *n* (%)				0.488
No	124 (14.4)	66 (15.3)	58 (13.4)	
Yes	739 (85.6)	365 (84.7)	374 (86.6)	
Immunity induction, *n* (%)				0.748
Thymoglobulin	820 (95)	408 (94.7)	412 (95.4)	
Basiliximab	43 (5)	23 (5.3)	20 (4.6)	
WBC, median (IQR), 10^9/L	7.1 (5.6, 9.3)	7.1 (5.4, 9.4)	7.1 (5.6, 9.2)	0.489
RBC, median (IQR), 10^{12}/L	3.1 (2.6, 3.7)	3.1 (2.6, 3.7)	3.1 (2.6, 3.6)	0.263
Neutrophil percentage, median (IQR), %	80.3 (74, 85.8)	80.3 (73.4, 86)	80.3 (74.4, 85.6)	0.680
Lymphocyte percentage, median (IQR), %	11.1 (6.6, 17.3)	11.1 (6, 17.9)	11.1 (7.2, 17)	0.182
Hemoglobin, median (IQR), g/L	92 (80, 108)	92 (80, 109)	92 (80, 105.2)	0.363
Platelet, median (IQR), 10^9/L	215 (168.5, 264)	215 (171, 264.5)	215 (164, 263.2)	0.485
Globulin, median (IQR), g/L	22.7 (20.2, 25.4)	22.7 (20.1, 25.5)	22.7 (20.2, 25.3)	0.926
Albumin, median (IQR), g/L	37.3 (34.3, 41.6)	37.3 (34.3, 41.9)	37.3 (34.3, 41.3)	0.263
Prealbumin, median (IQR), mg/L	286 (248, 328.5)	286 (249, 321.5)	286 (245.8, 333)	0.704
Alanine transaminase, median (IQR), U/L	16 (11, 25.5)	16 (12, 26)	16 (11, 25)	0.602
Aspartate aminotransferase, median (IQR), U/L	15 (12, 19)	15 (12, 19)	15 (12, 19)	0.846
Direct bilirubin, median (IQR), umol/L	2.6 (2, 3.5)	2.6 (2, 3.5)	2.6 (2.1, 3.5)	0.173
Total bilirubin, median (IQR), umol/L	7.7 (6.3, 10)	7.7 (6, 10)	7.7 (6.4, 10.1)	0.146
Urea, median (IQR), mmol/L	9.6 (7.6, 13.5)	9.6 (7.7, 13.1)	9.6 (7.6, 13.8)	0.905
Creatinine, median (IQR), umol/L	117 (93, 161.5)	117 (95, 162)	117 (91, 159.2)	0.381
Uric acid, median (IQR), umol/L	303.5 (251.5, 370)	303.5 (254.5, 372.1)	303.5 (249.8, 367.2)	0.270
Triglyceride, median (IQR), mmol/L	1.8 (1.6, 2.1)	1.8 (1.6, 2.2)	1.8 (1.6, 2.1)	0.814
Cholesterol, median (IQR), mmol/L	4.5 (4.1, 4.9)	4.5 (4, 4.9)	4.5 (4.1, 4.9)	0.847
Blood glucose, median (IQR), mmol/L	4.9 (4.5, 5.4)	4.9 (4.6, 5.5)	4.9 (4.5, 5.4)	0.675

TABLE 2 (Continued)

Variables	Total ($n = 863$)	Testing group ($n = 431$)	Training group ($n = 432$)	*P*-value
Albumin infusion:				0.982
No	261 (30.2)	131 (30.4)	130 (30.1)	
Yes	602 (69.8)	300 (69.6)	302 (69.9)	
RBC infusion:				0.805
No	361 (41.8)	178 (41.3)	183 (42.4)	
Yes	502 (58.2)	253 (58.7)	249 (57.6)	

recipients. Thus, a comprehensive classification system based on the selected factors can be developed and a novel nomogram may also be established by incorporating such factors. It is anticipated that recipients with infectious diseases can be recognized quickly by the nomogram and evidence-based use of prophylactic antibiotics can also be promoted.

Materials and methods

Summary of study methodology

This study was conducted in Renji Hospital, which is affiliated with Shanghai Jiaotong University School of Medicine. The hospital is one of the largest organ transplant centers in China and the number of kidney transplantation is about 400 per year. Information on kidney transplantation recipients between January 2018 and October 2021 were retrieved from the HIS by four attending physicians (R-Y C, S Z, S-Y Z and D-W Li). The design and implementation of the study carefully followed the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statement (11). The Ethics Committee of Renji Hospital provided an exemption from the requirement for informed consent, since only previously clinical data were used, and the privacy of the patients was protected. For each patient enrolled, an identification number was issued to ensure anonymity.

Study population, sample size, and disease definition

Patients aged between 18 and 80 years old who had received a kidney transplantation and were followed up regularly were included in the study. Those who met any of the following criteria were excluded: (I) had a chronic infectious disease; (II) urinary tract infection had been diagnosed in the donor; and (III) incomplete follow-up data. Thus, none of the recipients was prescribed antibiotics before transplantation.

All the patients included were divided into a training set and a testing set at a ratio of 1:1. Logistic regression model was applied for nomogram construction in our study. Based on the Events Per Variable (EPV) criterion and the sample size guideline for logistic regression from observational studies, a minimum sample size of 500 patients was recommend (12). More than one thousand adult kidney transplantation recipients were regular follow up during the study period and the sample size could fulfill the requirement.

Antibiotic prophylaxis is one of the most important components of our perioperative management process. Third generation cephalosporins were often prescribed to those with living related kidney transplantation for about 1 week. Those with allograft renal transplantation were often prescribed carbapenems during their hospital stay. Infectious diseases in kidney transplantation recipients included pneumonia, urinary tract infection, bacteremia, abdominal infection, central nervous system infection and skin and soft tissue infection. The definitions of these infectious diseases followed the diagnostic criteria of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) carefully (13). Pathogenic bacteria were isolated using cultures in the microbiological laboratory, and pathogenic viruses were identified using polymerase chain reaction (PCR). Recipients with hypoalbuminemia (defined as the serum albumin level was <35 g/L) received an albumin infusion before the operation (14), and those with renal anemia or hemoglobin levels lower than 70 g/L received a blood infusion (15, 16).

Clinical data collection and assessment

Data about the recipients was retrieved from the HIS and included the following aspects: (I) information on infectious diseases of the donors and kidneys; (II) information on infectious diseases of the kidney transplantation recipients; (III) clinical characteristics of the kidney transplantation recipients, including gender, age, comorbidity (chronic renal dysfunction, hypertension, and diabetes); (IV) antirejection medications prescription, including glucocorticoid, anti-proliferation, and immunosuppressive agents; and (V) laboratory tests of blood samples of the recipients during the follow-up period. The average follow-up period for the first 90 days after transplantation was once a week and then every other week over the next 180 days. Data from the last follow-up before infection was included in the study.

Statistical analysis

Data relating to continuous variables were expressed as median [interquartile range (IQR)] or mean ± standard deviation (SD). For categorical variables, percentages were calculated and Chi square tests were performed. Normally

distributed continuous variables were compared using the Student's *t*-test, whereas non-normally distributed variables were compared using the Wilcoxon rank sum test.

To identify the risk factors for infectious diseases in kidney transplantation recipients, the Least Absolute Shrinkage and Selection Operator (LASSO) regression model with the "lambda.min" criterion was applied for variable selection. Lasso algorithm allowed variables selection by forcing the coefficients of non-significant variables to shrink to zero through a penalty. In this process, potential confounding variables would be removed and only significant independent variables would be retained for outcome prediction (occurrence of infectious disease or not). It excluded non-essential variables and retained a subset of the most important variables for outcome prediction (occurrence of infectious disease or not). The prediction model was built by incorporating the variables selected by the LASSO model into a logistic regression equation. Model discrimination was assessed using receiver operating characteristic (ROC) curves, which included sensitivity, specificity, correctly classified rate, positive likelihood ratio, and negative likelihood ratio. A calibration curve to assess the goodness-of-fit, accuracy, and applicability of the predictive nomogram in the training and testing sets was generated. To reduce the risk of overfitting, the whole cohort was randomly divided into the training and testing sets at a ratio of 1:1. They were used for model development and unbiased assessment of model performance, respectively. Finally, a novel nomogram consisting of the selected predictors was established to provide an individualized risk of the infectious disease occurrence. Decision curve analysis (DCA) was adopted to evaluate the net benefit for the prediction model in comparison to default strategies, which assume that all or no observations received interventions (17, 18). We also converted the net benefit into the net reduction in interventions to show the reduction in the number of unnecessary interventions per 100 patients based on the prediction model. To achieve this, the graphs of net benefit and the net reduction in interventions were plotted against a range of clinically reasonable threshold probabilities for the training and testing sets.

Statistical analyses were performed using R (University of Auckland, New Zealand, Version 3.6.2) and SPSS 21.0 (IBM Corp., Armonk, NY, USA). A two-sided P-value < 0.05 was considered statistically significant.

Results

Clinical features of the study population

During the study period, 1,037 adult kidney transplantation recipients were initial screened and 863 of them were finally

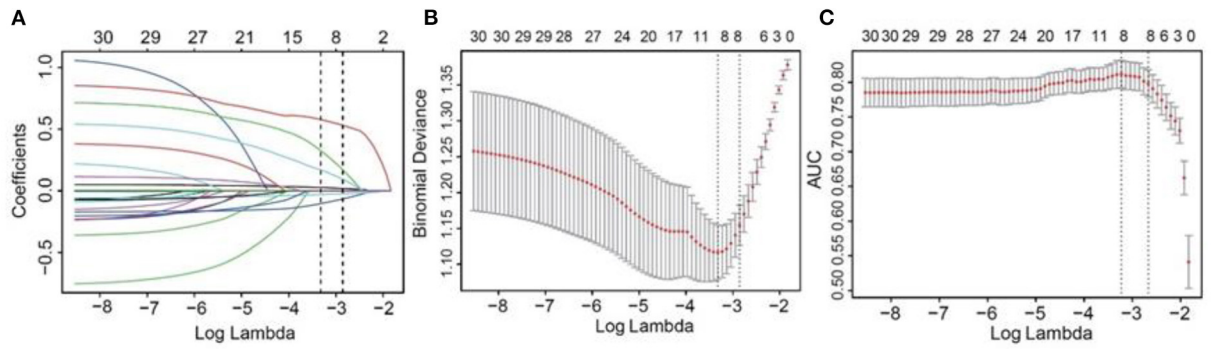

FIGURE 2
Selection of risk factors of infectious diseases using LASSO regression algorithm. A vertical line was plotted at the given lambda, selected by 10-fold cross-validation with minimum classification error and minimum classification error plus one standard error. For the optimal lambda that gives minimum classification error, 8 features with a non-0 coefficient were selected. **(A)** LASSO coefficient profiles of the candidate variables. **(B)** The binomial deviance with 95% CI (y-axis) was plotted against log (lambda) (bottom x-axis), when the number of included variables were changed (upper x-axis). **(C)** The AUCs with 95% CI (y-axis) were plotted against log (lambda) (bottom x-axis), when the number of included variables were changed (upper x-axis). LASSO, the Least Absolute Shrinkage and Selection Operator; CI, confidence interval; AUC, areas under the curve.

TABLE 3 Parameters of ROC curves for prediction of infectious diseases in training set and testing set.

Data set	Cutoff	ACC	SENS	SPEC	PPV	NPV	pDLR	nDLR	FSCR
Training set	0.50	0.78	0.76	0.80	0.76	0.81	3.85	0.29	0.76
Testing set	0.50	0.77	0.80	0.74	0.75	0.79	3.11	0.27	0.77

ACC, Overall accuracy of classification; SENS, Sensitivity; SPEC, Specificity; PPV, Positive predictive value; NPV, Positive predictive value; pDLR, Positive diagnostic likelihood ratio; nDLR, Negative diagnostic likelihood ratio; FSCR, F-score.

enrolled in our study. Among them, 407 (47.2%) had infectious diseases during the follow-up period, and most of them were viral infections (305, 74.9%) (Figure 1). The pathogenic viruses were identified by PCR and the majority of them were cytomegalovirus (CMV) and respiratory syncytial virus (RSV). No patient with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was detected. Among the 73 bacterial isolates, 7 were identified as Methicillin-resistant Staphylococcus aureus (MRSA), 29 were resistant to carbapenems and 33 were resistant to third generation cephalosporins.

The median time from discharge to infection occurrence was 62.3 (46.9, 90.6) days and the most common type of infection was pneumonia (289, 71.0%), followed by urinary tract infection (72, 17.7%). Moreover, 31 recipients had two or more sites infection. It revealed that most of the patients with infectious diseases needed red blood cell (RBC) transfusion (72.7%) and albumin (85.3%) infusion throughout the follow-up period due to anemia or hypoalbuminemia (Table 1).

Predictive factors identification and validation

The 863 patients included were randomly divided into a training and a testing set at a 1:1 ratio. Basic clinical data of kidney transplantation recipients in the training and testing sets are shown in Table 2, and most of the variables included were evenly distributed. A LASSO model with the "lambda.min" criterion was applied to select variables. Thirty variables were screened initially, and 8 of them were retained for subsequent prediction model construction (Figure 2).

The prediction model was built based on the 8 selected variables using a logistic regression equation. The parameters of the ROC curve at the optimal cut-off value according to different models were documented (Table 3; Figure 3). It was demonstrated that the area under the curve (AUC) was 0.83 and 0.81 in the training set and

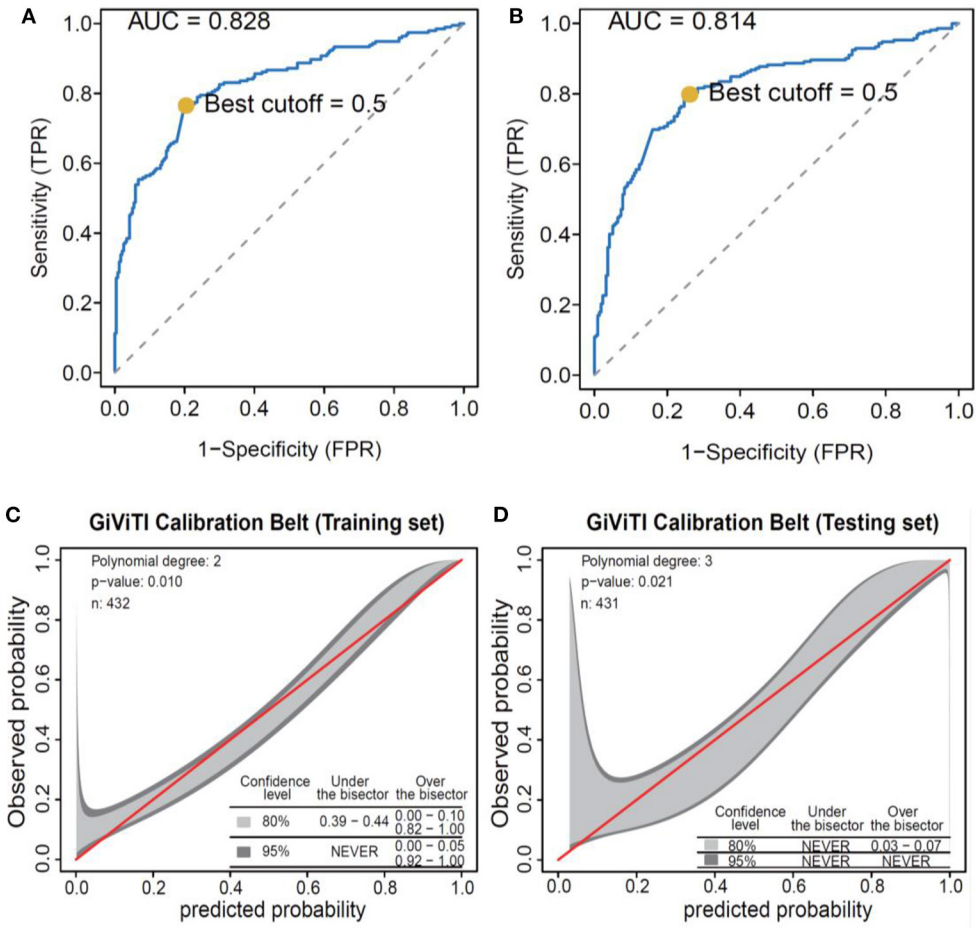

FIGURE 3
Performance of the logistic regression algorithm in infectious disease prediction. **(A)** ROC curves of the training set. **(B)** ROC curves of the testing set. **(C)** GiViTI calibration curves of the training set. **(D)** GiViTI calibration curves of the testing set. ROC, receiver operating characteristic.

