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Diagnostic accuracy of sepsis: Clinical scores combination and serum biomarkers for rapid diagnosis and prognosis

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Diagnostic accuracy of sepsis: Clinical scores combination and serum biomarkers for rapid diagnosis and prognosis

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Editorial: Diagnostic accuracy of sepsis: clinical scores combination and serum biomarkers for rapid diagnosis and prognosis

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Editorial on the Research Topic
Diagnostic accuracy of sepsis: clinical scores combination and serum biomarkers for rapid diagnosis and prognosis

Sepsis is a life-threatening syndrome triggered by infection, accounting for 17% of intra-hospital mortality increasing up to 26% in case of septic shock with estimated costs of over 24 billion dollars per year (1). Early diagnosis and appropriate treatment can significantly reduce sepsis mortality (1). However, unfortunatelyat present no gold standard for sepsis diagnosis has been defined (1).

The combination of clinical scores and biomarkers increases the diagnostic and prognostic accuracy of sepsis, improving patient clinical management (2). Various clinical variables and tools are used for sepsis screening, such as vital signs, signs of infection, systemic inflammatory response syndrome (SIRS) criteria, quick Sequential Organ Failure Score (q-SOFA) or Sequential Organ Failure Assessment (SOFA) criteria, National Early Warning Score (NEWS), or Modified Early Warning Score (MEWS) (2).

The Research Topic was aimed to provide further evidence about the role of clinical scores and serum biomarkers combination in increasing accuracy and timely sepsis diagnosis. In this Research Topic, eight original research papers and one systematic review with meta-analysis were published. All of them brought evidence that sepsis diagnosis, a syndrome whose timely diagnosis has a significant impact patient's prognosis, can be facilitated by the combination of clinical data, in the form of scores, and the dosage of biomarkers. In recent years, a lot has been published about new biomarkers useful for this purpose, but there is still no signature sepsis biomarker, unlike other diseases, such as troponin in myocardial infarction. This happens because sepsis is a polymorphic syndrome with various stages of severity, potentially involving with alteration of different homeostatic mechanisms and consequent different biomarkers involvement. Some of these biomarkers are more specifically altered during sepsis, mainly those involved in inflammation such as C-reactive protein (CRP), Procalcitonin (PCT), and presepsin.

In this regard, Park et al. reported data from a retrospective cross-sectional study on 757 patients with culture-proven bacterial infections. Data showed that PCT and presepsin

proved to be more promising biomarkers than CRP. Specifically, PCT showed the best performance in infection prediction, while presepsin yielded the best prognosis, proving their combination as a good tool to use in septic patients.

Within the Research Topic, two articles were included that evaluated biomarkers differently from those related to the inflammatory process, CRP, PCT, or presepsin, but related to organ damage or to innate or specific immunity activation. Zhao et al. in a retrospective cohort study on 456 patients with sepsis and sepsis-associated encephalopathy used the dosage of ammonium levels to correlate them to the prognosis. Authors reported a significant correlation of serum ammonia level with higher SOFA score and lactate but not with other prognostic factors such as hospital mortality or longer hospital stay, which, on the contrary, correlated significantly with Simplified Acute Physiology Score (SAPS II) and Charlson clinical score. These data confirmed that the combination of clinical scores and biomarkers can lead to rapid identification of patients at increased risk of death providing more targeted and effective monitoring.

Ma et al. performed a meta-analysis to investigate the accuracy of soluble-urokinase-type plasminogen activator receptor (SuPAR) in neonatal sepsis. This receptor is expressed on the membrane of immune cells, endothelial cells, and smooth muscle cells and is upregulated at sites of inflammation. It interacts and cooperates with many ligands and receptors, mainly integrins, to facilitate intracellular signaling, cell migration, cell adhesion, and tissue remodeling. SuPAR is released during inflammation or immune activation and although it is not disease-specific, its circulating levels reflect the severity and prognostic outcome of many infectious, inflammatory, and autoimmune disorders. The meta-analysis, while demonstrating the diagnostic potential of this biomarker, also highlighted that more high-quality studies are needed to confirm this data, given that the studies published so far are limited, there being only six.

Miyajima et al. analyzed parameters deriving from neutrophils cell population such as the Fluorescent light intensity (NE-SFL) and the Fluorescent light distribution Width (NE-WY) resulted in good indicators of sepsis compared to other biomarkers such as PCT, Interleukin 6 (IL-6), CRP and presepsin. In particular, it has been seen that NE-SFL and NE-WY are higher in patients with bacteremia and significantly associated with a high bacterial load as detected by the molecular PCR test. Furthermore, the levels of the two indicators significantly correlated with those of PCT and IL-6. The data of this study suggest that NE-SFL and NE-WY deriving from the analysis of cell population data may have a significant role in predicting severe bacterial infections.

An article about it was also included in the Research Topic a retrospective observational cohort study performed on 1,057 patients admitted to the Emergency Department after receiving antibiotic therapy for suspected sepsis (Sivayoham et al.). The aim of Sivayoham et al. was to risk-stratify sepsis patients for in-hospital mortality identifying the best risk-stratification tool for outcome at 180 days after admission. For risk stratification the following scores, Emergency Department suspected Sepsis (REDS) score, SOFA score, Red-flag sepsis criteria met, NICE high-risk criteria met, the NEWS2 score, and the SIRS criteria, were used. The results evidenced that 13.8% of patients died at hospital discharge and 27% died within 180 days, with overall survival of 74.4% at 180 days. Among the different scores the REDS and SOFA scores identified <50% of the population as high-risk and all tools except the SIRS criteria, were relevant for outcome at 180 days. These data suggest that although all the risk-stratification tools were useful for outcome at 180 days, REDS and SOFA scores were superior to other tools, while SIRS criteria were useless for this purpose.

Regarding the indicators of prognosis and mortality, the study by Yang et al. (3) was focused on patients with acute kidney injury (AKI) and Central Venous pressure (CVP) for volume status assessment. The authors investigated the optimal time window to obtain CVP preventing adverse outcomes. They showed that delayed CVP time assessment was associated with a greater risk of in-hospital mortality, while prompt CVP monitoring contributed to shorter length of ICU stays and fewer days of norepinephrine use, as well as better fluid management.

Recently, great importance has been given to the application of artificial intelligence algorithms in the medical field especially to improve patients' diagnosis and treatment. It was therefore useful to include in the Research Topic two articles in which the use of artificial intelligence algorithms represented examples of application in the diagnosis and prognosis of patients with sepsis.

Wang et al. performed metabolomics profiling in septic patients compared to healthy subjects and applied five different machine learning (ML) algorithms to analyze the obtained data. The authors demonstrated that the occurrence of sepsis determines metabolite dysregulation, especially of mannose-6-phosphate and sphinganine, which are positively correlated with biomarkers such as PCT, leukocyte count, CRP, and Interleukin-6. These data suggested how ML could represent a useful approach for precision medicine delivery.

ML approach was used also by Cheng et al. for sepsis in-hospital mortality prediction within 48 h from symptoms onset. These authors used dynamic changes in the patient's vital signs such as systolic and diastolic blood pressures, heart and respiratory rates, and body temperature, concluding that machine learning models were useful for mortality prediction within 6 to 48 h from admission.

Two studies were carried out in patients affected by COVID-19 were also included. The first by Al-Shudifat et al. (4) investigated the correlation between lung computed tomography (CT) data and demographics or vital signs findings. This cross-sectional study revealed that several factors could represent predictors for outcome and lung changes, such as age above 60 years old, the presence of dry or productive cough, and more than three antibiotic prescriptions. The second article was by Rivera-Fernandez et al. who performed a multicenter prospective cohort study investigating the role of PCT measurement on mortality in patients with COVID-19 and respiratory involvement. These authors showed that PCT elevation was observed in several measurements and significantly correlated to mortality. Moreover, PCT was high in more than 50% of non-survivors until the final day before death. The authors concluded that the serial assessment of procalcitonin in these patients could be useful for death risk stratification.

We hope that this Research Topic provides a stimulus and an upgrade to the scientific community to promote an increasingly integrated line of research between clinic and laboratory. This could

improve early and accurate diagnosis of serious and fatal diseases such as sepsis. This approach proves useful also in new infections, as COVID-19 has taught us.

Author contributions

SS and SA drafted the manuscript. CB, SS, and SA critically revised the manuscript for important intellectual content. 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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Performance of presepsin and procalcitonin predicting culture-proven bacterial infection and 28-day mortality: A cross sectional study

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Presepsin is a highly specific biomarker for diagnosing bacterial infections, but its clinical usefulness is not well validated. A retrospective cross-sectional study was conducted. Among the patients suspected bacterial infection or fulfilled the criteria of systemic inflammatory response syndrome (SIRS) and patients who underwent blood culture, presepsin, procalcitonin (PCT), and C-reactive protein (CRP) at the same time were included. Receiver operating characteristic (ROC) curve analysis and logistic regression were used to compare performance of three biomarkers. A total of 757 patients were enrolled, including 256 patients (33.8%) with culture-proven bacterial infection and 109 patients (14.4%) with bacteremia. The 28-day mortality rate was 8.6%. ROC curve analysis revealed that the area under the curve (AUC) of PCT was higher than that of presepsin for both culture-proven bacterial infection (0.665 and 0.596, respectively; $p = 0.003$) and bacteremia (0.791 and 0.685; $p < 0.001$). In contrast, AUC of PCT for 28-day mortality was slower than presepsin (0.593 and 0.720; $p = 0.002$). In multivariable logistic regression analysis, PCT showed the highest ORs for culture-proven bacterial infection (OR 2.23, 95% CI 1.55–3.19; $p < 0.001$) and for bacteremia (OR 5.18, 95% CI 3.13–8.56; $p < 0.001$), while presepsin showed the highest OR for 28-day mortality (OR 3.31, 95% CI 1.67–6.54; $p < 0.001$). CRP did not show better performance than PCT or presepsin in any of the analyses. PCT showed the best performance predicting culture-proven bacterial infection and bacteremia, while presepsin would rather be useful as a prognostic marker.

KEYWORDS

presepsin, procalcitonin, bacterial infections, sepsis, prognosis

Introduction

Untreated bacterial infections and bacteremia can cause major health problems with a mortality rate as high as 30% (1–3). Early recognition of bacterial infection and administration of empirical antibiotics are essential to improve prognosis for infected patients (4). However, differential diagnosis of bacterial infection from other non-infectious causes of systemic inflammation is often difficult, because fever and leukocytosis have poor sensitivity and specificity in many clinical settings (5, 6). Culture-based approaches remain the gold standard for diagnosis of bacterial infection including bacteremia, but they are time-consuming and results are not available for 12–48 h (6–8). Therefore, recent interest has focused on inflammatory biomarkers for early assessment of bacterial sepsis, including procalcitonin (PCT) and C-reactive protein (CRP) (9), however, these biomarkers could be elevated in non-infectious conditions (10, 11). Presepsin, the soluble fraction of cluster of differentiation 14 (CD14), is suggested as a novel biomarker for bacterial sepsis, which is released into circulation when monocytes are activated after binding with lipopolysaccharides (LPS) and LPS binding protein (12–14). Several studies showed that presepsin is a very good inflammatory biomarker for early diagnosis of sepsis and evaluation of sepsis prognosis (14–24). However, the clinical usefulness of presepsin is still controversial because its superior performance for predicting bacterial infection compared with PCT was observed in relatively small cohorts (14, 17) and most large-scale studies did not include a sufficient number of culture-proven bacterial infections (16, 25–29). To determine the clinical usefulness of presepsin, we evaluated its performance predicting culture-proven bacterial infection among patients with sepsis, in comparison with PCT and CRP.

Materials and methods

Patients and study design

The cross sectional study was conducted between January 2020 and October 2020 at Konkuk University Hospital, a 950-bed, community-based tertiary medical center in Seoul, Republic of Korea. We screened the electronic medical records (EMR) of adult patients (≥ 18 years) who were clinically suspected to have bacterial infection and fulfilled the systemic inflammatory response syndrome (SIRS) criteria. Among these patients, patients who underwent blood culture and presepsin at the same time were included. Bacteremia was defined as recovery of any pathogenic bacterial species in one or two sets of blood cultures. Microorganisms commonly considered as contaminants were excluded from the bacteremia group (30). Culture-proven bacterial infection was defined as isolation of pathogens from possible clinical specimens. This study was approved by the Institutional Review Board (IRB) of Konkuk University Medical Center (#2022–04-040) and performed in accordance with the Declaration of Helsinki. Informed consent was waived by the IRB of Konkuk University Medical Center because the EMR was reviewed retrospectively with de-personalized identification numbers.

Data collection

Data were collected from administrative, pharmaceutical, and laboratory computerized databases maintained by the medical information team at Konkuk University Medical Center. Clinical records were reviewed, and the following information was recorded: age, sex, infection type, blood culture results, Charlson's weighted index score (CWIs), 28-day mortality, and Quick Sequential Organ Failure Assessment (qSOFA) score. Infection type was clinically established based on clinical symptoms, imaging, and laboratory findings with or without isolation of bacteria from the presumed source (16, 31). The 28-days mortality was defined as death caused by any reasons within 28 days of the presepsin test. qSOFA scores were calculated by checking respiratory rate, Glasgow Coma Scale (GCS) score, systolic blood pressure recorded at the time of obtaining blood for presepsin and culture. Laboratory findings at the same time as presepsin test and blood culture including CRP and PCT were collected. The severity of illness in bacteremia was assessed using the Pitt bacteremia score, which has been validated in several previous studies (32).

Measurement methods

Plasma presepsin concentrations were measured using an automated chemiluminescent enzyme immunoanalyzer, PATHFAST system (LSI Medience Co., Tokyo, Japan). Presepsin in the sample binds to anti-presepsin antibodies to assemble an immunocomplex with ALP-labeled antibodies and mouse monoclonal antibody-coated magnetic particles. After 10-min incubation with a chemiluminescent substrate, luminescence was generated by the enzyme reaction, a photomultiplier detected, and presepsin concentration was calculated (13). We defined a cut-off value of 314 pg/mL according to the manufacturer's instructions. Serum PCT levels were measured with an electrochemiluminescence immunoassay (Brahms GmbH, Henningsdorf, Germany) in the Roche Cobas e-System (Roche Diagnostics, Basel, Switzerland). Serum separation tubes were used for CRP. CRP was measured using the latex immunoturbidimetric method with a CRP-Latex X2 (Denka Seiken Co., Tokyo, Japan) on a Toshiba 200FR

Autoanalyzer (Toshiba Medical Systems Co., Ltd., Tokyo, Japan) (33).

Statistical analyses

To compare clinical variables, the Mann-Whitney *U*-test was used for continuous variables, and chi-square and Fisher's exact tests were used for categorical variables. Age, sex, qSOFA, CWIs (which could be confounding factors), and three biomarkers were included in the multivariable logistic regression model. The area under the receiver-operating-characteristics (ROC) curve estimation was used to evaluate the diagnostic performances of the tested biomarkers and area under the curve (AUC) differences were calculated with the De Long test (34). Optimal cut-off values were derived from ROC curves using the point closest-to-(0,1) corner in the ROC plane which defines the optimal point as the minimizing the distance between the ROC curve and the (0,1) point (35), and sensitivity, specificity, and predictive values were estimated to predict culture-proven bacterial infection with or without bacteremia. The 28-day mortality rates were calculated based on these cut-off values. IBM SPSS Statistics version 20.0 for Windows (IBM, Armonk, NY, United States) was used for all statistical analyses.

Results

Study population and microbiology results

Of the 850 patients who were screened for this study, 93 who did not undergo blood culture and presepsin at the same time were excluded. A total of 757 patients were finally included in the study. Culture-proven bacterial infection with or without bacteremia was detected in 256 patients (33.8%). Bacteremia was detected in 109 (14.4%) patients. Gram-negative microorganisms were obtained in 84 samples and Gram-positive organisms in 27 samples. Two or more microorganisms were identified in five patients (4.5%) (**Supplementary Table 1**). When three biomarkers and Pitt bacteremia score were compared in patients with bacteremia according to microorganism type, no statistically difference was found between patients with Gram-positive, Gram-negative, and poly-microbial infections (**Supplementary Table 2**).

Comparison between bacteremia group and non-bacteremia group

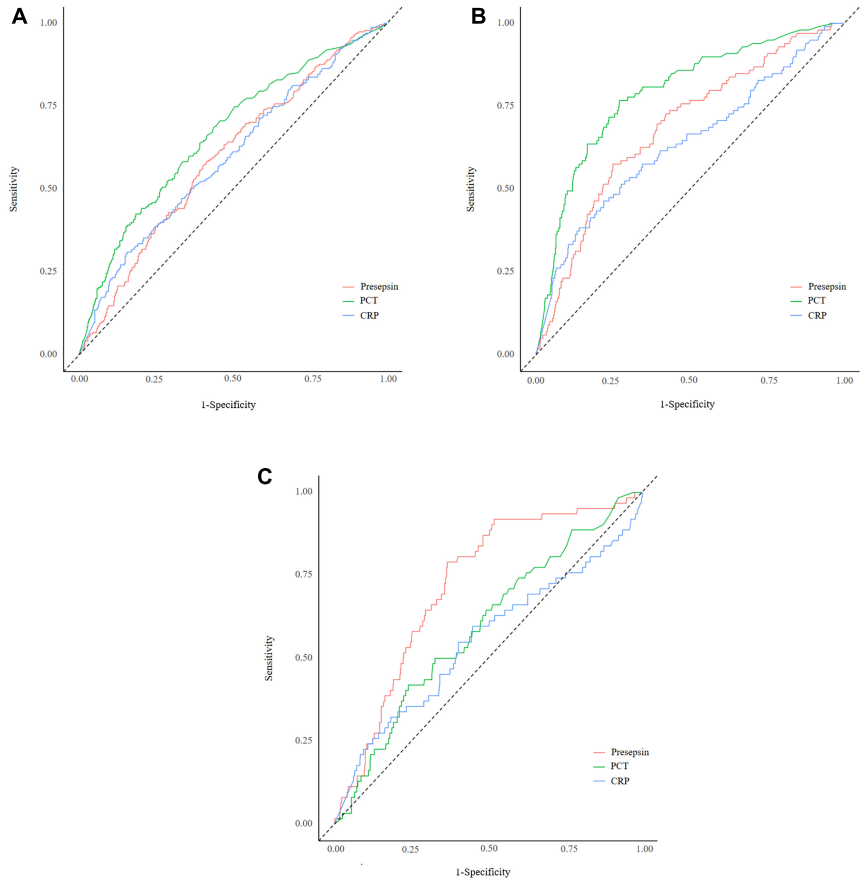
Demographic and clinical characteristics of the patients in the bacteremia and non-bacteremia groups are shown in **Table 1**. There were no differences between the bacteremia and non-bacteremia group regarding age, sex, CWI, and 28-day mortality. Patients with urinary tract infection (33.9 vs. 15.3%, $p < 0.001$) or skin, soft tissue, or bone infection (10.1 vs. 3.5%, $p < 0.001$) were more common in the bacteremia group, whereas the proportion of patients with pneumonia was higher in the non-bacteremia group (46 vs. 7.3%, $p < 0.001$). Presepsin, PCT, and CRP values were higher in the bacteremia group than the non-bacteremia group ($p < 0.001$) (**Table 1**).

Diagnostic accuracy of three biomarkers for predicting culture-proven bacterial infection, bacteremia, and 28-day mortality

ROC curves for presepsin, PCT, and CRP for predicting culture-proven bacterial infection, bacteremia, and 28-day mortality are shown in **Figure 1**. The ROC curve analysis for predicting culture-proven bacterial infection with or without bacteremia yielded an AUC value 0.596 (95% CI: 0.551–0.641) for presepsin, 0.665 (95% CI: 0.621–0.709) for PCT, and 0.581 (95% CI: 0.550–0.642) for CRP (**Figure 1A**). The AUC value of PCT was higher than that of presepsin ($p = 0.003$), while AUC value of presepsin was equal to that of CRP ($p = 0.996$). The cut-off values derived from ROC curves were 592.5 pg/mL for presepsin, 0.305 ng/mL for PCT, and 21.94 mg/dL for CRP. When we used a presepsin cut-off value of 592.5 pg/mL for culture-proven bacterial infection with or without bacteremia, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 57.42, 60.68, 42.73, and 73.61%, respectively.

ROC curve analysis for predicting bacteremia yielded an AUC value of 0.685 (95% CI: 0.628–0.741) for presepsin, 0.791 (95% CI: 0.742–0.840) for PCT, and 0.637 (95% CI: 0.572–0.701) for CRP. The AUC value of PCT was higher than that of presepsin ($p < 0.001$), while AUC value of presepsin was higher than that of CRP ($P < 0.001$) (**Figure 1B**). The cut-off values derived from ROC curves were 1028.5 pg/mL for presepsin, 1.23 ng/mL for PCT, and 24.2 mg/dL for CRP. When we used a presepsin cut-off value of 1028.5 pg/mL for diagnosing bacteremia, sensitivity, specificity, PPV, and NPV were 55.05, 76.54, 28.30, and 91.01%, respectively.

ROC curve analysis for predicting 28-day mortality yielded an AUC value of 0.72 (95% CI: 0.66–0.781) for presepsin, 0.593 (95% CI: 0.519–0.667) for PCT, and 0.522 (95% CI: 0.467–0.637) for CRP. The AUC value of presepsin was higher than that of PCT ($p = 0.002$) (**Figure 1C**). The cut-off values derived from ROC curves were 704.5 pg/mL for presepsin, 2.68 ng/mL for PCT, and 23.2 mg/dL for CRP. When we used a presepsin cut-off value of 704.5 pg/mL for predicting 28-day mortality, sensitivity,



(A) Culture-proven bacterial infections.

	AUC	Cut-off value	Sensitivity	Specificity	PPV	NPV
Presepsin	0.596	592.5 pg/mL	57.42	60.68	42.73	73.61
PCT	0.665	0.305 ng/mL	64.84	63.72	47.43	77.89
CRP	0.581	21.94 mg/dL	26.69	85.83	51.70	70.49

(B) Bacteremia.

	AUC	Cut-off value	Sensitivity	Specificity	PPV	NPV
Presepsin	0.685	1028.5pg/mL	55.05	76.54	28.30	91.01
PCT	0.791	1.23ng/mL	76.8	72.1	33.93	94.33
CRP	0.637	24.2 mg/dL	36.11	86.73	31.2	89.06

(C) 28-days mortality

	AUC	Cut-off value	Sensitivity	Specificity	PPV	NPV
Presepsin	0.72	704.5 pg/mL	78.46	64.74	17.29	96.97
PCT	0.593	2.68 ng/mL	40	79.77	15.66	93.40
CRP	0.522	23.2 mg/dL	30.77	83.24	14.71	92.75

ROC, receiver-operating-characteristics; PCT, procalcitonin; CRP, C-reactive protein; AUC, area under curve; PPV, positive predictive value; NPV, negative predictive value; PCT, procalcitonin; CRP, C-reactive protein.

FIGURE 1
ROC curves for predicting culture proven bacterial infections, bacteremia, and 28-day mortality along with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at the best cut-offs for the following parameters: presepsin, PCT, and CRP.

TABLE 1 Patient demographic and clinical characteristics.

Variables	Bacteremia (n = 109)	Non-bacteremia (n = 648)	p-value
Sex, male	56 (51.4)	356 (54.9)	0.557
Age (years)	70 (60–79)	72 (61–81)	0.618
Biomarkers			
Presepsin, pg/mL	1730.3 ± 1930.0	920.4 ± 1243.9	< 0.001
PCT, ng/mL	16.1 ± 17.2	3.9 ± 10.0	< 0.001
CRP, mg/dL	16.5 ± 11.6	11.1 ± 9.4	< 0.001
Source of infection			
Urinary tract	37 (33.9)	99 (15.3)	< 0.001
Pneumonia	8 (7.3)	298 (46)	< 0.001
Intra-abdominal	29 (26.6)	119 (18.4)	0.061
Skin, soft tissue, bone	11 (10.1)	23 (3.5)	0.005
Catheter associated	10 (9.2)	4 (0.6)	< 0.001
Neutropenic fever	13 (11.9)	67 (10.3)	0.741
CNS/deep neck	1 (0.9)	9 (1.4)	1.000
Not specified	0 (0)	29 (4.5)	0.015
Quick SOFA score	0 (0–2)	0 (0–1)	0.001
CWIs	1 (0–2)	1 (0–3)	0.697
28-day mortality	15 (13.8)	50 (7.7)	0.057

Data are expressed as numbers (%) of patients or means ± standard deviations. PCT, procalcitonin; CRP, C-reactive protein; CNS, central nervous system; SOFA, sequential organ failure assessment; CWI, Charlson's weighted index of comorbidity.

specificity, PPV, and NPV were 78.46, 64.74, 17.29, and 96.97%, respectively.

Independent predictors of culture-proven bacterial infection, bacteremia, and 28-day mortality

The cut-off values of three biomarkers derived from the ROC analysis were used for logistic regression analysis (**Table 2**). PCT (OR = 2.23, 95% CI: 1.55–3.19; $p < 0.001$), CRP (OR 1.62, 95% CI 1.08–2.44; $p = 0.020$), and qSOFA (OR 1.48, 95% CI 1.03–1.98; $p < 0.001$) were found to be the independent predictors of culture-proven bacterial infection, but presepsin was not. Presepsin (OR 2.28, 95% CI 1.41–3.70; $p < 0.001$) and PCT (OR 5.18, 95% CI 3.13–8.56; $p < 0.001$) were found to be the independent predictors of bacteremia, but CRP was not. In contrast, only presepsin among biomarkers was found to be the independent predictors of 28-day mortality (OR 3.31, 95% CI 1.67–6.54; $p < 0.001$), in addition to qSOFA (OR 3.14, 95% CI 2.36–4.18; $p < 0.001$) and CWIs (OR 1.19, 95% CI 1.05–1.34; $p = 0.006$).

Discussion

Early recognition of bacterial infection including bacteremia is very important for initiating antimicrobial therapy and improving clinical outcomes (27). Biomarkers play an essential role in early identification of bacterial infection, furthermore, bacteremia, sepsis, severe sepsis, and septic shock (10). PCT was regarded as a useful marker for diagnosis of bacterial infection. It could identify patients with sepsis in 96% and septic shock in 98% of cases, which seemed to be superior to SOFA score (36, 37). Moreover, PCT showed better diagnostic and prognostic role in case of gram-negative sepsis and septic shock than gram-positive and fungal sepsis. These superior performance of PCT may make it possible to tailor antimicrobial therapy early (38).

However, as research results showed PCT could be elevated in non-infectious conditions such as postoperative settings, cardiogenic shock, trauma, burn, acute pancreatitis and acute graft-vs.-host disease, efforts were made to find another ideal biomarker due to these limitations (10, 11). Presepsin, a new diagnostic biomarker for sepsis, is highly specific for diagnosing bacterial infections because its production is associated with bacterial phagocytosis and cleavage of microorganisms by lysosomal enzymes. It was proven to be secreted from granulocytes by infectious stimuli in an animal study (39).

Therefore, we evaluated the usefulness of presepsin to predict diagnosis of culture-proven bacterial infection and bacteremia in adult patients relative to other biomarkers. We retrospectively collected measurement the level of three biomarkers, presepsin, PCT and CRP in patients with suspected different infectious conditions on the day of occurrence of it and also collected final culture

TABLE 2 Independent factors for predicting culture-proven bacterial infection, bacteremia, and 28-day mortality.

	Variables	Adjusted OR (95% CI)	*p*-value
Culture-proven	Age	1.01 (1.00–1.02)	0.296
Bacterial infection	Sex	1.43 (1.03–1.98)	0.031
	Presepsin ≥ 592.5 pg/mL	1.25 (0.88–1.77)	0.220
	PCT ≥ 0.305 ng/mL	2.23 (1.55–3.19)	< 0.001
	CRP ≥ 21.5 mg/dL	1.62 (1.08–2.44)	0.020
	qSOFA	1.48 (1.03–1.98)	< 0.001
	CWIs	0.98 (0.91–1.06)	0.627
Bacteremia	Age	0.99 (0.97–1.00)	0.120
	Sex	1.31 (0.83–2.06)	0.249
	Presepsin ≥ 1028.5 pg/mL	2.28 (1.41–3.70)	< 0.001
	PCT ≥ 1.23 ng/mL	5.18 (3.13–8.56)	< 0.001
	CRP ≥ 24. 2 mg/dL	1.65 (0.98–2.75)	0.057
	qSOFA	1.21 (0.97–1.53)	0.097
	CWIs	0.94 (0.84–1.04)	0.213
28-day mortality	Age	1.02 (1.00–1.04)	0.094
	Sex	0.91 (0.49–1.66)	0.750
	Presepsin ≥ 704.5 pg/mL	3.31 (1.67–6.54)	< 0.001
	PCT ≥ 2.68 ng/mL	1.02 (0.52–1.98)	0.964
	CRP ≥ 23.2 mg/dL	1.47 (0.74–2.92)	0.273
	qSOFA	3.14 (2.36–4.18)	< 0.001
	CWIs	1.19 (1.05–1.34)	0.006

OR, odds ratio; PCT, procalcitonin; CRP, C-reactive protein; SOFA, sequential organ failure assessment; CWI, Charlson's weighted index of comorbidity.

results. All three biomarkers and qSOFA scores were associated with bacteremia in univariable analyses. Among pneumonia cases, eight patients had bacteremia (7.3%) while 298 patients did not (46%). It may be that, with pneumonia, it is only possible to diagnose the causative pathogen in 30–40% of cases using conventional diagnostic methods (40).

ROC curve analysis demonstrated that PCT was superior to presepsin and CRP as a diagnostic biomarker, and it had higher sensitivity and negative predictive value for predicting culture-proven bacterial infection and bacteremia. Numerous studies showed that presepsin is a good inflammatory marker for sepsis, wherein it showed better sensitivity, specificity, and diagnostic accuracy than PCT. However, only a few studies with a small number of patients focused on the role of presepsin for predicting bacterial infection including bacteremia and each study yielded conflicting results (25–28). Leli et al. conducted a study with 92 patients with suspected sepsis. Bacteremia was confirmed in 32 of 92 patients, and they showed that both presepsin and PCT had good diagnostic accuracy in predicting bacteremia, superior to CRP (25). Romualdo et al. produced similar results, wherein bacteremia was confirmed in 37 of 226 patients with SIRS and presepsin and PCT showed similar potential to differentiate between SIRS patients with and without bacteremia. The AUC value of presepsin for predicting bacteremia was higher than PCT (26). Imai et al. conducted a prospective study with 46 elderly patients and the AUC values were not different among presepsin and PCT (27). However, these studies evaluated the utility of presepsin for predicting bacteremia with a relatively small population. In contrast, our study evaluated 757 patients all with suspected infection from any origin. Among them, 256 patients had culture-proven bacterial infection, and 109 of those infected patients were confirmed to have bacteremia.

The optimal cut-off value for presepsin for diagnosing bacteremia in our study was 1028.5 pg/mL, relatively higher than other studies (14–16, 23). However, as with most prospective studies, patients who were suspected to have sepsis or septic shock at the time of admission were included, patients who had non-infectious etiologies that manifested like sepsis or septic shock or who had no bacterial infections may have been included. In a bacteremia study, the authors suggested an optimal presepsin cut-off value of 729 pg/mL, but this study only included 37 bacteremic patients (26). The retrospective cross sectional study design, characteristics of the single-center study, and patient diversity including hospitalized patients as well as hospitalization through the emergency department may have influenced the high cut-off value of presepsin.

Whether presepsin can distinguish between Gram-positive and Gram-negative bacterial infections is still controversial (21, 23, 41). It has been hypothesized that presepsin levels can differentiate type of bacterial origin from the fact that presepsin is a receptor of LPS, which is one of the components of Gram-negative bacteria (23). Although, there was a difference in presepsin levels between Gram-positive and Gram-negative bacterial infections, the difference was not statistically meaningful (947.5 vs. 1232.5, $P = 0.705$).

Some studies concluded that presepsin could be used to assess the severity of inflammatory disease without infection or viral disease. These studies showed that inappropriate monocyte or neutrophil activation due to systemic lupus erythematous flare-up had induced elevation of presepsin levels. These results also suggested that presepsin production was influenced by monocyte phagocytosis from a neutrophil extracellular trap (42, 43). Presepsin has also been suggested as a predictive biomarker of severity in COVID-19 infections. Severe COVID-19 infections could cause a systemic inflammatory reaction combined with elevated cytokines, such as monocyte chemoattractant protein 1 and macrophage inflammatory protein 1a. These cytokines may stimulate presepsin production (44, 45). Combining the results of previous studies and the result of our study, presepsin may be a specific marker for clinical situations involving monocyte activation rather than being specific to bacterial infection.

Taken together, presepsin could be a useful prognostic factor for 28-day-mortality rather than a predictor of bacterial infection.

This study had several limitations. First, there is a potential selection bias because the suspicion of bacterial infection was made freely by physicians. Second, we did not classify according to time of blood sample collection. It is known that presepsin specifically increases within a few hours of clinically suspected sepsis (15). However, because this was a cross-sectional study, it was not possible to compare each biomarker serially over time in clinical situations of suspected bacterial infection with or without bacteremia. Additional validation through cohort study is required. Third, we did not exclude patients with acute kidney injury (AKI) or end-stage kidney disease (ESRD), knowing that renal function could falsely increase presepsin levels. In our study, there were only 40 patients with AKI or ESRD, and additional evaluations were not performed.

In conclusion, among the evaluated biomarkers, PCT showed best performance predicting culture-proven bacterial infection and bacteremia while presepsin was more useful as a prognostic marker. Further studies are necessary to better understand the role of presepsin in various clinical settings, such as viral infection, fungal infection, and non-infectious inflammatory conditions.

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 the Institutional Review Board (IRB) of Konkuk University Medical Center (#2022–04-040). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JP, J-HK, H-WM, JY, and HK: conceptualization. JP and H-WM: investigation. H-WM: laboratory work and methodology. JP: supervision. JP, J-HK, and H-WM: writing—review and editing. All authors have read and agreed to the publication of this manuscript.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.954114/full#supplementary-material

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Machine learning models for predicting in-hospital mortality in patient with sepsis: Analysis of vital sign dynamics

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Purpose: To build machine learning models for predicting the risk of in-hospital death in patients with sepsis within 48 h, using only dynamic changes in the patient's vital signs.

Methods: This retrospective observational cohort study enrolled septic patients from five emergency departments (ED) in Taiwan. We adopted seven variables, i.e., age, sex, systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and body temperature.

Results: Among all 353,253 visits, after excluding 159,607 visits (45%), the study group consisted of 193,646 ED visits. With a leading time of 6 h, the convolutional neural networks (CNNs), long short-term memory (LSTM), and random forest (RF) had accuracy rates of 0.905, 0.817, and 0.835, respectively, and the area under the receiver operating characteristic curve (AUC) was 0.840, 0.761, and 0.770, respectively. With a leading time of 48 h, the CNN, LSTM, and RF achieved accuracy rates of 0.828, 0759, and 0.805, respectively, and an AUC of 0.811, 0.734, and 0.776, respectively.

Conclusion: By analyzing dynamic vital sign data, machine learning models can predict mortality in septic patients within 6 to 48 h of admission. The performance of the testing models is more accurate if the lead time is closer to the event.

KEYWORDS

sepsis, dynamic vital sign, mortality, prediction model, machine learning model

Highlights

- By analyzing dynamic vital sign data, machine learning models can predict mortality in septic patients within 6–48 h of admission.
- The performance of the machine learning models is more accurate if the lead time is closer to the event.

Introduction

Sepsis is the presence of an acute infection and new organ dysfunction. It can be life-threatening if not recognized and treated promptly (1). Despite advanced care, previous studies have demonstrated that sepsis remains a significant burden worldwide and is the most common cause of in-hospital deaths (2–4). Although outcomes have improved in recent decades, mortality remains high at approximately 25–30% (5). Furthermore, septic shock is associated with an even higher mortality rate of ∼40–50% (6). For patients at critical risk, increased awareness, aggressive treatment, and broad-spectrum empiric antibiotics significantly decrease the mortality risk (7). It is therefore imperative to rapidly and accurately stratify patients with sepsis and high in-hospital mortality.

In recent decades, medical artificial intelligence (AI) has been used to achieve clinical diagnoses and suggest treatments. A few examples where AI has shown promise for clinical diagnoses include diabetic retinopathy screening (8), skin lesion classification (9), and assist in detection of abdominal free fluid during focused assessment with sonography (10). In combination with machine learning algorithms and electronic health records (EHRs), clinical data sources enable us to rapidly generate prediction models and predict clinical outcomes. For instance, an AI model has been used to predict the mortality of patients diagnosed with COVID-19 (11), outcomes in trauma patients (12), and neurological outcomes of out-of-hospital patients after a cardiac arrest (13).

Machine learning methods can predict in-hospital mortality in sepsis patients in an intensive care unit (ICU) (14). At the time of sepsis onset, Barton et al. demonstrated that a machine learning algorithm with gradient-boosted trees increases the sensitivity and specificity of predicting sepsis occurrence over the commonly used systemic inflammatory response syndrome (SIRS), modified early warning score (MEWS), sequential organ failure assessment (SOFA), and quick sequential organ failure assessment (qSOFA) scoring systems (15). In addition, machine learning algorithms can predict the occurrence of severe sepsis and septic shock (14, 16). For predicting in-hospital mortality of ED patients with sepsis, Taylor et al. found that a machine learning approach outperformed existing clinical decision rules (17).

However, most previous prediction models for mortality require a large number of variables, including the underlying disease, laboratory data, and clinical parameters. The aim of our study was to build ML models for predicting the risk of in-hospital death in patients with sepsis within 48 h, using only dynamic changes in the vital sign.