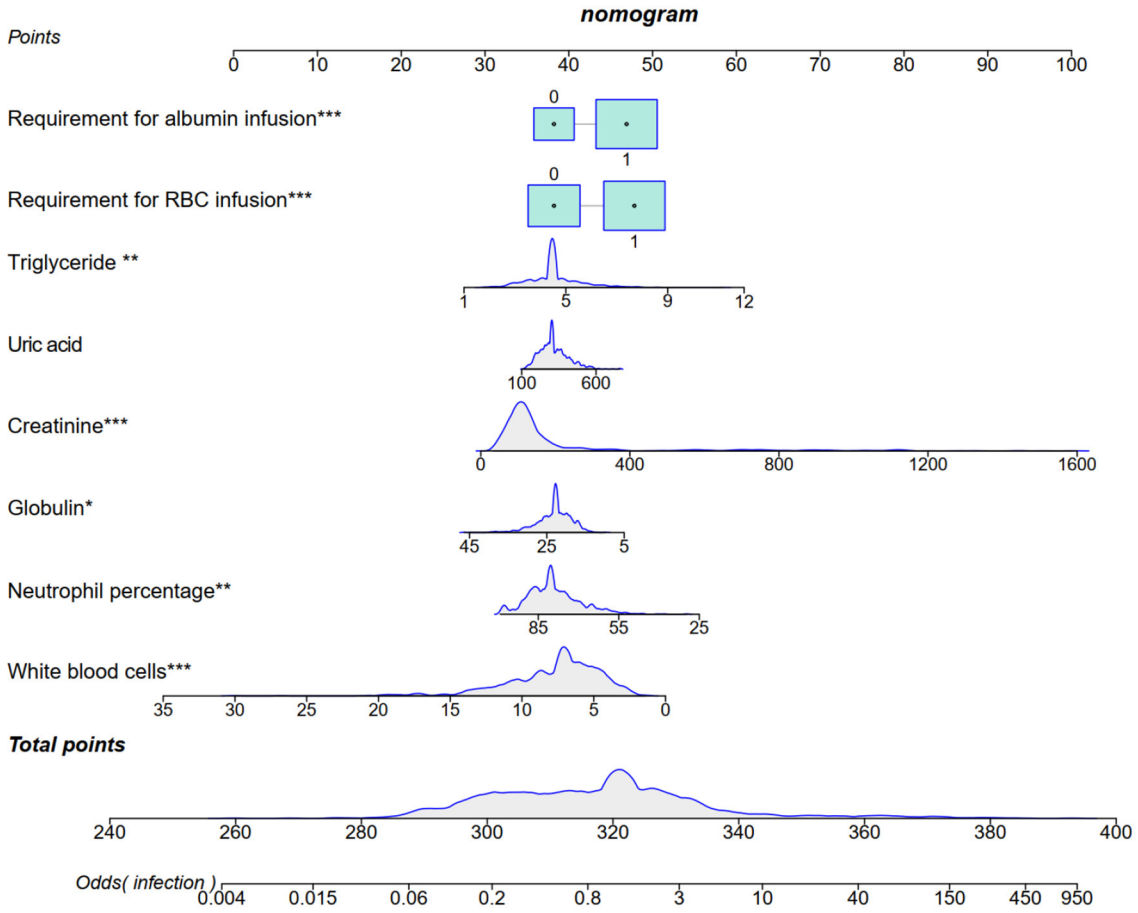

FIGURE 4
A nomogram to predict infectious diseases was developed using the predictors selected using LASSO. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. LASSO, Least Absolute Shrinkage and Selection Operator.

testing set, respectively, with high sensitivity (0.76 and 0.81, respectively), specificity (0.88 and 0.74, respectively), and F scores (0.76 and 0.77, respectively). Moreover, calibration curves revealed good agreements between predicted and observed probability for infectious diseases in both sets.

Estimating the efficacy probability of infectious diseases using the nomogram

We incorporated 8 selected predictors as prognostic features for the nomogram: requirements for albumin and RBC infusions, and levels of triglyceride, uric acid, creatinine, globulin, neutrophil percentage, and white blood cell (WBC). The nomogram can be utilized to predict individualized risk of infectious diseases in patients with kidney transplantation (Figure 4).

Assessment of the nomogram performance based on the DCA

The DCA indicated the superior net benefit of the prediction model compared with default strategies, which assume that all or no observations received interventions in the training and testing sets (Figures 5A,B). The results of DCA were also demonstrated by converting the net benefit into the reduction in interventions per 100 patients. As shown in Figures 5C,D, a clinical strategy based on the nomogram would decrease the number of unnecessary interventions at a wide range of threshold probabilities in the training and testing sets.

Discussion

A total of 863 recipients of kidney transplantations were enrolled in our study, and a novel nomogram consisting of 8

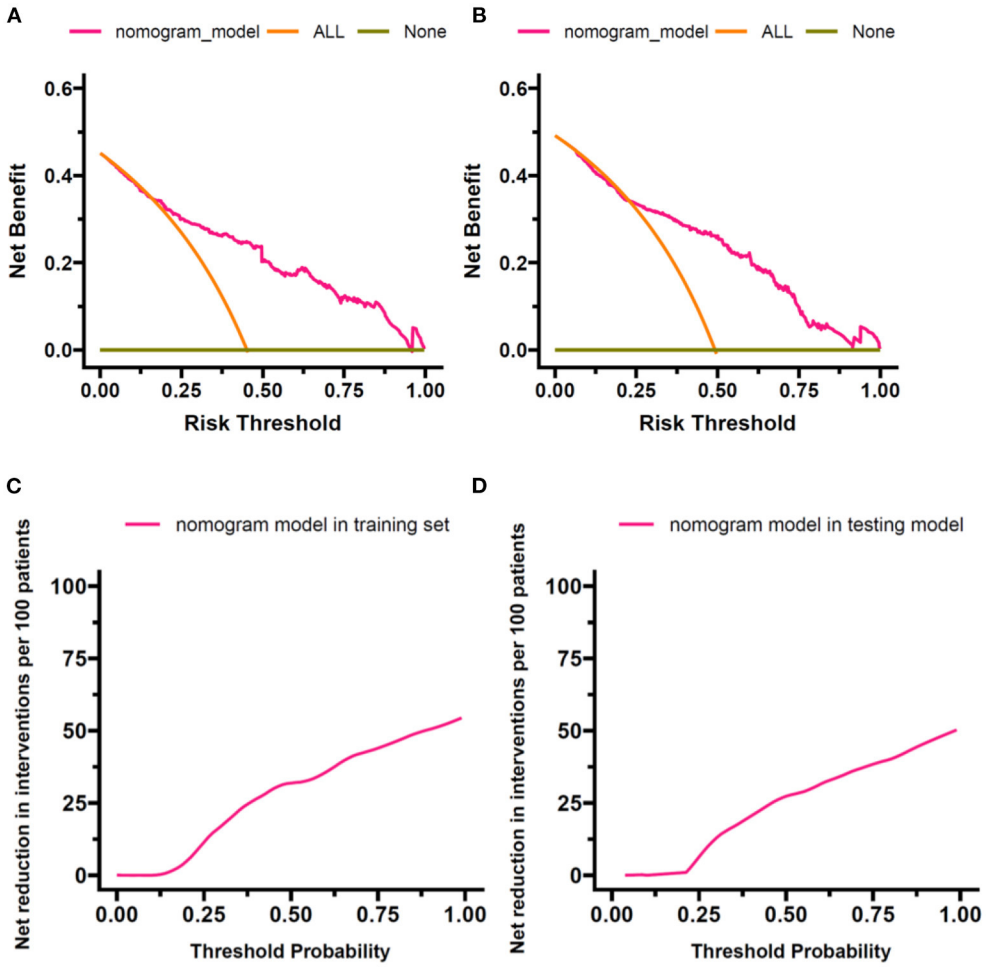

FIGURE 5
Results of DCA. DCA was performed to compare the nomogram-based decision with default strategies, which assume that all or no observations received interventions. **(A)** Net benefit against threshold probability in the training set. **(B)** Net benefit against threshold probability in the testing set. **(C)** Net reduction in interventions per 100 patients against threshold probability in the training set. **(D)** Net reduction in interventions per 100 patients against threshold probability in the testing set. DCA, decision curve analysis.

selected variables was established to predict the development of infectious diseases. Our study demonstrated that the requirements for albumin and RBC infusions, the levels of triglyceride, uric acid, creatinine, globulin, neutrophil percentage, and WBC should be carefully monitored during the follow-up period of the adult kidney transplantation recipients. It is indicated that the novel nomogram can improve the rational use of antibiotics and it may be helpful in decreasing the occurrence of antimicrobial resistance.

Kidney transplantation really improves the long-term outcomes in those with chronic renal failure, while opportunistic infection is still an enormous challenge. Thus, the recipients should be carefully evaluated for the risk of infection occurrence, and evidence-based prophylactic antibiotics prescription is recommended (19). Nearly half of the recipients enrolled in our study were infected during the 3-year follow-up period, and most of them were diagnosed with viral infections (74.9%), which included cytomegalovirus (CMV) and BK virus (20). The typical symptoms of viral infection are fever and fatigue. Above all, leukopenia and a low percentage of neutrophils can often be found from the complete blood count (CBC) (21). Therefore, it is reasonable that the count of WBC and the percentage of neutrophils in kidney recipients with infectious diseases are lower than those without infections.

Serum globulin level is one of the most important factors which can reflect the status of humoral immunity. The multiple roles of globulin in the host defense include regulation of the immune system, pathogen clearance, mucosal immunity, and toxin neutralization (22). Not surprisingly, the presence of posttransplant hypogammaglobulinemia is

known as an independent risk factor that may induce infectious diseases. Our study shows that the level of serum globulin is lower in the infected than the non-infected group, which is in line with a meta-analysis that included 18 studies (23).

Graft-versus-host disease (GVHD) is another major problem for kidney transplantation, which may present as high levels of uric acid, creatinine and renal vascular resistance (24). Thus, glucocorticoids and immunosuppressors should be prescribed, which may lead to further impairment of immune function and a higher probability of opportunistic infections. Nutritional status is another important indicator that cannot be neglected in assessing the risk of infection (25). It has already been demonstrated that a low concentration of serum albumin is strongly associated with reduced kidney function, which may also cause infection in kidney recipients (26). Thus, albumin infusion is needed for those with a low level of serum albumin during the follow-up period, which has been recognized as an independent risk factor for infection. Hemoglobin is a protein with multiple functions. It is not only responsible for carrying oxygen in an organism but also has implication for the genetic resistance to infection (27). The hemoglobin peptide library can produce different biological effects, which include antimicrobial hemoglobin-derived peptides. Then, the antimicrobial hemoglobin-derived peptides can produce antibacterial effects, thereby reducing inflammation caused by microbial infections (28, 29). Therefore, the requirement for RBC infusion was also included in our nomogram as a risk factor for infection.

Lipids are indispensable in the infection process, although they are usually associated with the metabolic and nutritional status of patients (30). In the present study, triglyceride disturbances were detected at the follow-up period and higher levels could be found in the recipients with infectious diseases. Triglyceride is usually degraded by lipoprotein lipase to produce free fatty acids, that may activate the nuclear factor-κB (NF-κB) role in the inflammatory response and resulting infectious diseases in kidney transplantation recipients (31, 32). Activated macrophages can inhibit lipoprotein lipase production to increase triglyceride levels by releasing TNF-α and IL-1 which may also be another factor that can promote the occurrence of infection (33).

A simple and novel nomogram consisting of 8 factors has been established in our study and it was also evaluated by ROC and benefit curves. Honestly, requirements for albumin and RBC infusions, the levels of triglyceride, uric acid, creatinine, globulin, neutrophil percentage, and WBC are not specific and the results of them are easily available in clinical practice. However, they can access the status of immunity and nutrition as well as the function of the allograft. Thus, the novel nomogram is worthy of clinical applications. Yet, limitations should still be mentioned. First, as the vast majority of infected kidney transplantation recipients have viral infections, the nomogram cannot be extrapolated to all range of infectious diseases. Second, invasive devices and catheter indwelling may disrupt the mucosal barrier and lead to the incidence of infectious diseases. However, some important confounding factors did not include in LASSO regression model of our study. Whether the recipients need mechanical ventilation or central venous catheter should be further analyzed in future research. Third, the type of infection is quite complex in adult kidney transplantation recipients which consists of simple and mixed infection. They were not further distinguished in our prediction model and studies with larger sample size are needed in the future. In addition, experimental validation of the nomogram through future studies is warranted.

Conclusions

Adult kidney transplantation recipients are high-risk hosts for infectious diseases. The novel nomogram consisting of 8 factors reveals good predictive performance and may promote the reasonable antimicrobial prescription. Thus, it is possible to decrease the burden of health economics and contain the trends of antimicrobial resistance. More external validations are required to confirm its effectiveness for further clinical application.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Renji Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conception and design: Y-TY, CZ, and X-DY. Administrative support: MZ. Provision of study materials or patients: R-YC, S-YZ, D-WL, and Y-TY. Collection and assembly of data: SZ. Data analysis and interpretation: SZ, CZ, and Y-TY. Manuscript writing and final approval of manuscript: All authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Application of oXiris-continuous hemofiltration adsorption in patients with sepsis and septic shock: A single-centre experience in China

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oXiris is a new, high-adsorption membrane filter in continuous hemofiltration adsorption to reduce the inflammatory response in sepsis. The investigators retrospectively reviewed patients with sepsis/septic shock who underwent at least one oXiris-treatment from November 2020 to March 2022. The demographic data, baseline levels before treatment, clinical datas, prognosis, and the occurrence of adverse events during treatment were recorded. 90 patients were enrolled in this study. The hemodynamic indices, sequential organ failure assessment score, lactate, inflammatory biomarkers levels were significantly improved at 12 h and 24 h after treatment. Procalcitonin and interleukin-6 reduction post-treatment of oXiris were most pronounced in infection from skin and soft tissue, urinary and abdominal cavity. Logistic regression analysis showed that pre-treatment sequential organ failure assessment score ($p = 0.034$), percentage decrease in sequential organ failure assessment score ($p = 0.004$), and age ($p = 0.011$) were independent risk factors for intensive care unit mortality. In conclusion, oXiris-continuous hemofiltration adsorption may improve hemodynamic indicators, reduce the use of vasoactive drugs, reduce lactate level and infection indicators. Of note, oXiris improve organ function in sepsis, which may result to higher survival rate.

KEYWORDS

oXiris, continuous hemofiltration adsorption, sepsis, septic shock, cytokine storm

Introduction

Sepsis is a life-threatening organ dysfunction syndrome because of a disordered host response after a host infection caused by pathogenic microorganisms (1). Sepsis has been confirmed to be associated with a >10% in-hospital mortality; septic shock is subset of sepsis with particularly severe circulatory, cellular, and metabolic abnormalities that carries a greater risk of death than sepsis alone, and it is associated with >40% in-hospital mortality (1). Although the concept and technology of treatment have developed rapidly, the incidence and case fatality rate of sepsis are still high, which has become challenge for the global medical community.

Sepsis is a highly heterogeneous clinical syndrome. Different host response and pathophysiological driving mechanisms of different patients result in complex but excessive immune activation and immunosuppression of sepsis, which has always been the central link in the pathophysiology of sepsis (2). Upregulation of pro-inflammatory and anti-inflammatory pathways causes a system-wide release of cytokines, mediators, and pathogenesis-related molecules, resulting in the activation of the coagulation and complement cascades (3). In septic shock, the dysregulated host response to infectious pathogens leads to a cytokine storm—the uncontrolled production and release of humoral pro- and anti-inflammatory mediators—causing cytotoxicity and promoting the development of organ dysfunction and increased mortality (4).

Continuous renal replacement therapy (CRRT) is the main form of RRT in the intensive care unit (ICU), because it has accurate volume control and stable acid-base and electrolyte correction and can achieve hemodynamic stability. Significant results have been achieved in severe patients, especially in the field of sepsis treatment (4, 5). In addition to renal replacement therapy, adsorption therapy seems to be more promising in the application of sepsis (3, 5, 6).

oXiris (Baxter, Meyzieu, France) is a filter that combines cytokine- and endotoxin-removal properties, renal replacement function, and antithrombotic properties (7). The peak of "cytokine casade" should be within the first a few hours of onset, and this should be the optimal intervening window for oXiris. It can reduce the levels and early harmful effects of circulating proinflammatory cytokines and endotoxins in the first few hours and days of septic shock therapy to improve patient outcomes (4, 7).

It has been shown that oXiris treatment in septic patients enables optimization of hemodynamic status, clears inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, and interferon-γ, and ultimately improves prognosis (8–11). However, the evidence-based use of oXiris for sepsis is still limited. This study aims to explore the clinical effect of continuous hemofiltration adsorption (CHFA) with oXiris filter in patients with sepsis/septic shock.

Materials and methods

Study population

The study protocol was approved by the Institutional Ethics Committee of the Second Xiangya Hospital of Central South University (No. 2022K040). We retrospectively collected data on 90 patients with sepsis/septic shock who received at least one oXiris-CHFA treatment at the Second Xiangya Hospital, Central South University, between November 2020 and March 2022. All the patients treated in ICU. The inclusion criteria were: (1) Patients with clinical diagnosis consistent with sepsis (meeting the 2016 Sepsis-3 definition) (1); and (2) men or women in the age range of 18–90 years. The exclusion criteria were: (1) immunodeficiency diseases such as tumors, connective tissue disease, and use of immunosuppressants in the last 3 months; and (2) pregnant or lactating patients.

Methods

All selected patients underwent titrated fluid resuscitation in strict accordance with the sepsis guidelines (2016 Sepsis-3) (1) and received vasoactive drugs, empiric/based antibiotics, mechanical ventilation, sedation, and analgesia. Baseline characteristics, primary site of infection, microbiological results, antibiotic and acute physiological and chronic health assessment (APACHE II) scores, initial creatinine level, and renal function grade [acute kidney injury (AKI)- stages 1–3 (12) or end-stage renal disease (ESRD)] were recorded. Details of baseline characteristics and infection and clinical outcomes are presented in Table 1.

All patients received at least one oXiris-CHFA treatment with the oXiris filter on a Prismaflex system (Baxter International, Deerfield, IL, United States). The mode was CHFA (CVVH/CVVHDF + oXiris adsorption), and 4 patients underwent hemoperfusion therapy (HA380, Jafron Biomedical Co., Zhuhai, China). Start timing, treatment dose, duration, and anticoagulation (citrate/heparin/no anticoagulant) were determined by the physician in charge according to the patient's specific situation. The oXiris-CHFA treatment duration lasted at least 24 h for each patient, except under special circumstances (such as death or abandoning treatment). The blood flow rate was maintained between 150 and 200 mL/min. Details of the CRRT prescriptions are presented in Table 2.

Data collection

For patients who had used oXiris-CHFA several times, we only recorded data before and after the first treatment, including heart rate (HR), respiratory rate (RR), mean arterial pressure (MAP), and norepinephrine (NE) level at 0, 12, and 24 h after treatment. The levels of procalcitonin (PCT), IL-6, and lactate and the sequential organ failure assessment (SOFA) scores were compared at 0, 12, and 24 h after treatment.

Study design and statistical methods

This study was designed for self-pairing, and SPSS software (version 22.0; IBM Corporation, Armonk, NY, United States) was used for statistical analysis of data. The distribution of measurement data was first tested, and normally distributed measurement data were expressed as mean ± standard

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TABLE 1 Baseline characteristics and infection and clinical outcomes of included patients.

Clinical data	
Male (*n*, %)	59 (65.6)
Age, years	63 [50.75–74]
SOFA total	14 [10-17]
APACHE II	24 [19-32]
Creatinine, μmol/L	172.00 [109.75–278.50]
Renal function at CRRT initiation (*n*, %)	
AKI Stage 3	24 (26.7)
AKI Stage 2	23 (25.6)
AKI Stage 1	32 (35.6)
ESRD	11 (12.2)
Norepinephrine (*n*, %)	84 (87.00)
Norepinephrine, μg/kg/min	0.6 [0.14–1.50]
Mechanical ventilation (*n*, %)	75 (83.3)
PaO_2/FiO_2 ratio	190.25 [112.85–287.00]
ECMO (*n*, %)	5 (5.5)
Lactate ≥2 mmol/L (*n*, %)	77 (85.56)
Lactate, mmol/L	5.15 [2.78–8.65]
Infection characteristics	
Site of infection (n, %)	
Pulmonary	28 (31.1)
Abdominal	33 (36.7)
Skin and soft tissue	5 (5.6)
Bacteremia	16 (17.8)
Urinary	8 (8.9)
Culture (*n*, %)	
Gram negative	45 (50.0)
Gram positive	17 (18.9)
Fungus	12 (13.3)
Not identified	16 (17.8)
Positive blood culture	27 (30)

SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; AKI, acute kidney injury; CRRT, continuous renal replacement therapy; ESRD, end-stage renal disease; ECMO, extracorporeal membrane oxygenation. Continuous variables are presented as median [interquartile range].

deviation, and repeated measurement ANOVA test was used for comparison among three groups. Non-normally distributed measurement data were expressed as median [interquartile range (IQR)]: Friedman's test was used for comparison among three groups, and Wilcoxon symbol rank test was used for comparison between two groups. A p value <0.05 was considered to indicate statistically significant differences. Based on patients' survival during ICU treatment, all patients were divided into survival and non-survival groups. Two independent non-normally distributed samples were compared by Mann–Whitney U test, and categorical variables were compared using either chi-square test or Fisher's exact test, as appropriate. Independent sample Kruskal-Wallis test was used to compare subgroups grouped according to baseline conditions and RRT parameters.

Results

Demographic data

A total of 90 patients (59 male; median age: 63 years; IQR: 50.75–74 years) with sepsis/septic shock were included from November 2020 to March 2022. Eleven patients had ESRD but required CRRT due to hemodynamic instability. Of the 79 patients with AKI, 32, 23, and 24 patients had stages 1, 2, and 3, respectively. On admission, the median APACHE II score was 24 (IQR: 19–23). The median dose of initial use of the vasoactive drug was 0.6 μg/kg/min (IQR: 0.14–1.50). The median oxygenation index was 190.25 mmHg (IQR: 112.85–287.00): 75 patients were undergoing mechanical ventilation for respiratory support at inclusion, and 5 patients were treated with extracorporeal membrane oxygenation (ECMO). The median lactate level was 5.15 mmol/L (IQR: 2.78–8.65), and 77 patients had lactate levels ≥2 mmol/L. Abdominal infection ($n = 33$) was the most common source of sepsis in this study, followed by pulmonary infection ($n = 28$). Gram-negative sepsis was found in 45 (50%) patients, followed by gram-positive ($n = 17$) and fungal ($n = 12$) sepsis. Further, 27 patients (30%) had positive blood cultures. Patient characteristics and the details of the infections are described in Table 1.

All patients were treated with 1–12 oXiris-CHFA [median = 2 (IQR: 1–2)] at the discretion of the attending physician. The median time between ICU admission and the start of oXiris was 18 h (IQR: 7.00–61.50), and in terms of treatment mode selection, 56 patients required CVVH, 30 required CVVHDF, and 4 required CVVH + HP. More than half of the patients (51.10%) required no anticoagulant, 43.30% had citrate anticoagulant, and only 5.60% had heparin anticoagulant therapy. The median prescribed treatment dose was 28.95 mL/kg/h (IQR: 26.81–38.89), and the median filter score was 21.15% (IQR: 19.03%−24.31%). Because some patients had also been treated with other filters, the median CRRT treatment time for each patient was 66.85 h (IQR: 37.63–132.50), and the oXiris treatment time was 38.5 h (IQR: 22.00–59.87). Details of the CRRT prescriptions are presented in Table 2.

Results after oXiris treatment

After 24 h of oXiris-CHFA, MAP increased by 9.1% ($p < 0.001$), NE dose decreased by 61.53% ($p < 0.001$), HR decreased by 21.31% ($p < 0.001$) and RR decreased by 21.74% ($p < 0.001$). In parallel to hemodynamic stabilization, blood lactate

TABLE 2 CRRT prescription in each patient.

CRRT parameter	
CRRT modality (*n*, %)	
CVVH	56 (62.2)
CVVH + HP	4 (4)
CVVHDF	30 (33)
Blood flow rate (*n*, %)	
150 mL/min	39 (43.3)
200 mL/min	51 (56.7)
Circuit anticoagulation (*n*, %)	
Citrate	39 (43.3)
Heparin	5 (5.6)
None	46 (51.1)
Prescribed therapeutic dose (mL/kg/h) (*n*, %)	28.95 [26.81–38.89]
≥30 mL/kg/h	38 (42.2)
<30 mL/kg/h	52 (57.8)
Filtration fraction (%)	21.15 [19.03–24.31]
Time between ICU admission and oXiris® initiation, h	18.00 [7.00–61.50]
Number of sessions per patient	2.00 [1.00–2.00]
Duration of CRRT treatment, h	66.85 [37.63–132.50]
Duration of oXiris-CHFA treatment, h	38.5 [22.00–59.87]

CRRT, continuous renal replacement therapy; CVVH, continuous venovenous hemofiltration; HP, hemoperfusion; CVVHDF, continuous venovenous hemodiafiltration; ICU, intensive care unit. Continuous variables are presented as median [interquartile range].

levels decreased by 37.86% after 24 h compared to the pre-treatment period ($p = 0.008$) (Table 3, Figure 1). The SOFA score was significantly decreased by 21.43% ($p < 0.001$) after 24 h of oXiris-CHFA treatment, and the median pre-/post-treatment SOFA score was 14 (10.00–17.00) vs. 11.00 (9.00–15) (Table 3, Figure 2).

In terms of infection index, PCT decreased by 48.79% ($p < 0.001$) after 24 h of treatment, and IL-6 decreased by 81.80% ($p < 0.001$) (Table 3, Figure 2). The ICU mortality rate was 34.4%, wherein 59 patients survived and 31 patients died. The median ICU stay time was 7.5 days (4.00–20.50), and the 30-day mortality rate was 44.4%. Hospital mortality rate was 35.6%.

Subgroup analysis and regression analysis

Initiation time and therapeutic dose of oXiris-CHFA

There were no statistically significant differences in ICU mortality rate between patients who received oXiris ≤24 h or >24 h ($p = 0.921$) and between patients whose prescription therapeutic dose was ≥30 mL/kg/h or <30 mL/kg/h ($p = 0.309$). There was no significant difference in SOFA scores between surviving and non-surviving groups [13.00 (10.00–16.00) vs. 14.00 (12.00–18.00), $p = 0.064$], but after 24 h of oXiris-CHFA, the surviving patients had significantly lower SOFA scores than non-surviving patients [10.00 (8.00–13.00) vs. 15.50 (12.00–17.00), $p < 0.001$] (Figure 3).

Site of primary infection

From the perspective of primary infection sites, the distribution of PCT and IL-6 percentage reduction was significantly ($p = 0.035$ and $p = 0.001$, respectively). The decline in PCT was most pronounced in skin and soft tissue infections (61.4%), followed by urinary (57.20%) and abdominal (43.63%) infections, and was least in blood (7.35%) and pulmonary infections (3.57%). The decrease in IL-6 was also the most pronounced in urinary tract infections (93.8%), followed by skin and soft tissue infection (80.42%), abdominal cavity infection (79.29%), and pulmonary infection (23.64%), and showed the least significant decrease in blood infection (0%) (Figure 4, Supplementary Table 1).

Other subgroup analyses

We divided the sample population into groups from different perspectives, such as renal function status at CRRT initiation, culture result type, CRRT method, blood flow velocity, and anticoagulation method, and compared whether there were differences in PCT, IL-6, SOFA score, the decline of lactate, vasoactive drug dosage, and the improvement of vital signs among the groups. The results showed that patients who started treatment at AKI1 stage had a greater decrease in IL-6 levels than those who started treatment later or had end-stage renal disease ($P = 0.027$) (Supplementary Table 1). Patients with a blood flow rate of 200 ml/min during CRRT showed more significant improvements in HR ($p = 0.012$) and RR ($p = 0.015$) than 150 ml/min (Supplementary Table 1). The results of subgroup comparison of different anticoagulation methods showed that the improvement of HR ($p = 0.009$) and RR ($p = 0.019$) was heparin (36.54%, 41.38%, respectively), no anticoagulation (23.79%, 30.73%, respectively), and citrate (11.74%, 11.88%, respectively) in order (Supplementary Table 1).

Regression analysis

We conducted a logistic regression analysis for patient age, pre-treatment SOFA score, percentage SOFA score decline, APACHE II score, pre-treatment creatinine level, total CRRT duration, oXiris duration, time from ICU to oXiris initiation, number of oXiris-filters use, percentage MAP increase, percentage HR decline, percentage RR decline, percentage PCT decline, and percentage IL-6 decline. The results showed that pre-treatment SOFA score ($p = 0.034$), percentage decrease in

TABLE 3 Hemodynamic and inflammatory biomarkers, metabolic changes, and blood platelet count during oXiris-CHFA treatment.

Parameter	Baseline ($n = 90$)	12 h ($n = 85$)	24 h ($n = 75$)	p value
MAP[a], mmHg	75.16 [66.00–84.00]	79.33 [73.00–85.84]	82.00 [77.67–88.67]	<0.001
Norepinephrine dosage[a], μg/kg/min	0.65 [0.14–1.50]	0.40 [0.12–1.00]	0.25 [0.00–0.70]	<0.001
HR[a], per min	122.00 [102.75–136.50]	101.00 [87.50–119.00]	96.00 [80.00–109.00]	<0.001
RR[a], per min	23.00 [19.75–29.00]	20.00 [16.50–21.00]	18.00 [16.00–20.00]	<0.001
SOFA[b]	14 [10.00–17.00]	–	11.00 [9.00–15]	<0.001
Lactate[a], mmol/L	5.15 [2.78–8.65]	3.6 [2.05–7.40]	3.20 [2.20–4.80]	0.008
Procalcitonin[a], ng/mL	23.70 [3.31–81.07]	12.9 [2.47–59.75]	11.90 [5.08–41.90]	<0.001
Interleukin-6[a], pg/mL	1986.50 [555.25–5000.00]	1245.00 [292.00–5000.00]	361.50 [138.75–1051.00]	<0.001
Blood platelet count[a], 10^9/L	83.50 [28.75–143.50]	–	44.00 [22.00–90.00]	<0.001

oXiris-CHFA, continuous hemofiltration adsorption with oXiris; MAP, mean arterial pressure; HR, heart rate; RR, respiratory rate; SOFA, Sequential Organ Failure Assessment. Non-normally distributed measurement data were expressed as median [interquartile range].