Methods

Study population and extraction samples

This is a retrospective observational cohort study conducted from January 1, 2006 to December 31, 2017. The study was approved by the IRB Review Board of the Chang Gung Medical Foundation (IRB number: 201801713B0; approved on 28 January 2019) in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was not required owing to the retrospective nature of the study.

We used data provided by the Chang Gung Medical Center, including five EDs that belonged to a single healthcare system and were geographically dispersed nationwide in Taiwan. Sepsis patients were extracted from the electronic database records of the Chang Gung Medical Center under the following conditions: (1) The age of the patient was over 17 years, (2) blood culture was obtained, and (3) antibiotics were prescribed in medical order. Sepsis patients were defined according to the Third International Consensus Definition of Sepsis (Sepsis-3) definition, that was an acute change in Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more consequent to the infection (1).

We excluded patients who had an out-of-hospital cardiac arrest because their high mortality rates could falsely affect the performance of the prediction models. Besides, we also excluded patients for the following reasons: (1) the length of the hospital stay was >3 days (2) the patients recorded less than three times when they stay in hospital, and (3) patients with incorrect data and format. The selection process and sample numbers were listed in the Supplementary Table 1.

The outcome was divided into two results: positive instances in which patients died in the hospital and negative instances in which patients survived. After cleaning problematic data such as those containing less than one record for every variable, and those having an error in terms of format, the number of positive instances was 19,434, and the other negative instances numbered 194,646. Supplementary Table 1 presents the detailed sample selection process. We found that the number of negative instances was 10-times greater than the number of positive instances. The number of surviving patients was 16-times the number of deceased patients. To resolve the imbalanced sample problems, we used random sampling in negative instances to balance the number of positive and negative instances. We use python programs which the system provides the function random choice(). The function random choice() can choose the instances from the negative instances randomly and the amount of the negative instances we requested.

Feature selection and data processing

To construct a mortality prediction tool, we adopted five vital signs: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), respiratory rate (RR), and body temperature (BP). Patient age and sex were also included. Vital signs were selected as objective predictor variables because they are routinely and frequently collected, regardless of the clinical situation, and the values are rarely affected by the examiner.

There were two different types of outcomes in this research. For these two outcomes, we extracted negative instances from all subjects who survived and positive instances from all subjects who died in the hospital. The data for up to 6–48 h prior to death were extracted as a positive instance, and the data for up to 6–48 h prior to survival or discharge were extracted as a negative instance, as shown in Supplementary Figure 1.

We mainly focused on four different lead times (k = 6, 12, 24, and 48) prior to the results, and we used machine learning and developed four models to predict the results k hours in advance.

The subsequent cleaning process ensured that the electronic data were ready for analysis and did not contain any errors. First, we removed problematic records. Second, to resolve the problems of missing data, the measured value of the vital sign variable was forward-filled following an initial measurement until the next available measurement. The choice was based on the clinical insight that measurements are taken more frequently during times of hemodynamic instability and less frequently when the patient appears stable. Third, the records were converted into z-scores using the computed means and standard deviations. These vital sign variables were normalized to [0, 1] for comparison. Fourth, the vital sign normalized values are divided into 255, according to the degree of the divided results, converted into grayscale, and transformed into an image for each patient.

Machine learning

To build appropriate models and develop an early warning system (EWS), we set the training, validation, and testing sets to a ratio of 6:2:2. The early warning system models developed using convolutional neural networks (CNN) was labeled as EWS_C, using long short-term memory (LSTM) was labeled as EWS_L, and using random forest (RF) was labeled as EWS_R, respectively.

CNNs are the most mature tools in graphical process in machine learning. It is a class of deep feed-forward artificial neural networks, and a CNN architecture is formed by a stack of distinct layers that transform the input volume into an output volume through a differentiable function (18). A few distinct types of layers contain a convolutional layer, a pooling layer, an activation layer, a fully connected layer, and a loss layer, the conceptual architecture of which is illustrated in Supplementary Figure 2 and Supplementary Table 2.

LSTM is effective for capturing the underlying temporal structures in time-series data. It consists of the following three gates: forget, input, and output gates. These three gates interact to control the flow of information. LSTM builds memory by feeding the previous hidden state as additional input in the subsequent step. This makes the model particularly suitable for modeling dynamics in vital sign data, which has a strong statistical dependency between medical events over the time intervals. LSTM enables the network to maintain the previous information of the hidden states as internal memory (19). The network architecture of the LSTMs are listed in Supplementary Table 3. Parameters of Random Forest model.

RF is an efficient, multi-class approach that is able to handle large attribute spaces, and has been widely used in several domains including real-time face recognition and bioinformatics (20). RF is an ensemble method used to construct many decision trees that are applied in the classification of a new instance based on a majority vote. Each decision tree node uses a subset of attributes that are randomly selected from the entire original set of attributes. The RF model parameters are listed in Supplementary Table 4.

The flow of the applied research method is described in the previous section. Supplementary Figure 3 presents the overall research flow diagram.

For the reliability and stability of our models in this research, our research adopted two validation methods. The first validation, we use k-fold cross-validation methods considered our sample sizes, we adopted k-fold = 5 for model tuning and yield a satisfying generalization performance. In k-fold cross-validation, we randomly spilt the training dataset in k folds without replacement, where k-1 folds are used for the models training and one folds is used for testing. This procedure is repeated k times and we obtain k models and performance estimates. Then, we calculate the average and 95% confidence interval performance of the models based on the different, independent folds to obtain a performance estimate that is less sensitive to the sub-partitioning of the training data. We listed the cross-validation results of EWS_C, EWS_L, and EWS_R in the Supplementary Tables 8–10, respectively.

The second validation part was reserved the data of 2017 as extra validation part. The EWS_C, EWS_L, and EWR_R were validated the data of 2017, and the results were listed in the validation part in the Supplementary Tables 5–7, respectively. From the 1-year clinical validation, our research results were more reliable and stability.

Statistical analysis

To accurately build the early warning system model, we compared it with other standard machine learning algorithms,

i.e., CNN, LSTM, and RF. We call these models EWS_C, EWS_L, and EWS_R, respectively. The model performance was assessed based on discrimination using the precision, recall, accuracy, receiver operating characteristic (ROC) curve, and derived area under the ROC curve (AUC).

Results

Dataset statistics

The symbols in this research included 28,530 positive instances and 194,646 negative instances. We also excluded (1) records from <72 h after admission to the ED, (2) cases with fewer than three records, and (3) incorrect data or an improper format. To summarize, k = 6, 12, 24, and 48 h in the numbers of excluded and included samples are listed in Supplementary Table 1.

We extracted the vital signs of the patients in k (where k = 6, 12, 24, and 48 h) prior to the time of death as a positive instance. By contrast, we extracted the vital signs of the patient k (h) prior to the time of survival as a negative instance. The smaller the lead time before the result is, the larger the number of patient cases. For example, when k = 6 h, there are 19,434 positive instances and 194,646 negative instances; in other cases, when k = 48 h, there are 17,123 positive instances and 125,102 negative instances.

Mortality prediction performance

We compared the models among these three methods: CNNs, LSTMs, and RF. To distinguish which model is more accurate and reliable and help doctors make decisions, we also compared four different lead time models, i.e., 6, 12, 24, and 48 h models.

In the first part, we used the CNN-based algorithm under different lead-time models for EWS_C. With EWS_C, the precision, sensitivity, and accuracy values of the training and validation models are summarized in Supplementary Table 5. In the validation model, we also provide AUC_COV and the confidence interval (CI) under different lead-time models. In addition, we provide the ROC curve of EWS_C in Figure 1A.

According to the validation models for EWS_C, the records show that the precision among these four different lead time models is above 0.85. The sensitivity was above 0.75 for EWS_C6 and EWS_C12, and the sensitivities for EWS_C24 and EWS_C48 were above 0.7. In addition, the accuracy for EWS_C was >0.8. In Figure 1A, we found that the AUC in the EWS_C training model at 6 h was the largest, reaching 0.92. We also found that the other lead times (12, 24, and 48 h) and their AUC were all above 0.8. In the testing of the EWS_C model for all lead times, the ROC curves are all above 0.8 (Figure 1B). In Supplementary Table 6 and Figure 1C, we summarize the training and validation results. We found that the precision, sensitivity, and accuracy are ∼0.8, 0.7, and 0.75. As shown in Figure 1D, the ROC curve for EWS_L testing was approximately 0.75. Supplementary Table 7 summarizes the results of the training and validation of EWS_R. We found that the validation of EWS_R had a precision of approximately 0.8, a sensitivity of ∼0.7, and an AUC of nearly 0.77. According to Figure 1E, for the ROC curve of EWS_R in the training model, the area of all lead times was over 0.8. In the testing model, the area of all lead times was over 0.7 (Figure 1F).

Figure 2 show the ROC curves of the three testing models for lead times of 6, 12, 24, and 48 h. Regardless of the lead time, we found that the AUC of EWS_C was the largest. For a 48-h lead time, the AUC was still over 0.8.

Discussion

In our study, machine learning models were used to predict the mortality in septic patients 48 h prior to death. The AUC of the testing models for a 48-h lead time is 0.83, 0.74, and 0.77 with the EWS_C, EWS_L, and EWS_R models, respectively. In general, the performance of the testing models is more accurate if the lead time is closer to the event. The AUC of the testing models under a 6-h lead time could achieve values of 0.84, 0.75, and 0.78 for EWS_C, EWS_L, and EWS_R, respectively. For all lead times, we found that the AUC of EWS_C had the best model performance among the ML models, with an AUC within the range of 0.82–0.85.

A wide array of rule-based scoring systems was developed to assess the severity of illness and risk stratification. Examples frequently used as severity assessment tools in the ICU are the simplified acute physiology score (SAPS) II (21), acute physiology and chronic health evaluation (APACHE) III and IV scores (22, 23), and the SOFA score (24). As a major limitation of the above systems applied in the ED, they require information that is often not readily available during a patient's time in the ED. Therefore, EWSs were developed to detect patients at risk of deterioration and predict catastrophic events in an ED. For the general ED population and patients with respiratory distress, the NEWS achieves the highest accuracy in mortality prediction (25). For patients with infection or sepsis, the MEDS and MEWS were the most utilized methods of assessment. In general, the MEDS (AUC of 0.73–0.871) achieves a better accuracy than MEWS (AUC of 0.596–0.73) in predicting in-hospital mortality (26–30). Other prognostic scores frequently used in an ED include a rapid emergency medicine score and the qSOFA, with an AUC range of 0.62–0.80 and 0.58–0.76, respectively (31, 32).

These EWSs were created mostly based on physiological measurements and clinical observations, including vital signs, level of consciousness, laboratory data, and other metrics,

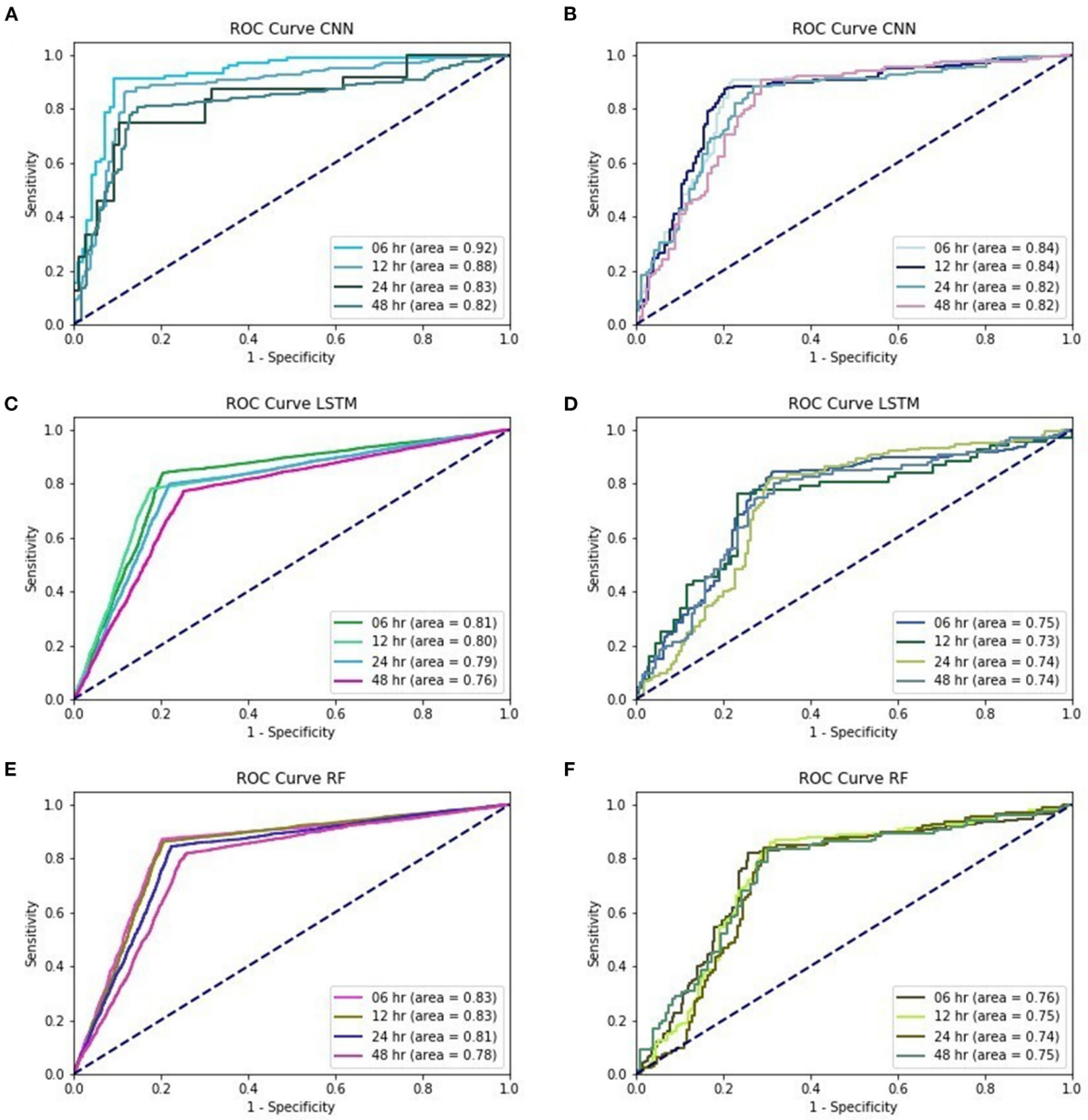

FIGURE 1
(A) training performances of ROC curve in EWS_C, **(B)** testing performances of ROC curve in EWS_C, **(C)** training performances of ROC curve in EWS_L, **(D)** testing performances of ROC curve in EWS_L, **(E)** training performances of ROC curve in EWS_R, **(F)** training performances of ROC curve in EWS_R.

depending on the selected modification tool. Thus, most of these scores are complex or disease-specific, leading to a poor early recognition of septic patients at risk of deterioration. Moreover, the differences between the observed and expected mortality may also be caused by inadequate diagnostic data, unreliable Glasgow coma scale (GCS) score assessment, regional differences, and changes in the effectiveness of therapy over time (33, 34).

With the progress and development of big data techniques, machine learning methods have attracted research attention in the past decade. Zhang et al. found that the least absolute shrinkage and selection operator technique achieves a good discrimination and calibration for mortality prediction in patients with severe sepsis (35). Using over 500 clinical variables, Taylor et al. demonstrated that the machine learning approach outperformed existing clinical decision rules, with

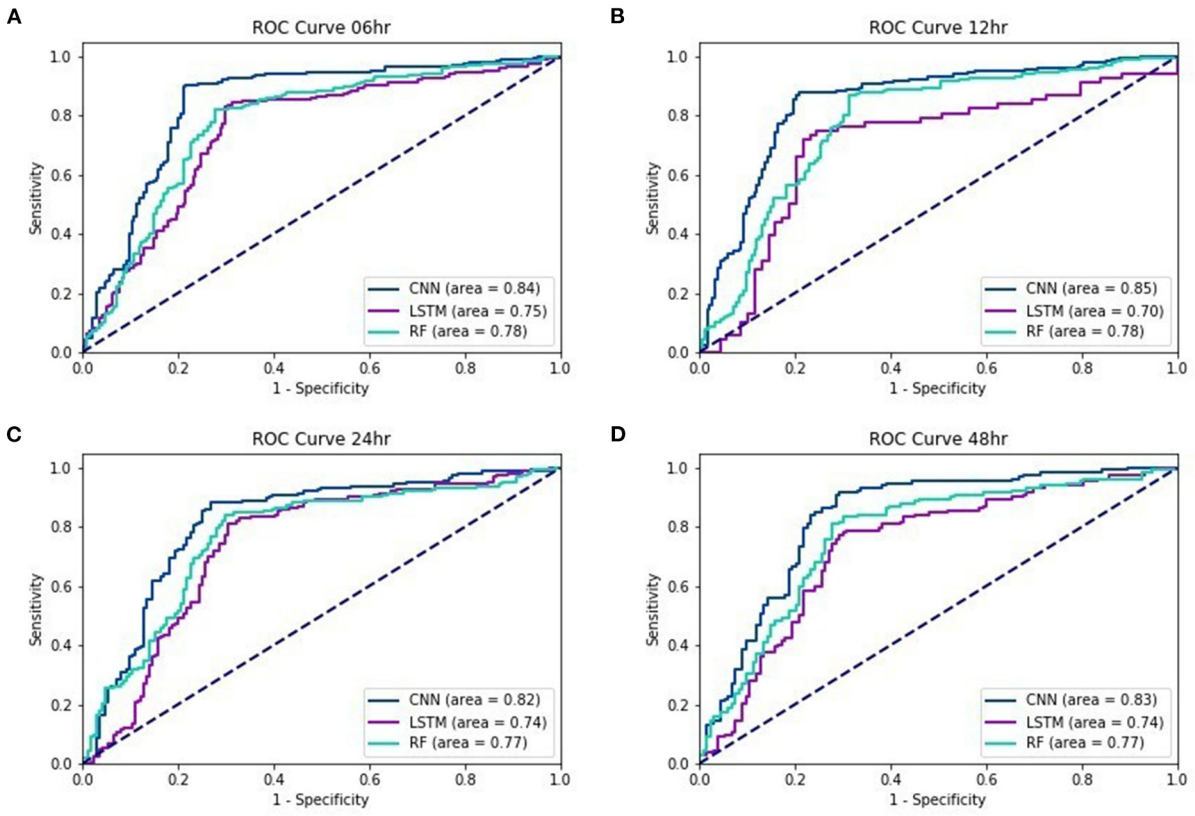

FIGURE 2
ROC curves of EWS_C, EWS_L, and EWS_R: **(A)** 6 **(B)** 12, **(C)** 24, and **(D)** 48 h lead times.

the RF model performing better than the LR model in terms of discrimination (17). Considering a total of 587 features, including demographics, vital signs, and laboratory results, Giannini et al. showed that an RF classifier can predict the impending occurrence of severe sepsis and septic shock with a low sensitivity of 26% and high specificity of 98% (16). Misra et al. indicated that using clinical and administrative data, machine learning models can be applied to predict septic shock within the first 6 h of admission, with a sensitivity of 83.9% and a specificity of 88.1% based on RF (36). Utilizing nine features combined with vital signs, chief compliances, and the emergency severity index, Klug et al. concluded that the gradient boosting model shows a high predictive ability for screening patients at risk of early mortality using data available at the time of triage in the ED (37). However, most of the previous machine learning methods for predicting the prognosis of patients with sepsis required numerous variables, including laboratory results, GCS, and clinical parameters.

Several studies highlight the value of dynamic vital sign changes for building predictive models. A pilot study used physiomarkers to generate 52 highly ranked features and build an eXtreme Gradient Boost classifier that could predict post-liver transplant patients 12 h before developing sepsis (38). Another observational cohort study yielded a total of 60 features from physiomarkers, and revealed predict severe sepsis 8 h prior to the event in critically ill children (39). Van Wyk et al. found that using continuous physiological data alone to generate a total of 132 features, random forest classifier could discriminate sepsis 5 h before the onset (40). Using five physiological data streams including HR, RR, and BP (systolic, diastolic, and mean), Mohammed et al. developed a support vector machine (SVM) classifier for predict sepsis up to interval of 17.4 h before sepsis onset, with an average test accuracy, sensitivity, specificity, and area under the receiver operating characteristics curve of 0.83, 0.757, 0.902, and 0.781, respectively (41).

However, using only physiological data, previous studies mostly focused on predict sepsis event. By contrast, our study focused on predict mortality. We included only seven input parameters in our study, including age, sex, and vital signs (BT, SBP, DBP, HR, and RR), available from the moment of triage to any time during hospitalization. Using data available in the

ED in real time, artificial intelligence can accurately predict mortality in septic patients 6–48 h prior to clinical recognition.

The proposed method has several advantages. First, vital signs had clear-cut values and were obtained through machine measurements, which reduced the expert judgment and limited variations in the healthcare providers. In addition, models that require hundreds of variables may lead to difficulty in encoding the databases and may have more missing values or data errors. Instead, we attempted to develop an uncomplicated model that requires simple input parameters that are routinely collected during daily practice. A simplified tool would be more easily implemented in resource-limited ED settings. Furthermore, the data used in our model were widely available in clinical practice. Lukaszewski et al. reported that neural networks can correctly predict patient outcomes of overt sepsis prior to clinical diagnosis with high sensitivity and selectivity (91.43 and 80.20%, respectively) (42). Because cytokines are not routinely measured, this tool is impractical in clinical practice. Instead, our study attempted to develop a simplified model with feasible and reliable input parameters that can be efficiently collected in place with limited medical resources.

Our study has several limitations. First, this was a retrospective study conducted in Taiwan. The sample was homogeneous and may have been subject to local practices, limiting its generalizability to other ethnicities. Second, we did not compare all available ML models and scoring systems, or their variations. There are hundreds of different ML models and variations; therefore, a comprehensive study is unfeasible. Application of the developed ML model to other datasets or populations requires a further clinical evaluation.

Conclusion

This study contributes to clinical areas using machine learning in-hospital mortality prediction models for sepsis patients in the ED. By analyzing dynamic vital sign data, machine learning models can predict mortality in septic patients within 6–48 h of admission. The CNN achieves the best model performance in comparison to the LSTM and RF approaches. In general, the performance of the testing models is more accurate if the lead time is closer to the event.

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 Chang Gung Medical Foundation. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

CK and C-MS conceived the study and assumed responsibility for the paper as a whole. I-MC and C-HL managed the data and including quality control. C-TK, F-CC, and C-CC provided statistical advice on the study design and analyzed the data. C-YC and C-TK chaired the data oversight committee. C-YC drafted the manuscript and all of the authors contributed substantially to its revision. All authors read and approved the final 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.964667/full#supplementary-material

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Evaluation of procalcitonin elevation during ICU stay and its relationship with mortality in ICU patients for COVID-19 with respiratory involvement. A multicenter prospective cohort study

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Introduction: A multicenter prospective cohort study studied patients admitted to the intensive care unit (ICU) by coronavirus-19 (COVID-19) with respiratory involvement. We observed the number of occasions in which the value of procalcitonin (PCT) was higher than 0.5 ng/ml.

Objective: Evaluation of PCT elevation and influence on mortality in patients admitted to the ICU for COVID-19 with respiratory involvement.

Measurements and main results: We studied 201 patients. On the day of admission, acute physiology and chronic health evaluation (APACHE)-II was 13 (10–16) points. In-hospital mortality was 36.8%. During ICU stay, 104 patients presented 1 or more episodes of PCT elevation and 60 (57.7%) died and 97 patients did not present any episodes of PCT elevation and only 14 (14.4%) died ($p < 0.001$). Multivariable analysis showed that mortality was associated with APACHE-II: [odds ratio (OR): 1.13 (1.04–1.23)], acute kidney injury [OR: 2.21 (1.1–4.42)] and with the presentation of one or more episodes of escalating PCT: [OR: 5.07 (2.44–10.53)]. Of 71 patients who died, 59.2% had an elevated PCT value on the last day, and of the 124 patients who survived, only 3.2% had an elevated PCT value on the last day ($p < 0.001$). On the last day of the ICU

stay, the sequential organ failure assessment (SOFA) score of those who died was 9 (6–11) and 1 (0–2) points in survivors ($p < 0.001$). Of the 42 patients who died and in whom PCT was elevated on the last day, 71.4% were considered to have a mainly non-respiratory cause of death.

Conclusion: In patients admitted to the ICU by COVID-19 with respiratory involvement, numerous episodes of PCT elevation are observed, related to mortality. PCT was elevated on the last day in more than half of the patients who died. Serial assessment of procalcitonin in these patients is useful because it alerts to situations of high risk of death. This may be useful in the future to improve the treatment and prognosis of these patients.

KEYWORDS

procalcitonin, COVID-19, respiratory involvement, ICU, sepsis—diagnostics

1 Introduction

The coronavirus-19 (COVID-19) pandemic has affected many patients with high mortality. Many patients have required admission to the intensive care unit (ICU), with mortality very high (1), and knowledge of the prognostic factors of these patients is very important.

Biomarkers are of great help in diagnosis and treatment in different fields of medicine (2–6). Procalcitonin (PCT) is being used as a marker of bacterial infection and to distinguish whether the cause of the clinical picture is bacterial or viral. And as stated by Lippi and Plebani (7), the production and release into circulation of procalcitonin from extra-thyroid sources are greatly amplified during bacterial infections, actively supported by increased concentrations of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6. However, the synthesis of this biomarker is inhibited by interferon (INF)-γ, the concentration of which increases during viral infections.

Several meta-analyses and systematic reviews have been performed on the usefulness of PCT for diagnosing bacterial sepsis in critically ill patients (8–10).

Increased PCT values are associated with a nearly 5-fold higher risk of severe SARS-CoV-2 infection (7), and serial procalcitonin measurement may play a role in predicting evolution toward a more severe form of the disease (7, 11). Han et al. (12) postulate that raised PCT observed in COVID-19 could be due either to bacterial co-infection, which is itself causing increased severity and driving systemic sepsis or as a direct marker of a more severe or widespread viral infection. Previous studies (13, 14) find associations between PCT values, severity, and clinical outcomes, especially for mechanical ventilation and all-cause mortality.

Patients admitted to the ICU for COVID-19 with respiratory involvement often require a prolonged stay (15). Patients with prolonged ICU stay present multiple problems that need to be detected and treated. Many studies evaluate the prognostic implications of PCT at a single time point in the evolution of patients with COVID-19 but the prognostic implications at different times during the ICU stay of these patients are less well studied (16). Furthermore, the interpretation of the significance of elevated PCT in ICU patients is difficult to generalize, since these patients frequently develop renal failure (17, 18) and the interpretation of elevated PCT levels is difficult in this group of patients.

We think that it is necessary to investigate further the diagnostic and prognostic implications of elevated PCT values in patients with COVID. We think that the assessment of PCT values at different times of ICU stay may help to increase our knowledge and assist in the management and treatment of these patients with high severity and mortality.

This study aims to analyze PCT levels on admission and during ICU stay in patients admitted to the ICU with respiratory involvement due to COVID-19 and to evaluate its possible prognostic implications.

2 Materials and methods

2.1 Design

A prospective multicentric cohort study was conducted and we studied all patients admitted to the ICU with respiratory involvement due to COVID-19 in five hospitals. Data were obtained from ICU patients from the following Spanish hospitals in Andalusia: "Hospital Universitario Jaén" in Jaén, "Hospital de San Agustín" in Linares (Jaén), "Hospital de la Serranía de Ronda" in Ronda (Málaga), "Hospital Infanta Margarita" in Cabra (Córdoba), and "Hospital de Montilla" in Montilla (Córdoba). The patients were admitted from 9th March 2020 until August 2021 in one hospital, until November

2020 in two hospitals, and until April 2020 in two other hospitals."

Coronavirus-19 infection was confirmed in 100% of the patients by a positive result by real-time reverse transcriptase polymerase chain reaction (PCR) for SARS-CoV-2 or detection of IgM or IgG antibodies for SARS-CoV-2, determining infection in the first 15 days and the positivity for IgM.

Admission of patients to the ICU was determined according to the usual criteria of each hospital. In general, in our ICUs the admission criteria were similar, admitting recoverable patients who required specific organic support treatment in the ICU or who required monitoring due to the risk of complications requiring treatment in the ICU. In the specific case of our patients, all of them were affected by COVID-19 with respiratory involvement, the main reason was the need for ventilatory support, with a high percentage of invasive mechanical ventilation.

We collected data on affiliation, previous pathology, and the severity of the process assessed on the first day of admission and the third day with sequential organ failure assessment (SOFA) (19) and acute physiology and chronic health evaluation (APACHE)-II (20).

Procalcitonin values were evaluated daily on certain occasions or in a very high percentage of the days they were in the ICU. A graph of PCT values during each patient's stay was created for each patient. The number of peaks or occasions during the evolution when the value was in a range above 0.5 ng/ml was observed. Independent peaks in the evolution of the same patient were considered when, after an increase of more than 0.5 ng/ml on one occasion during the evolution, a decrease of at least two-thirds of the maximum value was observed on the following days, followed by another increase (the maximum value of the increase also being greater than 0.5 ng/ml).

Acute kidney injury (AKI) was defined according to AKIN criteria (21): Stage 1–Increase in serum creatinine of more than or equal to 0.3 mg/dl or increase to more than or equal to 150–200% from baseline or diuresis less than 0.5 ml/kg/h for more than 6 h, Stage 2–Increase in serum creatinine to more than 200–300% from baseline or diuresis less than 0.5 ml/kg/h for more than 12 h and Stage 3–Increase in serum creatinine to more than 300% from baseline or creatinine greater than 4 mg/dl with an acute increase of at least 0.5 mg/dl or diuresis less than 0.3 ml/kg/h for 24 h or more or anuria for 12 h or more or the initiation of renal replacement therapy. The plasma creatinine value was measured in all patients on admission to the ICU. However, since many patients are admitted to the ICU with impaired renal function, we also calculated the estimated basal creatinine and considered the basal creatinine to be the lower of the two values.

The estimated basal creatinine value was calculated according to the formula (MDRD), defined as the ideal creatinine value for each patient, assuming a normal glomerular filtration rate of 75 ml/min/1.73 m^2: (Estimated basal creatinine = $(75/[186 \times (age^{-0.203}) \times (0.742$ if female$) \times (1.21$ if African-American$)])^{-0.887}$).

For most calculations, the patient was considered to have AKI if they were classified as stage 1, stage 2, or stage 3. The presence of AKI was assessed at different time points where the relationship between increased PCT and mortality was assessed (day 3 of ICU stay, last day of ICU stay, 2 days before ICU discharge, and 5 days before ICU discharge). And for the analysis of the relationship between the presence of 1 or more episodes of elevated PCT during the entire ICU stay, the presence of AKI was considered if at any time the patient presented AKIN criteria of stage 1, stage 2, or stage 3. If the patient presented different stages at different times in his evolution, we classified the patient in the worst stage when it was necessary to use the stage of renal involvement in the statistical analysis.

The treatment of patients conformed to the protocols published by country health authorities and the hospitals participating in the study. **Table 1** shows a summary of the therapeutics used by the patients.

2.2 Approval of the study by the institutional review board

The study protocol was approved by the Research Ethics Committee of Hospital de Jaén (1508-N-20).

2.2.1 Sample size calculation

For the calculation of the sample size, it was taken into account that the main conclusions would be obtained through multivariate analysis with logistic regression, assuming approximate mortality of 40% and knowing that an approximate number of 10 deceased patients were necessary for each variable included in the multivariable model. We calculated that with a sample of no less than 70 patients it was possible to include a maximum of three variables in the model (22). However, a larger sample would allow us to include more variables in the multivariate analysis if necessary and would increase the reliability of the statistical analysis and the generalizability of the results. For this reason, we will try to obtain a sample of about 200 patients.

2.3 Statistical analysis

Continuous variables are expressed as median (25th percentile–75th percentile). Qualitative variables were expressed as absolute and relative frequencies. The Mann–Whitney U-test was used for the comparison of continuous variables and the X^2-test for qualitative variables.

Multivariate analysis with multiple logistic regression was performed. Discrimination was assessed with the area under the

TABLE 1 Patients characteristic at ICU admission and ICU Interventions.

	Overall (N = 201)	Hospital survivors (N = 127)	No hospital survivors (N = 74)	
Age (years)	63 (56–72)	60 (51–70)	67 (62–74.75)	<0.001
Sex (male)	142 (70.6%)	88 (60.3%)	54 (73%)	0.58
Medical history				
Cardiological	114 (56.7%)	69 (54.3%)	45 (60.8%)	0.37
Respiratory	67 (33.5%)	43 (33.3%)	825 (33.8%)	0.95
Kidney	25 (12.4%)	12 (9.4%)	13 (17.6%)	0.93
Liver	5 (2.5%)	1 (0.8%)	4 (5.4%)	0.04
Hematological	23 (11.5%)	10 (7.9%)	13 (17.6%)	0.04
Oncological	22 (10.9%)	12 (9.4%)	10 (13.5%)	0.37
None	39 (19.4%)	28 (22%)	11 (14.9%)	0.21
APACHE II (points)	13 (10–16)	12 (9–14)	15 (12–18)	<0.001
SOFA (points)	5 (3–7)	5 (3–6)	6 (4–8)	<0.005
ICU days	12 (7–26)	11 (7–23)	15 (8–29)	0.09
Mechanical ventilation days (*)	7 (0–17.5)	0 (0–14.5)	19.5 (5–25)	<0.001
P/F ratio *(mm Hg)	145 (111–170)	150 (117–175)	140 (106–160)	0.09
Leukocytes (103/μl)	8,770 (6,390–13,200)	8,410 (5,985–13,055)	9,175 (7,009–13,795)	0.031
Neutrophils 1 day (103/μl)	7,355 (4,535–11,507)	7,055 (4,201–11,449)	7,742 (5,808–11,512)	0.166
Lymphocytes 1 day *(103/μl)	616 (402–957)	622 (406–962)	598 (393–954)	0.61
Lymphocytes 1 day stratification <600	96 (47.8%)	59 (46.5%)	37 (50%)	0.628
Neutrophils 1 day/Lymphocytes 1 day ratio (*)	12.28 (8.05–18.08)	11.56 (7.86–17.1)	13.3 (9.17–19.42)	0.16
Platelets (103/μl)	236 (160–305)	251 (166–320)	210 (151–274)	0.03
Creatinine (mg/dl)	0.91 (0.71–1.16)	0.90 (0.7–1.1)	0.95 (0.8–1.4)	0.03
Urea (mg/dl)	49 (36–67)	45 (34–60)	57 (41–86)	<0.001
LDH (*) (UI/L)	439 (345–593)	411 (320–510)	499 (406–706)	<0.001
AST (*) (UI/L)	41 (29–59)	41 (29–59)	41 (29.7–63)	0.90
ALT (*) (UI/L)	36 (25–61)	37 (25–60.2)	34.5 (24–62.2)	0.53
CK (*) (UI/L)	49 (31–99)	45 (29–85)	56 (41–121)	0.04
CRP (*) (mg/L)	130 (63–204)	123 (63–209)	144 (69–189)	0.97
PCT (ng/mL)	0.14 (0.04–5)	0.10 (0–0.41)	0.26 (0.1–0.5)	0.001
D dimers (*) (μg/L)	1,300 (774–2,321)	1,160 (754–2,023)	1,573 (982–3,815)	0.02
ICU interventions				
IPPV	129 (64.2%)	63 (49.6%)	66 (89.2%)	<0.001
Prono	91 (45.3%)	47 (37%)	44 (59.5%)	0.002
Tracheostomy	49 (24.6%)	26 (20.6%)	23 (31.5%)	0.086
corticosteroids	182 (90.5%)	115 (90.6%)	67 (90.5%)	0.998
Hemodiafiltration	18 (9%)	5 (3.9%)	13 (17.6%)	0.01
Vasopressors	122 (66.7%)	59 (46.5%)	63 (85.1%)	<0.001
Empirical antibiotic (*)	165 (82.9%)	99 (78%)	66 (91.7%)	0.14
Antiviral treatment	76 (37.8%)	45 (35.4%)	31 (41.9%)	0.362
Tozilucimab	74 (36.8%)	42 (33.1%)	32 (43.2%)	0.149

*N in those variables is less than the column totals and there are missing values.

receiver operating characteristic (ROC) curve and calibration with the Hosmer–Lemeshow test. We also calculated confidence intervals of the odds ratio (OR) by bootstrapping.

The statistical study was carried out with the SPSS and "R" using the "Rcmdr" package and the "Boot" for bootstrapping. We considered $p < 0.05$ as statistically significant.

3 Results

We studied 201 patients, APACHE-II was 13 (10–16) points, and the SOFA on the day of admission was 5 (3–7) points. On day 3, 198 patients were alive and SOFA on this day was 5 (3–8) points.

TABLE 2 Univariate analysis at different times of the ICU stay.