[a]Friedman's test for comparison between three groups.

[b]Wilcoxon symbol rank test for comparison between two groups.

p value <0.05 was considered as a significant difference.

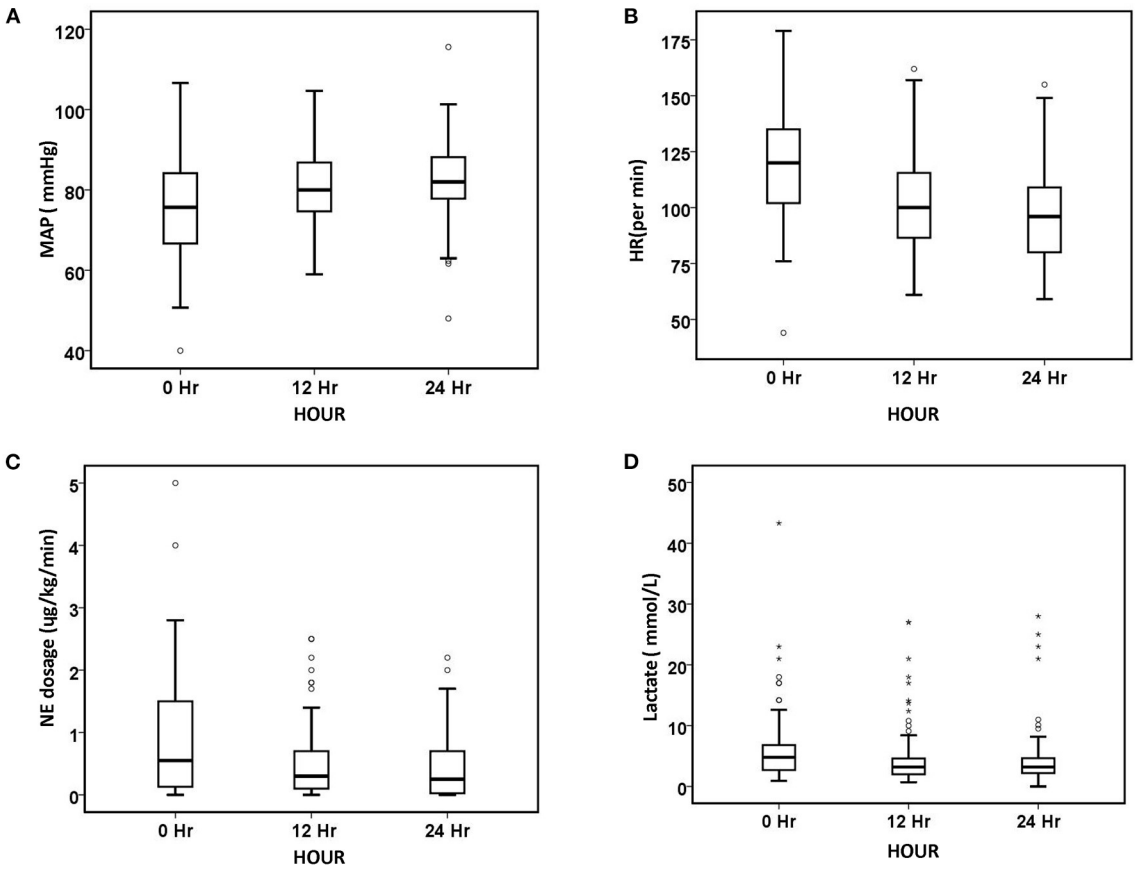

FIGURE 1
Changes in hemodynamic parameters and lactate level over 24 h. **(A)** MAP, **(B)** HR, **(C)** NE dosage, and **(D)** lactate. The number of patients at baseline, 12 h, and 24 h was 90, 85, and 75, respectively. The symbol * stands for outlier.

SOFA score ($p = 0.004$), and age ($p = 0.011$) were independently associated with ICU mortality rate. For every 1-unit increase in pre-treatment SOFA score, the risk of death increased by 27%; for every 1 year of age increase, and 8.6%, the risk of death increased by 8.6%; for every 1 increase in percentage decrease in SOFA score, a 6.6% reduction in risk of death.

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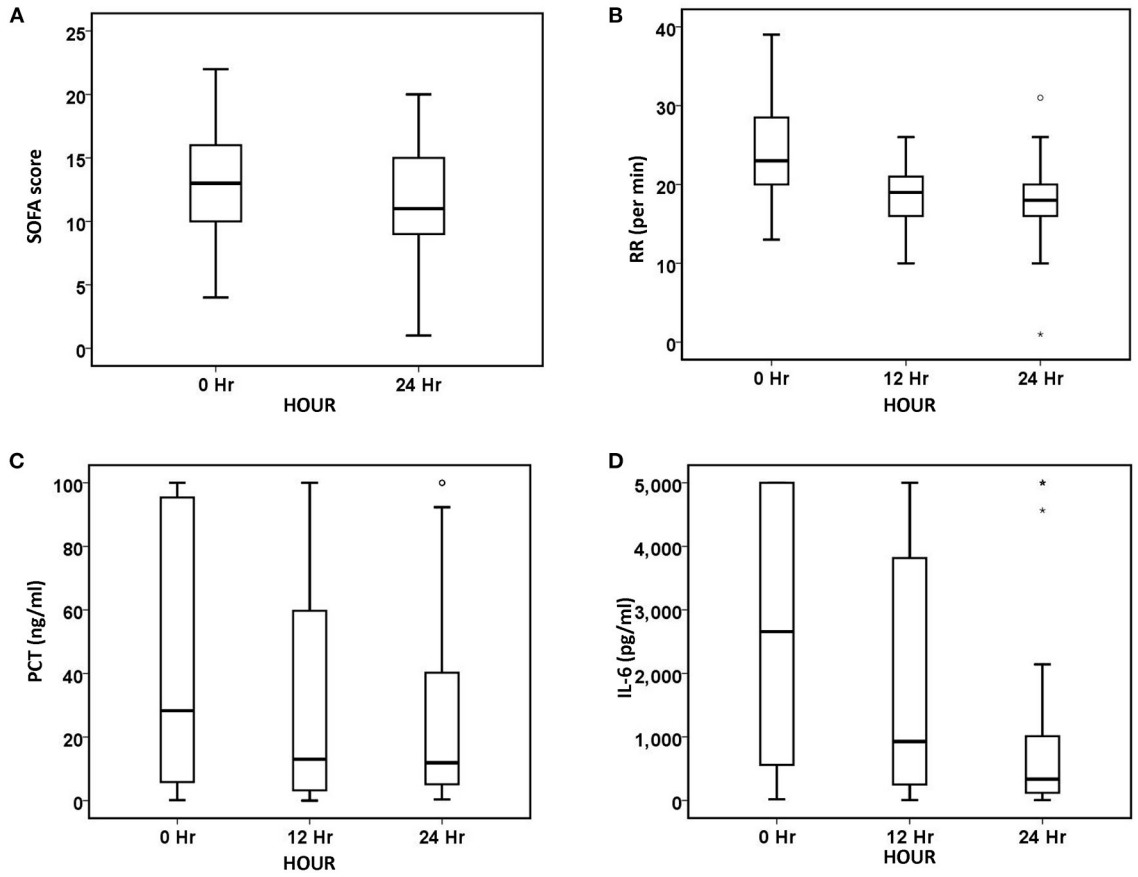

FIGURE 2
Changes in organ function and inflammatory biomarkers over 24 h. **(A)** SOFA, **(B)** RR, **(C)** PCT, and **(D)** IL-6. The number of patients at baseline, 12 h, and 24 h was 90, 85, and 75, respectively. The symbol * stands for outlier.

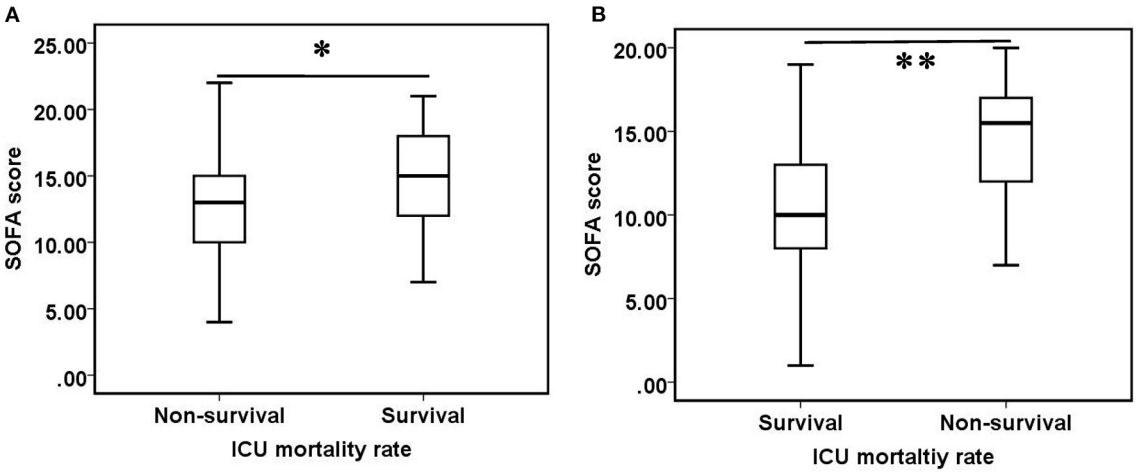

FIGURE 3
Comparison of SOFA scores between survival and non-survival groups. **(A)** Before oXiris-CHFA treatment (0 h), **(B)** after oXiris-CHFA treatment (24 h). *P = 0.064, **P < 0.001.

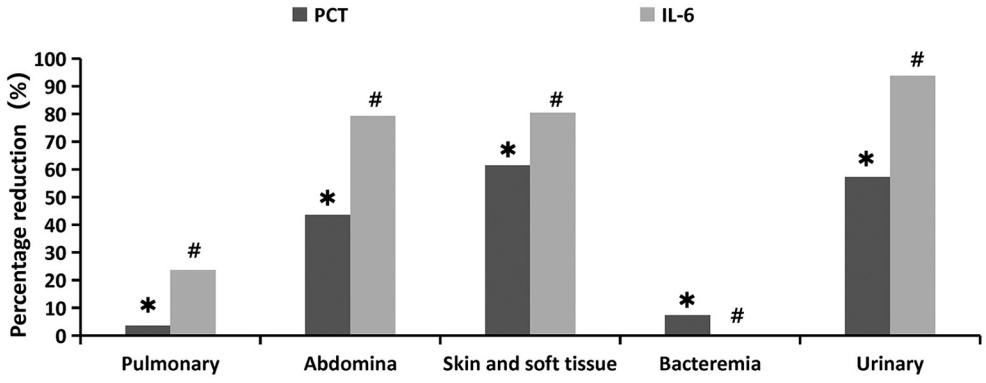

FIGURE 4
Percentage reduction of infection indicators at different primary infection sites 24 h after oXiris-CHFA treatment. *P = 0.035, #$p = 0.001$.

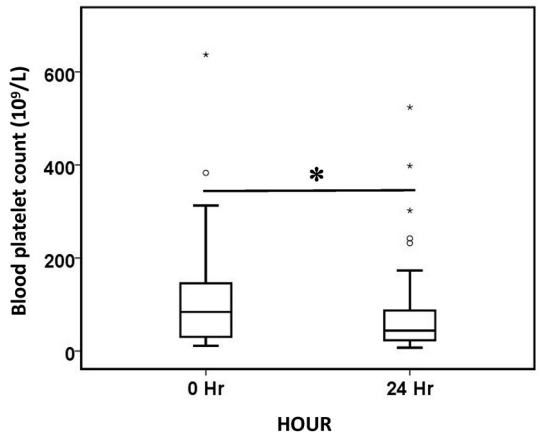

FIGURE 5
Comparison of blood platelet count before (0 h) and after (24 h) oXiris-CHFA treatment. *P < 0.001. The symbol * stands for outlier.

Adverse events

Blood platelet count decreased by 47.3% after 24 h of treatment (Figure 5). However, the decrease in platelets did not differ significantly between the different anticoagulation groups ($p = 0.054$), and it did not differ significantly among the different CRRT modality ($p = 0.905$). The majority of patients (83.3%) tolerated oXiris well; 15 patients experienced adverse events during treatment, with the most common being coagulation-related adverse events. 8 patients had high transmembrane pressure alarm in the treatment, 3 patients developed clotting deaeration chamber, 2 patients had hypotension during treatment, and 2 patients had abnormal pressure alarm at the arterial or venous end of the catheter.

Discussion

The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) defined sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection. This organ dysfunction can be identified as an acute change in the total SOFA score of 2 after infection. Septic shock is classified as a subtype of sepsis, defined as the need for a vasopressor to maintain MAP ≥65 mmHg despite adequate volume resuscitation, with serum lactate levels >2 mmol/L (18 mg/dL) (1).

According to a recent global survey, sepsis is a common disease worldwide. In 2017, 48.9 million cases of sepsis were reported worldwide, resulting in 11 million deaths, or 19.7% of all global deaths (13). Hospital mortality rate in patients with sepsis ranges from 15 to 30%, and the 1-year mortality rate is 35% (14–16). Septic shock has a higher risk of mortality, with hospital and 1-year mortality rates of 39–56 and 60%, respectively (15–17). Sepsis is not only an important public health problem (18, 19), it also presents a significant global economic burden (20).

Patients with sepsis undergo immune hyperactivation and experience a cytokine storm, which leads to multiple organ failure (3). Cytokine storm is a comprehensive term for generalized immune dysregulation characterized by systemic symptoms and systemic inflammation and multi-organ dysfunction (21). The severity of the cytokine storm is associated with patient prognosis in septic shock (22).

Measures to treat sepsis include antimicrobial application and infectious source control, optimization of hemodynamics (using fluid and vasoactive drugs), blood purification therapy, and immunomodulatory/targeted therapy. Different treatments may be required for each stage of sepsis (23), and timely intervention in the early stage of the cytokine storm has the potential to improve the prognosis of patients with sepsis.

The treatment targeting inflammatory mediators has become a new target in the treatment of sepsis, and the removal of systemic inflammatory mediators can be accomplished by blood purification therapy (3). Alleviating or eliminating endotoxin and cytokine storms in the body can be accomplished in various ways, including blood purification, which helps alleviate sepsis, improve patient hemodynamic status, and perhaps improve patient outcomes (3, 24–26).

Many different attempts have been made in the field of blood purification. For example, increasing the therapeutic dose, adjusting the interception molecular weight size, and using plasma exchange (27–31). A reasonable reason for the use of adsorption therapy in sepsis is the response to restoring balanced pro-inflammatory and anti-inflammatory mediators (32). Adsorption therapy is widely used in the treatment of sepsis and includes the use of cytokine adsorption columns (CytoSorb®) and polymyxin B adsorption columns (Toraymyxin®) (3, 32–35).

oXiris is a representative film material with high-adsorption film technology comprising three different layers (7). Its structure include AN69 copolymer hydrogel structures (to adsorb cytokines), multiple layers of polyethyleneimine (to adsorb endotoxins), and heparin grafting (to reduce local thrombogenicity). This unique design allows for a combination of four characteristics in one device: renal support, cytokine removal, endotoxin removal, and local anticoagulant therapy (7). It not only has high adsorption efficiency for cytokines and circulating endotoxin, but also has good blood compatibility, and can perform CHFA for patients without plasma separation. Recent studies have shown that the use of oXiris in patients with sepsis or septic shock can effectively reduce lactate levels, reduce concentrations of endotoxin and cytokines, optimize hemodynamics, reduce SOFA scores, and improve clinical outcomes (8, 9, 11, 36, 37). European Experience recommends oXiris in septic patients with unstable hemodynamic status, with or without AKI (38). The Asia Pacifica Experience also recommends that patients with sepsis or septic shock should be treated based on their hemodynamic indicators, microcirculation, and organ function, rather than AKI (39). Therefore, we believe that oXiris-based clinical research should be focused on patients' hemodynamic indicators and perfusion index and whether adverse reactions occurred in order to analyze the clinical outcome in terms of survival with oXiris treatment; this could provide a meaningful basis for the clinical treatment of sepsis.