	Total	Died	Lived	*p*
ICU stay	(*N* = 201)	(*N* = 74)	(*N* = 127)	
≥1 PCT elevation episode (a)	104 (54.7%)	60 (81.04%)	44 (34.6%)	<0.001
APACHE II (points)	13 (10–16)	15 (12–18)	12 (9–14)	<0.001
Acute kidney injury (*n*, %) (b)	100 (49.8%)	52 (73.9%)	48 (37.8%)	<0.001
AKIN stages (c)				<0.001
Stage 0 (*n*, %)	101 (50.2%)	22 (29.7%)	79 (62.2%)	
Stage 1 (*n*, %)	47 (23.4%)	12 (16.2%)	35 (27.6%)	
Stage 2 (*n*, %)	14 (7%)	10 (13.5%)	4 (3.1%)	
Stage 3 (*n*, %)	39 (19.4%)	30 (40.5%)	9 (7.1%)	
AKIN stage 0 during ICU stay	(*N* = 101)	(*N* = 22)	(*N* = 79)	
≥1 PCT elevation episode	36 (35.6%)	13 (59.1%)	9 (13.8%)	0.009
AKIN stage 1 during ICU stay	(*N* = 47)	(*N* = 12)	(*N* = 35)	
≥1 PCT elevation episode	21 (44.7%)	9 (75%)	12 (34.3%)	0.014
AKIN stage 2 during ICU stay	(*N* = 14)	(*N* = 10)	(*N* = 4)	
≥1 PCT elevation episode	12 (85.7%)	10 (100%)	2 (50%)	0.016
AKIN stage 3 during ICU stay	(*N* = 39)	(*N* = 30)	(*N* = 9)	
≥1 PCT elevation episode	35 (89.7%)	28 (93.3%)	7 (77.8%)	0.177
Admission	(*N* = 201)	(*N* = 74)	(*N* = 127)	
PCT elevation at admission (*n*, %)	42 (20.89%)	17 (22.97%)	25 (19.68%)	0.58
SOFA on admission day (points)	5 (3–7)	6 (4–8)	5 (3–6)	<0.005
Admission and SOFA >8 points	(*N* = 38)	(*N* = 22)	(*N* = 16)	
PCT elevation (*n*, %)	15 (39.47%)	8 (36.4%)	7 (43.8%)	0.65
Third-day after admission (d)	(*N* = 187)	(*N* = 68)	(*N* = 119)	
PCT elevation (*n*, %)	45 (24.1%)	26 (38.2%)	19 (*N* = 16%)	0.001
Acute kidney injury in third day (*n*, %)	64 (34.2%)	33 (48.5%)	31 (26.1%)	0.002
SOFA on the third day (points)	5 (3–8)	7 (5–9)	4 (3–6)	<0.001
Third-day after admission and SOFA ≥8 points	(*N* = 50)	(*N* = 29)	(*N* = 21)	
PCT elevation (*n*, %)	17 (34%)	12 (41.4%)	5 (23.8%)	0.196
Third day after admission and acute kidney injury	(*N* = 64)	(*N* = 33)	(*N* = 31)	
PCT elevation (*n*, %)	25 (39.1%)	18 (54.5%)	7 (22.6%)	0.21
SOFA on this day (points)	6 (4–8)	7 (6–9)	4 (3–8)	0.02
Third day after admission and non-acute kidney injury	(*N* = 123)	(*N* = 35)	(*N* = 88)	
PCT elevation (*n*, %)	20 (16.3%)	8 (22.9%)	27 (26.2%)	0.009
SOFA on this day (points)	4 (3–7)	7 (4–8)	3 (2–6)	<0.001
Five days before ICU discharge (e)	(*N* = 172)	(*N* = 65)	(*N* = 107)	
PCT elevation (*n*, %)	39 (22.7%)	29 (44.6%)	10 (9.3%)	<0.001
SOFA on this day (points)	3 (2–6)	6 (4–8)	2 (2–3)	<0.001
Acute kidney injury on this day (*n*, %)	40 (23.3%)	27 (41.5%)	13 (12.4%)	<0.001
Five days before discharge from ICU and acute kidney injury	(*N* = 40)	(*N* = 27)	(*N* = 13)	
PCT elevation (*n*, %)	21 (52.5%)	19 (70.4%)	2 (15.4%)	0.001
SOFA on this day (points)	4 (2–7)	6 (4–9)	2 (1–3)	<0.001
Five days before discharge from ICU and non-acute kidney injury	(*N* = 132)	(*N* = 38)	(*N* = 94)	
PCT elevation (*n*, %)	17 (12.9%)	10 (26.3%)	7 (7.4%)	0.003
SOFA on this day (points)	3 (2–6)	6 (5–8)	2 (2–4)	<0.001
Two days before ICU discharge (f)	(*N* = 197)	(*N* = 72)	(*N* = 125)	
PCT elevation (*n*, %)	41 (20.8%)	34 (47.2%)	7 (5.6%)	<0.001
SOFA points (points)	2 (1–6)	7 (7–9.25)	2 (1–2)	<0.001
Acute kidney injury on this day (*n*, %)	51 (25.9%)	38 (52.7%)	13 (10.4%)	<0.001
Two days before discharge from ICU and acute kidney injury	(*N* = 51)	(*N* = 38)	(*N* = 13)	
PCT elevation (*n*, %)	27 (52.9%)	25 (65.8%)	2 (15.4%)	0.002

(Continued)

TABLE 2 (Continued)

	Total	Died	Lived	*p*
SOFA on this day (points)	6 (2–10)	9 (5–10)	18 (1–2)	<0.001
Two days before discharge from ICU and non-acute kidney injury	(*N* = 146)	(*N* = 34)	(*N* = 112)	
PCT elevation (*n*, %)	14 (9.68%)	9 (26.5%)	5 (4.5%)	<0.001
SOFA on this day (points)	2 (1–4)	6 (5–8)	2 (1–2)	<0.001
Last day in ICU (g)	(*N* = 195)	(*N* = 71)	(*N* = 124)	
PCT elevation	46 (23.6%)	42 (59.2%)	4 (3.2%)	<0.001
Acute kidney injury on this day (*n*, %)	54 (27.69%)	45 (36.4%)	9 (7.3%)	<0.001
SOFA on this day (points)	2 (0–7)	9 (6–11)	1 (0–2)	<0.001
Last day in ICU and acute kidney injury	(*N* = 54)	(*N* = 45)	(*N* = 9)	
PCT elevation (*n*, %)	34 (63%)	33 (73.3%)	1 (11.1%)	<0.001
SOFA on this day (points)	9 (1–11)	10 (7–12)	1 (0–1)	<0.001
Last day in ICU and non-acute kidney injury	(*N* = 141)	(*N* = 26)	(*N* = 115)	
PCT elevation (*n*, %)	12 (8.5%)	9 (34.6%)	3 (2.6%)	<0.001
SOFA on this day (points)	1 (0–3)	8 (5–9)	1 (0–2)	<0.001

(a) PCT elevation: PCT greater than 0.5 ng/ml.
(b) Acute kidney injury was considered if the patient was classified as stage 1 or stage 2 or stage 3 of the AKIN classification at any time during the ICU stay.
(c) Stages of acute kidney injury according to AKIN classification during ICU stay.
(d) PCT on the third day was measured in 187 patients (there was one hospital where no patient was evaluated on the third day during the first months).
(e) Data of seven patients were missing and 22 patients were in ICU for less than 5 days.
(f) Data of two patients were missing and two patients were in ICU for less than 2 days.
(g) Data of six patients was missing.

The diagnosis of COVID-19 infection was made by serology in 8 cases and in the remaining cases by PCR. All patients presented respiratory pathology with chest X-ray findings on admission. Mortality was 35.3% in ICU and in-hospital was 36.8% (*N* = 74). ICU stay was 12 (7–26) days.

Table 1 shows the detailed characteristics of patients on admission to the ICU, both those who survived and those who died in the hospital. In-hospital mortality was statistically related to APACHE-II, SOFA, and age.

3.1 Analysis of PCT values during the stay in the ICU

During their ICU stay, the 45.3% of the patients did not present any episode of elevated PCT (PCT greater than 0.5 ng/ml), 27.9% presented an only episode and 26.9% presented 2 or more episodes of elevated PCT. The presence of one or more episodes of elevated PCT was statistically associated with mortality, OR: 8.08 (4.07–16.07), (Table 2).

Table 2 shows how mortality is also related to the severity assessed on the first day of admission to the ICU with the APACHE-II and the presence of AKI. Furthermore, the presence of increased PCT is related to mortality both in the total sample and in patients who develop AKI and in those who do not. There is also a statistically significant relationship between increased PCT and mortality in stages 0, 1, and 2 of the AKIN classification.

Multivariate analysis with logistic regression showed that in-hospital mortality was associated with the presentation of one or more episodes of rising PCT during their evolution [OR: 5.07 (2.44–10.53)] as well as with the severity assessed with the APACHE-II and with the presence of AKI (Table 3).

3.2 Relationship between PCT elevation at admission and mortality

On admission, 42 patients (20.9%) had PCT elevation and 17 (40.5%) died vs. 57 (35.8%) of the 159 patients who did not have PCT elevation ($p = 0.58$).

Subsequently, a similar analysis was performed, but restricted to patients with high SOFA values (≥8 points) on the first day of admission ($n = 38$). In these 38 patients, there was not statistically significant relationship between PCT elevation and mortality (Table 2). PCT was elevated in 15 patients and 8 of them died. The eight patients who died did so after an ICU stay of 7.5 (4–24) days.

3.3 Relationship between PCT elevation at day 3 of admission and mortality

On day 3 of admission, PCT was measured in 187 patients (there was one hospital where no patients were assessed on day 3 during the first months). 45 patients (24.1%) had an elevated

TABLE 3 Multivariate logistic regression models at different times of ICU stay.

Model and variables	OR	Confidence OR interval by bootstrapping	ROC area	Hosmer–Lemeshow test
ICU stay			0.81 (0.76–0.87)	7.85 ($p = 0.45$)
APACHE-II	1.13 (1.04–1.23)	(1.03–1.27)		
Acute kidney injury	2.21 (1.10–4.42)	(1.62–5.05)		
>1 episode of elevated PCT	5.07 (2.44–10.53)	(2.44–11.59)		
Third-day after admission			0.80 (0.73–0.86)	12.67 ($p = 0.124$)
Sofa on the third day	1.30 (1.14–1.48)	(1.14–1.51)		
Age	1.08 (1.04–1.11)	(1.04–1.12)		
PCT elevation	2.51 (1.13–5.57)	(1.14–5.87)		
Five days before discharge from ICU			0.92 (0.88–0.96)	12.85 ($p = 0.117$)
SOFA on day 5 before ICU discharge	2.05 (1.64–2.57)	(1.05–2.82)		
Age	1.10 (1.05–1.15)	(1.07–1.17)		
Acute kidney injury	9.73 (3.16–29.98)	(3.89–34.46)		
Two days before discharge from ICU			0.97 (0.95–0.99)	0.893 ($p = 0.999$)
SOFA on day 2 before ICU discharge	3.05 (2.11–4.41)	(2.39–6.23)		
Age	1.16 (1.08–1.25)	(1.09–1.35)		
Acute kidney injury	10.81 (2.21–52.88)	(1.76–137)		
PCT elevation on day 2 before ICU discharge	7.99 (1.32–48.25)	(1.01–169)		
Last day in ICU			0.991 (0.983–0.999)	4.74 ($p = 0.78$)
SOFA on the last day in ICU	2.95 (1.93–4.54)	(2.42–6.23)		
Age	1.18 (1.06–1.31)	(1.10–1.33)		
Acute kidney injury	53.41 (7.28–392)	(2.12–169)		
PCT elevation on the last day in ICU	38.83 (4.27–352.91)	(1.13–159.17)		

PCT. Of the 45 patients, 23 of them also had elevated PCT on the day of admission.

Of the 45 patients with elevated PCT, death occurred in 26 (57.8%) compared to 42 (29.6%) of the 142 patients who did not have elevated PCT ($p = 0.001$). Mortality was also related to the severity assessed with SOFA and to the presence of *AKI* on that day (Table 2).

Acute kidney injury was present in 64 patients whose PCT was assessed on day 3. When the relationship of mortality with both variables was studied jointly with multiple logistic regression, we found that it was statistically related to both PCT elevation at day 3 [OR: 2.70 (1.32–5.53)] and the presence of *AKI* [OR: 2.20 (1.15–4.24)].

Multivariate analysis with logistic regression showed that in-hospital mortality was associated with SOFA on day 3, age, and elevated PCT on day 3 [OR: 2.51 (1.13–5.57)], (Table 3). The presence of *AKI* on that day was not part of the model due to a lack of statistical significance ($p = 0.26$).

Subsequently, an analysis was performed but restricted to patients with high SOFA values (>8 points) on the third day of admission to the ICU ($n = 50$). In these 50 patients, there was not statistically significant relationship between elevated PCT and mortality (Table 2). PCT was elevated in 17 patients and 12 of them died. Furthermore, the 12 patients who died did so after an ICU stay of 18 (5–43) days.

3.4 Relationship between mortality with elevated PCT and SOFA in the last days of ICU stay

Subsequently, the last days of ICU stay were analyzed, specifically the last day or the day before discharge from ICU (discharge from ICU as alive or deceased), 2 days before and 5 days before that. On many occasions, patients are in the ICU on the last day for only a few hours and in these cases, laboratory tests were not performed on that day but were performed on the previous day. In these cases, we consider the PCT value on the last day to be the PCT value of the previous day.

The PCT value and SOFA score were evaluated in these three periods. A high PCT value was considered to be present on the last day of the stay or the day before (if the value had not been measured on the last day). If the PCT value had not been assessed on the last two days taken (this occurred in six patients), these patients were excluded from the analysis performed on the last day.

In the last 5 days, 56 patients had elevated PCT, and 47 of them died. Cultures were taken in 45 of the 56 patients. In 25 patients the cultures were positive for bacteria, in five for bacteria and fungi, in four for fungi only, and in 11 were negative. None of the nine positive-fungal cultures were positive in blood and all were in urine or respiratory secretions. And in

many of the cases the fungal-positive cultures were considered as colonization.

3.4.1 Evaluation of the last day of admission to the ICU

Mortality was statistically related to SOFA on the last day and elevated PCT on that day to the presence of AKI at that time (Table 2). Elevated PCT was statistically related to mortality in both patients with AKI and those without AKI (Table 2).

Multivariate analysis with logistic regression showed that in-hospital mortality was associated with SOFA on the last day, AKI, age and PCT elevation on the last day [OR: 38.83 (4.27–352.91)] (Table 3).

Of the 42 deceased patients with elevated PCT on the last day, cultures were taken in 36 patients and 23 (54.8%) had positive cultures, and all received antibiotics. Inadequate antibiotic treatment was observed in only two cases.

3.4.2 Evaluation two days before ICU discharge

Mortality was statistically related to SOFA 2 days before ICU discharge, the presence of AKI at that time, and elevated PCT on that day, (Table 2).

Multivariable logistic regression analysis showed that mortality was related to SOFA 2 days before ICU discharge, age, AKI, and elevated PCT on that day [OR: 7.99 (1.32–48.25)] (Table 3).

3.4.3 Evaluation five days before ICU discharge

The same analysis was performed 5 days before ICU discharge. Mortality was statistically related to SOFA 5 days before ICU discharge, the presence of a AKI and elevated PCT on that day, (Table 2).

Multivariate logistic regression analysis showed that mortality was related to SOFA on day 5 before ICU discharge, age, and the presence of AKI at that time (Table 3). There was no statistically significant relationship with increased PCT on that day ($p = 0.07$), OR: 2.92 (0.92–9.32).

3.5 Relationship between precipitating cause of mortality and elevated PCT in the last few days

Of the 74 patients who died, 71 were assessed for PCT on the last day. Of those 71 patients, in 42 patients the PCT was elevated on the last day, and 71.4% of them ($n = 30$) were considered to have a mainly non-respiratory cause of death. In 12 (28.6%) patients the precipitating cause of death was considered to be mainly respiratory, 23.8% were considered to be mainly septic shock and in 21.4% multi-organ failure.

Of the 71 patients who died and PCT was evaluated on the last day, in 29 patients the PCT was not elevated on the last day, and in 16 of them (55.2%) the cause was considered to be mainly respiratory.

3.6 Graphic representation of PCT values during the ICU stay

Figures 1, 2 graphically shows the evolution of PCT during all days of ICU stay in some of the patients of those in whom it was performed. Of note is the absence of graphs from one of the participating hospitals (63 patients). Figure 3 shows the ROC area of multiple logistic regression models about the evolution of PCT during all days of ICU stay of the patients.

These graphs show very clearly that the episodes of elevation of PCT were very persistent during the stay of patients in the ICU, both in deceased patients and in survivors. They also graphically show that episodes of PCT elevation are more frequent in the deceased than in the survivors. And we can also see how many of the patients who die present elevated PCT at the time of death.

4 Discussion

In this multi-center cohort study, we serially evaluated PCT values during ICU stay in patients admitted to the ICU for respiratory involvement due to COVID-19 and we studied the relationship of increased PCT values with mortality at different times of ICU stay.

The most striking findings of our study show that patients present numerous episodes of PCT elevation during their stay in the ICU and that they are related to mortality. And our study also shows that these elevations are a warning sign because many of the patients who die have elevated PCT values on the last day and that PCT elevations in the last days of ICU stay are statistically related to mortality. Similar episodes have been observed in many patients on admission and the third day and patients survive in many cases.

It highlights the numerous episodes of elevated PCT levels found, and that even in a quarter of the patients they occur on several occasions. Moreover, the presence of these episodes is statistically associated with mortality. Elevated PCT (7, 11) is associated with severe disease (defined as needing admission to an ICU or use of mechanical ventilation). The patients included in our study can be considered patients with the severe disease since they were admitted to the ICU and a high percentage required mechanical ventilation. Huang et al. (23) found that elevated PCT was associated with increased mortality [RR: 6.26 (1.75–22.42)], which coincides with our results in which we detected an association between mortality and one or more episodes of elevated PCT in patients with severe disease.

Our study also analyses the relationship between mortality and PCT elevation at various times during ICU stay. PCT elevation on the first day of ICU admission was not statistically related to mortality. This relationship was not statistically significant both in the total patients and in the subgroup of patients with the presence of multi-organ failure or with high

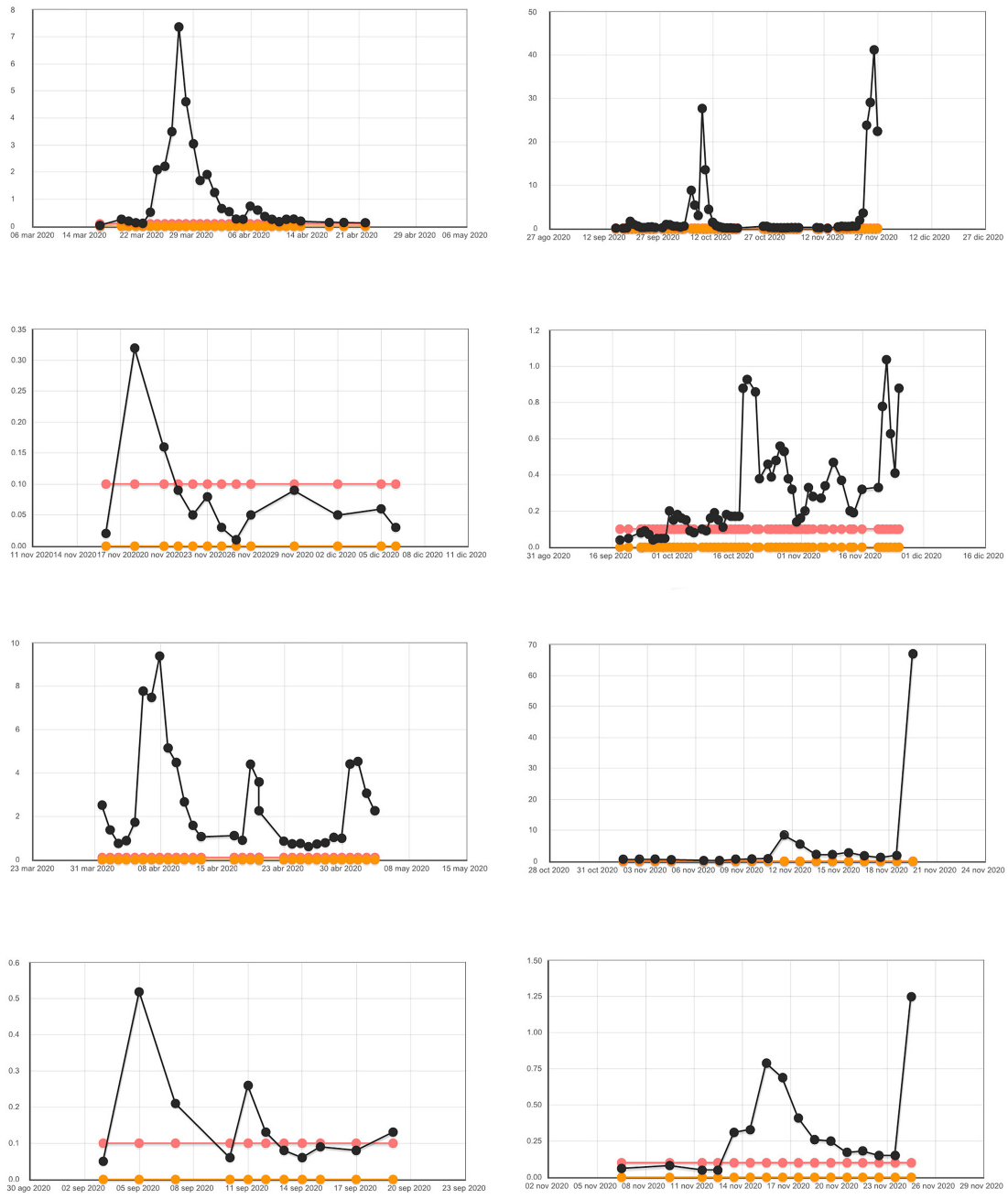

FIGURE 1
Evolution of PCT during all days of ICU stay in patients who died [lower and upper normal range of laboratory (0–0.05 ng/ml)]. We have considered the value of PCT was higher than 0.5 ng/ml as clinical significance.

SOFA scores. If our study had been limited to the analysis of the first day, we could have drawn erroneous conclusions, such as that elevated PCT in patients with COVID-19 is not associated with increased mortality. Possibly the use of prophylactic antibiotic treatment on admission to the ICU in a very large percentage of patients (82.9%) may explain why there is no relationship with mortality between PCT elevation on admission and mortality in our study. However, serial analysis of PCT values during the entire stay of these patients in the ICU has allowed us to see that the relationship between PCT elevation and mortality is statistically significant on the third day and in the last days of the ICU stay. And the relationship was statistically significant with mortality also in the multivariate analysis. The finding of elevated PCT elevation at these times complements the information on age and SOFA at these times.

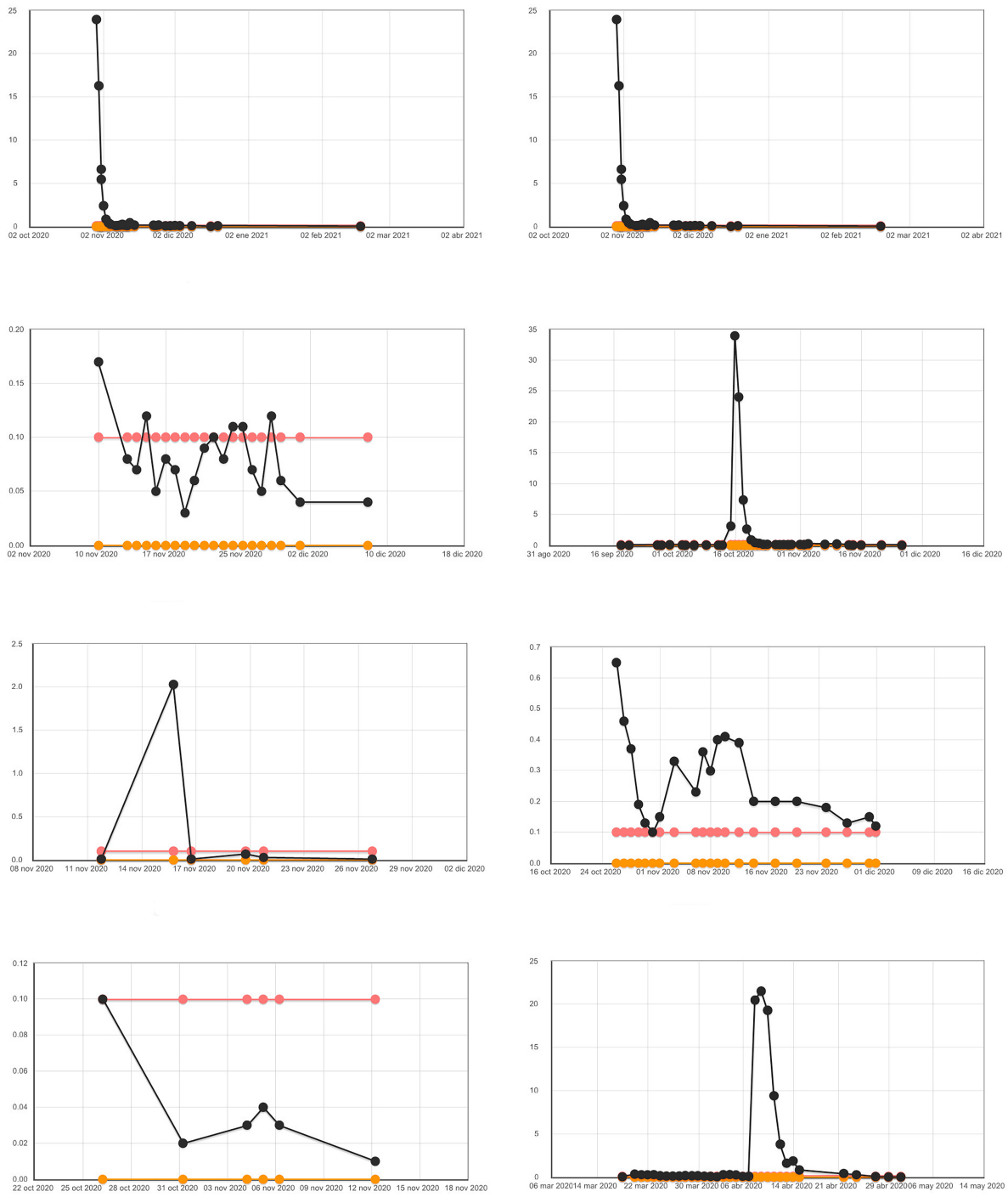

FIGURE 2
Evolution of PCT during all days of ICU stay in patients who lived [lower and upper normal range of laboratory (0–0.05 ng/ml)]. We have considered the value of PCT higher than 0.5 ng/ml as clinical significance.

Our findings are consistent with other studies such as that of Hu et al. (14) who found that in patients with high PCT values who recovered, PCT values decreased during recovery, but in those who died, serum levels of PCT increased as the disease worsened. They evaluated 95 patients of whom 12 were critically ill and six died. We evaluated a larger group of patients, all our patients are critically ill, and 74 of them died. This larger sample has allowed us to obtain statistically significant results, increase the confidence in the conclusions obtained, and also to be able to explore other different aspects. Not being able to know the

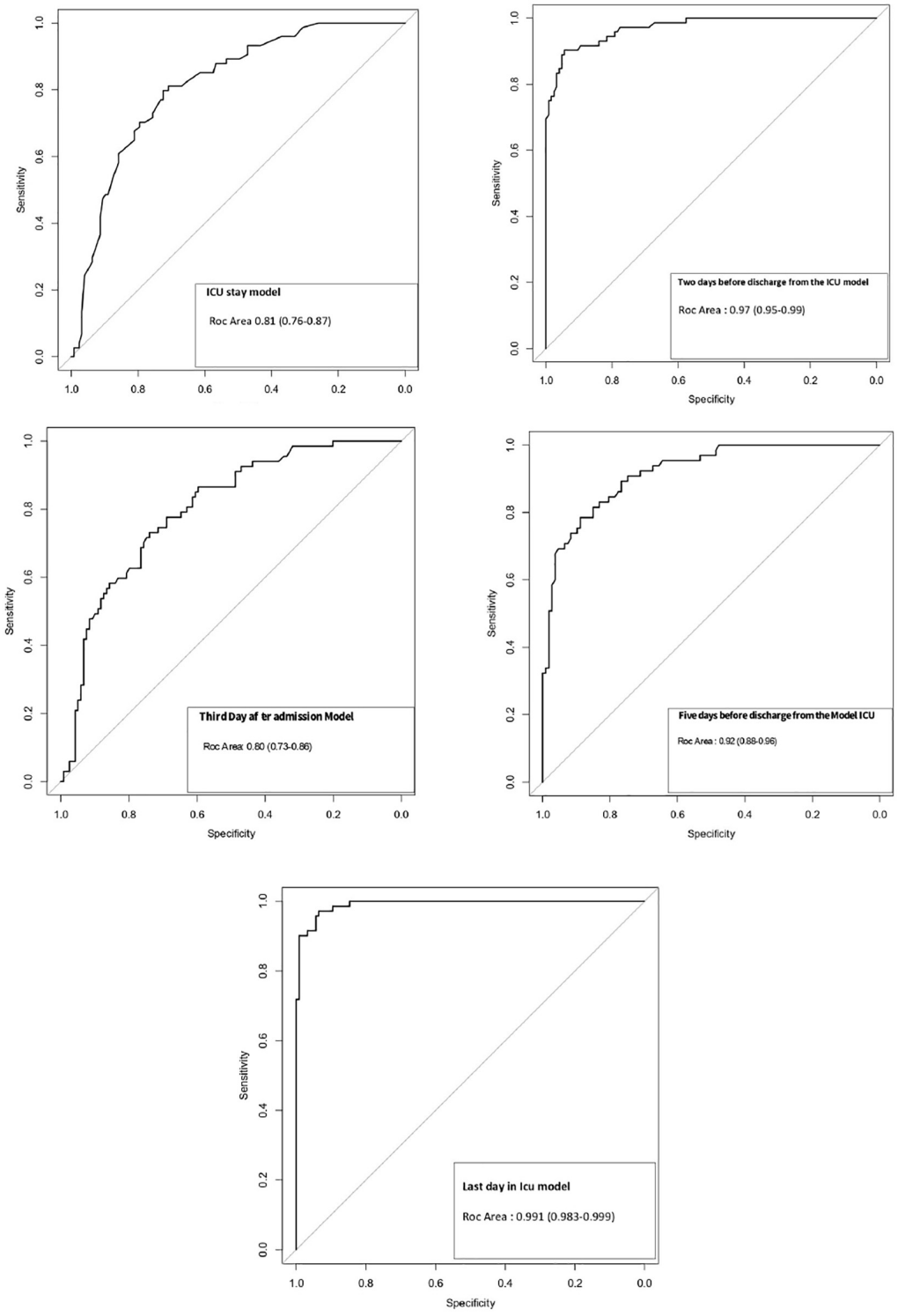

FIGURE 3
Receiver operating characteristic (ROC) area of multiple logistic regression models.

time of death of the patient entails an important problem in obtaining practical conclusions about the relationship we found between high PCT values in the last days of stay and mortality. We do not know *a priori* whether we are dealing with one of the frequent episodes of elevated PCT that the patient overcomes or one of the episodes that end with the patient's death. But it does

indicate that we must be alert to an elevated PCT because on many occasions it can lead to the death of the patient.

In-hospital mortality in our patients is high (36.8%) but similar to the mortality found by many authors. Armstrong et al. (1) in a meta-analysis with a very large number of patients found an ICU mortality of 41.6% (34.0–49.7%). The fact that our study is a multicenter study and our findings are compatible with previous studies with similar mortality figures makes us more confident that our results can be generalized and be of help for the management and treatment of other patients with this pathology. Although, logically, further research is needed to be able to draw valid and generalizable conclusions.

Although PCT is used as a marker for bacterial sepsis, there is still considerable doubt whether the elevated PCT levels observed in some patients with COVID-19 are due to a bacterial infection or are a direct marker for a more severe viral infection (12).

The use of detailed graphs of patients' PCT levels and their grouping according to death or survival, allows the reader a complementary and clearer view of the numerical data provided in our study. This makes it possible to observe the high number of cases with elevated PCT in both the deceased and the survivors. The pattern of evolution of PCT levels seen in our patients is more plausible in that it responds to episodes of bacterial infection that complicate the evolution of patients with COVID and that may occur on several occasions in the same patient.

Our data suggest that elevated PCT (in critically ill patients with multiorgan failure and COVID-19 infection) may indicate the presence of bacterial infection and should be treated as best as possible as it causes death in a high percentage of patients. PCT in many cases is indicative of sepsis of bacterial origin (8–10) and we believe that this interpretation can also be applied to our results and that elevated PCT is often due to bacterial infections. Furthermore, the high frequency with which the evolution of COVID patients admitted to the ICU is complicated by bacterial infection must be taken into account, which in some studies reaches percentages of more than 40% (24). The findings of our study show that in the last 5 days of ICU stay, cultures were taken in 45 of the 56 patients with elevated PCT and the cultures were positive for bacteria in 30 of the 45 patients in whom they were taken. Treatment of bacterial infection with antibiotics to which the causative germ is not sensitive is logically a major problem with very serious consequences. Delaying treatment of bacterial infection in ICU patients is detrimental and greatly increases mortality (25, 26) which is why empirical and early antibiotic treatment of bacterial sepsis forms part of the clinical practice guidelines (27).

The use of detailed graphs of PCT levels also allows us to see the high percentage of patients who die with PCT elevation in the last few days and the rapidity of PCT elevation in the short time before death. We have added to our article the PCT progression curves of a group of patients so that the reader can more clearly evaluate the results.

Procalcitonin levels may be increased in acute renal failure. Although this fact is under discussion, there are articles and meta-analyses (28, 29) that conclude that it is acceptable specificity in diagnosing bacterial infection in patients with renal impairment. However, we stratified the population according to renal function and found that elevated procalcitonin is associated with increased mortality in patients with normal renal function and patients with impaired renal function.

Another problem for a proper analysis of our results lies in the fact that the severe respiratory involvement of the patients may be seen by the attending physicians as the sole cause of the patients' death. This may be the cause of insufficient detection of aggravating factors that could be corrected and treated with the consequent better evolution of the patient. For this reason, we have tried to detect in our study which patients have died due to a deterioration of their respiratory condition and to see which patient's death is due to a cause other than respiratory deterioration. It has been observed that in many patients, at the time of death, there is an increase in PCT that the precipitating cause of death in many of these cases was not respiratory and that the precipitating cause of death was compatible with a deterioration of infectious origin. All these findings suggest that if detected and treated, it could sometimes prevent the death of the patient.

In our study, a large group of patients who died had elevated SOFA and elevated PCT levels with no response to therapeutic measures. This indicates that patients with elevated SOFA and high PCT are the highest-risk group to whom we need to pay special attention. One of the findings of our study is that in this group of patients, treatment measures are not generally ineffective with better response to therapy in the first days of the stay in the ICU.

These findings suggest that when the time of multi-organ failure is long, the response to therapeutic measures is lower (30, 31). Another possible cause is a higher frequency of limiting therapeutic measures because they are considered to be somewhat futile.

The results of our study show that PCT elevation is a frequent occurrence and that it helps to detect risk situations and can help to better interpret the patient's situation and the problems that may arise. And it can help in patient management, as has been seen in other situations (32).

4.1 Limitations

The number of patients in our study is not very large though it is sufficient to obtain statistically significant relationships on many occasions. The sample is sufficient to show that in patients admitted to the ICU for respiratory involvement by COVID-19, an increase in PCT is a warning sign that the patient is at risk

of death. Our study has a sufficient sample to demonstrate that episodes of increased PCT carry a high risk of death, although on many occasions patients survive this situation.

Our study suggests that PCT elevations are caused by a bacterial infection. We found that in the last 5 days of stay, cultures were positive for bacteria in 53% of the patients who had PCT elevations and in 67% of those who had cultures. We did not find positive cultures for bacteria in all cases of PCT elevation. Although it is not enough to affirm that PCT elevation is caused by a bacterial infection in all cases, we have found sufficient data to support that in many cases it is. Although future research is needed to shed more light on this aspect.

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 Research Ethics Committee of Hospital de Jaén (1508-N-20). The patients/participants provided their written informed consent to participate in this study.

Author contributions

RR-F and RP-M participated in all phases of the study (study design, data collection, statistical review, manuscript completion, and final review). EA-A participated in the statistical review and manuscript completion and prepared the figures and tables. All authors participated in the contribution of data collection.

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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.

Publisher's note

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Prognostic role of serum ammonia in patients with sepsis-associated encephalopathy without hepatic failure

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Objectives: Our previous study shows that serum ammonia in sepsis patients without hepatic failure is associated with a poor prognosis. The relationship between serum ammonia level and the prognosis of sepsis-associated encephalopathy (SAE) patients without hepatic failure remains unclear. We aimed to explore the relationship between serum ammonia levels and the prognosis of patients with SAE.

Materials and methods: This study is a retrospective cohort study. We collected 465 patients with SAE admitted to the intensive care unit (ICU) from Medical Information Mart for Intensive Care IV (MIMIC IV) from 2008 to 2019. Patients with SAE were divided into a survival group (369 patients) and a non-survival group (96 patients). We used the Wilcoxon signed-rank test and the multivariate logistic regression analysis to analyze the relationship between serum ammonia levels and the prognosis of patients with SAE. R software was used to analyze the dataset.

Results: The primary outcome was the relationship between serum ammonia level and hospital mortality of SAE. The secondary outcomes were the relationship between serum ammonia level and hospital stays, simplified acute physiology score (SAPS II), Charlson, Glasgow coma scale (GCS), sequential organ failure assessment (SOFA), and lactate level of SAE. The mortality of patients with SAE was 20.6%. The serum ammonia level was not significantly associated with hospital mortality, longer hospital stays, higher SAPS II and Charlson scores, and lower GCS of patients with SAE. The serum ammonia level was associated with higher SOFA scores and lactate levels in patients with SAE. The SAPS II and Charlson scores were independent risk factors for death in patients with SAE.

Conclusion: Serum ammonia level was associated with higher SOFA scores and lactate levels in patients with SAE. In addition, the SAPS II and Charlson

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scores can be used to assess the prognosis of patients with SAE. Therefore, we should closely monitor serum ammonia, SAPS II, and Charlson levels in patients with SAE.

KEYWORDS

serum ammonia, lactate, sepsis, sepsis-associated encephalopathy (SAE), simplified acute physiology score

1. Introduction

Sepsis-associated encephalopathy (SAE) is a common complication in patients with sepsis. It may occur in the acute phase of sepsis or after the patient survives and is discharged. SAE is manifested as changes in cognitive function and consciousness, including decreased attention, delirium, lethargy, coma, mood changes, long-term low quality of life, and dementia (1–3). The incidence of SAE is about 50% (4). The mortality risk of patients with SAE is significantly higher than that of patients with non-SAE (5). As SAE severity increases, the mortality rate is as high as 70% (6). In addition, patients with SAE have poor prognoses. Therefore, it is essential to seek potentially modifiable factors that affect the prognosis of patients with SAE.

Serum ammonia is a critical neurotoxic molecule (7). It is associated with the poor prognosis of patients with sepsis. Yazan Numan et al. found elevated ammonia levels can be a novel biomarker for sepsis (8). In a multi-center study, Jie Zhao et al. found that the area under the curve value of the ammonia level predicting the 28-day mortality was 0.813 in patients with sepsis (9). Our previous study also showed that serum ammonia levels without hepatic failure were associated with poor clinical outcomes in patients with sepsis, and the serum ammonia without hepatic failure group had higher short-term (hospital mortality: 59.8%; 30-day mortality: 47.7%) and long-term mortality (90-day mortality: 61.7%; 1-year mortality: 67.7%) (10). Amra Sakusic, Moldovan Sabov, and others found that 4.5% of patients with hyperammonemia in the ICU have a normal liver function, and 71% have encephalopathy (11). Alexandre Sanches Larangeira et al. found that serum ammonia levels of $>$ 100 μmol/L were associated with intracranial hypertension and higher mortality (12).

The relationship between serum ammonia level and hospital mortality of SAE is unclear. We hypothesize that serum ammonia levels are associated with a poor prognosis of patients with SAE and without hepatic failure.

Abbreviations: SAE, sepsis-associated encephalopathy; SAPS II, simplified acute physiology score; SOFA, sequential organ failure assessment; GCS, Glasgow coma scale; ICU, intensive care unit; MIMIC-IV, Medical Information Mart for Intensive Care IV.

2. Materials and methods

2.1. Patient

This study is a retrospective cohort study. We collected patients older than 18 years and stayed in the intensive care unit (ICU) for more than 24 h. The diagnosis process for patients with SAE is as follows: (1) Patients need to meet the diagnostic criteria of Sepsis 3.0. Sepsis was diagnosed with an acute change in the total sequential organ failure assessment (SOFA) score of $\geq$2 and documented or suspected infection complied with the Sepsis 3.0 criteria. The patients with infection sites or prescriptions of antibiotics and samples of bodily fluids for microbiological culture had suspected infection. In line with the existing literature, the microbiological sample must have been collected within 24 h when the antibiotic was first administered, and at the first occurrence of microbiological sampling, the antibiotic administration would be within 72 h (13). (2) In patients with sepsis, we collected serum ammonia and excluded patients diagnosed with acute and chronic liver disease. (3) In patients with sepsis, traumatic brain injury, encephalitis, intracranial infection, ischemic stroke, and metabolic encephalopathy caused by severe electrolyte imbalances or glycemic disturbances, pulmonary encephalopathy caused by excessive carbon dioxide partial pressure, hepatic encephalopathy, hypertensive encephalopathy, and other liver disease or kidney disease is affecting consciousness; mental disorders and neurological disease; chronic alcohol or drug abuse were excluded by us. The diagnosis of SAE is defined according to the following three aspects: the patient's Glasgow coma scale (GCS) score of $<$15, patients diagnosed with delirium according to the ICD code, and patients treated with haloperidol during hospitalization (4, 14, 15).

2.2. Data collection

Data were retrieved FROM Medical Information Mart for Intensive Care IV (MIMIC IV) from 2008 to 2019. MIMIC-IV is a publicly available database. Applying the MIMIC IV database requires one to become a credentialed user on PhysioNet and the completion of a training course in human subjects research. In addition, we need to sign the data use

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TABLE 1 Baseline characteristics and outcome of patient with SAE.

		Survival group $n = 369$	Non-survival group $n = 96$	P
Baseline variables				
Age, median (IQR)		64(55–72)	65(54.3–72.8)	0.351
Gender [*n* (%)]				
	Female	145(39.3)	48(50.0)	0.058
	Male	224(60.7)	48(50.0)	
Coexisting illness [*n* (%)]				
	Charlson	5(3-8)	6(4-9)	0.040
	Hypertension	50(13.6)	7(7.3)	0.096
	Diabetes	112(30.4)	22(2.9)	0.152
	Respiration	70(19.0)	21(21.9)	0.523
	Cardiovascular	100(27.1)	30(31.3)	0.420
	Renal	79(21.4)	17(17.7)	0.425
Site of infection [*n* (%)]				
	Intestinal	11(3.0)	3(3.1)	0.941
	Urinary	18(4.9)	6(6.3)	0.588
	Lung	15(4.1)	4(4.2)	0.964
	Catheter	5(1.4)	2(2.1)	0.602
	Skin and soft tissue	21(5.7)	4(4.2)	0.555
	Abdomiol cavity	11(3.0)	4(4.2)	0.558
Microbiology type [*n* (%)]				
	Klebsiella	45(12.2)	6(6.3)	0.097
	Acinetobacter baumannii	3(0.8)	1(1.1)	0.825
	Escherichia Coli	79(21.4)	9(9.4)	0.007
	Pseudomonas aeruginosa	24(6.5)	8(8.3)	0.528
	Staphylococcus aureus	6(1.6)	2(2.1)	0.759
	Enterococcus	148(40.1)	41(42.7)	0.644
Laboratory parameters, median (IQR)				
	Alanineamino transferase (IU/L)	40(20–41)	39.5(18.3 59.3)	0.694
	Aspartate aminotransferase (IU/L)	48(24–53.5)	48(31–59)	0.113
	Albumin(g/dL)	3.2(2.6–3.7)	1.7(0.8–3.3)	0.006
	Bilirubin(mg/dL)	1.3 (0.5– 1.7)	3.0(2.4– 3.4)	0.003
	White blood cell ($\times 10^{\wedge 9}$/L)	11.0(7.7–15.2)	11.4(7.8– 17.8)	0.329
	Neutrophils (%)	71.6(65.9–79.3)	74.6(68.9–83.0)	0.026
	Lymphocyte (%)	17.2(10.6–20.2)	14.9(6.1–20.6)	0.021
	Ammonia(μmol/L)	41(31–62)	42.5(26.3–65.8)	0.317
	Lactates (mmol/l)	1.7(1.2–2.4)	2.1(1.4–3.1)	0.002
Mechanical ventilation [*n* (%)]		186(50.4)	186(50.4)	64(66.7)

(Continued)

TABLE 1 (Continued)

		Survival group $n = 369$	Non-survival group $n = 96$	P
Renal replacement therapy [n (%)]		28(7.6)	13(13.5)	0.067
Score system, median (IQR)				
	SAPS II	37(29–46)	44(35.3–58.8)	<0.001
	SOFA	6(4–9)	8.5(6–12)	<0.001
	GCS	11(7–13)	8.5(4–13)	0.001
Use of vasopressors [n (%)]		127(34.4)	127(34.4)	55(57.3)
Length of hospital stays, days, median (IQR)		4.1(1.9–11.3)	4.1(1.9–11.3)	7.2(2.1–13.0)

agreement (DUA). The following CITI program course was completed: CITI 33690380. My registry form URL is https://physionet.org/settings/credentialing/. MIMIC IV was approved by the Institutional Review Boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. The requirement for individual patient consent was waived because the project does not impact clinical care, and all patient confidential information was anonymized. The MIMIC IV database (version 1.0) is publicly available at https://mimic-iv.mit.edu/. Any researcher who adheres to the data use requirements is permitted access to these databases. The codes are available at https://github.com/MIT-LCP/mimic-iv. We used the data of the patient's first stay in the ICU and retrieved the patient's relevant data through subject_id. The patient's age, gender, coexisting illness, site of infection, microbiology type, mechanical ventilation, renal replacement therapy, use of vasopressors, length of hospital stays, laboratory parameters, the worst laboratory parameters in the first 24 h of staying in the ICU, and the first 24-h Sequential Organ Failure Assessment (SOFA) score, Simplified Acute Physiology Score II (SAPS) score, and Glasgow Coma Scale (GCS) were extracted by R statistical software.

2.3. Statistical analysis

We used the Shapiro–Wilk test to evaluate whether the data were normally distributed. The continuous variables in this study were all skewed distributions. Continuous variables were expressed as the median (P 25, P 75) (interquartile range, IQR). Categorical variables were expressed as counts and proportions. We used the Wilcoxon signed-rank test to compare the continuous variables of the two groups of patients (Table 1), and the relationship between serum ammonia and GCS, SAPS II, SOFA, Charlson, lactate, and length of hospital stays (Figures 2, 3). We used the Pearson exact test to compare the categorical variables of the two groups, including gender, coexisting illness (hypertension, diabetes, respiration, cardiovascular, and renal), site of infection, microbiology type, mechanical ventilation, renal replacement therapy, and use of vasopressors (Table 1). A multivariate logistic regression analysis was used to explore the risk factors of mortality in patients with SAE as shown in Table 2. The data analysis in this study was used by R software.

3. Results

3.1. Baseline characteristics

Among 69,619 ICU patients, 19,658 patients met the diagnosis of sepsis 3.0. Serum ammonia was found in 1,377 of 19,658 patients with sepsis. After inclusion and exclusion criteria, 465 patients were diagnosed with SAE, divided into a survival group and a non-survival group according to hospital mortality. The survival group was 369 patients, and the non-survival group was 96 patients (Figure 1). Table 1 analyzes the baseline data and results of the survival group and non-survival group of patients with SAE. Comparing the survival group, the patients of the non-survival group had a higher Charlson score ($p = 0.040$), neutrophils [($p = 0.026$), lactates ($p = 0.002$), SAPS II ($p < 0.001$), and SOFA ($p < 0.001$), lower GCS score ($p < 0.001$), longer length of hospital stays ($p = 0.065$), and more non-survival patients with SAE used mechanical ventilation ($P = 0.004$), renal replacement therapy ($p = 0.067$), and vasopressors ($p < 0.001$). There was no significant difference in serum ammonia levels between the two groups.

3.2. Multivariate regression analysis of hospital mortality in patients with SAE

Multivariate regression analysis in patients with SAE found that serum ammonia levels were not related to the prognosis of patients with SAE, and SAPS II had a better predictive value for the mortality of patients with SAE ($p = 0.008$). However, the higher the Charlson SAE patients, the worse the prognosis ($p = 0.042$) (Table 2).

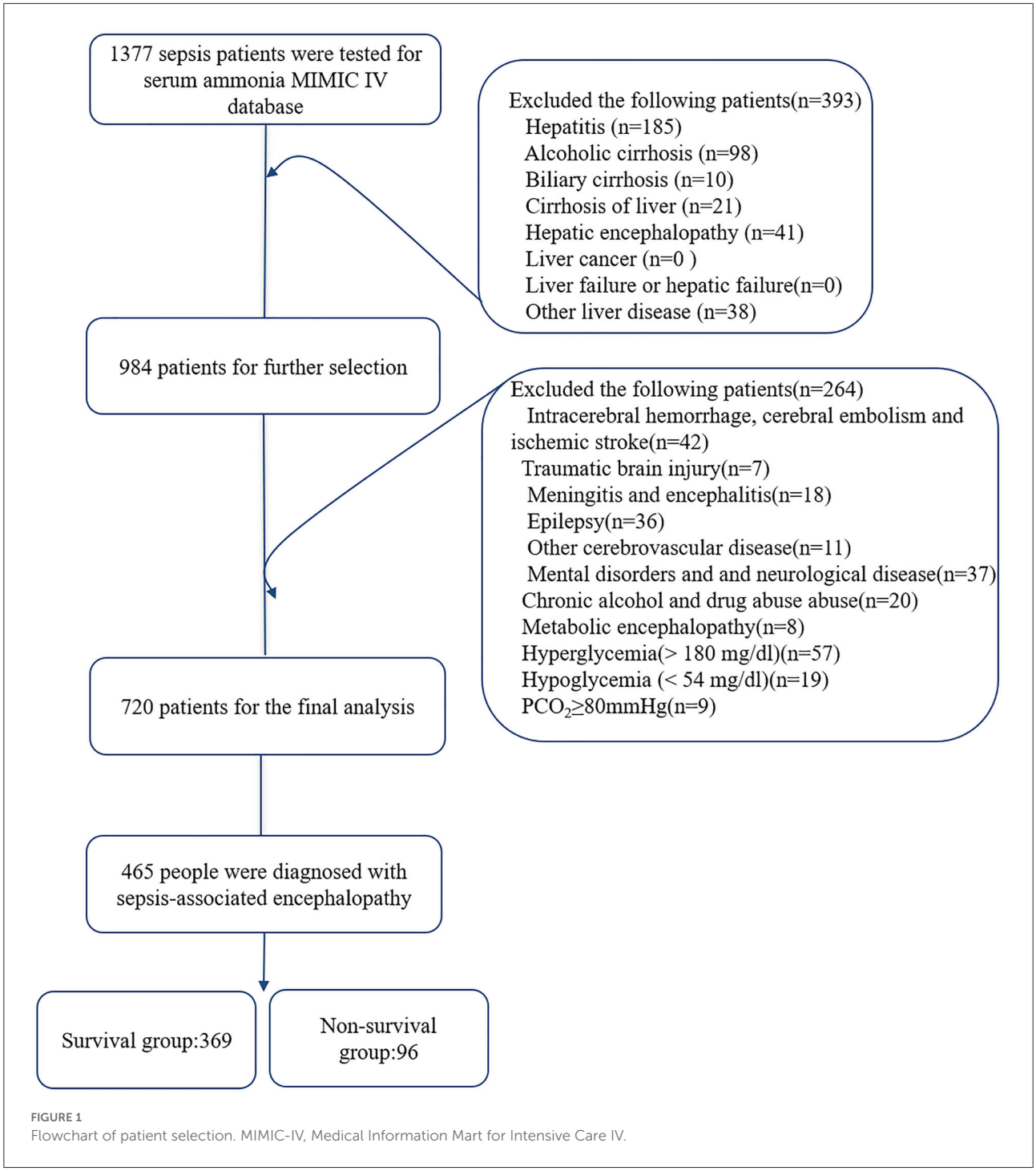

FIGURE 1
Flowchart of patient selection. MIMIC-IV, Medical Information Mart for Intensive Care IV.

3.3. The relationship between serum ammonia and disease severity scores of patients with SAE

According to the patient's GCS scores, the patients were divided into two groups: group 1: GCS score of patients ≥9 scores, group 2: GCS score of patients ≤8 scores. The Wilcoxon test was used to clarify the relationship between serum ammonia level and the GCS score. The study results in Figure 2 show that there is no difference in serum ammonia levels and GCS (p = 0.41) (Figure 2A).

We divided the patients into two groups according to the SAPS II score of the patients: group 1: SAPS II score of patients ≥40 scores and group 2: SAPS II score of patients ≤39

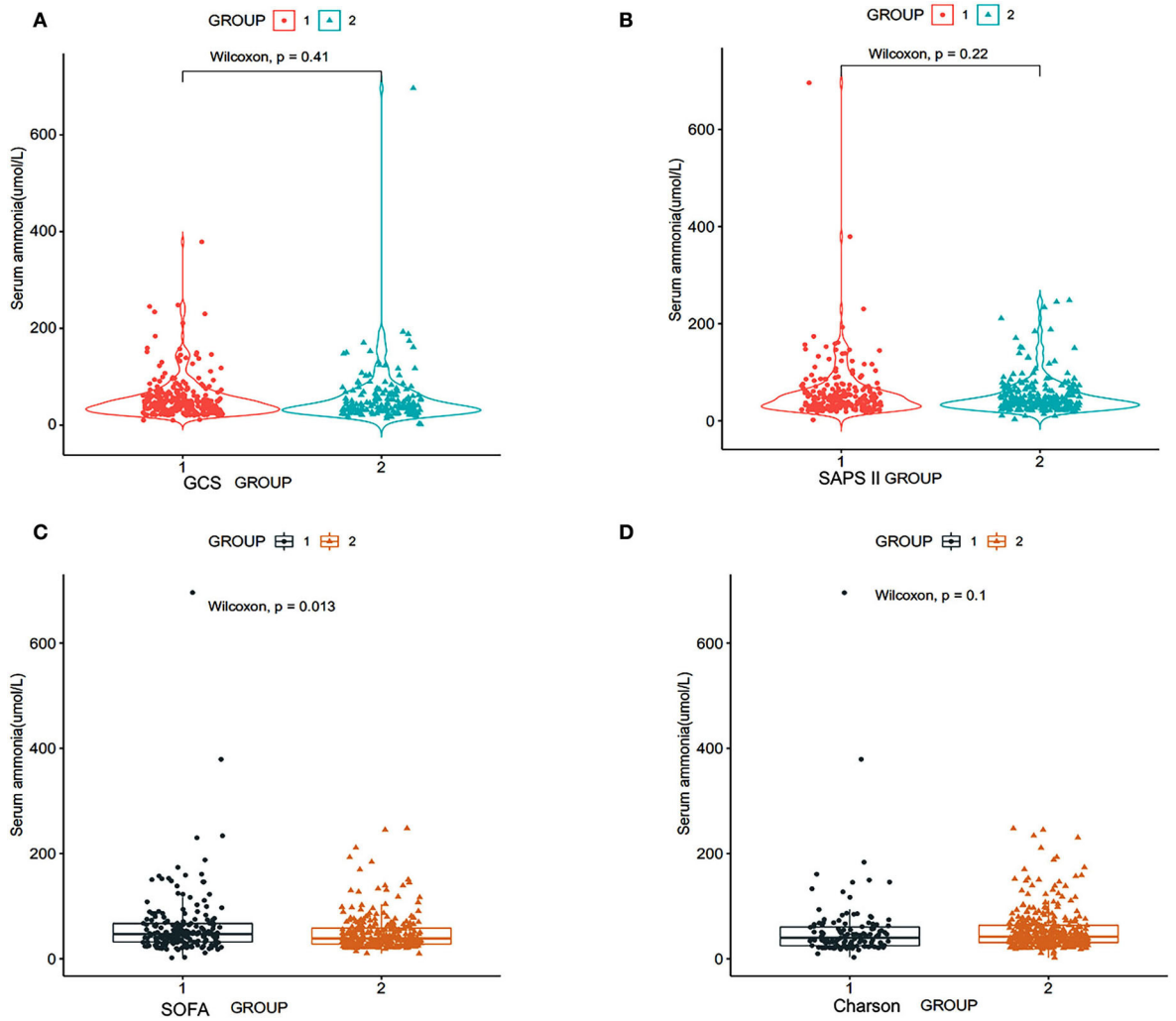

FIGURE 2
Relationship between serum ammonia and SOFA scores of patients with SAE, SAPS II, GCS, and Charlson scores. SOFA, sequential organ failure assessment; SAPS II, simplified acute physiology score; GCS, Glasgow coma scale (RStudio, Version 1.3.1056, USA). **(A)** Group 1: GCS score of patients ≥9 scores, group 2: GCS score of patients ≤8 scores; **(B)** group 1: SAPS II score of patients ≥40 scores, group 2: SAPS II score of patients ≤39 scores; **(C)** group 1: SOFA score of patients: ≥8 scores, group 2: SOFA score of patients ≤7 scores; **(D)** group 1: Charlson score of patients ≥8 scores, group 2: Charlson score of patients ≤7 scores.

scores. Figure 2B shows that there is no significant correlation between the serum ammonia level and SAPS II score ($p = 0.22$) (Figure 2B).

According to the SOFA score of the patients, we divided the patients into two groups: group 1: SOFA score of patients ≥8 scores and group 2: SOFA score of patients ≤7 scores. Figure 2C shows that serum ammonia levels may be related to higher SOFA scores ($p = 0.013$).

According to the Charlson score, the patients were divided into two groups: group 1: Charlson score of patients ≥8 scores and group 2: Charlson score of patients ≤7 scores. Figure 2D shows that serum ammonia level did not correlate with the Charlson score ($p = 0.22$).

3.4. The relationship between serum ammonia values, lactate values of patients with SAE, and length of hospital stays

According to the lactate level, the patients were divided into two groups: group 1: lactates level of patients ≥2 mmol/L and group 2: lactates level of patients ≤1.9 mmol/L. Figure 3A shows that serum ammonia levels may be related to lactate levels ($P = 0.044$).

According to the length of hospital stays, the patients were divided into two groups: group 1: length of hospital stays of patients ≥10 days and group 2: length of hospital stays of

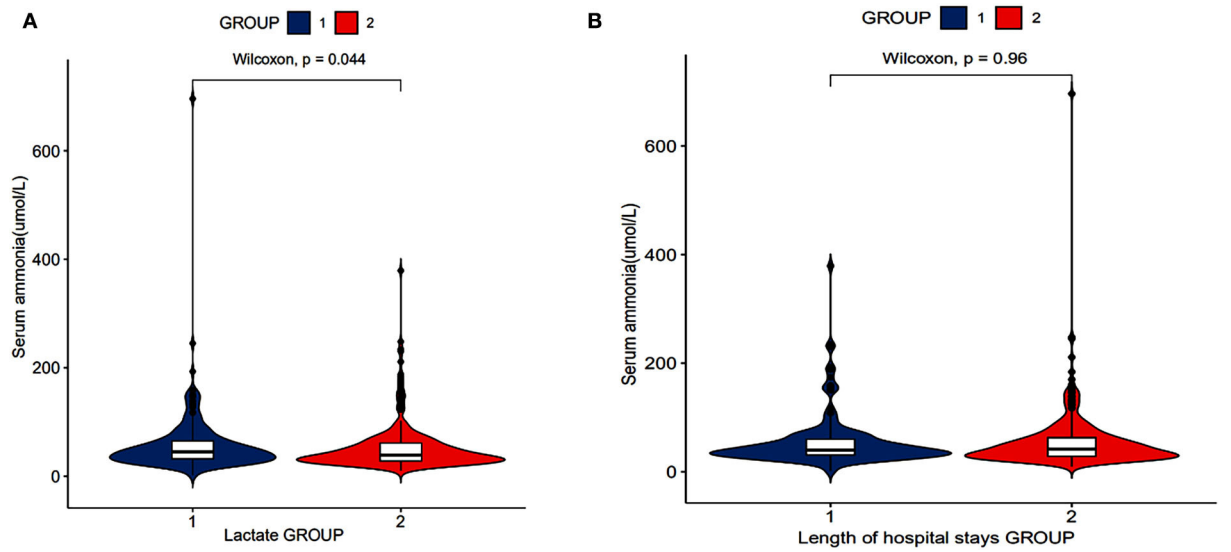

FIGURE 3
Relationship between serum ammonia and length of hospital stays, lactates of patients with SAE (RStudio, Version 1.3.1056, USA). **(A)** The relationship between serum ammonia levels and lactate levels. **(B)** The relationship between serum ammonia levels and length of hospital stays.

TABLE 2 **Multivariate logstic regression analysis of hospital mortality in SAE patients.**

		Wald chi-square value	Multivariate analysis			
			P	OR	95.0% CI	
					Lower	Upper
Sex [*n* (%)]		4.999	0.025	1.784	1.074	2.963
Charlson		4.144	0.042	1.086	1.003	1.175
Microbiology type [*n* (%)]						
	Klebslella	1.744	0.187	0.521	0.198	1.371
	Escherichia coli	6.151	0.013	0.365	0.165	0.809
Laboratory parameters, median (IQR)						
	Albumin(g/dL)	1.402	0.236	0.805	0.563	1.152
	Bilirubin(mg/dL)	5.077	0.024	1.142	1.017	1.282
	Neutrophils (%)	0.190	0.663	0.995	0.975	1.016
	Lymphocyte (%)	0.124	0.725	0.996	0.974	1.019
	Ammonia(μmol/L)	1.053	0.305	0.998	0.993	1.002
	Lactates (mmol/l)	0.315	0.575	1.037	0.913	1.178
Mechanical ventilation [*n* (%)]		00.107	00.744	1.108	0.599	2.048
Renal replacement therapy [*n* (%)]		0.096	0.757	0.878	0.387	1.994
Use of vasopressors [*n* (%)]		0.894	0.344	1.341	0.730	2.462
Score system						
	SAPS II	7.082	0.008	1.026	1.007	1.046
	SOFA	3.481	0.062	1.080	0.996	1.172
	GCS	3.578	0.059	0.938	0.878	1.002

patients ≤9.9 days. There was no significant difference in serum ammonia levels between the two groups (Figure 3B).

4. Discussion

Our cohort study shows that the non-survival group has higher Charlson, SAPS II, SOFA scores, and lactate levels; Charlson and SAPS II scores were independent risk factors for death in patients with SAE. Serum ammonia level was not associated with hospital mortality, longer hospital stays, higher SAPS II and Charlson scores, and lower GCS scores of patients with SAE without hepatic failure. However, it was associated with higher SOFA scores and lactate levels.

Our cohort study showed that the hospital mortality of non-surviving patients with SAE (20.6%) is lower than the results of Romain Sonneville et al. (50.3%) (4). It may be attributed to differences in the study population. The populations of our cohort study with acute and chronic liver disease were excluded. Although our study results show that the hospital mortality of patients with SAE is lower than in other studies, it is still at a high level. Non-survival patients with SAE had higher Charlson and SOFA scores, indicating that the more diseases in patients with SAE, the more severe organ dysfunction and the more prone to die. Non-survival patients with SAE had higher lactate levels, indicating that patients with SAE and poor perfusion were more prone to die. Zhiqiang Liu et al. found that the mortality rate of patients with sepsis in the higher lactate group was significantly higher than that of patients with sepsis in the lower lactate group (16). Our study results are consistent with their study. Yunlong Liu et al. (17) found that the sensitivity of lactate level of the 28-day mortality prediction of patients with sepsis was 0.826 (17). It shows that the worse the tissue perfusion, the more likely to die in patients with sepsis (18).

Multivariate regression analysis results show that SAPS II and Charlson scores were independent risk factors for death in patients with SAE. The relationship between SAPS II score and hospital mortality was developed using data from 137 intensive care units in 12 countries across Europe/North America (19). Amina Godinjak et al. found that SAPS II and Acute Physiology and Chronic Health Evaluation II scoring systems have the same good prognostic assessment capabilities for patients with sepsis (20). Our study shows that SAPS II had an excellent ability to assess the prognosis of patients with SAE. The Charlson scores showed a high ability to identify patients' survival (0.91) in a large healthcare database of more than 6 million hospitalized patients (21). The results of our cohort study confirm previous studies.

Our cohort study further demonstrated that higher SOFA scores and lactate levels might be related to serum ammonia levels in patients with SAE. In patients with liver failure, the patient's brain lactate levels increased significantly (22). The study by Chavarria et al. (23) found that the brain tissue and cerebrospinal fluid of rats with acute liver failure have higher levels of lactates (23). After treating brain astrocytes with NH4Cl for 24 h, the intracellular lactate level increased (22). Hyperammonemia increases the production of lactic acid in astrocytes by inhibiting the tricarboxylic acid cycle (24). Lactate causes astrocyte edema by regulating the pH value and the expression of aquaporin 4 in the brain (25). Ammonia could cause lactate levels to rise. Our study found that there is a correlation between serum ammonia and lactate levels in patients with SAE and without hepatic failure. Is there a relationship between serum ammonia in patients without hepatic failure and lactate levels in the cerebrospinal fluid of patients with SAE? Whether serum ammonia increases lactate levels needs to be confirmed by prospective studies in patients with SAE. Bodin Khwannimit et al. found that the area under the receiver operating characteristic curve of SOFA scores for predicting mortality in adults with sepsis and patients with septic shock was 0.880 (26). Jiayi Chen et al. (27) found that SOFA score was an independent risk factor for 28-day mortality in patients with SAE (27). Our study found that the SOFA scores of non-surviving patients with SAE are significantly higher than that of surviving patients with SAE (Table 1). Therefore, the SOFA scores may be an indicator for evaluating the prognosis of patients with SAE (Figure 2C). In addition, our further study found that serum ammonia levels are related to higher SOFA scores in patients with SAE. Therefore, we should closely monitor the changes in SOFA scores in patients with SAE.

There are several limitations in the study. First, the definition of SAE is based on the relevant retrospective analysis of the literature. Lack of imaging data may cause SAE's cohort to expand. Second, our study shows that the serum ammonia level is related to the SOFA score and lactates of patients with SAE. It is a retrospective study. We cannot prove its causality. Last, the condition of critically ill patients is critical and complex, and many confounding factors cannot be ruled out.

5. Conclusion

Non-survival patients with SAE had higher SOFA scores and lactate levels. Serum ammonia level is associated with higher SOFA scores and lactate levels in patients with SAE without hepatic failure. SAPS II and Charlson scores are valuable evaluation indicators for the poor prognosis of patients with SAE. Therefore, we should monitor the serum ammonia level, SOFA scores, SAPS II scores, and Charlson scores of patients with SAE and intervene in time.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://mimic-iv.mit.edu.

Code availability

Software application or custom code. The codes are available at https://github.com/MIT-LCP/mimic-iv.

Ethics statement

The establishment of the database was approved by the Institutional Review Boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center.

Author contributions

LZ, YL, SH, and KX designed the study, analyzed it, and drafted the article. BL, ZW, and RN acquired the data. LZ wrote the first draft of the manuscript. YL and KX revised the article and worked on the English and made the final version. All authors read and approved the final 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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Prognostic performance of the REDS score, SOFA score, NEWS2 score, and the red-flag, NICE high-risk, and SIRS criteria to predict survival at 180 days, in emergency department patients admitted with suspected sepsis – An observational cohort study

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Background: Patients admitted to hospital with sepsis are at persistent risk of poor outcome after discharge. Many tools are available to risk-stratify sepsis patients for in-hospital mortality. This study aimed to identify the best risk-stratification tool to prognosticate outcome 180days after admission *via* the emergency department (ED) with suspected sepsis.

Methods: A retrospective observational cohort study was performed of adult ED patients who were admitted after receiving intravenous antibiotics for the treatment of a suspected sepsis, between 1st March and 31st August 2019. The Risk-stratification of ED suspected Sepsis (REDS) score, SOFA score, Red-flag sepsis criteria met, NICE high-risk criteria met, the NEWS2 score and the SIRS criteria, were calculated for each patient. Death and survival at 180days were noted. Patients were stratified in to high and low-risk groups as per accepted criteria for each risk-stratification tool. Kaplan–Meier curves were plotted for each tool and the log-rank test performed. The tools were compared using Cox-proportional hazard regression (CPHR). The tools were studied further in those without the following specified co-morbidities: Dementia, malignancy, Rockwood Frailty score of 6 or more, long-term oxygen therapy and previous do-not-resuscitate orders.

Results: Of the 1,057 patients studied 146 (13.8%) died at hospital discharge and 284 were known to have died within 180days. Overall survival proportion was 74.4% at 180days and 8.6% of the population was censored before 180days. Only the REDS and SOFA scores identified less than 50% of the population as high-risk. All tools except the SIRS criteria, prognosticated for outcome at 180days; Log-rank tests between high and low-risk groups were: REDS score p <0.0001, SOFA score p <0.0001, Red-flag criteria p =0.001, NICE high-risk criteria p =0.0001, NEWS2 score p =0.003 and SIRS criteria p =0.98. On CPHR, the REDS [Hazard ratio (HR) 2.54 (1.92–3.35)] and SOFA [HR 1.58 (1.24–2.03)] scores out-performed

the other risk-stratification tools. In patients without the specified co-morbidities, only the REDS score and the SOFA score risk-stratified for outcome at 180days.

Conclusion: In this study, all the risk-stratification tools studied were found to prognosticate for outcome at 180days, except the SIRS criteria. The REDS and SOFA scores outperformed the other tools.

KEYWORDS

sepsis, septic shock, emergency department, clinical prediction rule, prognosis

Introduction

Sepsis, by definition, is a life-threatening condition (1). Worldwide, it is estimated to account for one in five deaths (2). Most studies on sepsis focus on the in-hospital or 28 day mortality rate. It is well recognised that patients admitted with sepsis who survive the index admission, continue to have an increased mortality rate in the ensuing months to years following discharge (3–12).

The majority of patients with sepsis in hospital are admitted as emergencies with community acquired sepsis (13). Early identification and treatment are the cornerstones of improving outcome in sepsis (14). This validates the crucial role of the emergency department (ED) in the management of sepsis. Identification of patients with sepsis or suspected sepsis can be carried out using a risk-stratification tool. Many risk-stratification tools have been advocated for use in the ED. However, little is known of the performance of these risk-stratification tools to prognosticate outcome at 180 days.

The risk-stratification tools are as follows: First, the Sequential Organ Failure Assessment (SOFA) score (15). The operational definition of Sepsis-3 (1), is the presence of two new points from baseline in the SOFA score. This is a cumulative score. Each of six organ systems is given an increasing score with increasing dysfunction. Increasing organ dysfunction is associated with an increased risk of mortality. The SOFA score is made up of physiological and laboratory variables. Second, the Red-flag criteria (16) are advocated for use by the United Kingdom (UK) Sepsis Trust. These criteria involve the presence of certain abnormal physiological parameters, a raised serum lactate or the recent use of chemotherapy. The presence of any of the criteria places the patient in a high-risk category for mortality. The Red-flag criteria are predominantly physiological variables. Third, the National Institute for Health and Care Excellence (NICE) guidance on the management of Sepsis, published in 2016 (17), recommend the use of certain the high-risk criteria, which are predominantly physiological variables. The presence of any of the high-risk criteria places the patient in the high-risk category. Fourth, the National Early Warning Score 2 (NEWS2) of ≥5 is recommended for use by the Royal College of Physicians (RCP) (18) to identify those who are likely to have sepsis or deteriorate. The NEWS2 is a cumulative score of the physiological variables which are given increasing values the further they deviate from normal values. It ranges from 0 to 20 points. Fifth, the Systemic Inflammatory Response Syndrome (SIRS) criteria used in the Sepsis-1 definition (19), uses a combination of three physiological parameters and the white cell count (WCC). The presence of two abnormal parameters places the patient in a high-risk category.

Lastly, the Risk-stratification of Emergency Department suspected Sepsis (REDS) score (20). This score has been externally validated in a small study (21). The REDS score combines physiological and laboratory variables. They are age, altered mental state, initial respiratory rate, initial systolic blood pressure (SBP), serum albumin, International Normalised Ratio (INR), lactate and refractory hypotension [the requirement of vasopressors to maintain a mean arterial pressure (MAP) >65 mmHg after an adequate fluid bolus]. The score ranges from 0 to 12. A score of three or more places the patient in a high risk category.

The ability of the afore-mentioned tools to risk-stratify ED suspected sepsis patients for survival at 180 days is not known. Furthermore, patients admitted with suspected sepsis often have several comorbidities that are known to be associated with mortality (20, 22). Any risk-stratification tool should work well in those with and without these comorbidities.

The primary aim of this study was to compare the prognostic performance of the REDS score, SOFA score, the Red-flag criteria, the NICE high-risk criteria, the NEWS2 score and the SIRS criteria to risk-stratify ED patients admitted with suspected sepsis, for survival at 180 days. The secondary aim was to study the performance of these tools in predicting outcome in those with and without the specified comorbidities.

Materials and methods

Setting, study design and time period

This retrospective single centre study was conducted in the ED of a large urban university teaching hospital in London, UK. The annual attendance of adult patients is over 130,000. The study period ran from 1st March to 3st August 2019, with the 180 day follow up period for the last patient ending on 26th February 2020. The final date of the study period was chosen such that the 180 day follow-up period did not over-lap with the commencement of the COVID-19 pandemic at the beginning of March 2020.

Data collection and participant selection

The ED adult sepsis registry contains routinely collected data for continuous monthly audit. For the period covered by this study, the registry contained all consecutive adult patients who attended the ED, received intravenous antibiotics for the treatment of suspected sepsis

and admitted to a hospital bed. The auditing clinicians (doctors) were trained to identify patients with suspected infection or sepsis from the contemporaneous clinical notes prepared by the clinicians treating the patient. The auditing clinicians entered the data in to an Excel spreadsheet. All laboratory results and outcome data were re-collected by a second researcher. The two sets of results were compared and any discrepancies were rechecked and corrected where necessary.

The outcome at 180 days was obtained from the Electronic Patient Record (EPR) and the clinical information technology (IT) system. These are two distinct systems. The EPR is connected to the National Health Service's national Personal Demographic Service (PDS). This meant that dates of death were readily available on the hospital's EPR system without the need to seek it from other external sources, as long as the death was registered somewhere in the UK. In the absence of a date of death, it could not be assumed that the patient was alive. If there was no date of death recorded on the EPR, the patient was censored on the last date they were known to be alive. The hospital pathology, radiology and clinic systems were searched for evidence that the patient was alive on day 180 from ED attendance. The patients' General Practise (GP) records were not accessed as we did not seek or obtain patients' consent. The cause of death was not identified and all-cause mortality was noted.

For patients with multiple attendances during the study period, only the final attendance was included in the study. All preceding attendances were excluded for such patients. Patients with missing results for blood tests that were required to calculate the different scores, were also excluded.