In this study, a total of 90 patients with sepsis (median APACHE II: 24) were included; this cohort included patients with both ESRD and AKI. The initial SOFA score (median SOFA: 14) suggested that most patients had failure of more than two organs.

After 24 h of oXiris-CHFA treatment, we observed a 9.1% increase in MAP, 61.53% decreased in NE dose, 61.53% decreased in HR, 21.74% decreased in RR, and 37.86% decreased in lactate, suggesting that this treatment may improve hemodynamics and microcirculation perfusion in patients, showing the effectiveness of treatment intuitively in clinical situations. Among the parameters examined, the changes in lactate and improvement of hemodynamic indicators also appeared in parallel, consistent with the pathophysiological mechanism seen in sepsis patients. After oXiris-CHFA treatment combined with standard flow sepsis fluid resuscitation treatment, most patients seemed to obtain satisfactory hemodynamic status in a relatively short time (24 h), and their tissue perfusion quickly improved along with correction of internal environmental disorders such as hyperlactemia and subsequent acidosis caused by tissue hypoxia.

In terms of organ function maintenance, oXiris-CHFA treatment resulted in a decrease in SOFA score by 37.86%, and SOFA score decreased from higher high level of 14 (10.00–17.00) to lower level of 11.00 (9.00–15), indicating that this treatment can play a positive role in organ function maintenance in septic patients. A SOFA score change over 2 points is a reliable predictor of in-hospital mortality within the ICU (40). This change was very significant in the first 24 h of the first use of oXiris, suggesting that the use of this treatment as soon as possible may shorten the time of organ dysfunction, reduce the use of supportive care such as ventilators, vasoactive drugs, and blood products, and further shorten the organ support time of patients and reduce medical costs. The subsequent subgroup analysis also showed that although the difference in SOFA scores between the pre-treatment survival and non-survival groups was not significant, the SOFA scores in the survival group after oXiris-CHFA treatment were significantly lower than those in the non-survival group, indicating that functional status of the organs and improved prognosis were better in the former group of patients.

In terms of infection index, PCT showed a significant decrease after treatment (48.79%), while the IL-6 decrease was more obvious (81.80%). AS a widely used biomarker of sepsis, PCT is a precursor of calcitonin with extreme low level in general. However, almost all tissues and organs secrete PCT in pathological conditions and its generation is regulated by bacterial toxins and cytokines (41). It is used to guide the diagnosis and antibiotic treatment of sepsis (42–44), and it is also used as an indicator to evaluate the severity of sepsis (45). Its elevated concentration and non-clearance are closely related to the all-cause mortality of sepsis (46). IL-6, a well-known proinflammatory factor in cytokine storm, is a product of T cells that stimulates B cells and enhances antibody production. Together with IL-1 and the inflammatory mediator TNF, it is the main regulator of inflammation and one of the few true pleomorphic cytokines (47, 48). A decrease in IL-6 predicts the success rate of antibiotic therapy for sepsis in nonsurgical patients (49). Moreover, the dynamic change of IL-6 is closely related to the individual patient mortality rate (22). The IL-6 and PCT tests have similar diagnostic values in distinguishing sepsis

from non-infectious systemic inflammatory response syndrome (50). Declines in the above two indicators show that after oXiris-CHFA treatment, the cytokine storm level of inflammatory cytokines in the body decreased significantly, and the systemic inflammatory response state was improved relative to that before treatment. The application of oXiris treatment in the early stage can help correct the high inflammation state of patients as soon as possible (within 24 h), reduce the resulting organ function damage, and reverse hemodynamic instability, thus improving the prognosis of patients.

In the present study, the ICU mortality, 30-day mortality, and hospital mortality rates were 34.4, 44.4, and 35.6%, respectively, consistent with previously reported mortality rates from sepsis and septic shock (14–17). Given that more-critical patients, often with more severe inflammatory responses, were included, the physician in charge preferred to use oXiris-CHFA; thus, all included patients had severe sepsis. This is confirmed by the higher SOFA score (14) and APACHE II score (24) at baseline, and we further discuss the factors influencing mortality rate in the subsequent subgroup analysis results. Due to the different timing of inclusion, although 77 (85.56%) had 2 mmol/L lactate before oXiris-CHFA and 84 patients (87.00%) needed NE to maintain blood pressure, the actual number of patients with septic shock may not have had adequate fluid resuscitation.

The median time from ICU admission to initiation of oXiris treatment was 18 h, somewhat earlier than the 21–46 h in several other studies (11, 37, 51). The ICU mortality rate was also lower than previously reported rates (37). A retrospective study showed that patients who were started on oXiris treatment within 3 h of adequate resuscitation had reduced vasopressor use, decreased SOFA scores, and increased MAP compared with those who were started on oXiris after 3 h of adequate resuscitation (52). However, in this study, there was no significant difference in ICU mortality rate between patients initiated with oXiris-CHFA within 24 h versus after 24 h after admission ($p = 0.921$). A detailed review of the medical history showed that some patients were admitted to the ICU with a first diagnosis of severe pneumonia or hemorrhagic shock, liver and kidney failure, or cardiac and respiratory arrest rather than sepsis. In the course of ICU hospitalization, sepsis and septic shock occurred. That is, there was no indication for initiating oXiris-CHFA therapy when the patient first entered the ICU. Moreover, there are many factors affecting patient mortality, and inflammatory adsorption treatment is only part of the comprehensive treatment.

By comparing the two groups of patients in this study with prescribed therapeutic doses ≥30 mL/kg/h and <30 mL/kg/h, it appeared that no prescribed therapeutic dose was directly associated with ICU mortality rate. We found that the prescribed therapeutic doses of the included patients were basically within the appropriate range of the prescribed therapeutic doses of 25–30 mL/kg/h (actual achieved therapeutic dose 20–25 mL/kg/h) recommended by the Kidney Disease Improving Global Outcomes (KDIGO) guidelines (12). oXiris-CHFA–mediated improvement in endotoxin and cytokine storms in septic patients occurred mainly through the adsorption of the membrane (7). This effect is mainly related to the membrane area and filter use time rather than therapeutic dose, which may also be a prime reason for the above result.

Sepsis is a clinical syndrome with great heterogeneity rather than a class of diseases with relatively consistent etiology and pathophysiology (3). Numerous factors affect the prognosis of sepsis patients and include multi-organ system support treatment, volume management, etiology treatment, and rehabilitation treatment, CRRT does not independently influence patient treatment.

The therapeutic effect of oXiris-CHFA varies based on the primary site of infection in sepsis. The top three primary infection sites of inflammatory indicators (PCT and IL-6 decline) in this study were urinary tract, abdominal cavity, and skin and soft tissue, indicating that oXiris-CHFA treatment may be more clinically effective for infections originating from these regions. This is also consistent with previous reports that treatment with oXiris significantly improved hemodynamics and inflammation in patients with sepsis/septic shock due to abdominal, urinary, and skin soft tissue infections (8, 11, 53). Binary logistic regression analysis showed that both initial status and severity of decline in SOFA score were independently associated with ICU mortality. This shows that the more severe the organ failure before treatment, the higher the risk of death; the better the organ function recovery after treatment, the lower the risk of death. This finding is consistent with previous studies (18, 54).

From our results, oXiris-CHFA treatment initiated in the early stage of sepsis (AKI stage 1) may reduce the levels of proinflammatory factors such as IL-6 more significantly than in the later stage (AKI stage 2–3). It can also be understood that the main purpose of oXiris-CHFA is not to replace the kidney, but to clear the early inflammatory storm, which is also consistent with many previous literatures (3, 38, 39). As for the effect of blood flow rate and anticoagulation on the improvement of vital signs, we all know that blood flow rate is closely related to the anticoagulation method, and also related to the basal state of the patient. In the case of heparin anticoagulation or no anticoagulation, the blood flow rate of CRRT is usually 200 ml/min, while the blood flow rate of citrate anticoagulation is usually <150 ml/min to ensure the anticoagulation effect. We believe that in CRRT patients with heparin and without anticoagulation, a higher flow rate can be used to correct the possible volume overload in these patients more quickly, resulting in more significant improvements in HR and RR.

Platelet counts decreased after oXiris-CHFA treatment, but this difference was not significant in subgroup analyses comparing anticoagulation ($p = 0.054$) and treatment modalities ($p = 0.905$). The reasons may be as follows. First,

cardiopulmonary bypass lines activate blood coagulation. Although heparin has been pre-grafted in the oXiris filter, other parts of the extracorporeal line do not have anticoagulation efficacy, so anticoagulation is still activated, resulting in the consumption of coagulation substances and consequent decrease of platelet count. The results of this study suggest that thrombocytopenia is not caused by inadequate anticoagulation or improper mode setting. Second, diseases with active bleeding in the primary site or severe infection cause platelet decline. The above two reasons have been similarly reported in previous studies of case reports (53). Further studies need to explore whether oXiris will directly leadly to a decrease in platelet count. Other adverse events were mainly thrombosis-related adverse events, but no bleeding-related adverse events were seen. Studies have also shown that fixed heparin has no significant systemic anticoagulant or adverse bleeding events (55). This also suggests that we should regulate anticoagulation according to the condition of oXiris treatment to reduce activation of the coagulation system and the consumption of coagulation factors and platelets.

This study included 90 patients with different causes of sepsis and septic shock with improvement in hemodynamic parameters, lactate levels, and organ function after oXiris-CHFA treatment; recorded changes in infection and inflammatory indicators and platelet changes; and finally recorded patient outcome. The present study has some limitations. First, this is a descriptive small case series, lacking a control group with a heterogeneous group of patients (i.e., infectious source, duration of antibiotic administration, resuscitation regimen) and multiple concomitant interventions (e.g., CRRT, antibiotics, ECMO, hemoperfusion, and vasopressors). This is probably the main limitation of this article. There are three reasons for the absence of control in this study: (1) All sepsis patients in our hospital during the same period (from November 2020 to now) have been treated with oXiris. (2) If historical control is selected, given the rapid update of sepsis guidelines in recent years, especially in fluid resuscitation and hemodynamic management, which have a great impact on the results of this study, the control group can provide limited reference. (3) This study focuses on the changes in hemodynamics, perfusion level and organ function of patients before and after oXiris use, and whether oXiris has different effects on patients with different primary infections. Second, due to the retrospective study design, PCT or IL-6 results were missing in some cases. Third, some patients eventually abandoned treatment for discharge owing to non-medical factors, which may have had an impact on the final outcome. Finally, whether improvements in hemodynamic and metabolic parameters might be achieved only by infection control and CRRT itself, and not necessarily by oXiris-CHFA treatment. oXiris-CHFA treatment can be used as an adjuvant treatment for sepsis patients, but further randomized controlled trials with a larger sample size are needed.

Conclusions

In patients with sepsis or septic shock, oXiris-CHFA treatment was associated with a significant improvement in hemodynamic measures, significantly decreased vasoactive drug dosage, reduced lactate level and infection measures, and decreased SOFA score after treatment. The SOFA score was an independent risk factor for ICU mortality. However, improvement of SOFA score after oXiris-CHFA treatment was scarcely reported and we provided convincing evidence in the present study. In terms of the primary site of infection, patients with skin and soft tissue, urinary tract, and abdominal cavity infections benefitted the most from treatment with oXiris-CHFA. The results of this study show that the efficacy and safety of oXiris-CHFA treatment are relatively high. It's worth noting that we observed no evidence that a therapeutic dose of $\geq$30 mL/kg/h improves survival rate of patient, and the decrease in platelet count may be multifactorial.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of the Second Xiangya Hospital of Central South University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YZ and JL helped in conceptualization, writing of the original draft, data curation, formal analysis, methodology, organization of results, assisted with conceptualization, fund acquisition, supervision, and writing-review and editing. YP, DZ, and XX performed data collection. YZ, CW, and LO analyzed the data.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Epidemiology, management, and outcome of infection, sepsis, and septic shock in a German emergency department (EpiSEP study)

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Background: The adjacent conditions infection, sepsis, and septic shock are among the most common causes of treatment in the emergency department (ED). Most available data come from intensive care units (ICU) and include nosocomial infections acquired during hospitalization. Epidemiological data from German EDs are not yet available, although the ED is one of the first points of contact for patients. The aim of this study was to investigate the epidemiology, causes, diagnosis, mortality, and treatment of patients with infections in the ED.

Materials and methods: In this retrospective, single-center observational study, routinely collected data from the patient data management system and from the hospital information system were analyzed. All adult patients who presented to the ED in connection with an infection during the study period from 01/01 to 28/02/2019 were included. Exclusion criteria were age $\leq$ 17 years and incomplete records. Three groups (I. Infection, II. Sepsis, and III. Septic shock) were defined according to SEPSIS-3 definitions.

Results: During the study period, a total of 6,607 patients were treated in the ED. Of these patients, 19.3% (n = 1,278) had an infection (mean age 56 $\pm$ 23 years, 50% female). The sites of infection were distributed as follows: Respiratory tract 35%, genitourinary tract 18%, maxillofacial/ears/nose/throat 14%, intraabdominal 13%, soft tissues 10%, central nervous system 1%, other cause 3%, or unknown cause 6%. Infection only, sepsis and septic shock were present in 86, 10, and 3%, respectively. There were significant differences

in vital signs as well as in the various emergency sepsis scores across the predefined groups [I vs. II vs. III: SOFA (pts.): 1 ± 1 vs. 4 ± 2 vs. 7 ± 3 ($p < 0.0001$), systolic blood pressure (mmHg): 137 ± 25 vs. 128 ± 32 vs. 107 ± 34 ($p < 0.05$), heart rate (bpm): 92 ± 18 vs. 99 ± 23 vs. 113 ± 30 ($p < 0.05$), respiratory rate (min-1): 18 ± 4 vs. 20 ± 7 vs. 24 ± 10 ($p < 0.05$)]. In the three groups, blood cultures were obtained in 34, 81, and 86%, of cases, respectively and antibiotics were administered in the ED in 50, 89, and 86%, of cases respectively. The 30-day mortality rate in the three groups was 1.6, 12.0, and 38.1%, respectively.

Conclusion: This study is the first to show the incidence, management, and outcome of patients classified as infection, sepsis, and septic shock in a German ED. The findings of our real-world data are important for quality management and enable the optimization of treatment pathways for patients with infectious diseases.

KEYWORDS

epidemiology, infection, sepsis, septic shock, emergency department

Introduction

Infections, sepsis, and septic shock are among the most common causes of treatment in the emergency department (ED) (1–3). The recognition, diagnosis, and initial treatment of patients with infections, sepsis, and septic shock represent a challenge for all involved in medical care that should not be underestimated (4, 5). For this reason, there are international and national recommendations for the management of sepsis (6–8).

However, while local infection can be treated well in the ED, patients with sepsis and the associated life-threatening organ dysfunction show a considerably less favorable course of disease associated with high mortality (1, 2, 4, 9).

Therefore, it is relevant to know the source of the most common infections, sepsis, and septic shock even in the ED (10). It is important to note that the source and frequency of infectious diseases treated in the ED do not necessarily have to correspond to those found in sepsis or septic shock. Compared to the quality of national and international epidemiological knowledge from the Intensive Care Unit (ICU) data on infection, sepsis and septic shock in the ED are extremely sparse (6).