Measurements

For each patient entered on to the ED Adult Sepsis register, the date and time of arrival, age, initial vital signs, Glasgow Coma Score (GCS), presence of new altered mental state, results for the white cell count, urea, creatinine, bilirubin, albumin, the point-of-care lactate, the presence of refractory hypotension, the lactate after the fluid bolus (if measured), the international normalised ratio (INR), the use of coumarin or direct oral anticoagulants (DOACs) were routinely entered in to the database from the clinical IT system and contemporaneous notes. Baseline GCS, platelet count, bilirubin and creatinine were also collected. The outcome at discharge and the final diagnosis, if it was an infection or not, were also recorded. If it was an infection, the organ that was infected was noted, if known. Conveyance to the hospital by ambulance and the admission to the intensive care unit (ICU) were also noted.

'Specified comorbidities'

The presence of the following comorbidities was noted: dementia, malignancy, inability to live independently [care home residency, a minimum three times a day care package or need help with activities of daily living -these correspond to a Rockwood Frailty score (23) of 6 or more], the use of long-term oxygen therapy (LTOT) and any previous do-not-resuscitate decisions. These comorbidities are referred to as 'specified comorbidities' throughout the manuscript. Whilst the presence of any of these specified comorbidities do not in themselves exclude patients from escalation of treatment, we have previously reported that 80% of patients who died without admission to ICU had at least one of these comorbidities (20). We have also reported that 48% of those admitted from the ED with an infection or suspected sepsis and 70% of these patients who go on to die in hospital, have one or more of these specified comorbidities prior to admission (24). Patients with the specified co-morbidities are less likely to be for full escalation of treatment. As the majority of deaths occur in those with the specified co-morbidities, it is important to identify if the risk-stratification tools identify those who are at high-risk of death amongst those without the specified co-morbidities. This would identify a role and purpose for the tools beyond the patient's co-morbidities and provide a more accurate reflection of the risk-stratification.

The REDS score, the baseline and admission SOFA score, the change in SOFA score from baseline, the presence and number of Red-flag criteria, the presence and the number of NICE high-risk criteria, the initial NEWS2 score and the number of SIRS criteria, were calculated for each patient.

Calculation of the SOFA score: Arterial blood gases are not measured in every patient in the ED. Therefore, the respiratory component of the SOFA score was replaced by the SaO_2/FiO_2 using a previously validated scoring system (25). Patients on LTOT were given a score of 1 point for their respiratory component of their baseline SOFA score. Patients with a MAP of <70 mmHg on arrival or after an intravenous fluid bolus were given a score of 1 point for their MAP and those with refractory hypotension were given a score of 3 points for this component of the SOFA score. Baseline MAP was assumed to be normal for all patients. Patients who had a minimum two point increase from the baseline SOFA score were deemed to be at high-risk of mortality.

With regard to the NICE high-risk criteria, we were unable to determine the number of hours the patient had been anuric as this was poorly documented. In addition, we did not study the moderate-high risk criteria as some of these criteria were inconsistently documented.

Outcome measure- The primary end-point was survival at 180 days from admission.

Data analysis

Once the data collection was checked and complete, it was anonymised and analysed. The data was stratified in to high-risk and low-risk groups as defined by each of the risk-stratification tools. Kaplan–Meier curves were then plotted for the high-risk and low-risk groups of each risk-stratification tool.

A Cox proportional-hazard regression (CPHR) was also performed for direct comparison of the risk-stratification tools. The risk stratification tools had a receiver operator characteristic (ROC) curve constructed and the area under the ROC (AUROC) curve calculated for the outcome at hospital discharge and at 180 days. The AUROC curves were compared. For the purposes of constructing a ROC curve, the number of criteria met was used for the Red-flag criteria, the NICE high-risk criteria and the SIRS criteria were used. The admission SOFA score was used to construct the ROC curve.

The study population was split in to those with and without the specified comorbidities and the prognostic performance of each risk-stratification tool was studied in each population. The prognostic performance of the REDS score was studied further by splitting the

whole population and those with and without the specified comorbidities, in to the different score-bands.

Statistics

MedCalc® Statistical Software version 20.018 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2021) was used for statistical analysis. Statistical significance was defined as $p < 0.05$. Continuous variables were tested for normality using the Kolmogorov–Smirnov test. Normally distributed continuous data are presented as a mean and standard deviation. Data not normally distributed are presented as a median and interquartile range. Categorical data are presented as percentages. Differences in categorical data was assessed using the Chi-square test. The survival curves for high and low-risk groups within each scoring system were compared using the Log-rank test and the Hazard ratio. The six risk-stratification tools were compared using the Cox-proportional hazard regression. The 'Enter' method was used. Variables were entered if $p < 0.05$ and removed if $p > 0.1$. The difference in AUROC curves was assessed by the DeLong method (26).

Sample size and missing data

We have previously reported in-hospital mortality rates of 5% in the low-risk (REDS scores 0–2) group and 21% for the high-risk (REDS scores 3–12) group (20). Thus, survival rates of 95 and 79%, respectively. We have also reported that the high and low risk REDS scores are equally distributed through the patient population, giving a 1:1 ratio (24). The estimated sample size for these survival rates, with a two sided alpha level of 0.01 with a power of 99%, would be 448. The type I error level and power were recalculated once the 180 day survival rates were known.

Patients who were missing data to calculate the REDS score or the SOFA scores were excluded. Patients on warfarin or a DOAC were scored 0 for INR in the REDS score, as this would be clinical practise.

Results

Of the initial study population of 1,628 admissions (Figure 1), 158 were excluded as they were repeat encounters for a small group of patients. A further 413 patients were excluded due to missing variables. Of the remaining 1,057 patients, 146 died in hospital (mortality rate of 13.8% and survival rate at discharge of 86.2%) and a total of 284 were known to have died by day 180. The survival rate for the study population as a whole was 78.4% at 90 days and 74.4% at 180 days. The median age of the study population was 73 years and males made up 50% of the population. The baseline characteristics are presented in Table 1. Patients with at least one of the specified co-morbidities made up 46.1% of the study population, 76% (111 of 146) of the in-hospital deaths, and 73.9% (210 of 284) of deaths at 180 days.

None of the continuous variables were normally distributed. Respiratory infections were the primary source of infections in 41% of the study population. Conveyance to hospital by ambulance occurred in 79.9% of the population. Admission to the ICU occurred in 8.6% of patients. Censoring was applied to 8.6% of the study population, where the outcome beyond the last date they were known to be alive within the follow-up period, was not known.

The survival rate at 180 days of the low-risk REDS score group was 83.5% and that for the high-risk group was 59.1%.The ratio of low risk to high risk was 1.08 (508/549). The sample size required with these parameters would be 343, for an alpha level of 0.01 and power of 99%.

The proportion of high-risk populations as stratified by the different risk-stratification tools were as follows: the REDS score (scores 3–12) 508 (48.1%), SOFA score (increase of 2 points) 380

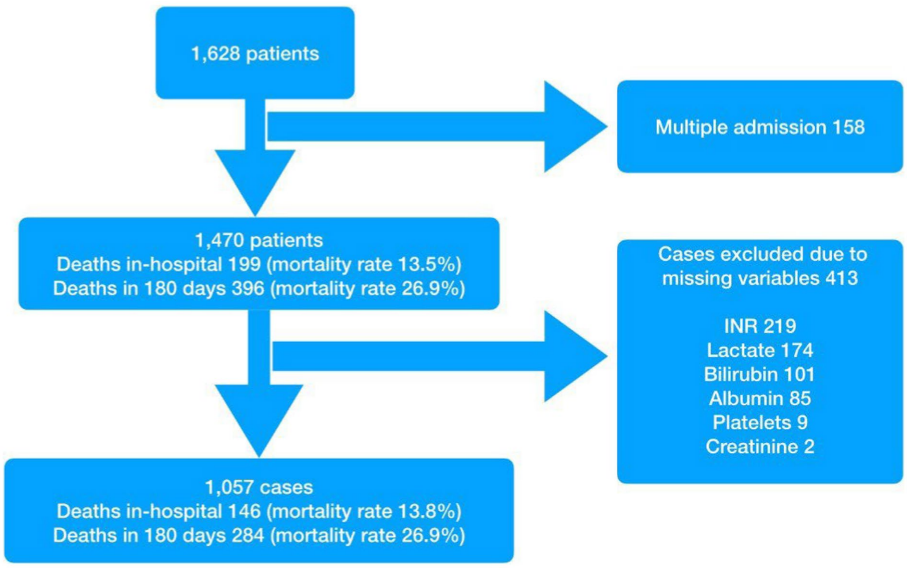

FIGURE 1
Patient flow.

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TABLE 1 Baseline variables of the study population.

	Number (percentage) or Median [Inter-Quartile Range]
Demographics	
Number	1,057
Age (years)	73 [58–83]
Males	529 (50.0%)
Deaths- In-hospital	146 (13.8%)
Deaths -in 180 days	284 (26.9%)
Initial vital signs	
Respiratory rate (breaths/min)	22 [18–28]
Heart rate (beats/min)	103 [86–118]
Systolic blood pressure (mmHg)	125 [108–144]
Temperature (degrees centigrade)	37.2 [36.6–38.2]
Glasgow Coma Score	15 [14–15]
Refractory hypotension	34 (3.2%)
Initial blood results	
White cell count	12.2 [8.2–16.1]
Neutrophil count	9.6 [6.2–13.3]
International Normalised Ratio (INR)	1.2 [1.1–1.4]
C-reactive protein	65 [21–152]
Albumin (g/L)	32 [28–36]
Lactate (mmol/L)	1.6 [1.1–2.4]
Treatments	
Time to antibiotics (minutes)	66 [38–159]
Antibiotics within an hour of arrival	492 (46.5%)
Volume of IVF commenced (mls)	1,000 [1000–2000]
Final source of infection	
Respiratory	433 (41%)
Urogenital	191 (18.1%)
Abdomen	68 (6.4%)
Soft tissue	73 (6.9%)
Unknown or multiple sites	143 (13.5%)
Other	2 (0.2%)
Ear Nose & Throat	7 (0.7%)
Device	3 (0.3%)
Central Nervous System	5 (0.5%)
No infection	132 (12.5%)
Scores	
REDS score	2 [2–4]
SOFA score	1 [0–3]
Red-flag criteria met	1 [1–2]
NICE guideline high-risk criteria met	1 [0–1]
NEWS2 score	5 [3–8]
SIRS criteria	2 [1–3]
Co-morbidities	
Dementia	143 (13.5%)
Malignancy	180 (17%)
CH resident/Live-in carer/ Minimum TDS care package	269 (25.4%)
Long-term oxygen therapy	26 (2.5%)
Previous DNAR order	104 (9.8%)
Any of the above 5 specified co-morbidities	487 (46.1%)
Other data	
Number alive but censored before 180 days	91 (8.6%)
Number arrived by ambulance	845 (79.9%)
Number admitted to the intensive care unit (ICU)	91 (8.6%)
Hospital length of stay (days)	6 [3–12]

IVF, Intravenous fluid; REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome; CH Care Home; TDS ter die sumendum (three times a day); DNAR, Do Not Attempt Resuscitation.

(36%), Red-flag criteria 858 (81.2%), the NICE high-risk criteria 702 (66.4%), the NEWS2 score (scores ≥5) 630 (59.6%), and the SIRS criteria (≥2 criteria) 752 (71.1%).

Table 2 illustrates the survival fractions in the high and low risk categories of the different stratification tools. The survival rates for the low risk group was highest for the REDS score. This was similar to the survival rate for those identified as low-risk by the Red-flag criteria. The survival rate for the low-risk group was lowest as stratified by the SIRS criteria. The REDS score and the SOFA score had the lowest survival fraction for their respective high-risk groups. All other scoring systems had similar survival fractions in their high-risk groups.

The Kaplan–Meier survival curves for the study population by the different risk-stratification tools, together with the log-rank test for the difference in survival between the high and low-risk categories are illustrated in Figure 2. All risk-stratification tools except the SIRS criteria, were able to prognosticate for outcome at 180 days. The hazard ratio between high and low risk groups for the different risk-stratification tools was greatest for the REDS and the SOFA scores. The hazard ratios for the Red-flag criteria, NICE high-risk criteria and the NEWS2 scores were similar. The SIRS criteria were not prognostic for survival at 180 days with no difference in survival fractions in the high and low risk categories on log-rank test, $p = 0.98$, and a hazard ratio of 1.00 (95% CI 0.78–1.30), see Table 2. Cox proportional hazard regression of the six risk-stratification tools showed that the performance of the REDS and SOFA scores were better than the other risk-stratification tools (Table 3).

The AUROC curve (Figure 3) for the REDS score for in-hospital mortality and mortality at 180 days was 0.70 (95% CI 0.67–0.72). The AUROC curve for the admission SOFA score however, decreased

TABLE 2 Survival proportion at 180days by risk-stratification tool.

Risk-stratification tool	Survival proportion and standard error of low-risk group	Survival proportion and standard error of high-risk group	Logrank test Significance	Hazard ratio with 95% confidence interval
REDS score	0.835 (0.017)	0.591 (0.022)	$p < 0.0001$	2.89 (2.29–3.66)
SOFA score	0.780 (0.017)	0.602 (0.026)	$p < 0.0001$	2.31 (1.80–2.96)
Red-flag criteria	0.811 (0.029)	0.694 (0.016)	$p = 0.001$	1.63 (1.22–2.19)
NICE high-risk criteria	0.794 (0.022)	0.677 (0.018)	$p = 0.0001$	1.64 (1.28–2.09)
NEWS2 score	0.762 (0.021)	0.685 (0.019)	$p = 0.0032$	1.43 (1.13–1.81)
SIRS criteria	0.714 (0.027)	0.717 (0.017)	$p = 0.9788$	1.00 (0.78–1.30)

REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome.

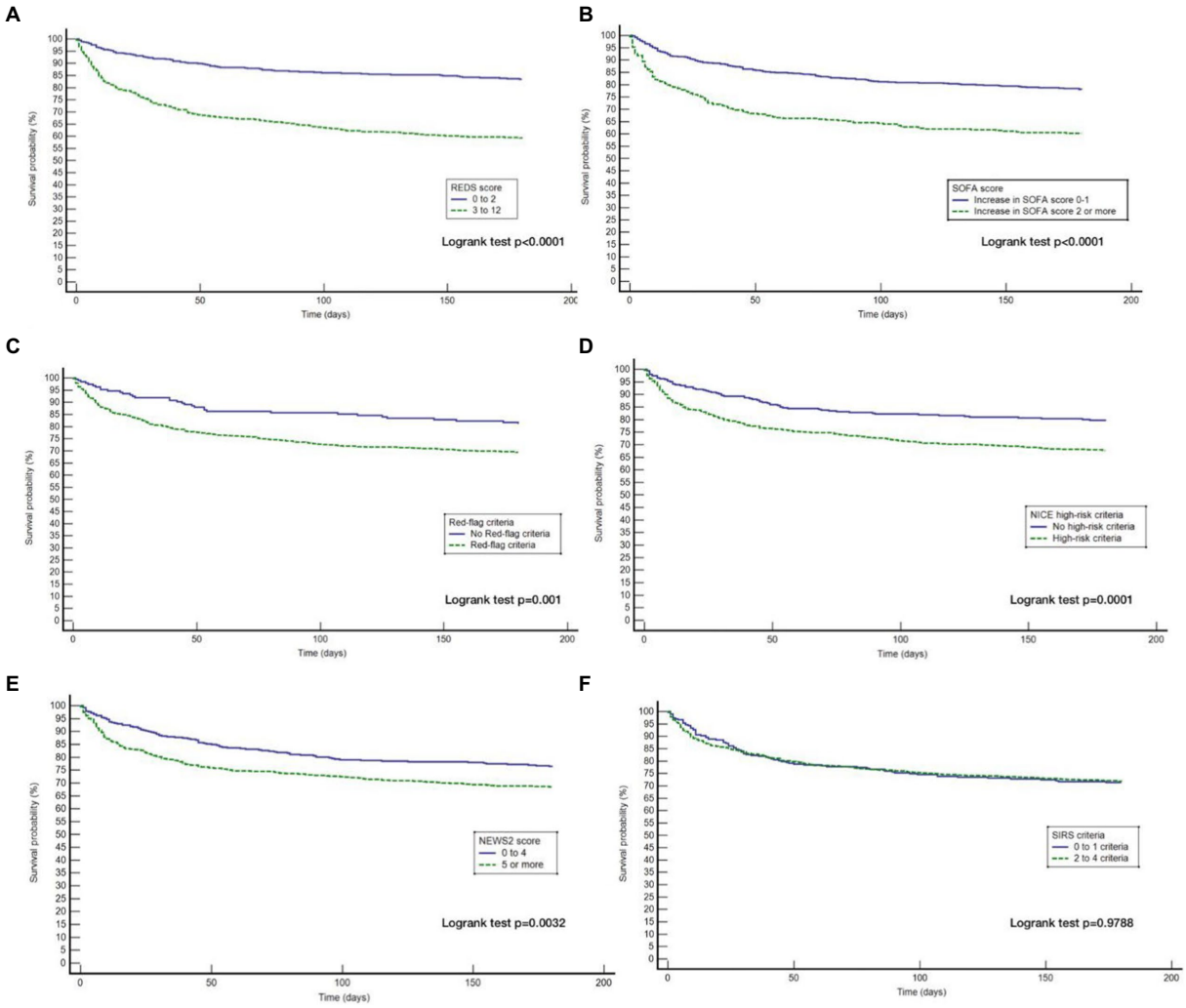

FIGURE 2
Kaplan–Meier curves for 180day outcome comparing high and low-risk criteria as stratified by the different risk-stratification tools. REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS=Systemic Inflammatory Response Syndrome; **(A)** Kaplan Meier curves for 180day survival as stratified by the REDS score; **(B)** Kaplan Meier curves for 180day survival as stratified by the SOFA score; **(C)** Kaplan Meier curves for 180day survival as stratified by the Red=flag criteria; **(D)**, Kaplan Meier curves for 180day survival as stratified by the NICE criteria; **(E)** Kaplan Meier curves for 180day survival as stratified by the NEWS2 score; **(F)** Kaplan Meier curves for 180day survival as stratified by the SIRS criteria.

substantially from 0.73 (95%CI 0.70–0.76) for in-hospital mortality to 0.67 (95%CI 0.64–0.70), for mortality at 180 days. The AUROC for the REDS and SOFA scores were both greater than each of the other four scores, for in-hospital mortality (Table 4). The results were similar for mortality at 180 days, except for the difference in the AUROC curve

TABLE 3 Cox proportional hazard regression of the six risk-stratification tools.

Risk-stratification tool	*b*	Standard error	Wald	*p*	Exp (*b*) (95% confidence interval)
REDS score	0.9306	0.141	42.995	$p < 0.0001$	2.54 (1.92–3.35)
SOFA score	0.4578	0.1262	13.2040	$p = 0.0003$	1.58 (1.24–2.03)
Red-flag criteria	0.0043	0.2435	0.00031	$p = 0.9859$	1.00 (0.62–1.62)
NICE high-risk criteria	0.1921	0.2157	0.7936	$p = 0.3730$	1.21 (0.79–1.85)
NEWS2 score	−0.0674	0.1788	0.1421	$p = 0.7062$	0.93 (0.66–1.33)
SIRS criteria	−0.1030	0.1470	0.4908	$p = 0.4836$	0.90 (0.68–1.20)

REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome.

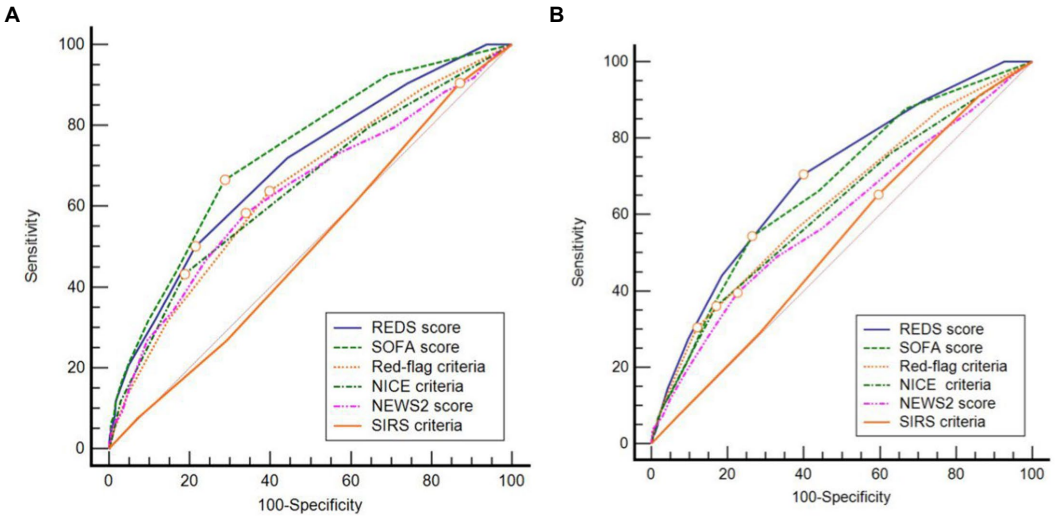

FIGURE 3
Receiver operator characteristic curves for **(A)** in-hospital and **(B)** 180day mortality. REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome.

between the SOFA score and the red-flag criteria, this no longer reached statistical significance.

Although the overall mortality rate at hospital discharge was 13.8%, analysis of the study population divided in to those *with* and *without* the specified comorbidities, reveals that the mortality rate at hospital discharge was significantly greater for those *with* the specified comorbidities at 22.8%, compared to 6.1% for those *without* the specified comorbidities, $p < 0.0001$. Similarly, the survival rate at 180 days for those *with* the specified comorbidities was 57.9% and those *without* the specified comorbidities was 87.7%. Kaplan–Meier survival analysis of patients *without* any of the specified comorbidities revealed that only the REDS and SOFA scores were prognostic for outcome at 180 days (Figure 4). This was confirmed on CPHR (Table 5). Similar analysis of those *with* the specified comorbidities showed that all risk-stratification tools except the Red-flag criteria and the SIRS criteria were prognostic (Figure 5). However, on CPHR the REDS score and the SOFA score outperformed the other scores (Table 6).

The REDS score was divided in to score-bands of 0–2, 3–4, 5–6 and 7–12. The Kaplan–Meier survival curves for score bands are illustrated in Figure 6 for the whole population (Figures 6A,B) and those *with* (Figures 6C,D) and *without* (Figures 6E,F) the specified comorbidities. In the population *without* the specified comorbidities, the in-hospital mortality rate in those with a REDS score of 5–6 was 10.4% (Figure 7), but the survival proportion was 70.8% at 180 days (Figure 6F). In the same population, the in-hospital mortality rate for those with a REDS score of 7–12 was 35.7% (Figure 7) and the survival rate was 50.5% at 180 days (Figure 6F).

For the population of patients *with* the specified comorbidities and a REDS score of 7–12, a survival proportion of 0.33 was reached within 7 days (Figure 6D). Of note, none of the 57 patients with a REDS score of 0 on presentation died in the 180 day follow-up period.

The survival proportions together with the hazard ratios of the latter three bands in comparison to the band 0–2, in the whole population, and in those with and without the specified comorbidities are presented in Table 7.

TABLE 4 Area under receiver operator characteristic (AUROC) curve for mortality at hospital discharge and at 180days for all risk-stratification tools; and the significance of the difference when compared with the AUROC curve of the respective REDS score.

Risk-stratification tool	AUROC and 95% Confidence interval for in-hospital mortality	Significance of the difference in AUROC curve compared to the REDS score	AUROC and 95% Confidence interval for mortality at 180days	Significance of the difference in AUROC curve compared to the REDS score
REDS score	0.70 (0.67–0.72)	Not applicable	0.70 (0.67–0.72)	Not applicable
SOFA score	0.73 (0.70–0.76)	$p = 0.13$	0.67 (0.64–0.70)	$p = 0.20$
Red-flag criteria	0.64 (0.62–0.68)	$p = 0.02$	0.63 (0.60–0.66)	$p = 0.0001$
NICE criteria	0.64 (0.62–0.67)	$p = 0.04$	0.62 (0.59–0.65)	$p < 0.0001$
NEWS2 score	0.64 (0.61–0.67)	$p = 0.01$	0.59 (0.56–0.62)	$p < 0.0001$
SIRS criteria	0.50 (0.47–0.53)	$p < 0.0001$	0.53 (0.49–0.56)	$p < 0.0001$

REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome.

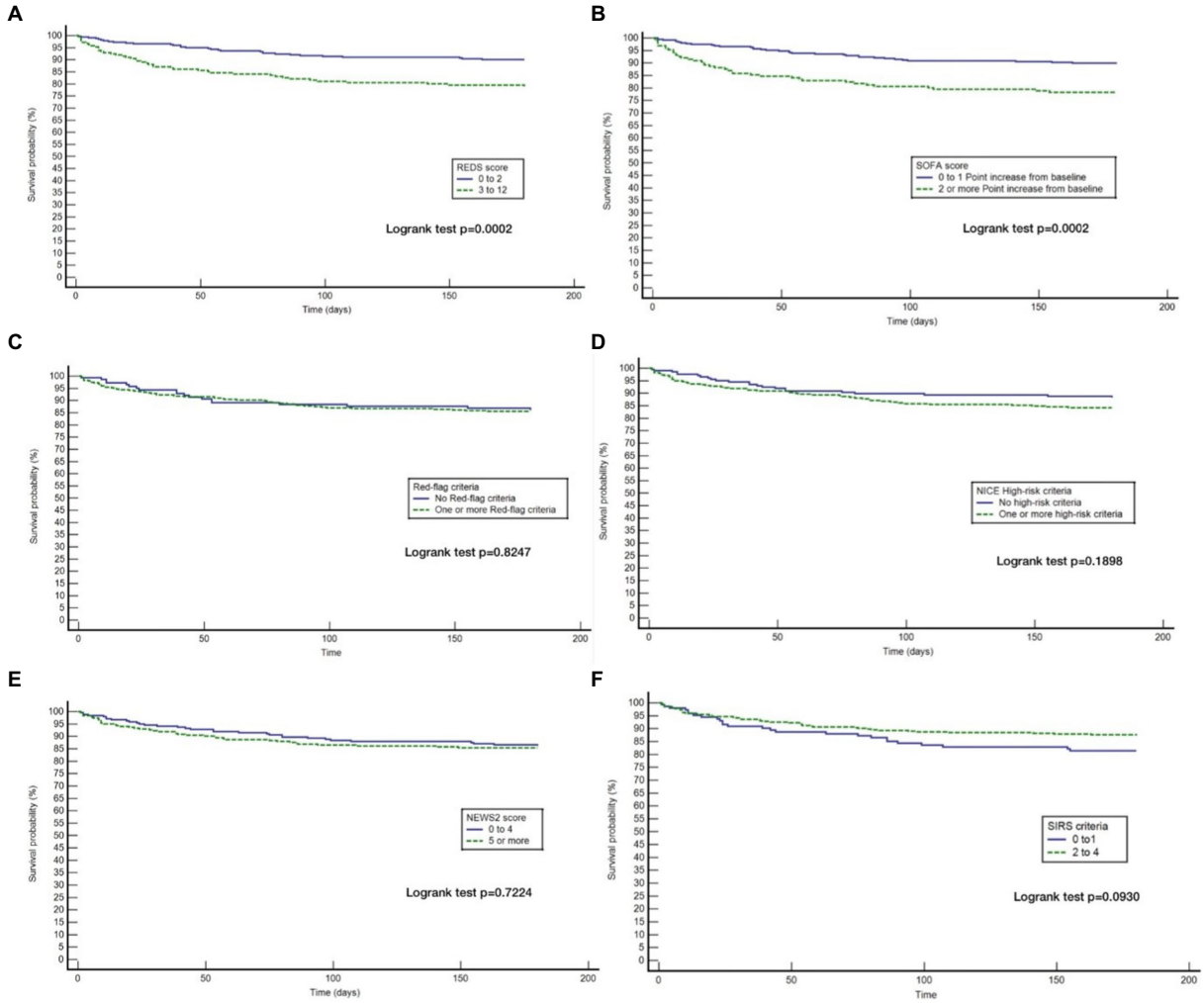

FIGURE 4
Kaplan Meier curves for 180day survival for those *without* specified* co-morbidities stratified by the different tools. **(A)** Kaplan Meier curves for 180day survival as stratified by the REDS score; **(B)** Kaplan Meier curves for 180day survival as stratified by the SOFA score; **(C)** Kaplan Meier curves for 180day survival as stratified by the Red=flag criteria; **(D)** Kaplan Meier curves for 180day survival as stratified by the NICE criteria; **(E)** Kaplan Meier curves for 180day survival as stratified by the NEWS2 score; **(F)** Kaplan Meier curves for 180day survival as stratified by the SIRS criteria. *Specified comorbidities, presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision.

TABLE 5 Cox proportional hazard regression of the six risk-stratification tools in the population *without* specified* comorbidities.

Stratification tool	*b*	Standard error	Wald	*p*	Exp (*b*) (95% confidence interval)
REDS score	0.7125	0.2665	7.157	$p = 0.0075$	2.04 (1.21–3.44)
SOFA score	0.6393	0.2561	6.2302	$p = 0.0126$	1.90 (1.45–3.13)
Red-flag criteria	−0.9914	0.5564	3.1755	$p = 0.0747$	0.37 (0.12–1.10)
NICE high-risk criteria	0.8871	0.5666	2.4516	$p = 0.1174$	2.43 (0.80–7.37)
NEWS2 score	−0.1935	0.3475	0.3100	$p = 0.5777$	0.82 (0.42–1.63)
SIRS criteria	−0.3786	0.2790	1.8420	$p = 0.1747$	0.68 (0.40–1.18)

REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome. *Specified comorbidities = presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision.

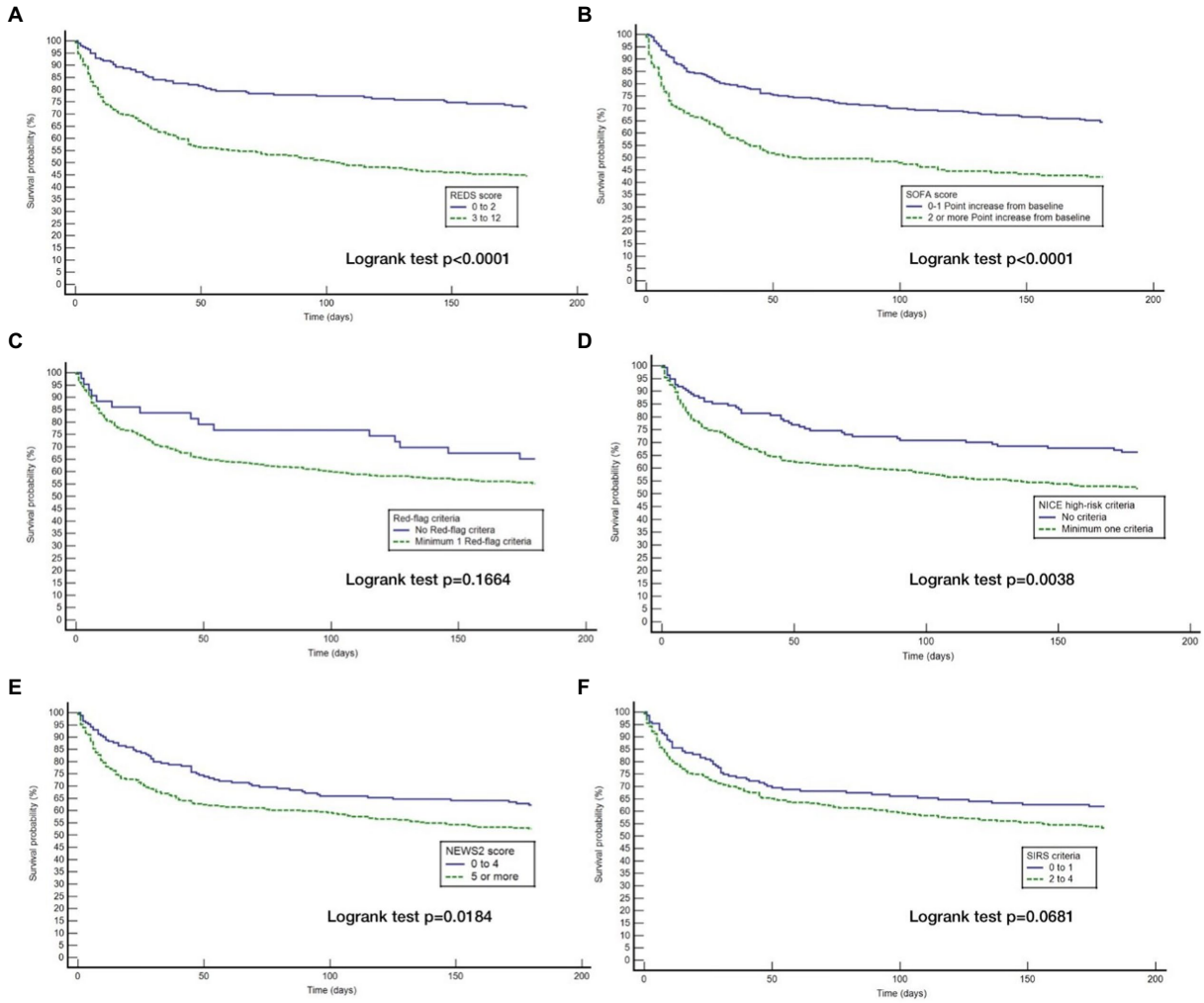

FIGURE 5
Kaplan Meier curves for 180day survival for those *with* specified* co-morbidities stratified by the different tools. **(A)** Kaplan Meier curves for 180day survival as stratified by the REDS score; **(B)** Kaplan Meier curves for 180day survival as stratified by the SOFA score; **(C)** Kaplan Meier curves for 180day survival as stratified by the Red=flag criteria; **(D)** Kaplan Meier curves for 180day survival as stratified by the NICE criteria; **(E)** Kaplan Meier curves for 180day survival as stratified by the NEWS2 score; **(F)** Kaplan Meier curves for 180day survival as stratified by the SIRS criteria. *Specified comorbidities=presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision.

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TABLE 6 Cox proportional hazard regression of the six risk-stratification tools in the population *with* specified* comorbidities.

Stratification tool	*b*	Standard error	Wald	*p*	Exp (*b*) (95% confidence interval)
REDS score	0.7811	0.1742	20.0986	$p < 0.0001$	2.18 (1.55–3.07)
SOFA score	0.5667	0.1479	14.6840	$p = 0.0001$	1.76 (1.32–2.36)
Red-flag criteria	−0.1430	0.3136	0.2079	$p = 0.6484$	0.87 (0.47–1.60)
NICE high-risk criteria	0.1763	0.2339	0.5679	$p = 0.4511$	1.19 (0.75–1.89)
NEWS2 score	−0.1583	0.2091	0.5735	$p = 0.4489$	0.85 (0.57–1.29)
SIRS criteria	0.1787	0.1774	1.0145	$p = 0.3138$	1.20 (0.84–1.69)

REDS, Risk-stratification of Emergency Department suspected Sepsis; SOFA, Sequential Organ Failure Assessment; NICE, National Institute for Health and Care Excellence; NEWS2, National Early Warning Score 2; SIRS, Systemic Inflammatory Response Syndrome.*Specified comorbidities = presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision.

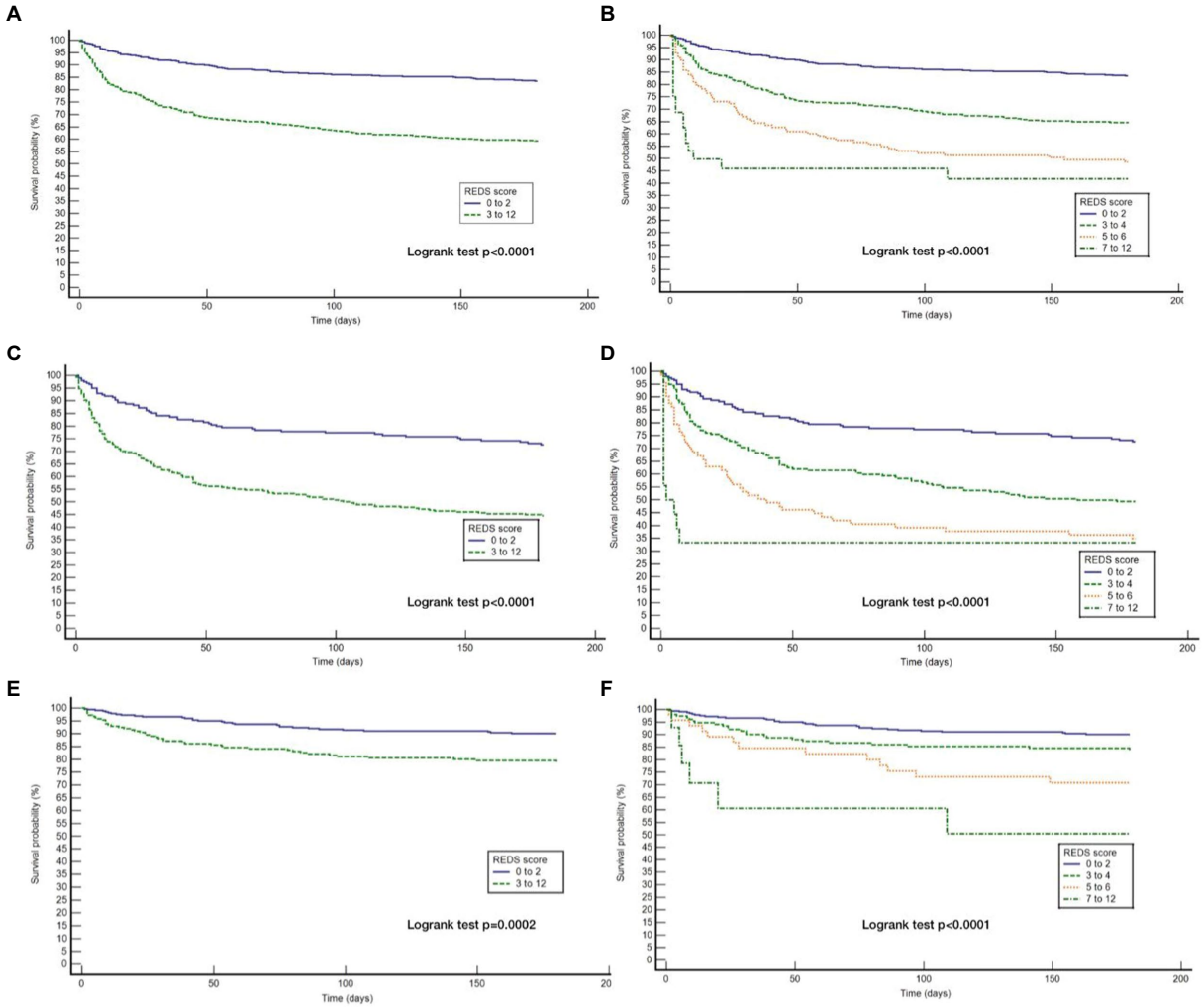

FIGURE 6
Kaplan Meier curves for 180day survival for the whole study population and those with and without the specified* co-morbidities by REDS score band. *Specified comorbidities=presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision **(A)** Kaplan Meier curves for the study cohort stratified by REDS score of 0–2 and 3–12; **(B)** Kaplan Meier curves for the study cohort stratified by REDS score of 0–2, 3–4, 5–6 and 7–12; **(C)** Kaplan Meier curves for those WITH the specified comorbidities stratified by REDS score of 0–2 and 3–12; **(D)** Kaplan Meier curves for those WITH the specified comorbidities stratified by REDS score of 0–2, 3–4, 5–6 and 7–12; **(E)** Kaplan Meier curves for those WITHOUT the specified comorbidities stratified by REDS score of 0–2 and 3–12; **(F)** Kaplan Meier curves for those WITHOUT the specified comorbidities stratified by REDS score of 0–2, 3–4, 5–6, and 7–12.