The collection of appropriate real-life data is the basis for future optimization of training and care concepts, early detection, development of guidelines and standard operating procedures (SOP), and patient safety in the ED. The aim of this retrospective, single-center study was therefore to compare epidemiology, management, and outcome of patients with infection, sepsis, or septic shock in a German ED.

Materials and methods

Study design and population

In this retrospective, mono-centric observational study, all adult patients admitted to the ED of the University Hospital Düsseldorf for infection, sepsis, or septic shock between 01/01 and 28/02/2019 were included. The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf, Germany (2020-973).

Setting

More than 44,000 patients are treated annually in the ED of the University Hospital of Düsseldorf, Germany. The ED is the first point of contact for almost all non-scheduled emergency patients. Only patients requiring urgent intervention (e.g., ST-segment elevation infarct) bypass the ED according to local protocols. The ED is part of a level I trauma center for the treatment of severely injured patients by a dedicated trauma team in accordance with national recommendations (11). Out-of-hospital care is provided by a two-tier emergency medical service (EMS) staffed with paramedics and emergency physicians. At our facility, patients are cared for in the ED by a team of nurses, residents, and senior physicians with expertise in emergency and critical care medicine. There are twelve regular cabins, four resuscitation rooms and a decision unit with twelve monitored beds in the ED.

Data collection

Demographical and medical care data were anonymously aggregated from the patient data management system (COPRA®, COPRA System GmbH, Berlin, Germany) and the hospital information system (MEDICO®, Cerner Deutschland GmbH, Itstein, Germany) by database query and transferred to a spreadsheet program (Microsoft® Office 365, version 16.37, Microsoft Corporation, Redmond, WA, USA). The analysis included age, sex, weight, height, infectious diseases, comorbidities, site of infection, in-hospital treatment (e.g., fluid resuscitation, laboratory tests, blood cultures, antibiotic therapy, therapeutic measures), vital signs (e.g., systolic blood pressure, respiratory rate, oxygen saturation by pulse oximetry, body temperature), transfer location (e.g., normal ward, ICU), and outcomes (length of stay in the ED, length-of-hospital-stay, 30-day mortality). The time of measurement of the data evaluation in relation to the 1 h-bundle refers to 1 h after admission to the ED.

Study definitions and emergency medicine sepsis scores

Patients were divided into three groups: I: Infection alone (without sepsis or septic shock), II: sepsis, III: septic shock based on the current SEPSIS-3 definition (6). Accordingly, sepsis is defined as a life-threatening organ dysfunction due to dysregulation resulting from infection. Organ dysfunction is determined by an acute 2-point change in SOFA score (6, 12). Septic shock is defined by catecholamine requirement to maintain a mean arterial blood pressure (MAP) of above 65 mmHg and a lactate level greater than 2 mmol/L despite adequate volume substitution (30 ml/kg) (6).

The following scores were calculated for all patients enrolled: Quick Sequential Organ Failure Assessment score (qSOFA) (6), SIRS (13), Prehospital Early Sepsis Detection (PRESEP) (14), modified National Early Warning Score (MEWS) (15), Sepsis-related organ failure assessment (SOFA) (12), and Mortality in Emergency Department Sepsis (MEDS) (16).

Statistical analysis

Data are presented as numbers and percentage, mean ± standard deviation (SD), median with interquartile ranges, as appropriate. The chi-squared test was applied for categorical data, and the Student's *t*-test for metric data. All tests used were two-sided, and statistical significance was set at $p < 0.05$. Microsoft Excel 2011 (Microsoft, Redmond, WA, USA) and DataGraph 4.5.1 (Visual Data Tools Inc. 2006–2022) were used for statistical analyses and to prepare figures.

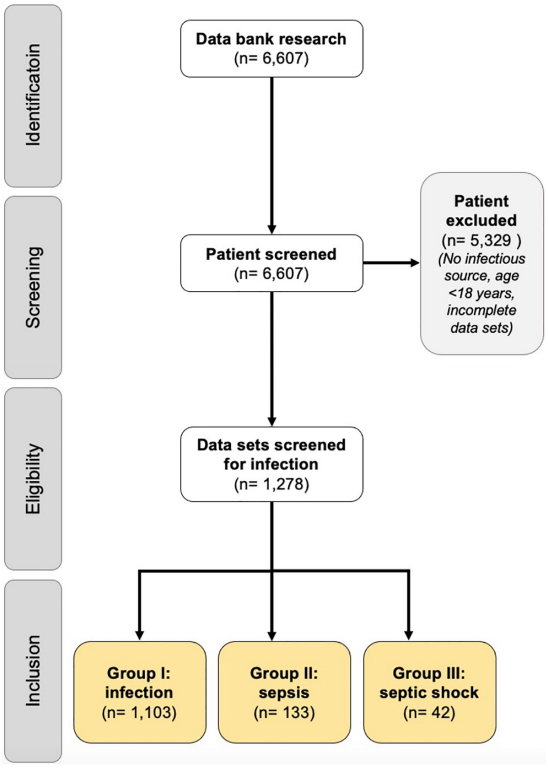

FIGURE 1
Prisma chart. A total of 6,607 patients were detected from the patient management system database as a potential study population. These 6,607 patients were screened for inclusion and exclusion criteria, resulting in the inclusion of 1,278 patients with documented infection focus. A total of 5,329 patients had to be excluded from the study because of relevant exclusion criteria (e.g., age < 18 years. Incomplete records, no infection focus). The study population of 1,278 patients was divided into infection (group I), sepsis (group II), and septic shock (group III) groups.

Results

During the 2-month study period, a total of 6,607 patients of all ages were treated in the ED. Patients with incomplete records and patients ≤ 17 years were excluded. After detailed screening and individual case examination, the final data set consisted of 1,278 patients (19.3%) with an infectious disease. Of these patients, 1,105 (86.5%) patients had an infection (group I), 133 (10.4%) had sepsis (group II), and 42 (3.3%) had septic shock (group III). The participant flow chart is shown in Figure 1. In relation to all ED visits during the study period, these results correspond to an incidence of infection alone, sepsis and septic shock of 16.7, 2.0, and 0.6%, respectively.

Patient characteristics

An overview of patient characteristics is provided in Table 1. The age of the patients increased significantly across the three predefined groups. In comparison to group I, the frequency of pre-existing concomitant conditions and life-limiting comorbidities, increased in groups II and III. The different emergency medicine sepsis scores steadily increased across the three predefined groups (Table 1).

Vital signs

The variability of vital signs in the three groups is shown in Figure 2. The shock index increases with the severity of the disease, with increasing tachycardia and hypotension (Table 1). Meanwhile, the variability of the measured body temperature and respiratory rate increases (Table 1).

Source of infection

The sources of infection in the groups I–III are shown in Figure 3. The predominant site of infection in all groups was the respiratory tract. Compared to group I, the proportion of respiratory infections doubled in patients with septic shock. The second most common source of infection was the genitourinary tract Infections of soft tissue, maxillofacial/ears/nose/throat were predominantly found in group I, suggesting a less frequent cause of sepsis and septic shock. The proportion of intra-abdominal infections decreased slightly with increasing sepsis severity from 13 to 9.5%. Despite extensive diagnostic investigations, the source of infection remained elusive in 5.0, 8.3, and 9.5% of patients in group I, II, and III, respectively.

Diagnostic workup and emergency interventions

Diagnostic procedures performed during treatment in the ED are presented in Table 2. The distribution of focus in the three groups was also reflected in the samples collected in the ED. Urine status, and, in the case of sepsis or septic shock, urine culture was obtained most frequently. With increasing severity of the disease, the diagnostic effort for the focus search increased significantly. Similar to the predominant respiratory source of infections, point-of-care testing (POCT) for influenza A/B and RSV was also performed in all three groups.

While only 16.3% of patients in group I underwent computed tomography (CT), CT was performed in 57.1% of patients in group III. Also, the use of sonography increased from 23% in group I to 53% in group II to 55% in group III.

As expected, the frequency of antibiotics administered orally decreased from 23.1 to 5.3% and 0% in groups I, II, and III, respectively. The opposite was observed for the frequency of administration of intravenous antibiotics within the first hour after admission to the ED (groups I-III: 5.3, 8.3, and 14.3%, respectively).

Guideline-based therapy according to the 1-h bundle and other ED emergency interventions increased in patients with sepsis and septic shock (Table 2).

Relocation sites and outcomes

Cohort-specific relocations sites and outcomes are shown in Table 3. Significant differences for the three groups are evident in the subsequent follow-up treatment. While 51% of patients from group I can still be treated as outpatients, the respective percentage in group III is 0%. Conversely, 1.5% from group I, 14.3% from group II, and 59.5% from group III required intensive care.

Length of stay (LOS) in the ED for patients with infection, sepsis, and septic shock was 500 ± 505, 867 ± 507, and 666 ± 475 min, respectively. Hospital LOS was 5 ± 9, 12 ± 14, and 11 ± 15 days in groups I–III, respectively.

The 30-day mortality increased significantly from 1.6% in patients with infection, to 12.0 in patients suffering from sepsis and 38.1% from septic shock. As many as four patients with septic shock (9.5%) died in the ED. In contrast, one patient with sepsis (0.8%) and no patient with infection died in the ED. During the whole hospital stay, 1.7% from group I died, as well as 12.0% from group II and another 38.1% from group III.

Discussion

In the present EpiSEP study, we show for the first time the significant differences in epidemiology, management and outcome of patients with infection alone, sepsis and septic shock in the ED. In the study cohort, which included more than 6,000 ED visits, one in five ED patients suffered from an infection during the study period. Using the SEPSIS-3 definition (6), 10.4% of the patients with infections suffered from sepsis, and 3.3% from septic shock. Our study thus shows for the first time care data and approximate incidence rates of infections, sepsis and septic shock in ED patients.

Despite considerable advances in medicine, sepsis is a condition that continues to be associated with high inpatient mortality, being the third leading cause of death in non-surgical ICU and the leading cause of death in non-cardiac and surgical ICU (17–19). Previous epidemiological studies on sepsis and septic shock were mainly conducted in the ICU setting (9, 18, 20–22) (Table 4).

There, a significant proportion of infections are of nosocomial origin, so that the source of infection is much more

TABLE 1 Patient characteristics of the patients suffered from infection, sepsis, and septic shock in the emergency department.

	All (n = 1,278)	Group I infection (n = 1,103)	Group II sepsis (n = 133)	Group III septic shock (n = 42)
Age (years, mean ± SD)	56 ± 23	**53 ± 23***	**69 ± 19****	72 ± 13
Male sex [n (%)]	637 (49.8)	546 (49.5)	67 (50.4)	25 (59.5)
Hospital admission by				
EMS [n (%)]	520 (40.7)	**390 (35.4)***	**92 (69.2)****	**38 (90.5)*****
Walking emergency [n (%)]	600 (46.9)	**568 (51.5)***	**29 (21.8)****	**3 (7.1)*****
Family doctor [n (%)]	91 (7.1)	**88 (8.0)***	3 (2.3)	0 (0.0)
MET [n (%)]	25 (2.0)	20 (1.8)	4 (3.0)	1 (2.4)
Interhospital transfer [n (%)]	13 (1.0)	10 (0.9)	3 (2.3)	0 (0.0)
Medical specialist [n (%)]	29 (2.3)	27 (2.4)	2 (1.5)	0 (0.0)
Residence				
Home [n (%)]	1167 (91.3)	**1023 (92.7)***	111 (83.5)	**33 (78.6)*****
Nursing home [n (%)]	110 (8.6)	**79 (72)***	22 (16.5)	**9 (21.4)*****
Pre-existing conditions				
COPD [n (%)]	119 (9.3)	**85 (7.7)***	22 (16.5)	**12 (28.6)*****
Kidney disease [n (%)]	164 (12.8)	**114 (10.3)***	39 (29.3)	**11 (26.2)*****
with dialysis [n (%)]	42 (3.3)	**21 (1.9)***	16 (12.0)	**5 (11.9)*****
Heart failure [n (%)]	54 (4.2)	**42 (3.8)***	11 (8.3)	1 (2.4)
Immunosuppression [n (%)][1]	149 (11.7)	**115 (10.4)***	26 (19.5)	8 (19.0)
Liver cirrhosis [n (%)]	19 (1.5)	**13 (1.2)***	5 (3.8)	1 (2.4)
Diabetes mellitus [n (%)]	207 (16.2)	**153 (13.9)***	39 (29.3)	**15 (35.7)*****
with insulin [n (%)]	81 (6.3)	63 (5.7)	13 (9.8)	5 (11.9)
Malnutrition [n (%)]	34 (2.7)	23 (2.1)	6 (4.5)	**5 (11.9)*****
Tumor disease [n (%)]	133 (10.4)	**100 (9.1)***	22 (16.5)	**11 (26.2)*****
Chemo-/Radiotherapy [n (%)]	49 (3.8)	38 (3.4)	9 (6.8)	2 (4.8)
Hematological diseases [n (%)]	126 (9.9)	**85 (7.7)***	31 (23.3)	**10 (23.8)*****
Transplantation [n (%)]	53 (4.1)	**40 (3.6)***	10 (7.5)	3 (7.1)
HIV [n (%)]	13 (1.0)	11 (1.0)	2 (1.5)	0 (0.0)
None [n (%)]	731 (57.2)	**695 (63.0)***	29 (21.8)	**7 (16.7)*****
Laboratory values				
Creatinine (mg/dl, median, IQR)	0.92 (0.73–1.26)	**0.87 (0.71–1.12)***	1.41 (0.96–2.15)	**1.67 (1.14–3.01)*****
Bilirubine (mg/dl, median, IQR)	0.50 (0.34–0.79)	**0.47 (0.33–0.74)**	0.63 (0.44–1.06)	**0.62 (0.37–1.05)*****
Leucocyts (X1000/µl, median, IQR)	10.2 (7.40–14.05)	**10.0 (7.4–13.73)***	11.7 (6.80–14.60)	**12.8 (10.10–17.73)*****
Thrombocyts (X1000/µl, median, IQR)	245 (189–306)	**250 (200–307)***	186 (141–285)	**225 (166.25–277.50)*****
Lactate (mmol/l, median, IQR)	1.5 (1.10–2.10)	**1.4 (1.00–2.00)***	**2.0 (1.30–2.50)****	**3.5 (1.68–5.45)*****
Vital signs (median, IQR)	**admission**	**discharge**	**admission**	**discharge**	**admission**	**discharge**	**admission**	**discharge**
SBP (mmHg; median, IQR)	133 (119–147)	119 (103–141)	**134 (122–148)***	**124 (107–145)***	**123 (109–144)****	110 (99–139)	100 (86–132)	**102 (94–134)*****
HR (bpm; median, IQR)	92 (80–105)	82 (72–96)	**90 (80–102)***	**80 (70–92)***	**99 (87–102)****	**86 (71–103)****	**110 (97–125)*****	**91 (80–123)*****
SI (min/mmHg; median, IQR)	0.7 (0.6–0.8)	0.7 (0.6–0.9)	**0.7 (0.6–0.8)***	**0.6 (0.5–0.8)***	**0.8 (0.7–1.0)****	**0.8 (0.6–1.0)****	**1.1 (0.8–1.4)*****	**0.9 (0.8–1.0)**
SpO2 (%,; median, IQR)	97 (95–99)	96 (94–98)	97 (95–99)	96 (94–98)	96 (93–98)	**96 (94–99)****	**94 (91–98)*****	**95 (93–99)*****
RR (min-1; median, IQR)	18 (15–20)	22 (18–27)	**18 (15–19)***	22 (18–27)	**18 (16–21)***	23 (19–28)	**20 (16–29)*****	22 (17–27)
Temp (°C; median, IQR)	36.9 (36.3–37.3)	37 (36.3–37.8)	36.9 (36.4–37.6)	37 (36.4–38)	36.9 (36.2–38)	37.3 (36.4–37.8)	36.6 (36–38)	36.2 (35.7–37.5)
GCS (points; median, IQR)	15 (15–15)	n.a.	15 (15–15)*	n.a.	**15 (14–15)****	n.a.	**14 (9–15)*****	n.a.

(Continued)

TABLE 1 (Continued)

	All (n = 1,278)	Group I infection (n = 1,103)	Group II sepsis (n = 133)	Group III septic shock (n = 42)
Emergency medicine Sepsis scores				
qSOFA (pts; median, IQR)	0 (0–0)	**0 (0–0)***	1 (0–1)	**1 (1–2)*****
SOFA (pts; median, IQR)	1 (0–2)	**0 (0–1)***	**3 (2–5)****	**7 (5–8)*****
SIRS (pts; median, IQR)	1 (1–2)	**1 (0–2)***	2 (1–3)	**2 (2–3)*****
MEWS (pts; median, IQR)	1 (1–3)	**1 (0–2)***	**3 (1–4)****	**5 (3–6)*****
PRESEP (pts; median, IQR)	2 (0–4)	**2 (0–3)***	**3 (2–5)****	**5 (3–6)*****
MEDS (pts; median, IQR)	3 (0–8)	**3 (0–6)***	**8 (5–11)****	**13 (10–15)*****

N, number; SD, standard deviation; pts, points; RSV, respiratory syncytial virus; EMS, emergency medical services; MET, medical emergency team; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; SBP, systolic blood pressure; HF, heart rate; bpm, beats per minute; SI, shock index; SpO2, oxygen saturation by pulse oximetry; RR, respiratory rate; Temp, temperature tympanal; GCS, Glasgow Coma Scale; IQR, interquartile range; qSOFA, quick sequential organ failure assessment; SOFA, sepsis-related organ failure assessment score; SIRS, systemic inflammatory response syndrome; MEWS, modified early warning score; PRESEP, prehospital early sepsis detection score; MEDS, mortality in emergency department sepsis score.
p is significant, if $p < 0.05$; * = $p_{I,II} < 0.05$; ** = $p_{II,III} < 0.05$; *** = $p_{I,III} < 0.05$.
The bold values represent significant results.

frequently determined by previous operations, interventions or prolonged invasive ventilation. Moreover, intensive care patients often require more specific therapeutic measures than patients who present to the ED for the first time with symptoms that may initially be unspecific. In the Extended Prevalence of Infection in Intensive Care (EPIC) I study, showed that 45% of ICU patients were treated due to one or more infections. Of these, only 14% were community-acquired, whereas 10% were hospital-acquired, and 21% ICU-acquired (23). EPIC III came up with similar results in terms of ICU-acquired infections (21). The prospective, multicenter German Incidence of severe sepsis and septic shock (INSEP) study even described that 57% of sepsis cases were nosocomial-associated, and, of these, 50% were ICU-acquired (9). Consequently, these epidemiological figures from studies in the intensive care unit cannot be transferred to the ED. Although according to the recommendations of the Surviving Sepsis Campaign (SSC) guidelines (7) sepsis should be recognized as soon as possible, there are no comprehensive epidemiologic studies on infection, sepsis, and septic shock in the ED.

In a comparison of our epidemiologic data with the most common ICU studies on sepsis (9, 18, 20–22) (**Table 4**), we were able to show that the weighting of the focal distribution differs significantly apart from the respiratory and genitourinary tracts. It suggests that abdominal and bloodstream infections are significantly more common in ICU than in the ED. This is probably due to nosocomial acquired infections in particular. Although the dominant infection focus in the ED is represented by the respiratory tract, it appears to be a disproportionately frequent focus in EPISEP compared with other ED studies (**Table 5**). The reason for this could be the seasonal influence in the EpiSEP study. In the EpiSEP study, soft tissue infections also occur significantly less frequently than in all other ED studies included in **Table 5**, which may be due to the fact that our dermatology department has its own ED. The proportion of unknown infection sites also seems to be lower in ICUs than in ED patients, which is probably due to the more aggressive diagnostics. These differences indicate that sepsis appears to present even more heterogeneously in the ED than in intensive care units. Future guidelines should take this into account in order to optimize early diagnosis and treatment already in the ED.

As shown in **Table 5**, sepsis and septic shock seem to be very heterogeneous regarding the source of infection in the ED (24–28). Different approaches to identify patients with sepsis and septic shock confound the true incidence of these conditions. This is often based on now outdated sepsis definitions or on the inclusion of a study population based on ICD-10 coding. Overall, the respiratory tract clearly dominates in septic shock in the study comparison (24–28). Nevertheless, the question remains as to where the differences in the source of infection between EpiSEP (61.9% respiratory tract in septic shock) and, for example, ARISE (32.8% respiratory tract in septic shock) originate (24).

With reference to the epidemiological findings of the EpiSEP study, patients of the infection group were younger than these suffering from sepsis and septic shock, whereas the mean age of the latter was the same or older such as in the EGDT River's study (29), ARISE (63 ± 17) (24), ProCESS (60 ± 16) (26), and ProMISE (66 ± 15) (30).

Consistent with the results of other studies (22–25), patients in the EpiSEP study showed significant changes in vital signs as a function of disease severity: Compared with patients with infection alone, patients with sepsis or septic shock were more hypotensive, presented with tachycardia, and had a higher respiratory rate as well as lower shock index and oxygen saturation. In line with the patients suffering from septic shock in the ARISE study (24), patients of the EpiSEP study

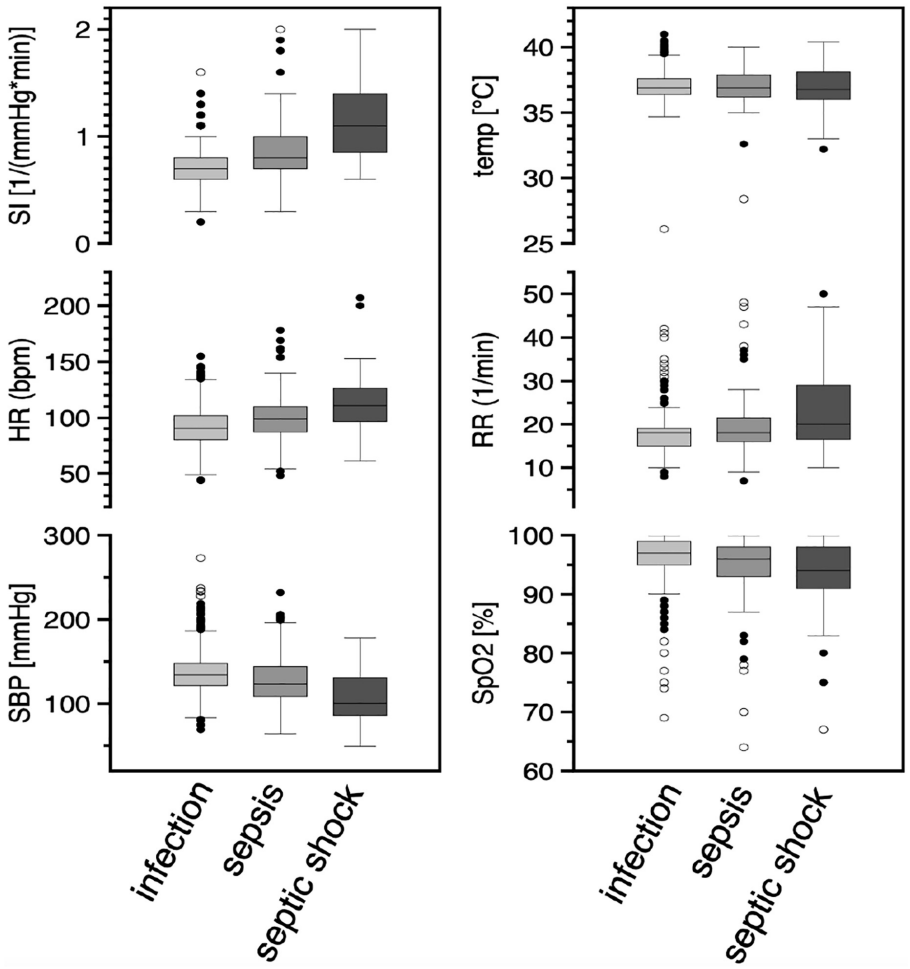

FIGURE 2
Vital signs in the three subgroups of patients. Results were illustrated as box-and-whisker-plot with 25 and 75%-quantiles (box) median (bar), 1.5X interquartile range and outliers (circles) in the three subgroups (I: infection n = 1,103, II: sepsis n = 133, III: septic shock n = 42). Abbreviations: SI, shock index; HR, heart rate; SBP, systolic blood pressure; temp, temperature tympanal; RR, respiratory rate; SpO2, oxygen saturation by pulse oxymetry.

with septic shock showed a comparable mean lactate level of 4.4 ± 3.8 mmol/l.

The group comparison showed a significant discrimination of the three groups by the emergency medicine sepsis scores (Table 1). The SOFA score used by the guideline for the detection of sepsis is not immediately available at the time of admission due to parameters such as the Horovitz quotient or necessary laboratory values. Furthermore, at least 21.8% in group II and 7.1% of the patients with a septic shock arrived in the ED as a "walking emergency." This shows the importance of a structured assessment and the use of scores to recognize critically ill patients at ED admission.

We found that the infection sites in the three subgroups of the EpiSEP study differ significantly. The leading causes of infection in the EpiSEP study were respiratory tract disease, genitourinary tract disease, maxillofacial/ears/nose/throat area and intra-abdominal causes, and soft tissue infections. The high proportion of patients with infection focus in the maxillofacial/ears/nose/throat area is the prime example that these focuses are very relevant in an ED but does not seem to represent a relevant focus for sepsis and septic shock. In addition, there are more patients in the ED whose infectious focus could not be clearly identified during the ED stay.

Patients with severe sepsis and septic shock in the four mentioned ED studies showed the following causes: respiratory tract 31.9–39.5%, urogenital tract 17.3–27.2%, intraabdominal 5.9–15.7%, and other causes in 26.9–40.0% (22–25). Based on a comparison of different ICU studies (Table 5), with increasing disease severity the respiratory tract is the dominant focus in sepsis and septic shock, the other causes are more or less comparable (9, 18, 20–22).

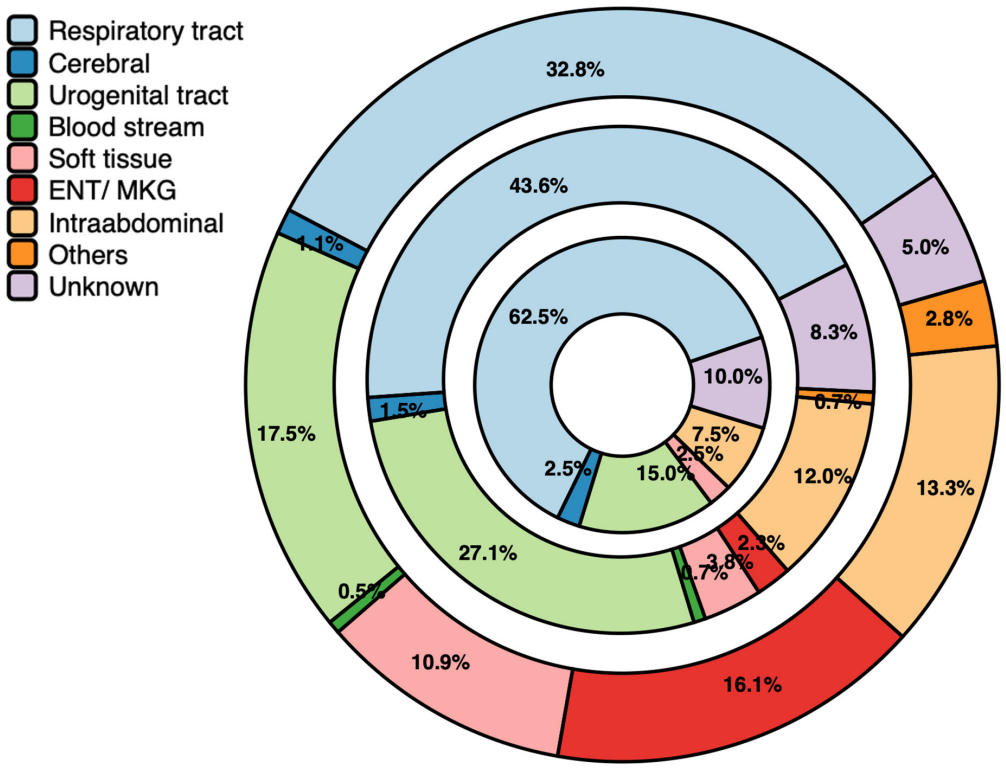

FIGURE 3
Sources of infection. Results were illustrated as circles represented the distribution in percentage (%) of the infection sites in the three subgroups (I: infection *n* = 1,103, outer circle; II: sepsis *n* = 133, middle circle; III: septic shock *n* = 42, inner circle).

The 1-h bundle of the SSC included (1) measurement of lactate level, (2) collection of blood culture before administration of antibiotics, (3) early administration of broad-spectrum antibiotics, (4) initiation of rapid administration of crystalloid solution, (5) application of vasopressors (7). While these 5 items were not fulfilled or only partially fulfilled in the infection group, the degree of fulfillment was higher in the EpiSEP group with sepsis and septic shock. The chosen time of measurement, 1 h after admission, suggests that the actual guideline adherence with fulfillment 1 h after diagnosis, should be significantly higher. Nevertheless, future timely documentation is essential for accurate evaluation of guideline adherence.

As recommended by SCC, measurement of lactate in the EpiSEP study was performed in more than 94.7–95% in patients suffering from sepsis and septic shock (**Table 2**). Positive blood cultures are associated with more frequent multiorgan failure and higher mortality. Therefore, the obligatory recruitment of blood cultures in the ED with subsequent possible isolation of a pathogen sets the course for an empirical adjustment of antibiotic therapy during the course (31). The compliance of our ED treatment with the SSC guidelines in the subgroup of patients suffer from sepsis and septic shock can be considered as very high as the proportion of performed blood cultures was 81–88%, and the administration of broad-spectrum antibiotics after blood culture recruitment was performed in 71–76% of cases. It is well known that the initial administration of broad-spectrum antibiotics, must be reevaluated promptly in the early follow-up. Antibiotic administration occurred within 1 h in 8.3% in sepsis and 14.3% in septic shock (**Table 2**). The validity of these data is limited by the time of measurement that was chosen (1 h after admission). Even assuming that guideline adherence would be significantly better if the measurement time point was 1 h after diagnosis in accordance with guidelines, this remains a result to be critically evaluated. The resulting optimization potential must be evaluated in the future by real-time documentation to be able to set the results in relation to guideline adherence.

According to the recently published studies by Permpikul et al. (32) on early vasopressor therapy in septic shock, we administrated vasopressors in a very high percentage of 83.3%.

The relevance of sepsis diagnosis is particularly underpinned in patients with septic shock, as delaying an initiation of treatment significantly reduces the likelihood of patient survival (33), so that initiation of adequate treatment in the ED should also occur as soon as possible. With increasing disease severity, the number of invasive procedures (e.g., central venous access, arterial line) performed also increased in accordance with

TABLE 2 Diagnostic workup performed in the emergency department.