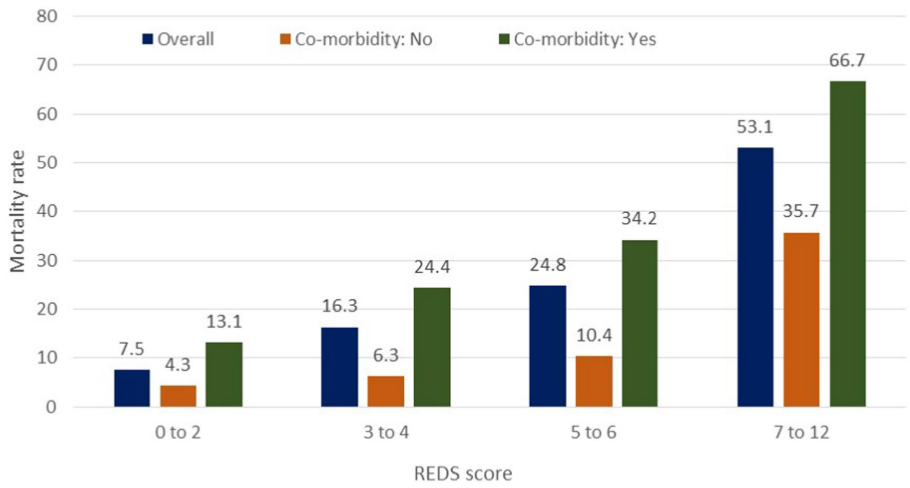

FIGURE 7
In-hospital mortality rates by the REDS score in patients with and without specified* co-morbidities. *Specified comorbidities=presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision.

TABLE 7 Survival proportion and hazard ratio compared to low-risk group at 180days by the REDS score bands, in all patients, those with specified* co-morbidities and those without specified* comorbidities.

REDS score band	Survival proportion (standard error) All patients	Hazard ratio with 95% confidence interval compared to low-risk group [REDS score 0–2] *All* patients	Survival proportion (standard error) *With* specified comorbidities	Hazard ratio with 95% confidence interval compared to low-risk group [REDS score 0–2] With specified comorbidities	Survival proportion (standard error) *Without* specified comorbidities	Hazard ratio with 95% confidence interval compared to low-risk group [REDS score 0–2] Without specified comorbidities
0–2	0.835 (0.017)	Not applicable	0.726 (0.032)	Not applicable	0.901 (0.017)	Not applicable
3–4	0.643 (0.026)	2.45 (1.90–3.17)	0.493 (0.036)	2.17 (1.62–2.91)	0.839 (0.030)	1.73 (1.03–2.91)
5–6	0.486 (0.047)	4.08 (2.72–6.11)	0.349 (0.056)	3.38 (2.19–5.23)	0.708 (0.068)	3.30 (1.40–7.79)
7–12	0.418 (0.091)	6.90 (2.81–16.95)	0.333 (0.111)	5.64 (2.05–15.61)	0.505 (0.150)	7.73 (1.22–48.85)

*Specified comorbidities = presence of any one of the following: dementia, malignancy, care home residency or a minimum three times a day care package, on long-term oxygen therapy (LTOT) or a previous do-not-resuscitate decision.

Discussion

In this study, we have shown that the REDS score, the SOFA score, the Red-flag criteria, the NICE criteria and the NEWS2 score prognosticate outcome at 180 days, in ED patients admitted with suspected sepsis. The SIRS criteria did not prognosticate outcome at 180 days. The REDS score and the SOFA score outperformed the Red-flag criteria, the NICE criteria, the NEWS2 score and the SIRS criteria to prognosticate outcome at 180 days. The AUROC of the REDS score was maintained between hospital discharge and 180 days. The 180 day survival proportion for patients with REDS scores of 0–2, 3–4, 5–6, and 7–12 were 83.5, 64.3, 48.6, and 41.8%. The REDS and SOFA scores are the only scores amongst those studied that recognised less than 50% of the population as high-risk.

We are not aware of any other British study exploring 180 day outcome after discharge in the non-ICU setting. Unwin et al studied the SIRS criteria and the Red-flag sepsis criteria, the NICE criteria and SOFA score, as risk-stratification tools to predict outcome at 90 days (11). The study population included ED and ward patients over three 24 h periods, whilst our population consisted of only ED patients. The overall survival at 90 days was 74.7% which was similar to our survival rate of 78.4% at 90 days and 74.4% at 180 days. Unwin *et al* found the log-rank test to be significant for outcome at 90 days for the SOFA score, the NICE criteria and the SIRS criteria. But they did not find the log-rank test to be significant for the Red-flag criteria for outcome at 90 days. A study by Borgonovo et al.,(27) looked at the prognostics value of the SIRS criteria in patients admitted with acute decompensated cirrhosis with and without and infection. Whilst infection itself was independently associated with mortality at 90 days, they did not find the presence of the SIRS criteria to be associated with mortality at 90 days, in those with an infection. In fact, we too have previously reported that the SIRS criteria were not prognostic for in-hospital mortality (24).

The NEWS2 score, consisting entirely of physiological variables, is well recognised to prognosticate outcome in hospital and therefore, recommended by the RCP to identify patients who are likely to have sepsis or deteriorate. It is used across many hospitals as a common tool to measure acuity. It has the advantage of being a common language based on bedside observations. Our study also found the NEWS2 score to be prognostic at 180 days, but it performed less well than the REDS and SOFA scores on CPHR. Similarly, the Red-flag sepsis criteria and the NICE high-risk criteria were also prognostic as they are based heavily on the NEWS2 score. And as seen with the NEWS2 score they performed less well when compared to the REDS and SOFA scores. The NEWS2 score, the Red-flag criteria and the NICE high-risk criteria are heavily weighted by physiological variables and had similar performance characteristics. The REDS and SOFA scores however combine physiological variables together with laboratory results and performed better than the scores based predominantly on physiological variables.

The Red-flag sepsis criteria and the NICE criteria were published with a view to deliver antibiotics within an hour of recognition. In the ED it would mean within an hour of arrival. Blood results are not usually available within an hour. However, the most recent guidance from the Surviving Sepsis Campaign (14) is to deliver antibiotics within an hour if shock is present or if sepsis is definite or probable. If shock is not present or the patient could have another condition, the recommendation is to perform investigations and if found to have an infection or sepsis, to deliver antibiotics within 3 h of recognition. We too have previously found that the time to antibiotics is critical in those with refractory hypotension, with a number needed to treat of four, but not in those without refractory hypotension (28). We have also suggested that a SBP of <100 mmHg could be used to identify patients who are likely to develop refractory hypotension. For all other patients we could review the blood results to determine if they are likely have an infection before delivering antibiotics. This would not only help with antimicrobial stewardship but also enable the use of the better risk-stratification tools such as the REDS or SOFA scores, which require blood results.

It is clear that a significant proportion of the study population (46.1%) had one or more of the specified co-morbidities. This group of patients were also disproportionately represented in both, the in-hospital and the 180 day mortality. The likely reason for this is that escalation of treatment may not have been appropriate in this population as a whole, although individuals would have been treated on their merit. The purpose of studying the risk stratification tools in those without the specified co-morbidities is to identify a group of patients who are less likely to have treatment limitations and thus a better reflection of risk-stratification. In this group, only the REDS and SOFA scores were found to stratify for survival at 180 days.

Increasing REDS scores were associated with progressively worsening survival rates at 180 days. This suggests that identifying these patient in the ED can help manage expectations of family members in addition to serving as an opportunity to implement enhanced care. Although the REDS score has been externally validated in a small study (21), it needs to be externally validated in a large study.

Limitations

Whilst our study has several strengths such as a large sample size, no missing variables in the study population and minimal censored individuals, there are some limitations. First, it is a single centre study. This limits its generalisability until externally validated. Second, it is a retrospective study which may have been biassed by a variable that has not been accounted for. We hope by studying a population that was greater than required, we would have mitigated any unknown bias that may have occurred. Third, we did not study the patients who were not admitted although it is unlikely they were septic when discharged. Fourth, we limited our follow-up to 180 days. We do not know if the scores are prognostic beyond this point. Fifth, we included all patients irrespective of their final diagnosis. So patients who did not have an infection may have biassed our results, but this group formed only 12.5% of the study population. We did not exclude this population as the final diagnosis of no infection will not be known at the point of admission. Sixth, we did not study the treatments implemented. We acknowledge that this may have had an impact on outcome.

Conclusion

The REDS score, SOFA score, Red-flag criteria, the NICE high-risk criteria and the NEWS2 score were all able to prognosticate outcome at 180 days. However, the REDS and SOFA scores outperformed the other scores studied. The SIRS criteria did not prognosticate for outcome at 180 days. The REDS and SOFA scores were the only tools that were able to stratify patients for 180 day outcome in those without the specified comorbidities.

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 Ethics approval: This study of routinely collected anonymised data did not include an intervention and did not change the normal process of care. It is a retrospective observational study from a single centre and therefore not generalizable. In accordance with the National Health Service (NHS) Health Research Authority (HRA) and Medical Research Council (MRC) guidance such studies do not require formal ethics approval (29). In accordance the national Health Research Authority guidance around the General Data Protection Regulation and the Data Protection Act of 2018, patient consent is not required for the analysis of anonymised data (30, 31). This study was registered with the Clinical Effectiveness and audit office of St George's University Hospital, under the registration code AUDI000933. The exploration

of patient encounters with the hospital *via* the hospital's clinical IT system to study the outcome in the follow-up period after the index admission, was approved by the hospital's Caldicott guardian. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

NS designed the study and was involved in gathering and checking of the data together with AH, TS, PD, and DC. AR critically reviewed for intellectual content. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

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The accuracy of soluble urokinase-type plasminogen activator receptor for the diagnosis of neonatal sepsis: a meta-analysis

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Background: Neonatal sepsis is one of the major causes of morbidity and mortality in newborns. However, atypical clinical manifestations and symptoms make the early diagnosis of neonatal sepsis a challenge. Relatively high-serum soluble urokinase-type plasminogen activator receptor (suPAR) has been implicated as a diagnostic biomarker for adult sepsis. Therefore, the meta-analysis is intended to explore the diagnostic value of suPAR for neonatal sepsis.

Methods: The PubMed, Cochrane Library, Embase, Web of Science, China National Knowledge Infrastructure, China Biological Medicine Disk, and Wanfang databases were retrieved from inception to 31 December 2022 to collect diagnostic accuracy studies about suPAR for neonatal sepsis. Two reviewers independently screened the literature, extracted data, and assessed the risk of bias in the included studies using the quality assessment of diagnostic accuracy studies-2 (QUADAS-2) tool. Then, a meta-analysis was performed using Stata 15.0 software.

Results: A total of six articles involving eight studies were included. The results of the meta-analysis showed that the pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were 0.89 [95%CI (0.83–0.93)], 0.94 [95%CI (0.77–0.98)], 14 [95%CI (3.5–55.2)], 0.12 [95%CI (0.08–0.18)], and 117 [95%CI (24–567)], respectively. The area under the curve (AUC) of summary receiver operator characteristic (SROC) curves was 0.92 [95%CI (0.90–0.94)]. Sensitivity analysis confirmed the stability of the results, and publication bias was not observed. Fagan's nomogram results demonstrated the clinical availability of the findings.

Conclusion: Current evidence suggests that suPAR has potential diagnostic value for neonatal sepsis. Owing to the limited quality of the included studies, more high-quality studies are needed to verify the above conclusion.

KEYWORDS

neonatal sepsis, soluble urokinase-type plasminogen activator receptor, suPAR, diagnosis, meta-analysis

1. Introduction

While neonatal care has evolved over the years and neonatal sepsis-related deaths have fallen, it remains an important cause of mortality for neonates, especially for preterm infants (1). The incidence of septic shock has been reported to reach 1.3% among patients in neonatal intensive care units (NICUs) (2). If early diagnosis and appropriate treatment are not made in time, it is very easy for organ failure to occur in children, and it is even a fatal threat to neonates. Although blood culture is considered the gold standard for diagnosing sepsis, this process cannot produce immediate results, and a large blood sample is required to provide optional results (3). Common biomarkers, such as C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6), are generally associated with inflammation, and thus, their specificity for infection is low and could be affected by many other reasons (4). Consequently, challenges remain in achieving early recognition, accurate diagnosis, and standardized management of neonatal sepsis.

Currently, various biomarkers, biological molecules that are characteristic of normal or pathogenic processes and can be easily and objectively measured, have been proposed as being of potential use for sepsis diagnosis, therapeutic guidance, and/or prognostication (5, 6). Urokinase-type plasminogen activator receptor (uPAR) is a single-strand transmembrane glycoprotein that is expressed in neutrophils, monocytes macrophages, T cells, dendritic cells, and other inflammatory and immune cells. The natural immune response and the inflammatory process can be affected by uPAR through its influence on chemotaxis and phagocytosis of pathogens, and its interaction with extracellular matrix components, such as vitronectin and integrins. After being cleaved and released from the cell membrane, uPAR is recognized as a soluble receptor (7) that can be found in various bodily fluids, including blood, urine, cerebrospinal fluid, and saliva. Back in 1995, elevated plasma suPAR levels were reported in a small group of septic intensive care unit (ICU) patients (8). Since then, a growing body of evidence has shown that suPAR blood levels increase in conditions with severe inflammation and immune activation, such as infectious, autoimmune, and neoplastic diseases (9). Additionally, suPAR appears to discriminate better than some other biomarkers among patients with different severities of illness (10). Recently, numerous studies have shown that an early increase in suPAR levels predicts severe respiratory failure (11), acute kidney injury (12), and death in patients with coronavirus disease 2019 (COVID-19). Based on these findings, Kyriazopoulou et al. designed the suPAR-guided Anakinra treatment for Validation of the risk and Early Management Of severe respiratory failure by COVID-19 (SAVE-MORE) study to evaluate the efficacy and safety of early initiation of anakinra treatment in hospitalized patients with moderate or severe COVID-19 (13). This study was approved by the US emergency use authorization (EUA) and the food and drug administration (FDA). As a long-term inflammatory biomarker, suPAR has attracted widespread attention.

The diagnostic value of suPAR in neonatal sepsis has been reported in the literature; however, there are significant variations among different studies. The present meta-analysis aims to explore the accuracy of suPAR in diagnosing neonatal sepsis and provide evidence-based support for whether suPAR can be used as an early diagnostic marker of neonatal sepsis.

2. Materials and methods

2.1. Search strategy

Our meta-analysis was designed according to the preferred reporting items for systematic reviews and meta-analyses guidelines for diagnostic test accuracy (PRISMA-DTA), which are shown in the Supplementary material. We retrieved the PubMed, Cochrane Library, Embase, Web of Science, China National Knowledge Infrastructure (CNKI), China Biological Medicine Disk (CBM), and Wanfang Data databases using the search terms 'suPAR', 'Urokinase Plasminogen Activator Receptor', 'soluble', 'newborn', 'premature infant', 'sepsis', 'neonatal sepsis', and so on. To enhance the recall and precision ratio, we used the combination of medical subject headings (MeSH) and entry terms mainly in our primary search. The date of our last search was set at 31 December 2022.

2.2. Study selection

Two researchers (JM and XC) independently selected the literature and cross-checked and negotiated with a third party (LH) in case of differences. The following criteria were applied to identify studies for inclusion in our meta-analysis: (1) original report published on the accuracy of suPAR in diagnosing neonatal sepsis, including case-control and cohort studies; (2) the researchers were able to extract information from the 2×2 contingency table so that true positive (TP), true negative (TN), false positive (FP), and false negative (FN) values could be directly or indirectly obtained; (3) the subjects were newborns within 28 days after birth; and (4) without language restrictions. The exclusion criteria were as follows: (1) the outcome was inconsistent with the criteria of neonatal sepsis; (2) duplicate publication; (3) conference papers, abstracts, and lectures; and (4) literature unable to extract data of diagnostic four grid table.

2.3. Definitions

Neonatal sepsis definitions do not align with those used for adults and children, as many clinicians still rely on microbiological results rather than organ dysfunction (14). The signs and symptoms of sepsis are non-specific and include temperature instability (usually with fever), irritability, lethargy, tachypnea, grunting, hypoxia, poor feeding, tachycardia, poor perfusion, and hypotension (15). Clinical sepsis is defined as the presence of at least two typical clinical signs along with two laboratory abnormalities. Culture-proven sepsis requires a positive microbial blood culture.

Overall, clinicians need to be aware of the differences in sepsis definitions in neonates and the challenges of diagnosing sepsis based on non-specific symptoms. A more comprehensive approach, including both clinical and laboratory findings, can help accurately identify neonatal sepsis and guide appropriate treatment.

2.4. Data extraction

Based on the preset inclusion and exclusion criteria, the data we extracted mainly included the first author's name, publication year,

country, type of sample, gestation age, birth weight, sepsis onset, reference standard, sample size, TP, FP, FN, and TN.

2.5. Quality assessment

The quality assessment of diagnostic accuracy studies-2 (QUADAS-2) tool was used to assess the quality of the selected studies. This test comprises four key domains that discuss patient selection, index test, reference standard, and flow of patients through the study, as well as the timing of the index tests and reference standard (flow and timing) (16). Two review authors (XW and JL) individually conducted the assessment and cross-checked each other's work. Any disagreements were resolved through discussion or by seeking the opinion of a third author (CR) to reach a consensus.

2.6. Statistical analysis

All analyses were undertaken using Stata 15.0 statistical software. Hierarchical summary receiver operating curves (HSROCs) along with Spearman's correlation coefficient were used for estimating the heterogeneity caused by the threshold effect. Furthermore, the statistical heterogeneity among the research results was analyzed by Cochran Q statistic, and the I^2-test was used to quantitatively judge the size of heterogeneity. When study heterogeneity was statistically significant ($I^2 \geq 50\%$ or $p \leq 0.05$), the random effect model was used; otherwise, a fixed effects model was used (17). The obvious heterogeneity was treated by sensitivity analysis. To evaluate suPAR potential and accuracy in neonatal sepsis diagnosis, sensitivity (Sen), specificity (Spe), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), pretest probability, post-test probability, and area under the curve (AUC) of summary receiver operating characteristic (SROC) were used. Deeks' funnel plot asymmetry test was used to evaluate publication bias (18).

3. Results

3.1. Study characteristics

After a preliminary search, a total of 26 studies were identified, and 17 of them were weeded out by our exclusion criteria. Reading the title, abstract, and full text, the remaining six articles were included in our study (7, 19–23). Figure 1 shows the selection progress. The six articles, including eight studies, involved 212 neonates with sepsis, of which 141 explicitly mentioned their blood culture was positive. They also included 161 infected neonates without sepsis and 231 healthy neonates. The characteristics of the six articles incorporated into our study are displayed in Table 1.

3.2. Quality assessment

The risk of bias and the applicability of the included study were assessed using QUADAS-2. The outcomes are illustrated in Figure 2. Four studies (7, 19, 21, 23) used a prospective study design that avoided inappropriate exclusion. The remaining articles with case–control design may exaggerate diagnostic accuracy. As shown, the high risk of bias was mainly detected in the domain of the index test since the included studies did not use pre-specified thresholds but the optimal ones on the ROC curve in their analysis.

3.3. Heterogeneity analysis and diagnostic accuracy

We first performed heterogeneity analysis by using the HSROC model to estimate, which is shown in Figure 3. Spearman's correlation analysis of sensitivity and (1-specificity) logarithm showed that the correlation coefficient was 0.314 ($p = 0.544$), indicating that there was no threshold effect. However, the sensitivity and specificity of I^2 were above 50%, which means there was heterogeneity between studies so the random effect model was used for statistical analysis. The results are displayed in Figure 4. As it turns out, the overall sensitivity and specificity of the eight studies were 0.89 [95%CI (0.83–0.93)] and 0.94 [95%CI (0.77–0.98)], respectively. The PLR was 14 [95%CI (3.5–55.2)], the NLR was 0.12 [95%CI (0.08–0.18)], and the DOR was 117 [95%CI (24–567)]. The SROC curve analysis of suPAR test accuracy in neonatal sepsis diagnosis revealed an AUC of 0.92 [95%CI (0.90–0.94)] (Figure 5).

3.4. Sensitivity analysis

To evaluate the reliability and robustness of the analysis results, we rejected individual studies in turn and remerged with the remaining research. The result showed that it has little impact on the amount of merger effect regardless of which study has been excluded (Figure 6). In other words, our research results are relatively stable, and the analysis results are highly reliable.

3.5. Clinical utility of the index test

The Fagan graph was plotted to find out valuable clinical utility. Fagan's nomogram indicated that, if the result of a diagnostic test was positive, the probability that the neonates suffered sepsis would increase from the pretest risk of 20 to 78%. If the result was negative, the probability that the newborn was affected with sepsis decreased from a pretest risk of 20 to 3% (Figure 7).

3.6. Publication bias

The Deeks' funnel plot asymmetry test on the six included studies showed that there was no obvious asymmetry ($p = 0.77$), and it indicated that the pooled results were not influenced by the publication bias if the value was 0.05 as a standard test (Figure 8).

4. Discussion

The soluble form of uPAR, known as suPAR, maintains a stable serum level regardless of harvesting time, diet (24), biological circadian rhythm (25), and repeated freezing dissolution. In recent

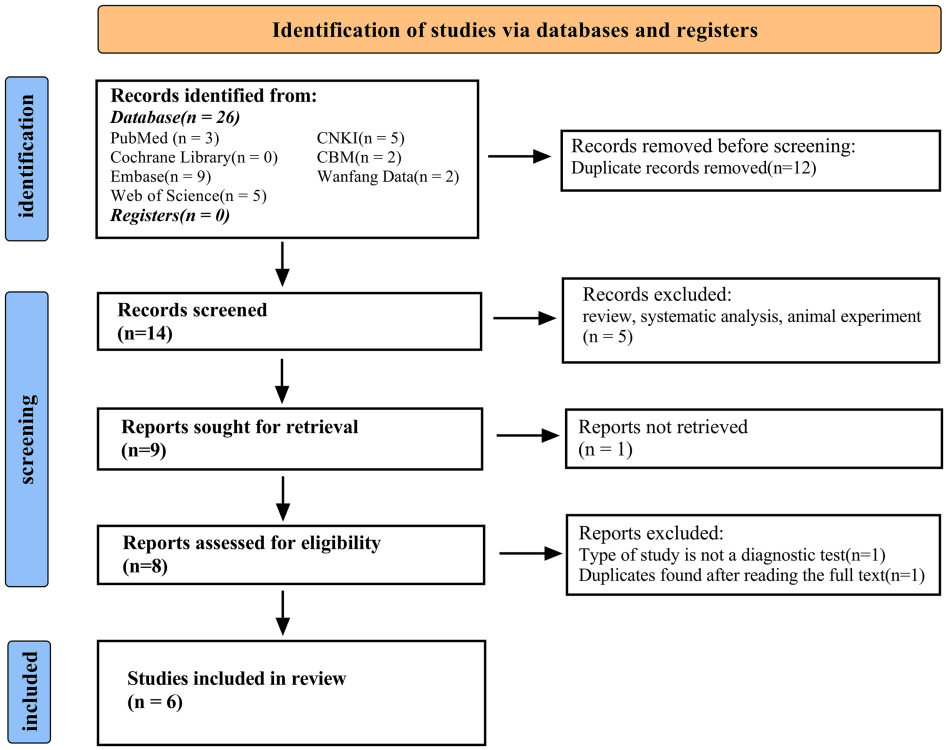

FIGURE 1
PRISMA (preferred reporting items for systematic reviews and meta-analyses) flow diagram and exclusion criteria.

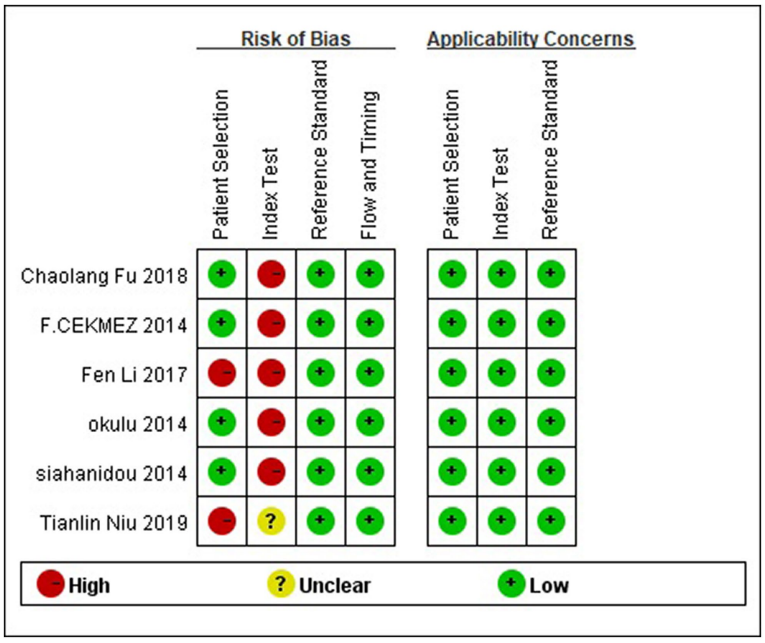

FIGURE 2
Quality assessment of diagnostic accuracy studies-2 (QUADAS-2) tool.

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TABLE 1 Characteristics of the included studies.

Author and year	Country	Type of sample	Gestation age(w)	Birth weight(g)	Sepsis onset	Reference standard	Sample size	TP	FP	FN	TN	Threshold (ng/ml)
Cekmez (19)	Turkey	Neonate	P 36.1 ± 2.7	2,420 ± 368	Mixed	Culture-proven sepsis	60	38	1	2	19	13.63
			C 36.0 ± 2.3	2,520 ± 280								
Okulu (21)	Turkey	Neonate	P 31.8 ± 4.2	1689.6 ± 914.5	Late	At least 3 sepsis-related clinical signs	66	8	0	2	56	11.3
						CRP > 1 mg per 100 ml						
			C 33 ± 2.4	1983.8 ± 535.5		At least two other altered serum parameters in addition to CRP						
						blood culture; positive or negative						
Siahanidou (7)	German	Term	P 39 ± 1.0	3,135 ± 351	Mixed	Culture-proven sepsis	65	8	5	5	47	4.79
			C 38.6 ± 1.0	3,216 ± 406								
Li (22)	China	Preterm	P 31.9 ± 0.5	1904 ± 48	Late	Culture-proven sepsis	85	36	0	4	45	10.9
			C 32.4 ± 0.2	2048 ± 61		Clinical sepsis						
Fu (23)	China	Term	P 37 ~ 42周	-	Early	Culture-proven sepsis	438	28	275	3	132	12.01
			C-			Clinical sepsis		25	69	6	338	suPAR 12.01 sICAM 349.50
Niu (20)	China	Neonate	P 32.5 ± 10.0	1906.3 ± 248.9	Mixed	Culture-proven sepsis	150	68	9	7	66	10.76
			C 31.2 ± 9.8	2107.0 ± 298.4				70	3	5	7	hs-CRP 10 m/l suPAR 10.76

The '-' means that this term is not mentioned in the article.
P, patients in the case groups; C, control group; TP, the patients' number of reference standard positives with a positive index test; FP, the patients' number of reference standard negatives with a positive index test; FN, the patients' number of reference standard positives with a negative index test; TN, the patients' number of reference standard negatives with a negative index test.

years, suPAR has been shown to play important regulatory roles in various immunological functions and has been extensively studied as a modern inflammation marker. Several observational studies have suggested that the increased serum levels of suPAR are associated with a variety of systemic inflammatory disorders, such as infection by the human immunodeficiency virus-1 and diffuse carcinomatosis (26). A meta-analysis by Huang et al. (27) on adult sepsis has proved that suPAR has moderate diagnosis and prognosis value.

Nevertheless, these results most certainly cannot be directly extrapolated to neonatal patients with sepsis due to the widely different conditions, age, developmental stage, and overall state of the organism struck by neonatal sepsis (28). To the best of our knowledge, this present meta-analysis is the first reported investigation of the diagnostic value of suPAR in neonatal sepsis.

Our results indicated that detecting suPAR in neonatal sepsis had high sensitivity and specificity. The pooled data from eight studies showed that the AUC of suPAR in diagnostic value was 0.92. Currently, C-reactive protein (CRP) is the most studied biomarker (29), and in a recent meta-analysis of CRP for neonatal sepsis (30), the pooled sensitivity was 0.74 and specificity was 0.62. A systematic review of 1,959 patients reported that the sensitivity and specificity of PCT were 81% [95%CI (74–87%)] and 79% [95% CI (69–87%)], respectively (31). Compared with the previous meta-analysis, our results found that suPAR exhibited higher specificity than CRP and PCT, suggesting that it can better identify non-sepsis neonates and is more distinguishable than other biomarkers in newborns with different inflammatory diseases. The DOR of this research is 117, indicating that suPAR has high diagnostic efficiency by combining results from different studies into summary estimates with increased precision (32). In this study, positive and negative likelihood ratios were also selected as measurement indicators of diagnostic efficiency. With a PLR and NLR of 14 and 0.12, respectively, on the one hand, the

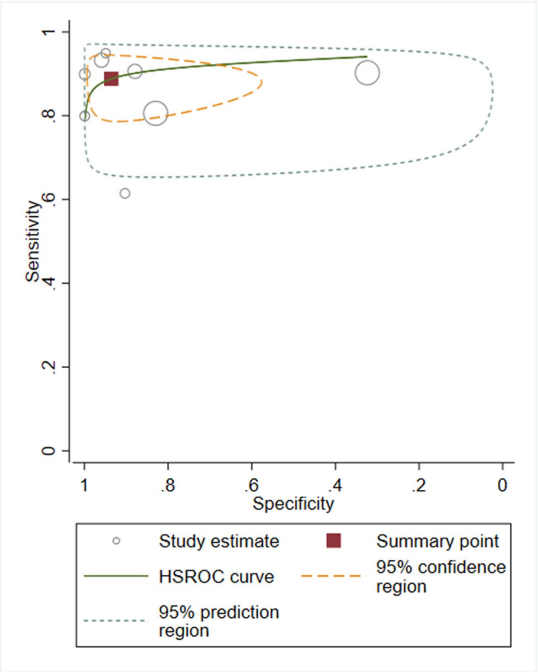

FIGURE 3
Hierarchical summary receiver operator characteristic (SROC) curve.

positive rate of suPAR in neonates with sepsis is 14 times higher than without sepsis, and cases of suPAR showing positive should accept further examination to confirm the diagnosis. On the other hand, if suPAR is negative, the probability of neonatal sepsis is 12%, which means it has a good elimination effect. Considering all these results, it appears that suPAR has outstanding accuracy in diagnosing neonatal sepsis. However, whether suPAR can be used as a final diagnostic index is still inconclusive. Furthermore, a few studies have suggested that high suPAR plasma levels closely correlate with morbidity and mortality in septic patients, demonstrating its value as a prognostic biomarker in systemic inflammation and sepsis (33, 34). One of the studies we included also confirmed that the level of first-day suPAR can help predict the prognosis of neonatal sepsis (22).

As the forest plot illustrates, there is some heterogeneity in the estimates of sensitivity and specificity, which may reduce the robustness of the results to some extent. At present, blood culture is still the 'gold standard' for the diagnosis of neonatal sepsis, but its positive rate is low due to factors such as blood collection, culture conditions, and antibiotic treatment. As a result, most sepsis diagnoses in the included studies were based on clinical diagnosis, with three studies clearly stating that positive blood culture was used as the standard for sepsis inclusion. However, there was not enough data to support the subgroup analysis of blood culture-positive and clinically diagnosed sepsis. Fortunately, the threshold effect, as a potential

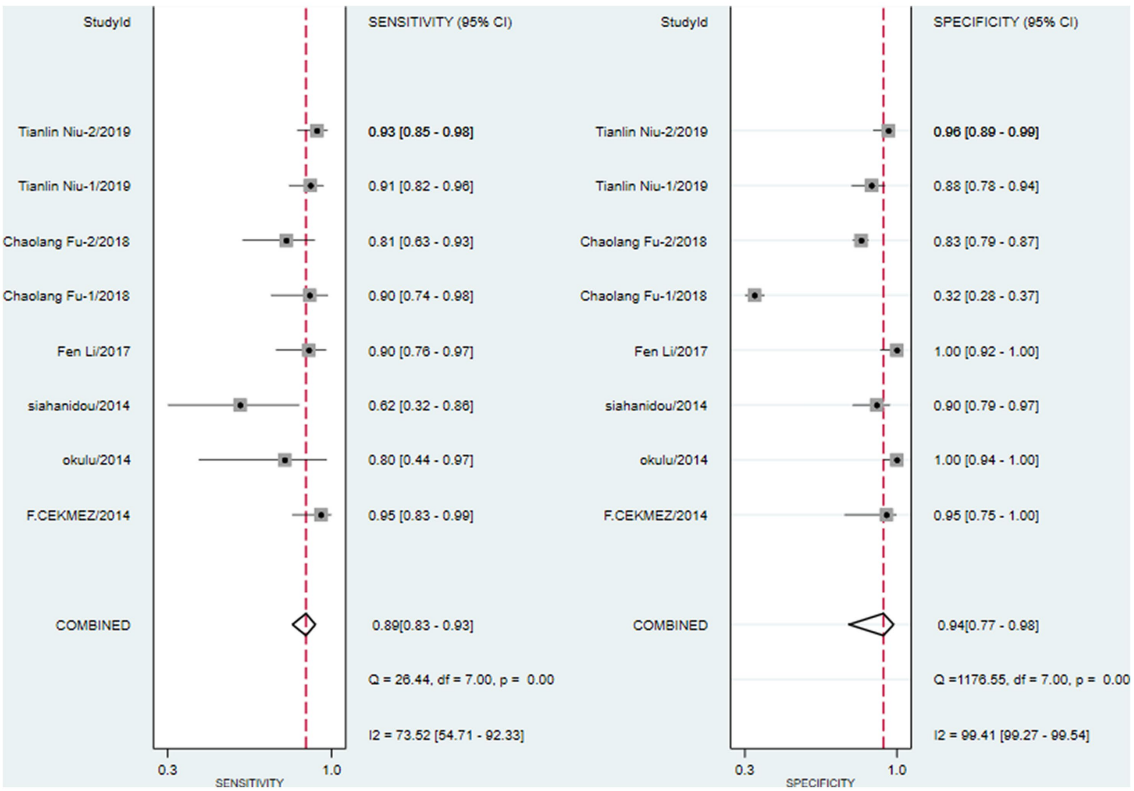

FIGURE 4
Forest plots for pooled sensitivity and specificity of neonatal sepsis diagnosis by soluble Urokinase-type plasminogen activator receptor (suPAR).

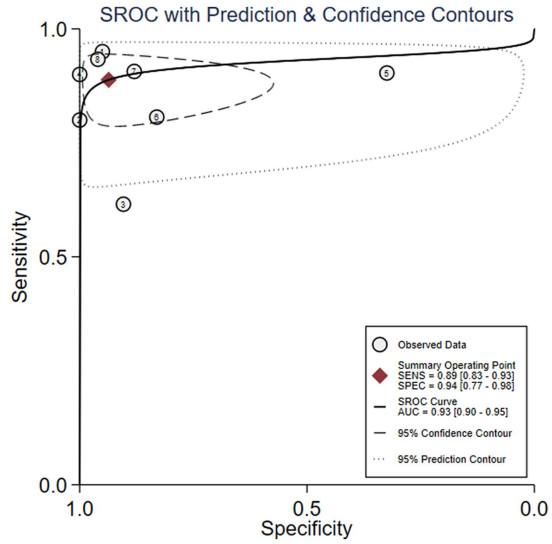

FIGURE 5
Soluble Urokinase-type plasminogen activator receptor (suPAR) symmetrical summary receiver operator characteristic (SROC) curve for all eight studies.

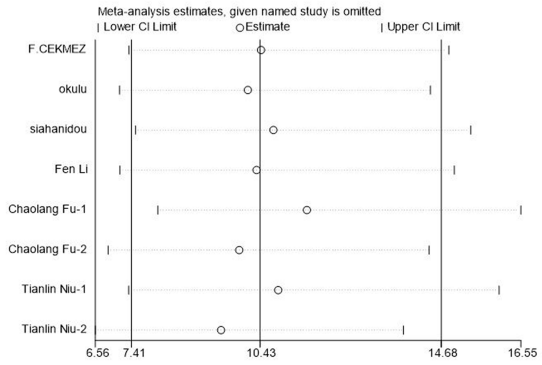

FIGURE 6
Sensitivity analysis of soluble Urokinase-type plasminogen activator receptor (suPAR) for neonatal sepsis diagnosis.

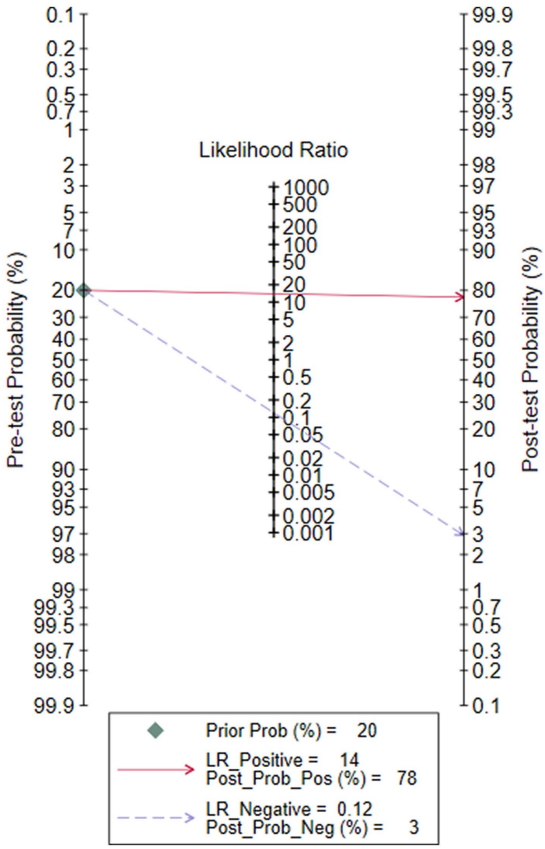

FIGURE 7
Fagan's nomogram for calculating the post-test probabilities of soluble Urokinase-type plasminogen activator receptor (suPAR) for neonatal sepsis diagnosis.

influencing factor, was found not to exist. Beyond that, other factors that may cause heterogeneity include gestational age, birth weight, blood sample processing conditions, and suPAR detection methods. However, the included studies did not provide sufficient data to explore the potential association of these factors. Therefore, to ensure the highest possible accuracy for the diagnostic efficiency of suPAR, prospective studies with rational design, high quality, large sample sizes, and long-term follow-up should be considered as much as possible.

The diagnostic threshold plays a crucial role in disease diagnosis. Despite the increasing number of studies on the diagnosis of neonatal sepsis by suPAR, the normal range of its serum has not yet been determined. Small sample studies show that the adult blood suPAR level is 1.2–4.0 ng/ml, while the newborn blood suPAR level is 3.7–10.8 ng/ml (7, 21, 35, 36). The critical level of plasma suPAR in the six works of literature included in this meta-analysis was 4.79–13.63 ng/ml, which is consistent with previous research both domestically and internationally. However, except for Niu et al.'s study, none of the studies included in this meta-analysis predetermined the diagnostic threshold, which may have a certain impact on the results. Therefore, future research should pay attention to exploring the correlation between suPAR and neonatal sepsis and determining its optimal critical value. Although this study is heterogeneous, it still provides a valuable reference for future research.

There are several limitations to the current meta-analysis. First, the limited number of included studies may have an impact on the result of the meta-analysis. Second, our review did not investigate the diagnostic value of suPAR when used in conjunction with other biomarkers. Third, it was difficult to obtain the raw data for each included study, which restricts us to explore the prognosis value of suPAR in neonatal sepsis. More importantly, owing to the uniqueness of neonatal infection, the relationship between the cutoff level of suPAR and age after birth in full-term and preterm infants needs to be further studied. Finally, sources of heterogeneity in the results should still be considered carefully. Nevertheless, no significant publication bias was found in this study, and the sensitivity analysis results did not change significantly, indicating that the research conclusions are reliable to a certain extent.

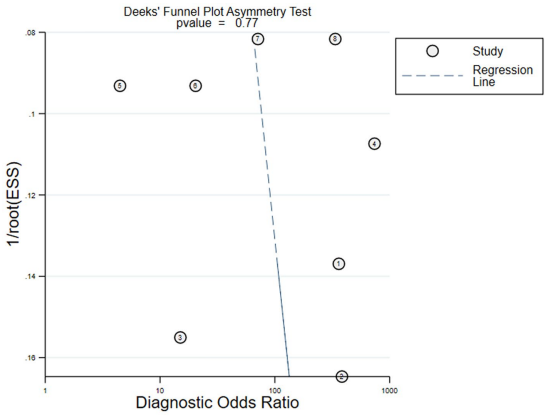

FIGURE 8
Deeks' funnel plot for identifying publication bias.

5. Conclusion

The existing evidence shows that suPAR has a high diagnostic value for neonatal sepsis and has a certain clinical guiding role in reducing neonatal sepsis mortality. In clinical application, symptomatic newborns who test negative for suPAR cannot be ruled out for neonatal sepsis. Clinical practice is needed to determine whether symptomatic newborns who test positive for suPAR have neonatal sepsis. Based on the existing research defects, more prospective studies with reasonable design and long-term follow-up are needed to clarify the diagnostic value of suPAR in neonatal sepsis.

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 authors.

Author contributions

JM and XC planned and designed the direction of this study. They are also the original drafters of this paper. XW, JL, JM, and XC participated in retrieving the literature. LG and YS collected the data. JM, XC, XW, and LH implemented the data processing and analyzing. In the end, JM, XC, LH, and CR completed the final reviewing and editing. 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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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1169114/full#supplementary-material

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Presepsin: gelsolin ratio, as a promising marker of sepsis-related organ dysfunction: a prospective observational study

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Introduction: We aimed to facilitate the diagnosis and prognosis of sepsis-related organ dysfunction through analyzing presepsin (PSEP) and gelsolin (GSN) levels along with a novel marker, the presepsin:gelsolin (PSEP:GSN) ratio.

Methods: Blood samples were collected from septic patients at the intensive care unit (ICU) at three time points (T1-3): T1: within 12h after admission; T2: second day morning; T3: third day morning. Sampling points for non-septic ICU patients were T1 and T3. PSEP was measured by a chemiluminescence-based POCT method while GSN was determined by an automated immune turbidimetric assay. Data were compared with routine lab and clinical parameters. Patients were categorized by the Sepsis-3 definitions. PSEP:GSN ratio was evaluated in major sepsis-related organ dysfunctions including hemodynamic instability, respiratory insufficiency and acute kidney injury (AKI).

Results: In our single center prospective observational study, 126 patients were enrolled (23 control, 38 non-septic and 65 septic patients). In contrast to controls, significantly elevated (p <0.001) admission PSEP:GSN ratios were found in non-septic and septic patients. Regarding 10-day mortality prediction, PSEP:GSN ratios were lower (p <0.05) in survivors than in non-survivors during follow-up, while the prognostic performance of PSEP:GSN ratio was similar to widely used clinical scores (APACHE II, SAPS II, SOFA). PSEP:GSN ratios were also higher (p <0.001) in patients with sepsis-related AKI than septic non-AKI patients during follow-up, especially in sepsis-related AKI patients needing renal replacement therapy. Furthermore, increasing PSEP:GSN ratios were in good agreement (p <0.001) with the dosage and the duration of vasopressor requirement in septic patients. Moreover, PSEP:GSN ratios were markedly greater (p <0.001) in patients with septic shock than in septic patients without shock. Compared to septic patients requiring oxygen supplementation, substantially elevated (p <0.001) PSEP:GSN ratios were observed in septic patients with demand for mechanical ventilation, while higher PSEP:GSN ratios (p <0.001) were also associated with extended periods of mechanical ventilation requirement in septic patients.

Conclusion: PSEP:GSN ratio could be a useful complementary marker besides the routinely used SOFA score regarding the diagnosis and short term mortality prediction of sepsis. Furthermore, the significant increase of this biomarker may also indicate the need for prolonged vasopressor or mechanical ventilation requirement of septic patients. PSEP:GSN ratio could yield valuable information

regarding the extent of inflammation and the simultaneous depletion of the patient's scavenger capacity during sepsis.

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KEYWORDS

sepsis-3, organ dysfunction, prognosis, presepsin, gelsolin, presepsin:gelsolin ratio, novel biomarker

Introduction

Sepsis is still a leading cause of mortality at the intensive care unit (ICU) despite the availability of modern treatment modalities (1, 2). Recent epidemiological studies suggest an increasing incidence along with a slightly decreasing mortality rate (3–5). As stated in the latest sepsis-3 definitions, sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (6).

Any vital organ system could be affected during the development of sepsis, therefore the most important manifestations of organ dysfunctions include hemodynamic instability, respiratory insufficiency, acute kidney injury (AKI), acute liver failure, thrombocytopenia and altered mental state (6, 7). The currently used Acute Physiology and Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II) and Sequential Organ Failure Assessment (SOFA) scores have major advantages regarding the prognosis of critically ill patients. However, these clinical prediction scores may have limitations due to the heterogeneity of sepsis itself (6–9).

Serum procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP) are commonly utilized inflammatory markers during the clinical evaluation of septic patients, yet the role of biomarkers remains unspecified in the sepsis-3 definitions (6, 7). Apart from hs-CRP and PCT, approximately 200 promising sepsis biomarkers have been examined to date, however, no single marker had adequate sensitivity and specificity for the diagnosis and prognosis of sepsis (9–11). On the other hand, a multi-marker approach involving novel sepsis biomarkers (e.g., presepsin, IL-6) could be useful regarding this issue.

Presepsin (PSEP) is a 13-kDa soluble fragment of the 55-kDa cluster of differentiation marker protein 14 (CD14), which is the receptor for lipopolysaccharide (LPS) and LPS-binding protein complexes (12, 13). PSEP measurement was found to be valuable in the early diagnosis of sepsis and the evaluation of sepsis severity compared with other inflammatory conditions (e.g., trauma, burning, surgeries) (14, 15). According to several multicentric studies, the diagnostic cut-off levels of PSEP varied among 400–600 pg/ml for sepsis, while PSEP was also useful regarding the prognosis of septic patients (16–19). Furthermore, numerous studies showed increased PSEP concentrations in different conditions involving renal dysfunction (e.g., chronic kidney disease, sepsis-related AKI) (20–24).

Gelsolin (GSN; MW = 83 kDa) is an essential component of the so-called extracellular actin scavenger system, due to its protective role by sequestering of liberated actin in the circulation while also modulating the immune response (25–28). As a result, significantly lower serum GSN levels were detected in various inflammatory diseases (29–31). A previous study conducted in our institute also suggested that increased serum actin:GSN ratios correlated with higher mortality rates in patients with severe sepsis (32).

We hypothesized that the simultaneous measurement of PSEP and GSN levels could yield valuable information regarding the diagnosis and prognosis of sepsis and sepsis-related organ dysfunctions. Therefore, we investigated a new potential marker: the presepsin:gelsolin (PSEP:GSN) ratio.

The primary objectives of our study were the followings:

- Comparing PSEP:GSN ratios of control, non-septic and septic patients
- Analyzing the diagnostic performance of PSEP:GSN ratio in non-septic vs. septic patients
- Examining the 10-day mortality prediction of PSEP:GSN ratio in sepsis.

The secondary objectives of our study were as follows:

- Investigating PSEP:GSN ratio in sepsis-related hemodynamic instability based on the dosage and the duration of vasopressor requirement
- Analyzing PSEP:GSN ratio in sepsis-related respiratory insufficiency based on the requirement for oxygen supplementation vs. mechanical ventilation
- Evaluating PSEP:GSN ratio in the diagnosis of sepsis-related AKI.

Abbreviations: ICU, intensive care unit; APACHE II, Acute Physiology and Chronic Health Evaluation II score; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment score; SSC, Surviving Sepsis Campaign; MODS, multiple organ dysfunction syndrome; PiCCO, Pulse Index Continuous Cardiac Output; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; RRT, renal replacement therapy; KDIGO, Kidney Disease Improving Global Outcomes; hs-CRP, high-sensitivity C-reactive protein; PCT, procalcitonin; PSEP, presepsin; GSN, gelsolin; PSEP:GSN, presepsin:gelsolin ratio.

Materials and methods

Study design

A previous study was performed in our institute investigating urinary actin in control, septic and sepsis-related AKI patients (33). Besides healthy control individuals, non-septic patients needing

ICU hospitalization after major surgical interventions (e.g., esophageal or pancreatic cancer surgery, cardiac surgery) and acutely diagnosed septic patients were enrolled consecutively in our single center prospective observational study conducted between January 2018 and February 2020 at the Department of Anesthesiology and Intensive Therapy (Medical School, University of Pécs, Hungary). Detailed information was given to all patients or their next-of-kin regarding our study protocol while written consent was obtained from all. Exclusion criteria were patients under 18 years of age, unobtainable or withdrawn consent, end-stage renal disease requiring chronic dialysis or kidney transplantation and patients with malignancies in palliative care. The study protocol was registered retrospectively at ClinicalTrials.gov (NCT05060679) and was approved by the Regional Research Ethics Committee of the University of Pécs (no. 4327.316-2,900/KK15/2011) conforming to the 7th revision of the Helsinki Declarations (2013).

Control individuals were recruited as outpatients from the Department of Ophthalmology (Medical School, University of Pécs, Hungary). Exclusion criteria were lack of consent, infectious disease, kidney disease or acute inflammation (hs-CRP >5 mg/L).

Definitions

- Sepsis: The diagnosis of sepsis was determined after admission based on the sepsis-3 definitions (6). Inclusion criteria for sepsis were the followings: a suspected or microbiologically confirmed infection and at least 1 vital organ dysfunction shown in increased SOFA score (>2). Non-septic patients also could have had elevated admission SOFA scores, yet these patients' clinical status was not associated with the presence of an infection. Therapeutic approaches of sepsis were based on the international guidelines of the actual Surviving Sepsis Campaign (SSC) (7, 34).
- Sepsis-related hemodynamic instability: Following the classification of the SOFA score for the cardiovascular system, patients were categorized based on their worst daily values (monitored hourly) of vasopressor (mostly norepinephrine) requirement into low (≤0.1 μg/kg/min) and high (>0.1 μg/kg/min) dose groups, while patients were also divided based on shorter (≤5 consecutive days) and longer (>5 consecutive days) vasopressor requirement during ICU stay. Patients with septic shock were identified as stated in the sepsis-3 definitions (6).
- Sepsis-related respiratory insufficiency: Patients were categorized based on their requirement for oxygen supplementation (e.g., face mask ($FiO_2 \geq 50\%$), high-flow nasal oxygen therapy) and mechanical ventilation (invasive ventilation after endotracheal intubation), while the latter group was also divided based on shorter (≤7 consecutive days) and longer (>7 consecutive days) requirement for mechanical ventilation during ICU stay (6, 7). Patients needing mechanical ventilation were further divided based on the development of (at least) moderate acute respiratory distress syndrome (ARDS) according to the Berlin definition (35).
- Sepsis-related AKI: Patients with elevated serum creatinine levels and/or decreased urine output within 24 h after admission were considered to have AKI based on the Kidney Disease Improving Global Outcomes (KDIGO) classification (36). Therapeutic interventions of sepsis-related AKI followed the aforementioned SSC and KDIGO guidelines (7, 34, 36).
- Multiple organ dysfunction syndrome (MODS): Septic patients were regarded to have MODS if they developed at least 2 or more vital organ dysfunctions (e.g., hemodynamic instability, respiratory insufficiency, altered mental state, AKI, acute liver failure, thrombocytopenia) during follow-up based on significantly elevated SOFA scores (at least ≥2 points for every organ dysfunction).

Patients with sepsis-related acute liver failure and thrombocytopenia were not investigated (although initially planned), as these complications occurred only in <20% of the septic study population.

As the majority of mechanically ventilated septic patients received propofol or dexmedetomidine sedation during the early stages of respiratory failure, we had limitations regarding the accurate assessment of the patients' level of consciousness using the Glasgow Coma Scale.

First-day values of SAPS II, APACHE II and SOFA scores were calculated for assessing disease severity. Patients were categorized as survivors and non-survivors using 10-day mortality data.

Sampling

Similarly to our previous study, blood samples were collected from septic patients at the ICU at three time points (T1-3): T1: within 12 h after admission; T2: second day morning of follow-up; T3: third day morning of follow-up (33). Sampling points for non-septic patients were the first (T1) and third (T3) postoperative morning. Arterial blood was obtained from every non-septic and septic patient from arterial catheter using plastic blood collection tubes with accelerator gel (5 ml) for serum samples, glucose/lactate and EDTA-anticoagulated tubes (4 ml) for plasma samples (BD Vacutainer, Franklin Lakes, NJ, United States). Not more than one sample (venous blood) was collected from controls. Anticoagulated blood samples were centrifuged immediately (10 min, 1,500 g) while for native blood, tubes were centrifuged after coagulation (10 min, 1,500 g), then plasma and serum aliquots were stored without preservatives at −70°C until analysis.

Laboratory analysis

Serum parameters including total protein (se-TP), albumin, bilirubin, kidney function markers (se-urea, se-creatinine) along with plasma lactate, platelet count (PLT), and inflammatory parameters (white blood cell count (WBC), hs-CRP, PCT) were measured using automated routine procedures at our accredited laboratory (Department of Laboratory Medicine, Medical School, University of Pécs, Hungary; NAH-9-0008/2021). Serum gelsolin (GSN) was measured by an automated immune turbidimetric assay [Cobas 8,000/c502 module (Roche Diagnostics GmbH, Mannheim, Germany)] developed and validated in our laboratory (37, 38).

Determination of plasma presepsin levels and presepsin:gelsolin ratio

PSEP concentrations were measured using an automated Point of Care instrument (PATHFAST; LSI Medience Corporation,

Tokyo, Japan) based on a chemiluminescent enzyme immunoassay technique with a detection range of 20–20,000 pg/ml (39). Tests were performed according to the manufacturer's instructions and the performance of the method was checked by bi-level controls. PSEP:GSN ratio was calculated as the ratio of PSEP to GSN concentrations.

Statistical analysis

The IBM SPSS Statistics for Windows, Version 22 (IBM Corp., NY, United States) software was used for statistical analysis. Since our data did not show normal distribution by the Kolmogorov–Smirnov and Shapiro–Wilk tests, we performed non-parametric tests. The control, non-septic and septic patient groups were compared using Chi-square or Fischer's exact test for qualitative data and Mann–Whitney U or Kruskal–Wallis tests for quantitative data. Friedman's ANOVA with *post hoc* Dunn tests along with Wilcoxon tests were carried out to compare the quantitative data of different time points in every patient group. Diagnostic and prognostic performance of laboratory and clinical parameters were evaluated by receiver operating characteristic (ROC) curves. Spearman's rank correlation test was applied for investigating relationships between quantitative variables. Quantitative data were presented as medians and interquartile ranges (IQR) while qualitative data as frequencies and percentages (%). Values of $p < 0.05$ were considered as statistically significant. The significance level was adjusted according to the Bonferroni correction during the analysis of multiple comparisons. The MedCalc Statistical Software, Version 20 (MedCalc Software Ltd., Ostend, Belgium) was used for performing the DeLong tests when comparing the individual ROC curves with each other.

Results

Patients' demographic and laboratory data

In the present study, a total of 126 patients (23 control, 38 non-septic, 65 septic) were enrolled consecutively. In addition, 37 more patients (11 control, 7 non-septic, 19 septic) were excluded during the recruitment period of our study. Admission demographic, laboratory and clinical data are shown in Table 1. A moderate difference ($p < 0.017$) was found between the patient groups regarding age and some of the listed of comorbidities. A significant difference ($p < 0.001$) was observed between the control, non-septic and septic patient groups in se-TP, se-albumin, hs-CRP, PSEP and GSN levels along with PSEP:GSN ratios. Admission values of se-urea, se-creatinine, WBC and PLT were also different ($p < 0.017$) as well in the non-septic and septic groups compared with those of the controls. APACHE II, SAPS II and SOFA scores along with PCT levels were higher ($p < 0.001$) in septic patients than in non-septic patients. Major therapeutic requirements of 38 non-septic patients were the followings: all patients received adequate fluid resuscitation, yet 23 (60.5%) had temporary low dose vasopressor requirement; 36 (94.7%) needed oxygen supplementation, 2 (5.3%) received temporary mechanical ventilation, 5 (13.2%) had temporary AKI-1 stage kidney injury and nobody required renal replacement therapy (RRT) during follow-up.

Septic patients' clinical data

Major therapeutic requirements of 65 septic patients were the followings: 54 (83.1%) needed vasopressor support, 48 (73.8%) were treated with mechanical ventilation [median (IQR) Horowitz quotient: 157 (117–207) mmHg], 17 (26.2%) received oxygen supplementation [median (IQR) Horowitz quotient: 333 (273–412) mmHg], 53 (81.5%) required hydrocortisone supplementation. Mechanically ventilated patients received propofol or dexmedetomidine sedation during the early stage of severe respiratory failure. Furthermore, only 11 (16.9%) septic patients developed liver failure, while also 11 (16.9%) septic patients had thrombocytopenia.

All of the 45 sepsis-related AKI patients were given adequate fluid resuscitation, however, 16 (35.5%) of them with the most severe condition also received – besides fluid replacement and vasopressor therapy – invasive hemodynamic monitoring (PiCCO). In addition, 7 (58.3%) AKI-1, 7 (53.8%) AKI-2 and 14 (70.0%) AKI-3 stage septic patients were treated with diuretics (mostly furosemide). Furthermore, 15 (75.0%) AKI-3 stage septic patients required some form of RRT: 6 (40.0%) received intermittent hemodialysis (IHD) and 9 (60.0%) were treated with continuous renal replacement therapy (CRRT).

Septic patients were further divided based on the occurrence of MODS. Relevant data of septic patients with MODS ($n = 41$) and without MODS ($n = 24$) are presented in Supplementary Data Sheet 1 and in Supplementary Table 1 as well.

Monitoring presepsin:gelsolin ratio in control, non-septic and septic patients

An elevating trend was found in PSEP:GSN ratios between the control and non-septic patients at T1 (median: 1.7 vs. 9.9 ng/mg, $p < 0.001$), while septic patients showed higher PSEP:GSN ratios than non-septic patients at T1 (median: 9.9 vs. 105.9 ng/mg, $p < 0.001$) and T3 (median: 9.6 vs. 110.8 ng/mg, $p < 0.001$). There was no significant change in the kinetics of PSEP:GSN ratios during follow-up regarding the non-septic (T1, T3 median: 9.9 vs. 9.6 ng/mg, $p = 0.151$) and septic (T1, T2, T3 median: 105.9 vs. 97.2 vs. 110.8 ng/mg, $p = 0.487$) patient groups (Figure 1A). The diagnostic performance of first-day PSEP:GSN ratios in sepsis was assessed using ROC analysis. For distinguishing all non-septic ICU patients from septic patients, area under the curve (AUC) value of first-day PSEP:GSN ratio ($p < 0.001$) was found to be acceptable in contrast to SOFA ($p < 0.001$) and PSEP ($p < 0.001$; Figure 1B; Table 2).

Usefulness of presepsin:gelsolin ratio regarding the 10-day mortality prediction in sepsis

PSEP:GSN ratios were significantly lower in survivors compared with non-survivors at T1 (median: 80.6 vs. 322.7 ng/mg, $p = 0.007$), T2 (median: 88.4 vs. 349.4 ng/mg, $p = 0.01$) and T3 (median: 56.3 vs. 320.6 ng/mg, $p = 0.007$) as well (Figure 2A). Regarding 10-day mortality prediction, AUC values of first-day PSEP:GSN ratio ($p = 0.007$) and PSEP ($p = 0.023$) were comparable to APACHE II

TABLE 1 Patients' admission demographic, laboratory and clinical data.

	Control (n =23)	Non-sepsis (n =38)	Sepsis (n =65)	p value
Age (years)	52 (48–56)	64 (56–72)	68 (57–73)	<0.001[a,b]
Males, n (%)	13 (56.5)	26 (68.4)	43 (66.2)	0.409
Major comorbidities, n (%)				
Cardiovascular disease	10 (43.5)	33 (86.8)	51 (78.5)	0.002[a,b]
Type-2 diabetes mellitus	5 (21.7)	12 (24.9)	19 (29.2)	0.488
Chronic kidney disease	0 (0)	3 (7.9)	8 (12.3)	0.284
Pulmonary disease	2 (8.7)	11 (28.9)	12 (18.5)	0.218
Immunological disease	1 (4.3)	2 (5.2)	2 (3.1)	0.625
Malignancy	0 (0)	10 (26.3)	18 (27.7)	0.009[a,b]
Admission laboratory data				
se-TP (g/L)	76.1 (72.2–77.7)	51.7 (47.3–57.4)	47.6 (40.3–50.3)	<0.001[a,b,c]
se-albumin (g/L)	49.2 (46.9–51.1)	34.3 (29.2–38.5)	23.4 (19.5–27.7)	<0.001[a,b,c]
se-urea (mmol/L)	4.6 (4.0–5.5)	4.4 (3.5–5.8)	15.1 (9.9–24.9)	<0.001[b,c]
se-creatinine (μmol/L)	76 (70–86)	73 (62–99)	159 (99–285)	<0.001[b,c]
se-bilirubin (μmol/L)	11.2 (6.7–15.9)	7.3 (5.4–14.1)	9.7 (5.1–21.9)	0.169
WBC (G/L)	7.2 (6.4–7.9)	14.1 (12.1–16.2)	16.4 (10.6–22.7)	<0.001[a,b]
PLT (G/L)	262 (249–300)	165 (132–205)	197 (139–301)	0.004[a,b]
hs-CRP (mg/L)	1.3 (0.6–2.5)	102.8 (72.6–141.6)	284.2 (172.8–382.1)	<0.001[a,b,c]
PCT (ng/ml)	–	0.7 (0.3–2.1)	10.9 (4.5–48.7)	<0.001[c]
PSEP (pg/ml)	127 (89.5–159)	329 (209.5–442.5)	1,185 (501–3,073)	<0.001[a,b,c]
GSN (mg/L)	78.5 (75.1–89.1)	34.3 (28.7–40.3)	11.2 (6.1–20.8)	<0.001[a,b,c]
PSEP:GSN ratio (ng/mg)	1.7 (1.1–2.1)	9.9 (5.5–14.3)	105.9 (41.1–322.7)	<0.001[a,b,c]
Admission clinical data				
APACHE II score	–	7 (6–8)	20 (15–24)	<0.001[c]
SAPS II score	–	20 (17–26)	46 (36–55)	<0.001[c]
SOFA score	–	5.5 (3–7)	10 (8–12)	<0.001[c]
ICU treatment days	–	2 (1–3)	8 (4–14)	<0.001[c]

Continuous variables are shown as median (25th–75th percentiles) and categorical variables are expressed as a number (percentage). Mann–Whitney U and Chi-square tests were used for data comparison between patient groups. Level of significance was adjusted to $p < 0.017$ (according to the Bonferroni correction). Superscript lowercase letters refer to *post-hoc* analyses: [a]$p < 0.017$ between control and non-sepsis; [b]$p < 0.017$ between control and sepsis; [c]$p < 0.017$ between non-sepsis and sepsis groups. TP: total protein; WBC: white blood cell count; PLT: platelet count; hs-CRP: high-sensitivity C-reactive protein; PCT: procalcitonin; PSEP: presepsin; GSN: gelsolin; PSEP:GSN: presepsin:gelsolin ratio; APACHE II: Acute Physiology and Chronic Health Evaluation II score; SAPS II: Simplified Acute Physiology Score II; SOFA: Sequential Organ Failure Assessment score; ICU: intensive care unit.

(p <0.001), SAPS II (p =0.001) and SOFA (p =0.002) scores (Figure 2B; Table 2).

Presepsin:gelsolin ratio in sepsis based on requirements of vasopressor support

In contrast to septic patients with no vasopressor requirement, proportionately elevated PSEP:GSN ratios were found in septic patients with lower (≤0.1 μg/kg/min) and higher (>0.1 μg/kg/min) doses of norepinephrine requirement at T1 (median: 17.4 vs. 70.9 vs. 307.1 ng/mg, $p < 0.001$), T2 (median: 16.4 vs. 83.9 vs. 336.1 ng/mg, $p = 0.001$) and T3 (median: 19.1 vs. 54.5 vs. 249.1 ng/mg, $p = 0.016$; Figure 3A). Thus, patients with septic shock showed significantly increased PSEP:GSN ratios compared to septic patients without septic shock at T1 (median: 59.2 vs. 317.8 ng/mg, $p < 0.001$), T2 (median: 45.9 vs. 349.3 ng/mg, $p < 0.001$) and T3 (median: 53.2 vs. 254.1 ng/mg, $p < 0.001$; Figure 3B). Furthermore, septic patients with demand for vasopressor support longer than 5 consecutive days had substantially higher PSEP:GSN ratios than septic patients with shorter (≤5 days) vasopressor requirement at T1 (median: 66.7 vs. 247.4 ng/mg, $p < 0.001$), T2 (median: 54.9 vs. 323.1 ng/mg, $p < 0.001$) and T3 (median: 48.9 vs. 243.9 ng/mg, $p < 0.001$) as well (Figure 3C). For distinguishing all patients with septic shock from patients without septic shock, first-day AUC values were the following: PSEP:GSN ratio: 0.824 ($p < 0.001$); SOFA: 0.818 ($p < 0.001$). Derived cut-off values were: PSEP:GSN ratio: 161.2 ng/mg (sensitivity: 70.4%; specificity: 78.9%); SOFA: 10.5 (sensitivity: 70.4%; specificity: 76.3%; Figure 3D). For discerning septic patients with shorter (≤5 days) and longer (>5 days) vasopressor support, first-day AUC values were as follows: PSEP:GSN ratio: 0.821 ($p < 0.001$); SOFA: 0.698 ($p = 0.013$). Derived cut-off values were: PSEP:GSN ratio: 91.7 ng/mg (sensitivity: 93.1%; specificity: 68.0%); SOFA: 9.5 (sensitivity: 89.7%; specificity: 56.0%; Figure 3E).

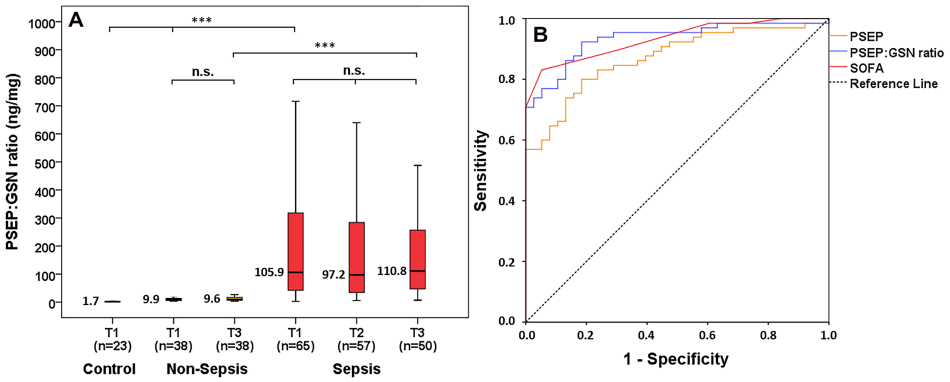

FIGURE 1
PSEP:GSN ratio in control, non-septic and septic patients. PSEP:GSN ratios of control, non-septic and septic patients during follow-up **(A)**. Receiver operating characteristic (ROC) curves of admission laboratory parameters for distinguishing non-sepsis from sepsis **(B)**. Time points: T1: within 12h after admission; T2: second day; T3: third day. PSEP, presepsin; PSEP:GSN, presepsin:gelsolin ratio; PCT, procalcitonin; SOFA, Sequential Organ Failure Assessment score. n: sample number; n.s.: not significant. ***p <0.001.

TABLE 2 Receiver operating characteristic (ROC) curve analysis of ICU patients.

Variable	AUC (95% CI)	Standard error*	Sens. (%)	Spec. (%)	Cut-off value	p value
Non-sepsis (n =38) versus sepsis (n =65)						
PSEP (pg/ml)	0.870 (0.803–0.937)	0.034	80.0	81.6	479.5	<0.001
PSEP:GSN ratio (ng/mg)	0.933 (0.886–0.981)	0.024	92.3	81.6	16.3	<0.001
SOFA score	0.933 (0.887–0.978)	0.023	83.1	94.7	7.5	<0.001
Comparison of ROC curves (DeLong test significance levels)						
PSEP versus PSEP:GSN (p = 0.007); PSEP versus SOFA (p = 0.049); PSEP:GSN versus SOFA (p = 0.988)						
Survivors (n =47) versus non-survivors (n =18) in sepsis (10-day mortality)						
PSEP (pg/ml)	0.683 (0.545–0.821)	0.071	72.2	59.6	1186.0	0.023
PSEP:GSN ratio (ng/mg)	0.719 (0.576–0.862)	0.073	72.2	70.2	161.2	0.007
APACHE II score	0.784 (0.659–0.908)	0.063	77.8	78.7	21.5	<0.001
SAPS II score	0.778 (0.660–0.897)	0.061	72.2	76.6	49.5	0.001
SOFA score	0.745 (0.619–0.827)	0.064	77.8	70.2	10.5	0.002
Comparison of ROC curves (DeLong test significance levels)						
PSEP versus PSEP:GSN (p = 0.351); PSEP versus APACHE II (p = 0.139); PSEP versus SAPS II (p = 0.197); PSEP versus SOFA (p = 0.403); PSEP:GSN versus APACHE II (p = 0.415); PSEP:GSN versus SAPS II (p = 0.483); PSEP:GSN versus SOFA (p = 0.763); APACHE II versus SAPS II (p = 0.915); APACHE II versus SOFA (p = 0.542); SAPS II versus SOFA (p = 0.556)						
Septic non-AKI (n =20) versus sepsis-related AKI (n =45)						
PSEP (pg/ml)	0.897 (0.820–0.974)	0.039	80.0	80.0	705.0	<0.001
PSEP:GSN ratio (ng/mg)	0.782 (0.670–0.894)	0.057	84.4	65.0	53.6	<0.001
se-creatinine (μmol/L)	0.925 (0.858–0.992)	0.034	88.9	90.0	139.5	<0.001
Comparison of ROC curves (DeLong test significance levels)						
PSEP versus PSEP:GSN (p = 0.015); PSEP versus se-creat (p = 0.524); PSEP:GSN versus se-creat (p = 0.018)						

Receiver operating characteristic (ROC) curve analysis of admission laboratory and clinical parameters for distinguishing non-sepsis from sepsis and predicting 10-day mortality in sepsis along with differentiating septic non-AKI from sepsis-related AKI. AUC, area under the curve; 95% CI, 95% confidence interval; Sens., sensitivity; Spec., specificity; ICU, intensive care unit; AKI, acute kidney injury; PSEP, presepsin; PSEP:GSN, presepsin:gelsolin ratio; PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II score; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment score. *DeLong et al., 1988.

Presepsin:gelsolin ratio in sepsis based on requirements of respiratory support

Septic patients with demand for mechanical ventilation showed significantly greater PSEP:GSN ratios than septic patients with oxygen supplementation requirement at T1 (median: 26.9 vs. 173.2 ng/mg, p <0.001), T2 (median: 30.5 vs. 129.5 ng/mg, p = 0.002) and T3 (median: 25.4 vs. 198.5 ng/mg, p = 0.001; Figure 4A). In contrast to septic patients supported with oxygen supplementation, this elevating trend of PSEP:GSN ratio was even more explicit among septic patients

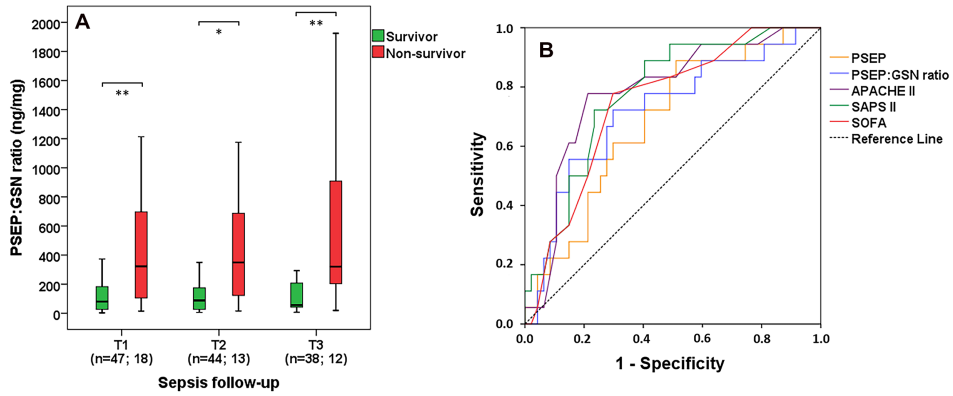

FIGURE 2
Survival data and predictive power of PSEP:GSN ratio. PSEP:GSN ratio in survivor and in non-survivor septic patients based on 10-day mortality during follow-up **(A)**. Receiver operating characteristic (ROC) curves of admission parameters for predicting 10-day mortality in sepsis **(B)**. Time points: T1: within 12h after admission; T2: second day; T3: third day. PSEP, presepsin; PSEP:GSN, presepsin:gelsolin ratio; APACHE II, Acute Physiology and Chronic Health Evaluation II score; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment score. n: sample number. *p <0.05; **p <0.01.