	All (n = 1,278)	Group I infection (n = 1,103)	Group II sepsis (n = 133)	Group III septic shock (n = 42)
Sampling				
Sputum [n (%)]	16 (1.3)	**9 (0.8)***	4 (3.0)	**3 (7.1)*****
Urine [n (%)]	672 (52.6)	**541 (49.0)***	100 (75.2)	**31 (73.8)*****
Urine culture [n (%)]	340 (26.6)	**251 (22.8)***	69 (47.6)	**20 (47.6)*****
Stool sample [n (%)]	29 (2.3)	**21 (1.9)***	7 (5.3)	1 (2.4)
Drain secretion [n (%)]	24 (1.9)	**15 (1.4)***	6 (4.5)	**3 (7.1)*****
Liquor [n (%)]	11 (0.9)	7 (0.6)	2 (1.5)	**2 (4.8)*****
POCT Influenza A/B, RSV [n (%)]	403 (31.5)	**320 (29.0)***	63 (47.4)	**20 (47.6)*****
Imaging				
Chest x-ray [n (%)]	558 (43.7)	**421 (38.2)***	102 (76.7)	**35 (83.3)*****
TTE [n (%)]	194 (15.2)	**134 (12.1)***	42 (31.6)	**18 (42.9)*****
Abdominal sonography [n (%)]	351 (27.5)	**257 (23.3)***	71 (53.4)	**23 (54.8)*****
Computed tomography [n (%)]	249 (19.5)	**180 (16.3)***	**45 (33.8)****	**24 (57.1)*****
Antibiotic therapy				
Oral [n (%)]	262 (20.5)	**255 (23.1)***	7 (5.3)	**0 (0.0)*****
Intravenous [n (%)]	441 (34.5)	**294 (26.7)***	111 (83.5)	**36 (85.7)*****
After blood cultures [n (%)]	420 (32.9)	**294 (26.7)***	94 (70.7)	**32 (76.2)*****
Compliance to 1-h bundle[#]				
Fluid resuscitation [n (%)]	28 (2.2)	**12 (1.1)***	**9 (6.8)****	**7 (16.7)*****
Laboratory investigation [n (%)]	1124 (88.1)	**952 (86.3*)**	131 (98.5)	**441 (97.6)*****
Blood cultures [n (%)]	518 (40.5)	**374 (33.9)***	108 (81.2)	**36 (87.8)*****
Lactate measurement [n (%)]	940 (73.6)	**776 (70.4)***	126 (94.7)	**38 (95.0)*****
Vasopressor for MAP ≥ 65 [n (%)]	39 (3.1)	**0 (0.0)***	**4 (3.0)****	**35 (83.3)*****
Antibiotic therapy within 1 h [n (%)]	75 (5.9)	58 (5.3)	11 (8.3)	**6 (14.3)*****
Oxygen therapy				
Mask [n (%)]	83 (6.5)	**48 (4.4)***	**22 (16.5)****	**13 (30.9)*****
CPAP/NIV [n (%)]	5 (0.4)	4 (0.4)	0 (0.0)	1 (2.4)
Invasive ventilation [n (%)]	12 (0.9)	**2 (0.2)***	**3 (2.3)****	**7 (16.7)*****
Instrumentation				
Central venous access [n (%)]	55 (4.3)	**11 (1.0)***	**11 (8.3)****	**33 (78.6)*****
Arterial line [n (%)]	74 (5.8)	**15 (1.5)***	**23 (17.3)****	**36 (85.7)*****
Catecholamines [n (%)]	39 (3.1)	**0 (0.0)***	**4 (3.0)****	**35 (83.3)*****

N, number; POCT, point of care testing; RSV, respiratory syncytial virus; ECG, electrocardiogram; TTE, transthoracic echocardiography; MAP, mean arterial blood pressure; CPAP/NIV, continuous positive airway pressure/non-invasive ventilation.

[#]Time of measurement: 1 h after admission to the emergency department.

p is significant, if $p < 0.05$; * = $p_{I,II} < 0.05$; ** = $p_{II,III} < 0.05$; *** = $p_{I,III} < 0.05$.

The bold values represent significant results.

the literature (22–25), although a significantly higher rate of vasopressors and a lower rate of ventilation support were found than in the comparative studies.

In the literature, about one-third of patients entering an ICU are admitted through the ED (21, 28). In the EpiSEP study, half of all ED patients suffering from infection (50.5%) are admitted to the hospital, and the majority (40.5%) were admitted to general wards, only a minority of 5.5% were admitted to ICU. The cases admitted to the normal ward are therefore disregarded in the most common infection and sepsis studies. In our study, 97% of patients with sepsis were admitted as inpatients, but of these only 14.3% went to the ICU. Of the septic shock group, 100% were admitted as inpatients, of which the following proportions were admitted to the normal ward, ICU, or were external transferred: 7, 59.5, 14%, respectively. A total of 9.5% died already in the ED. These data supported the hypothesis that a large proportion of hospital admitted ED patients with sepsis were transferred to the normal ward and are thus excluded from the previous ICU studies. The 30-day mortality rate in the three groups was 1.6, 12.0, and 38.1%, respectively. These findings corresponded to the results of other sepsis studies (22–25).

TABLE 3 Relocations sites and outcomes of the patients suffered from infection, sepsis, and septic shock in the emergency department.

	All (n = 1,278)	Group I infection (n = 1,103)	Group II sepsis (n = 133)	Group III septic shock (n = 42)
Primary relocation site after ED				
Intensive care unit [n (%)]	61 (5.5)	**17 (1.5)***	**19 (14.3)****	**25 (59.5)*****
Stroke unit [n (%)]	14 (1.1)	10 (0.9)	3 (2.3)	1 (2.4)
Intermediate Care [n (%)]	10 (0.8)	8 (0.7)	2 (1.5)	0 (0.0)
Catheter laboratory [n (%)]	4 (0.3)	2 (0.2)	1 (0.8)	**1 (2.4)*****
Operation theater [n (%)]	11 (0.9)	8 (0.7)	2 (1.5)	1 (2.4)
Normal ward [n (%)]	500 (39.1)	**402 (36.4)***	**95 (71.4)****	**3 (7.1)*****
Interhospital transfer [n (%)]	41 (3.2)	30 (2.8)	**5 (3.8)****	**6 (14.3)*****
Discharge at home [n (%)]	569 (44.5)	**566 (51.2)***	3 (2.3)	**0 (0.0)*****
Discharge against medical aid [n (%)]	41 (3.2)	40 (3.6)	1 (0.8)	0 (0.0)
Other outpatient clinics [n (%)]	21 (1.6)	20 (1.8)	1 (0.8)	0 (0.0)
Outcomes				
Admission to				
ICU [n (%)]	61 (5.5)	**17 (1.5)***	**19 (14.3)****	**25 (59.5)*****
Normal ward [n (%)]	500 (39.1)	**402 (36.4)***	**95 (71.4)****	**3 (7.1)*****
Interhospital transfer [n (%)]	41 (3.2)	30 (2.8)	**5 (3.8)****	**6 (14.3)** ***
Discharge at home [n (%)]	569 (44.5)	**566 (51.2)** *	3 (2.3)	**0 (0.0)** ***
Death in ED [n (%)]	5 (0.4)	**0 (0.0)***	**1 (0.8)****	**4 (9.5)*****
Death in-hospital [n (%)]	51 (4.0)	**19 (1.7)***	**16 (12.0)****	**16 (38.1)*****
30-day-mortality [n (%)]	50 (3.9)	**18 (1.6)***	**16 (12.0)****	**16 (38.1)*****
LOS ED (min, median, IQR)	374 (205–693)	**340 (188–597)***	**718 (438–1284)****	**539 (292–779)*****
LOS ICU (days, median, IQR)	3 (2–7)	3 (1–10)	3 (2–5.75)	5 (2–8)
LOS hospital (days, median, IQR)	0 (0–8)	**0 (0–6.0)***	9 (0–6)	**5.5 (0–15.0)*****

n, number; ICU, intensive care unit; ED, emergency department; LOS, length-of-stay; min, minutes.
p is significant, if $p < 0.05$; * = $p_{I,II} < 0.05$; ** = $p_{II,III} < 0.05$; *** = $p_{I,III} < 0.05$.
The bold values represent significant results.

Limitations

The major limitation of our investigation is that it is a single-center retrospective study. However, the reliability of the results with over 1,100 patients in group I (infection alone) seems to be sufficiently large. However, the number of patients with sepsis, although identified from an initial cohort of over 6,000 ED patients, appears to be borderline low. Further multicenter studies involving a large number of study centers and a prospective study design should investigate the underlying epidemiology and causes of infection, sepsis and septic shock in the ED setting in a larger cohort. A further limitation is, that due to the local form of organization, some patients are treated in other outpatient clinics (e.g., ophthalmology, dermatology, gynecology), so infections in these patients may be underrepresented in the EpiSEP study cohort. In addition, children, as long as they are not critically ill or injured were treated in the pediatric ED of our institution. In the EpiSEP study these pediatric patients were excluded in order to avoid distortion. Supplementary, elective patients with a possible focus on infection are not included in our study. A further possible bias is that our university hospital is a specialized center for patients suffering from severe diseases (e.g., cancer, hematological diseases) and immunosuppressive state (e.g., heart and kidney transplantation).

Also, one may criticize that the underlying seasonal factors (winter season due to January and February) led to a seasonal bias in the study results, possible overrepresenting respiratory causes of infection, sepsis and septic shock. The validity of the data regarding the 1 h-bundle is limited by the fact that there was no exact time of documentation for the diagnosis "sepsis," so that we only used values within the first hour after admission to the ED.

Conclusion

The EpiSEP study shows important care data on patients with infection, sepsis, and septic shock in an German ED. By using vital signs and clinical findings for identification, the study approximates the actual incidence rates of sepsis and septic shock in the ED and emphasizes the importance of sepsis detection and structured diagnosis and therapy.

TABLE 4 Comparison of EpiSEP with intensive care unit-studies on epidemiology and causes of infection, sepsis, and septic shock.

Source (1)	EpiSep study 1 ED, Germany			EPIC II 1,265 ICUs in 75 countries (667 ICUs in Western Europe, 2007) (18)	EPIC III 1,150 ICUs in 88 countries (479 ICUs in Western Europe, 2017) (21)	SPICE-ICU 22 ICUs, Japan (20)		INSEP-study 434 ICUs, Germany (9)		MEDUSA 44 ICUs, Germany (22)	
	Infection (*n* = 1,103)	Sepsis (*n* = 133)	Septic shock (*n* = 42)	Infection (*n* = 7,087)	Infection (*n* = 8,135)	Sepsis-2 (*n* = 530)	Sepsis-3 (*n* = 569)	Sepsis (*n* = 211)	Septic shock (*n* = 190)	Intervention group (*n* = 2,596)	Control group (*n* = 1,587)
Respiratory tract	362 (34.9)	58 (43.6)	26 (61.9)	4503 (63.5)	4893 (60.1)	200 (37.7)	208 (36.6)	141 (66.8)	111 (58.4)	1078 (41.6)	688 (43.4)
Urogenital tract	193 (17.5)	36 (27.1)	6 (14.3)	1011 (14.3)	1138 (14)	91 (17.2)	101 (17.8)	17 (8.1)	8 (4.2)	314 (12.1)	216 (13.6)
ENT/OMF	178 (16.1)	3 (2.3)	0 (0.0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Intra-abdominal	146 (13.3)	16 (12.0)	4 (9.5)	1392 (19.6)	1490 (18.3)	111 (20.9)	119 (20.9)	59 (28.0)	77 (40.5)	974 (35.7)	568 (35.8)
Soft tissue	120 (10.9)	5 (3.8)	1 (2.4)	467 (6.6)	518 (6.4)	5 (0.9)	5 (0.9)	20 (9.5)	16 (8.4)	207 (8.0)	148 (9.3)
Unknown	55 (5.0)	11 (8.3)	4 (9.5)	n.d.	n.d.	22 (4.2)	24 (4.2)	n.d.	n.d.	96 (3.7)	50 (3.3)
Others	31 (2.8)	1 (0.8)	0 (0.0)	540 (7.6)	529 (6.5)	14 (2.6)	16 (2.8)	n.d.	n.d.	19 (0.7)	17 (1.1)
Cerebral	12 (1.1)	2 (1.5)	1 (2.4)	208 (2.9)	314 (3.9)	11 (2.1)	13 (2.3)	n.d.	n.d.	43 (1.7)	22 (1.4)
Blood stream	6 (0.5)	1 (0.8)	0 (0.0)	1071 (15.1)	1239 (15.2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d. n.d.

ED, emergency department; ICU, intensive care unit; ENT, ear/nose/throat; OMF, oral maxillofacial; n.d., no data.

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TABLE 5 Comparison of EpiSEP study with studies from emergency department on epidemiology and causes of infection, sepsis, and septic shock.

Source	EpiSEP study 1 ED, Germany			ARISE–study 51 EDs, Australia, New Zealand, Finland, Hong Kong, Republic of Ireland (24)		ProMISE 56 EDs, England (25)		ProCESS-study 31 EDs, United States (26)			Epidemiology of emergency department sepsis data from the National Health Informatics Project, Taiwan (27)		The impact of the Sepsis-3 definition on ICU admission of patients with infection 1 ED, Germany (28)
	Infection (*n* = 1,103)	Sepsis (*n* = 133)	Septic shock (*n* = 42)	Septic shock EGDT (*n* = 793)	Septic shock usual care (*n* = 798)	Septic shock EGDT (*n* = 625)	Septic shock usual care (*n* = 626)	Sepsis protocol-based EGDT (*n* = 439)	Sepsis protocol-based standard-therapy (*n* = 446)	Sepsis usual care (*n* = 456)	Sepsis ED admitted (*n* = 493,397)	Sepsis non-ED-admitted (*n* = 763,287)	Infection (*n* = 916)
Respiratory tract	362 (32.8)	58 (43.6)	26 (61.9)	289 (36.5)	262 (32.8)	228 (36.5)	207 (33.1)	140 (31.9)	152 (34.1)	151 (33.1)	277,945 (56.3)	398,504 (52.2)	(56.8)
Urogenital tract	193 (17.5)	36 (27.1)	6 (14.3)	148 (18.7)	160 (20.1)	108 (17.3)	117 (18.7)	100 (22.8)	90 (20.2)	94 (20.6)	193,060 (39.1)	234,313 (30.7)	(24.6)
ENT/OMF	178 (16.1)	3 (2.3)	0 (0.0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Intraabdominal	146 (13.2)	16 (12.0)	4 (9.5)	63 (8.0)	61 (7.6)	40 (6.4)	51 (8.1)	69 (15.7)	57 (12.8)	51 (11.2)	32,082 (6.5)	41,052 (5.4)	(7.5)
Soft tissue	120 (10.9)	5 (3.8)	1 (2.4)	90 (11.4)	76 (9.5)	39 (6.2)	39 (6.2)	25 (5.7)	33 (7.4)	38 (8.3)	34.058 (6.9)	28,931 (3.8)	(5.6)
Unknown	55 (5.0)	11 (8.3)	4 (9.5)	52 (6.6)	72 (9.0)	76 (12.2)	77 (12.3)	57 (13.0)	47 (10.5)	66 (14.5)	n.d.	n.d.	n.d.
Others	31 (2.8)	1 (0.8)	0 (0.0)	52 (6.6)	72 (9.0)	21 (3.4)	37 (5.9)	28 (6.4)	31 (7.0)	26 (5.7)	n.d.	n.d.	(5.5)
Cerebral	12 (1.1)	2 (1.5)	1 (2.4)	13 (1.6)	6 (0.8)	12 (1.9)	9 (1.4)	3 (0.7)	3 (0.7)	4 (0.9)	n.d.	n.d.	n.d.
Blood stream	6 (0.5)	1 (0.8)	0 (0.0)	75 (9.5)	86 (10.8)	97 (15.5)	86 (13.7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

ED, emergency department; ICU, intensive care unit; ENT, ear/nose/throat; OMF, oral maxillofacial; n.d., no data.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethikkommission, Medizinischen Fakultät, Heinrich-Heine-Universität, Düsseldorf, Gebäude 14.82, Ebene 01, Raum 101. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

NW, MB, and MM conceived the study, analyzed the data, and drafted the manuscript. MB supervised the project as a whole. NW, LB, OH, MB, and MM collected the data. MM, VK, OH, and PK substantively revised the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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