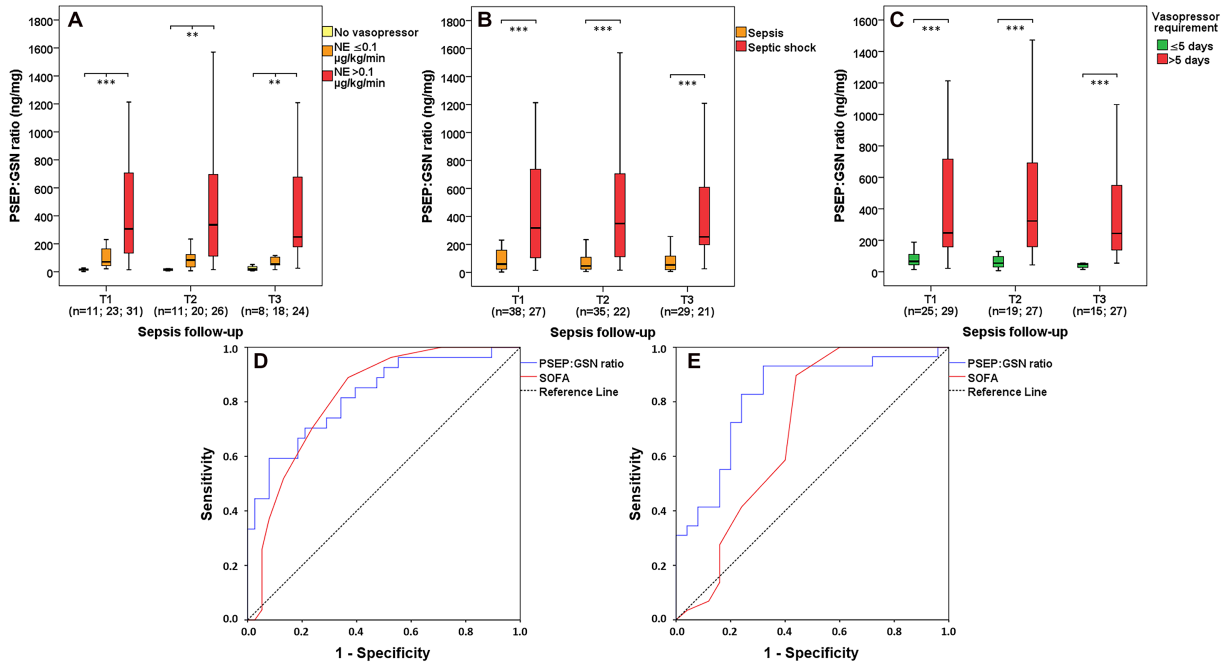

FIGURE 3
PSEP:GSN ratio in septic patients based on vasopressor requirement. PSEP:GSN ratios of septic patients with different doses of vasopressor requirement during follow-up **(A)**. PSEP:GSN ratios of patients with sepsis and septic shock **(B)** during follow-up. PSEP:GSN ratios of septic patients needing shorter (≤5days) and longer (>5days) vasopressor support during follow-up **(C)**. Receiver operating characteristic (ROC) curves of admission parameters for distinguishing sepsis from septic shock **(D)** along with discerning septic patients' shorter (≤5days) and longer (>5days) vasopressor requirement **(E)**. Time points: T1: within 12h after admission; T2: second day; T3: third day. NE, norepinephrine; PSEP, presepsin; PSEP:GSN, presepsin:gelsolin ratio; PCT, procalcitonin; SOFA, Sequential Organ Failure Assessment score. n: sample number. **p <0.01; ***p <0.001.

treated with mechanical ventilation, if they developed moderate or severe stage ARDS during follow-up (T1 median: 26.9 vs. 94.2 vs. 554.8 ng/mg, p = 0.007; T2 median: 30.5 vs. 89.1 vs. 567.3 ng/mg, p < 0.001; T3 median: 25.4 vs. 58.6 vs. 273.6 ng/mg, p = 0.029; Figure 4B). Furthermore, septic patients needing mechanical ventilation longer than 7 consecutive days had significantly higher PSEP:GSN ratios than septic patients with shorter (≤7 days) demand for mechanical ventilation at T1 (median: 80.6 vs. 307.1 ng/mg, p = 0.002), T2 (median: 62.1 vs. 336.1 ng/mg, p < 0.001) and T3 (median: 52.2 vs. 224.3 ng/mg, p = 0.004) as well (Figure 4C). For

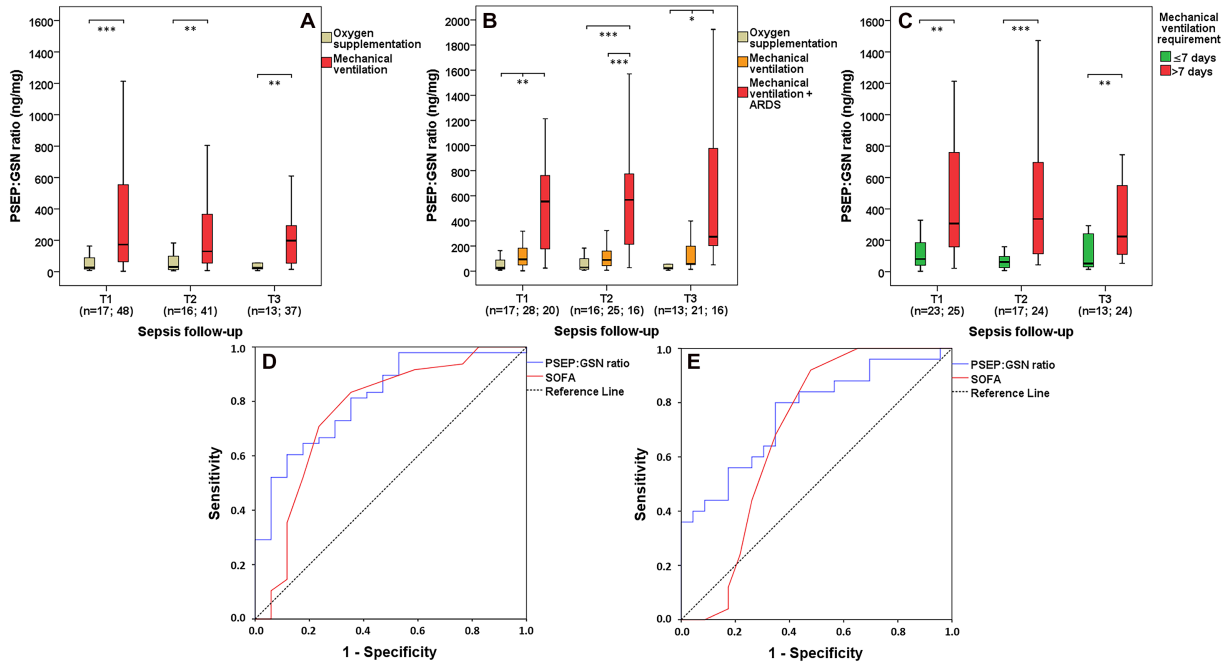

FIGURE 4
PSEP:GSN ratio in septic patients based on requirements of respiratory support. PSEP:GSN ratios of septic patients with requirements of oxygen supplementation and mechanical ventilation **(A)**, with the latter group having ARDS **(B)** during follow-up. PSEP:GSN ratios of septic patients having shorter (≤7days) and longer (>7days) requirement of mechanical ventilation during follow-up **(C)**. Receiver operating characteristic (ROC) curves of admission parameters for distinguishing septic patients needing oxygen supplementation from mechanical ventilation **(D)** along with discerning septic patients' shorter (≤7days) and longer (>7days) requirement of mechanical ventilation **(E)**. Time points: T1: within 12h after admission; T2: second day; T3: third day. ARDS, acute respiratory distress syndrome; PSEP, presepsin; PSEP:GSN, presepsin:gelsolin ratio; PCT, procalcitonin; SOFA, Sequential Organ Failure Assessment score. n: sample number. *p <0.05; **p <0.01; ***p <0.001.

differentiating septic patients with oxygen supplementation from patients with mechanical ventilation requirement, first-day AUC values were the following: PSEP:GSN ratio: 0.814 (p <0.001); SOFA: 0.763 (p =0.001). Derived cut-off values were: PSEP:GSN ratio: 68.8 ng/mg (sensitivity: 72.9%; specificity: 70.6%); SOFA: 9.5 (sensitivity: 70.8%; specificity: 76.5%; Figure 4D). For distinguishing septic patients with shorter (≤7 days) from longer (>7 days) demand for mechanical ventilation, first-day AUC values were as follows: PSEP:GSN ratio: 0.762 (p =0.002); SOFA: 0.692 (p =0.023). Derived cut-off values were: PSEP:GSN ratio: 134.3 ng/mg (sensitivity: 80.0%; specificity: 65.2%); SOFA: 10.5 (sensitivity: 68.0%; specificity: 65.2%; Figure 4E). Additional data regarding the ROC curve analysis of septic patients are presented in Supplementary Table 2.

Monitoring presepsin:gelsolin ratio in septic non-AKI and sepsis-related AKI patients

Sepsis-related AKI patients had substantially higher PSEP:GSN ratios than septic non-AKI patients at T1 (median: 43.6 vs. 176.1 ng/mg, p <0.001), T2 (median: 27.5 vs. 145.1 ng/mg, p <0.001) and T3 (median: 49.5 vs. 185.4 ng/mg, p =0.009) as well (Figure 5A). Furthermore, PSEP:GSN ratios were even more increased between patients in AKI-1 and AKI-3 stage at T1 (median: 85.8 vs. 419.5 ng/mg, p =0.006) and T2 (median: 87.6 vs. 308.8 ng/mg, p =0.011), while a difference was also observed between patients in AKI-2 and AKI-3 stage at T1 (median: 111.1 vs. 419.5 ng/mg, p =0.043; Figure 5B). For discerning all sepsis-related AKI patients from septic non-AKI patients, AUC value of first-day PSEP:GSN ratio (p <0.001) was slightly lower than PSEP (p <0.001) and se-creatinine (p <0.001; Figure 5C; Table 2).

Correlations

Quantitative data from all sample collection time points were used for calculating correlations. PSEP:GSN ratio showed strong correlation (p <0.001) with PSEP (ρ =0.924). Moderate correlations (p <0.001) were found between PSEP:GSN ratio and se-urea (ρ =0.720), se-creatinine (ρ =0.611), hs-CRP (ρ =0.573), PCT (ρ =0.576) and WBC (ρ =0.452), along with APACHE II (ρ =0.759), SAPS II (ρ =0.743) and SOFA (ρ =0.741) clinical scores. PSEP:GSN ratio showed negative correlations (p <0.001) with se-TP (ρ =−0.439), se-albumin (ρ =−0.667), and GSN (ρ =−0.853). In addition, PSEP had a moderate correlation (p <0.001) to se-screatinine (ρ =0.694). No further associations were observed with other inflammatory or clinical parameters.

Discussion

One of the main focuses of our study was to describe the time course of PSEP:GSN ratio among non-septic and septic patients. In

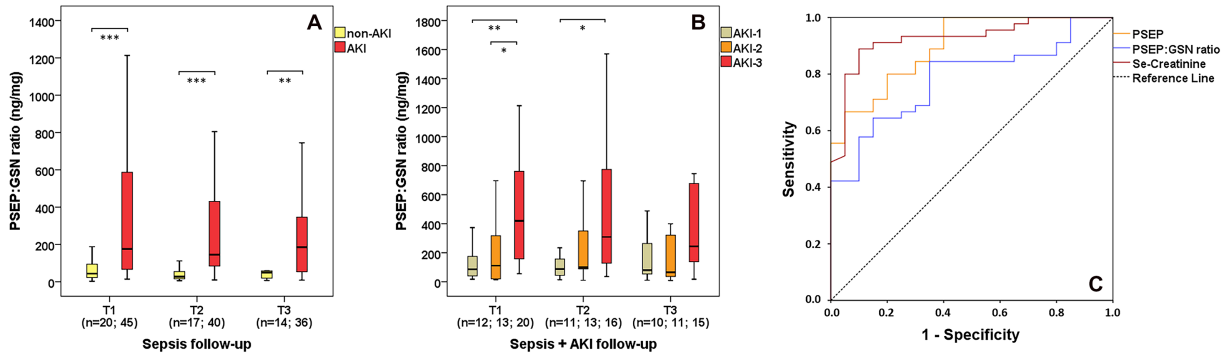

FIGURE 5
PSEP:GSN ratio in sepsis-related AKI. PSEP:GSN ratios of septic non-AKI and sepsis-related AKI patients **(A)** during follow-up. PSEP:GSN ratios of the individual sepsis-related AKI stages **(B)** during follow-up. Receiver operating characteristic (ROC) curves of admission laboratory parameters for distinguishing septic non-AKI from sepsis-related AKI state **(C)**. AKI, acute kidney injury; PSEP, presepsin; PSEP:GSN, presepsin:gelsolin ratio; PCT, procalcitonin. Time points: T1: within 12h after admission; T2: second day; T3: third day. n: sample number. *p <0.05; **p <0.01; ***p <0.001.

contrast to controls, significantly elevated PSEP:GSN ratios were detected in non-septic and septic patients. First-day PSEP:GSN ratios showed good performance compared with SOFA score and PSEP levels regarding the diagnosis of sepsis.

Moderate correlations were observed between PSEP:GSN ratio and the conventional inflammatory markers (hs-CRP, PCT). Regarding 10-day mortality data, PSEP:GSN ratios were substantially lower in survivors than non-survivors during follow-up, while the prognostic performance of PSEP:GSN ratio was similar to the widely used clinical scores (APACHE II, SAPS II, SOFA). These results suggest that PSEP:GSN ratio could also be a useful marker regarding the short-term mortality prediction of sepsis, yet the prognostic performance of PSEP levels was markedly inferior as opposed to the conventional clinical prognostic scores. Our results are slightly inconsistent with evidence from other multi-center studies showing better prognostic performance of PSEP in sepsis (14–16, 19, 40).

Regarding sepsis-related hemodynamic instability, increasing PSEP:GSN ratios were in good agreement with the dosage and the duration of vasopressor requirement in septic patients. Moreover, PSEP:GSN ratios were also higher in patients with septic shock than in septic patients without shock. First-day PSEP:GSN ratios also showed acceptable performance in relation to the SOFA score regarding the diagnosis of septic shock and the length of vasopressor requirement in sepsis. Concerning sepsis-related respiratory insufficiency, significantly elevated PSEP:GSN ratios were observed in patients needing mechanical ventilation compared with patients receiving oxygen supplementation. This increase was even more explicit in mechanically ventilated patients with (at least) moderate ARDS, while higher PSEP:GSN ratios were also associated with prolonged mechanical ventilation requirement in septic patients. First-day PSEP:GSN ratios performed relatively well in contrast to the SOFA score regarding the requirement and duration of mechanical ventilation in sepsis. Our results suggest that PSEP:GSN ratio could be a useful complementary marker besides the routinely used SOFA score, as the elevation of this parameter seems to have a good correlation with the progress of inflammation while also providing information about the patient's actin scavenger capacity. Furthermore, the substantial increment of PSEP:GSN ratio may also indicate the need for prolonged organ support treatment in sepsis (6, 7).

As we previously observed elevated urinary actin levels in sepsis-related AKI, we also found that PSEP:GSN ratios were higher in sepsis-related AKI patients compared with septic non-AKI patients, especially in AKI-3 stage septic patients needing RRT (33). This tendency was the same when investigating PSEP levels among control, non-septic and septic patients. In accordance with previous studies, our results show a similarly increasing tendency of PSEP levels in sepsis and in sepsis-related AKI (14–21).

However, first-day se-creatinine had better performance than PSEP and PSEP:GSN ratio in the diagnosis of sepsis-related AKI. As se-creatinine only reflects the decreased glomerular filtration rate, our results suggest that PSEP:GSN ratio provides a more complex information regarding the patient's condition and the overall organ dysfunction during sepsis. Therefore a growing body of evidence indicates that GSN (or its fragments) could also appear in the urine. Some studies found elevated urinary GSN levels (using western blot) in animal models of cisplatin/gentamicin-induced AKI, while urinary GSN was also investigated in patients with focal segmental glomerulosclerosis and rheumatoid arthritis as well (41–43). As far as we are aware, this is the first study to examine PSEP:GSN ratio in sepsis, therefore we did not have any other study for reference in this field. Additional investigation with extended case numbers may clarify the usefulness of PSEP:GSN ratio in the diagnosis of sepsis-related AKI.

Since GSN has a protective role by being an actin scavenger protein, numerous studies reported decreasing serum GSN concentrations in various clinical conditions (e.g., trauma, acute liver failure, myocardial infarction, sepsis) (27, 29–31). Our previous studies also showed declining serum GSN levels in sepsis and septic shock which were associated with increasing mortality rates (32, 37, 38). As a result, we also found significantly elevated PSEP:GSN ratios in septic patients, especially in severe sepsis-related organ dysfunctions (hemodynamic instability, respiratory insufficiency, AKI).

Our study has some limitations. To the best of our knowledge, PSEP:GSN ratio had not been explored before in sepsis, thus we aimed to be the first to examine this interesting area of clinical research. Therefore, no sample size or statistical power calculations were carried out prior to the study. We had limited capacities for consecutive patient enrollment, since our study was carried out as a single center study (16 bedded central

ICU). Septic patients with severe organ dysfunction were more frequently admitted to our ICU being a regional center for critical care. Non-parametric tests (e.g., Mann–Whitney U test) may reduce the power of comparison, however, they could be applied adequately despite working with unequal sample sizes among control, non-septic and septic patient groups. The majority of patients were admitted at night or in the late afternoon before the actual first-day sample collection resulting in a slightly variable time interval (within 12 h) before taking the first sample. It is difficult to establish the timing of organ dysfunction in septic patients, therefore, the onset of sepsis-related organ dysfunctions was determined within 24 h after ICU admission. We are aware of the concern that outpatients are a difficult control group for ICU patients, yet we aimed to establish a reference range for PSEP:GSN ratios in patients without inflammation.

In the future, we should extend the number of critically ill patients due to the heterogeneity of sepsis while also prolonging the sample collection period to 5–10 days as well. Since there are no commercially available rapid diagnostic kits for GSN measurements, the development of an efficient point of care test would facilitate the prompt determination of PSEP:GSN ratio in routine clinical practice.

In conclusion, the present study demonstrated the diagnostic and prognostic utility of PSEP:GSN levels among non-septic and septic patients while also investigating the latter group based on the occurrence of sepsis-related organ dysfunctions including hemodynamic instability, respiratory insufficiency and AKI. Its diagnostic performance was acceptable in differentiating sepsis vs. septic shock and oxygen supplementation vs. mechanical ventilation requirement compared with the routinely used SOFA score. Furthermore, its prognostic ability was also promising regarding the length of vasopressor and mechanical ventilation requirement in sepsis which could help clinicians in the assessment of the patients' condition. PSEP:GSN ratio could yield valuable information regarding the extent of inflammation and the simultaneous depletion of the patient's scavenger capacity during sepsis. Further investigations with extended sampling periods and larger study populations are warranted to clarify the importance of PSEP:GSN ratio in sepsis.

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 the Regional Research Ethics Committee of the University of Pécs (no. 4327.316-2900/KK15/2011) conforming to the 7th revision of the Helsinki Declarations (2013). The patients/participants provided their written informed consent to participate in this study.

Author contributions

DR took responsibility for sample and data analysis along with drafting the manuscript. PK was responsible for conceptualization and study design. ZH-S and BS participated in sample and data collection while providing assistance during blood sample analysis. AM, GW, TK, and DM were responsible for funding acquisition, approving data analysis and revision of the manuscript. 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1126982/full#supplementary-material

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Circulating sepsis-related metabolite sphinganine could protect against intestinal damage during sepsis

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Introduction: Sepsis is intricately linked to intestinal damage and barrier dysfunction. At present times, there is a growing interest in a metabolite-based therapy for multiple diseases.

Methods: Serum samples from septic patients and healthy individuals were collected and their metabonomics profiling assessed using Ultra-Performance Liquid Chromatography-Time of Flight Mass Spectrometry (UPLC-TOFMS). The eXtreme Gradient Boosting algorithms (XGBOOST) method was used to screen essential metabolites associated with sepsis, and five machine learning models, including Logistic Regression, XGBoost, GaussianNB(GNB), upport vector machines(SVM) and RandomForest were constructed to distinguish sepsis including a training set (75%) and validation set(25%). The area under the receiver-operating characteristic curve (AUROC) and Brier scores were used to compare the prediction performances of different models. Pearson analysis was used to analysis the relationship between the metabolites and the severity of sepsis. Both cellular and animal models were used to HYPERLINK "javascript:;" assess the function of the metabolites.

Results: The occurrence of sepsis involve metabolite dysregulation. The metabolites mannose-6-phosphate and sphinganine as the optimal sepsis-related variables screened by XGBOOST algorithm. The XGBoost model (AUROC=0.956) has the most stable performance to establish diagnostic model among the five machine learning methods. The SHapley Additive exPlanations (SHAP) package was used to interpret the XGBOOST model. Pearson analysis reinforced the expression of Sphinganine, Mannose 6-phosphate were positively associated with the APACHE-II, PCT, WBC, CRP, and IL-6. We also demonstrated that sphinganine strongly diminished the LDH content in LPS-treated Caco-2 cells. In addition, using both in vitro and in vivo examination, we revealed that sphinganine strongly protects against sepsis-induced intestinal barrier injury.

Discussion: These findings highlighted the potential diagnostic value of the ML, and also provided new insight into enhanced therapy and/or preventative measures against sepsis.

KEYWORDS

sepsis, serum metabolomics, machine learning, sphinganine, intestinal barrier function

1 Introduction

Sepsis is a widespread acute disease that causes severe multi-organ dysfunction syndrome (MODS) and circulatory failure (1, 2). The injuried intestinal induced by sepsis, which further aggravates septic development, eventually results in severe infection, and even death (3, 4). Currently, the primary protective measures against sepsis-induced intestinal injury are modulation of the intestinal flora disorder, along with the early initiation of enteral nutrition. However, the associated therapeutic effect is relatively unsatisfactory (5). Given the previous evidences, protecting the intestine from sepsis-induced damage is crucial to the prevention and therapy of sepsis itself.

Recent investigations highlighted a strong role of patient metabolism in modulating cellular function, and this is intricately linked to the development and pathogenesis of multiple diseases (6). Several circulating metabolites have been identified as possible diagnostic and prognostic indicators of different diseases (7, 8). A European research team, for example, reported that multiple serum metabolites, phosphatidylcholines, sphingomyelins, triglycerides, amino acids, and cholesteryl esters, are heavily altered in Hepatocellular carcinoma (HCC), and that several of these metabolites exhibit enhanced diagnostic sensitivity and specificity, compared to alpha-fetoprotein(AFP) (9). Notably, the protective function of several metabolites have been identified. One such example isγ-aminobutyric acid(GABA), which suppresses Reactive Oxygen Species(ROS) generation and monocyte adhesion to protect cells and tissues against cardiovascular disease (10).

The diagnostic indicators of sepsis mainly include body temperature, heart rate, respiratory rate, white blood cell count, serum C-reactive protein (CRP), and procalcitonin (PCT) and other biochemical indicators. However, these indicators often lack specificity in many cases and cannot determine whether a patient has sepsis. To improve the diagnostic accuracy of sepsis, researchers are currently exploring new diagnostic indicators, such as cell surface receptors, cytokines, metabolites, etc. Over the past decade, machine learning (ML) has gained remarkable interest in biomedical research for its potential to provide computer-aided diagnoses of various diseases (11). Machine learning techniques can enhance the predictive power of disease prediction models, notably the blood pressure neural network, which can be used to exploit genomic information for the discovery of molecular markers, as well as to aid in the identification of distinctive methylation sites in stomach cancer (12). Herein, we employed metabolomics to compare between the serum samples of septic patients and healthy individuals. Machine learning was used to screen differential metabolites and construct a diagnostic model to predict the diagnostic value of metabolites in sepsis. Lastly, we also explored the metabolite-mediated protection of intestinal barrier using both *in vitro* and *in vivo* experimentations.

2 Materials and methods

2.1 Clinical sample collection

Human samples were retrieved from healthy individuals (n =13), who were the volunteer population from health check-up center and septic patients (n = 13), who sought treatment at the Shanghai Fifth People's Hospital. This research received ethical approval from the aforementioned institution (Reference No. 2019-118), and informed consent from the legal guardians of study subjects. The following septic paitents were included in analyses: those with (i) sepsis diagnosis, based on the Third International Consensus Definitions for Sepsis and Septic Shock (13); (ii)between the age of 18 and 80; and (iii) hospitalized in our department within 12 h of sepsis onset. Among septic patients excluded from analyses were those infected with the human immunodeficiency virus, and complicated with hematologic malignancies, or those who underwent immunosuppressive therapy within 1 month of the start of this investigation. Pregnant and lactating females were also eliminated from the study analyses. The following healthy individuals were included in our analyses: (a) age and gender matched with septic patients; (b) with no abnormality in biochemical indexes, which was confirmed in the health examination. Among the healthy individuals who were eliminated from this study were: those with (a) prior sepsis or other severe infections; (b) prior hematological malignancies or other solid tumors; and (c) complicated with inflammatory disease.

Serum samples (n=13) were collected within 12h of admission, and healthy individual samples (n=13) were taken following admission. All samples underwent a 10 min centrifugation at 1,500 r/min, prior to storage at −80°C.

2.2 Metabolomics analysis of serum

Diluted serum samples in 1-l aliquots were inserted into a Waters Ultra-Performance Liquid Chromatography-Time of Flight Mass Spectrometry(UPLC-TOFMS) machine (Milford, MA). Chemical components underwent separation at 35°C *via* an Acquity UPLC BEH C18 column (Waters). During a 10-minute run, the adjusted mobile-phase flow rate was 0.5 ml/min, and aqueous acetonitrile gradient contained 0.1% formic acid (0% acetonitrile for 0.5 min, 20% acetonitrile by 5 min, 95% acetonitrile by 9 min, followed by equilibration at 100% water for 1 min prior to subsequent administration). The Waters QTOF Premier mass spectrometer was adjusted to positive electrospray ionization. The capillary and cone voltages were maintained at 3 kV and 20 V, respectively. The source and desolvation temperatures were at 120°C and 350°C, respectively. Nitrogen was employed as the cone (50 l/h) and desolvation gas (600 l/h), whereas, argon was used as the collision gas. The flight mass spectrometry duration was calibrated using sodium formate solution (range m/z 100-1000), and observed in real time using intermittent administration of the lock mass sulfadimethoxine ([M + H]+ = 311.0814 m/z). Mass chromatograms and mass spectrum information were retrieved and assessed in the centroid format with the MassLynx program (Waters).

2.3 ML analysis

We used sequential linear regression models to establish correlations among the variables present in the dataset. Then

extreme gradient boosting (XGBoost)was employed for relevant metastatic agent identification using python 3.7. The data set was randomly split into two data sets: a training (75%) data set, which was used to develop the models, and an internal validation (25%) data set, which was used to validate the constructed models. We utilized the following five representative ML classifier algorithms for model construction in the training data set: Logistic Regression, extreme gradient boost (XGBoost), GaussianNB(GNB), upport vector machines(SVM) and RandomForest. To ensure maximum use of data, we did use a crossvalidation method. The accuracy, precision (also called positive predictive value) and F1-score (F1) were calculated for each ML model to be evaluated and compared in the validation cohort. Through comprehensive evaluation of multiple evaluation indicators, the best performing model among the five ML models after using 5 cross- validations, was defined as the optimal model and selected for further prediction analysis. Finally, we performed calibration curve to evaluate the consistency of the optimal model.To build trust with healthcare professionals and make the decision-making process of machine learning transparent, it is important to understand how the model works. One way we did this was by using the SHAPELY Additive explanations (SHAP)values method to improve the interpretability of the best-performing model. SHAP values help us understand how each feature contributes to the model's output and how they affect the final prediction.

2.4 Mouse models

We acquired 6-8 week-old C57BL/6 mice, weighing between 20-23 g, from the Animal Center of East China Normal University (Shanghai, China), and housed them in plastic boxes with ad libitum standard rodent food and water. The room temperature was adjusted between 20-22°C, with a 12-hour light/dark cycle. All animal protocols received ethical approval from the East China Normal University (Shanghai, China). Following 1-week of adaptive feeding, mice were arbitrarily separated into four groups as follows, mice were randomly divided into 4 groups, 20 mice per group [Control, Sepsis, Sepsis+sphinganine(10mg/kg, 15mg/kg and 20mg/kg, respectively), sphinganine 15mg/kg]. Sphinganine was dissolved in vehicle (10% dmso and 90% saline [1:9]), was administered intraperitoneally at 6 h and 12 h after surgery and puncture. At 24 h post-surgery, mice were euthanized and samples of fresh stool, blood and main organs were isolated.were collected immediately.

2.5 Cell culture and treatment

Human colorectal adenocarcinoma (Caco-2) cells were maintained in Eagle's Minimum Essential Medium with 10% heat-inactivated fetal bovine serum (FBS, Gemini Bioproducts) and 1% non-essential amino acids from the American Type Culture Collection (Invitrogen, Manassas, VA, USA). Caco-2 cells (1×10^6 cells/well) were grown in 6-well plates, prior to treatment with Salmonella enterica serotype Typhimurium lipopolysaccharide (LPS) (Sigma). With increasing dosage experimentation, we established the optimal LPS dosage to be 1 mg/mL for a duration of 48 h. Hence, our cell cultures underwent LPS stimulation, in presence or absence of 10μM mannose-6-phosphate (Selleck, USA), and 5μM, 10μM, and 20μM sphinganine (Selleck, USA), respectively.

2.6 Intestinal histomorphological analysis

To conduct histological analysis, intestinal tissues underwent a 24-hour fixation in 10% neutral-formalin in PBS, followed by paraffin-embedding, then slicing into 4m thick sections, and staining with hematoxylin and eosin (H&E). Finally, IA pathologist, unaware of the specifics of this investigation, employed a light microscope (Olympus CX30, Japan) to assess the intestinal mucosal morphological damage.

2.7 Serum cytokine levels analysis

Blood samples were collected immediately following mice sacrificed, underwent a 10-minute centrifugation at 3,000 rpm at 4°C for serum extraction, and supernatants were maintained at -80°C till further analyses. Murine ELISA kits (88-7064, Thermo Fisher, Austria; EK280/3-01, MuLTI SCIENCE, Shanghai) were employed for D-lactic acid, Interleukin (IL)-1β, and IL-6 detection, following kit protocols.

2.8 Immunofluorescent assessment of tight junction

Following fixation and permeabilization in methanol or acetone at 20°C, intestinal tissues were overnight (ON) exposed to primary antibodies at 4°C, then treated with FITC-labeled secondary antibody for 1 hour at RT. Following nuclear counterstaining, the slices were treated to mounting media with 4,6-diamidino-2-phenylindole (DAPI), prior to visualization and image capture under a fluorescence microscopy. DAPI and FITC images were captured from the same tissue section.

2.9 Quantitative PCR

Murine intestinal samples were collected, and flash-frozen in liquid nitrogen, before storage at -80°C till further analyses. Total RNA isolation was conducted using TRIzol (15596026, Invitrogen, Carlsbad, CA, USA), and quantification *via* the Universal SYBR FAST qPCR Kit Master Mix (2x) (KAPA Biosystems, USA). The qPCR reaction parameters were as follows: 10 minutes at 95 °C, 45 cycles for 10 seconds at 95 °C, and 60 seconds at 59 °C, then 15 seconds at 95 °C, 15 seconds at 72 °C, and 15 seconds at 95 °C. Relative gene expression of Zonula occludens-1 (*ZO-1*), *Occludin*, and *Gapdh* were assessed *via* the $2^{-\Delta\Delta Ct}$ formula. The employed primer sequences are as follows: *ZO-1* forward 5′-GAGCAAGC CTCC-5′-GAGCAAGCCTCC-5′-GAGCAAGCCTCC-5′-GAGCA ATGCACATA-3′, reverse 5′-TCAGTTTCGGGTTTCCTT-3′; *Occludin* forward 5′-CAACGGCAAAGTGAATGGCA-3′, reverse 5′-CTTTCCTTCGTGGGAGTC-3′; *Gapdh* forward 5′-TGTGAA

CGGATTTGGCCGTA-3′, reverse 5′-GATGGTGATG GGTTT CCCGT-3′.

2.10 Western blot analysis

Murine intestines underwent lysis in lysis buffer, and protein quantification was performed *via* a BCA kit (Beyotime, China). Equal protein amounts were then electrophoresed on SDS/PAGE in a Bio-Rad Mini-PROTEAN apparatus, prior to transfer to PVDF membranes (Bio-Rad, Marnes-la-Coquette, France), which then underwent a 1-hour blocking in 5% nonfat milk (w/v) at RT, with subsequent ON exposure to primary antibodies at 4°C. The employed primary antibodies are listed as follows: anti-Occludin antibody (13409-1-AP); anti-ZO-1 antibody (21773-1-AP); and anti-GAPDH antibody (60004-1-Ig). All aforementioned antibodies were used in a 1:1000 dilution, and were purchased from Proteintech, USA. Subsequently, the separated proteins were treated with secondary antibodies: HRP-goat anti-mouse IgG (115-035-003) and HRP-goat anti-rabbit IgG (111-035-144) from Jackson ImmunoResearch. Protein band visualization was done with ECL chemiluminescence imaging system, and quantification *via* ImageJ software (Version 1.50i; National Institutes of Health, Bethesda, MD, USA). Finally, we calculated the IntDen (target protein)/IntDen (GAPDH) ratios.

2.11 Lactate dehydrogenase cytotoxicity assay

Target cell cytotoxicity was assessed based on the cellular LDH release, using an LDH cytotoxicity detection kit, following kit directions (TaKaRa, Japan). The LDH release percentage was computed as follows: % release =100×(experimental LDH release–spontaneous LDH release)/(maximal LDH release–spontaneous LDH release). 1% Triton X-100-treated cells were employed as positive controls for maximal LDH release.

2.14 Statistical analysis

Data are provided as mean maximal LDH 100ed on thees of Health, Bethesda, Msessed with the one-way analysis of variance (ANOVA), and inter-group comparisons were assessed using the t-test. After feature selection and data preprocessing, we developed 5 popular ML-based models to predict sepsis. Overall performance of each model was assessed *via* the accuracy, precision, and F1-measure. The best performing model was applied to the further interpretation. Finally, SHAP summary analysis, SHAP dependence analysis was utilized for model explainability. Statistical analyses were conducted using SPSS statistical software version 24.0 (IBM Corp., Armonk, NY, USA), R statistical software version 3.6.1 (R Project for Statistical Computing, Vienna, Austria), and Python software version 3.6.6 (Python Software Foundation, Wilmington, DE, USA). All statistical tests were two-sided, and *P*-values less than 0.05 were considered to be statistically significant.

3 Results

3.1 Alterations in serum metabolome among septic patients

To detect alterations in serum metabolome during early sepsis, we conducted LC-MS analysis, which identified 507 metabolites among 26 analyzed serum samples. The PCA scatter plots (Figure 1A) demonstrated that the metabolomics analysis was of high quality, with clustered QC samples. Based on our KEGG network enrichment analysis of differentially regulated metabolites between the sepsis and healthy cohorts (Fisher exact test), there were marked alterations in multiple signal transduction networks, like those involving tryptophan, glycine, serine and threonine, and pyrimidine metabolisms (Figure 1B). Using volcano plot filtering, we next revealed marked differentially regulated metabolites between the two cohorts (Figure 1C). A heatmap of the metabolites illustrated that the differentially regulated metabolites were heavily clustered in each cohort (Figure 1D). The pearson correlation analysis method was employed to examine variations in the metabolite data between the two different groups, with the aim of identifying any significant associations or correlations (Figure 1E).

3.2 Model performance

The variables that showed statistically significant differences in the single-factor analysis were subjected to multi-factor analysis using linear regression (Figure 2A). To score the variable sets, the XGBOOST algorithm was utilized. The scoring process involved adding variables sequentially, starting with Sphinganine, L-2-Hydroxyglutaric acid, Mannose 6-phosphate, p-Aminobenzoic acid, 2,4-Dinitrophenol, 3-Hydroxyphenylacetic acid, Ortho-Hydroxyphenylacetic acid, 3-Methyl-L-tyrosine, D-Galacturonate, and Pyrrole-2-carboxylic acid. The order of variables in each set was determined by their importance, which was estimated prior to scoring. The best set of variables identified through this process was Sphinganine and Mannose 6-phosphate (Figures 2B, C). The XGBoost model outperformed the other models with a higher AUROC compared with other 4 models, indicating better performance (Figures 2D, E; Tables 1, 2). Based on the AUROC of the 5 models, we made a forest plot of the AUC score of the multiple models. 5 models were seen after using 5 cross- validations, results showed that the XGBoost model e has the most stable performanc (Figure 2F). Based on the above aspects, we can conclude that the XGBoost model(AUROC=0.956) significantly outperformed 4 other machine learning models. The calibration plots of the five models are shown in Figure 2G. DCA indicated that the XGBoost model could serve as the best diagnostic tool for sepsis in Figure 2H. The SHAP package was utilized to analyze the XGBoost model, which demonstrated the impact of each feature on the sample and identified both positive and negative influences. The resulting bar chart displayed the correlation between the feature value's magnitude and its predicted impact (Figure 2I).

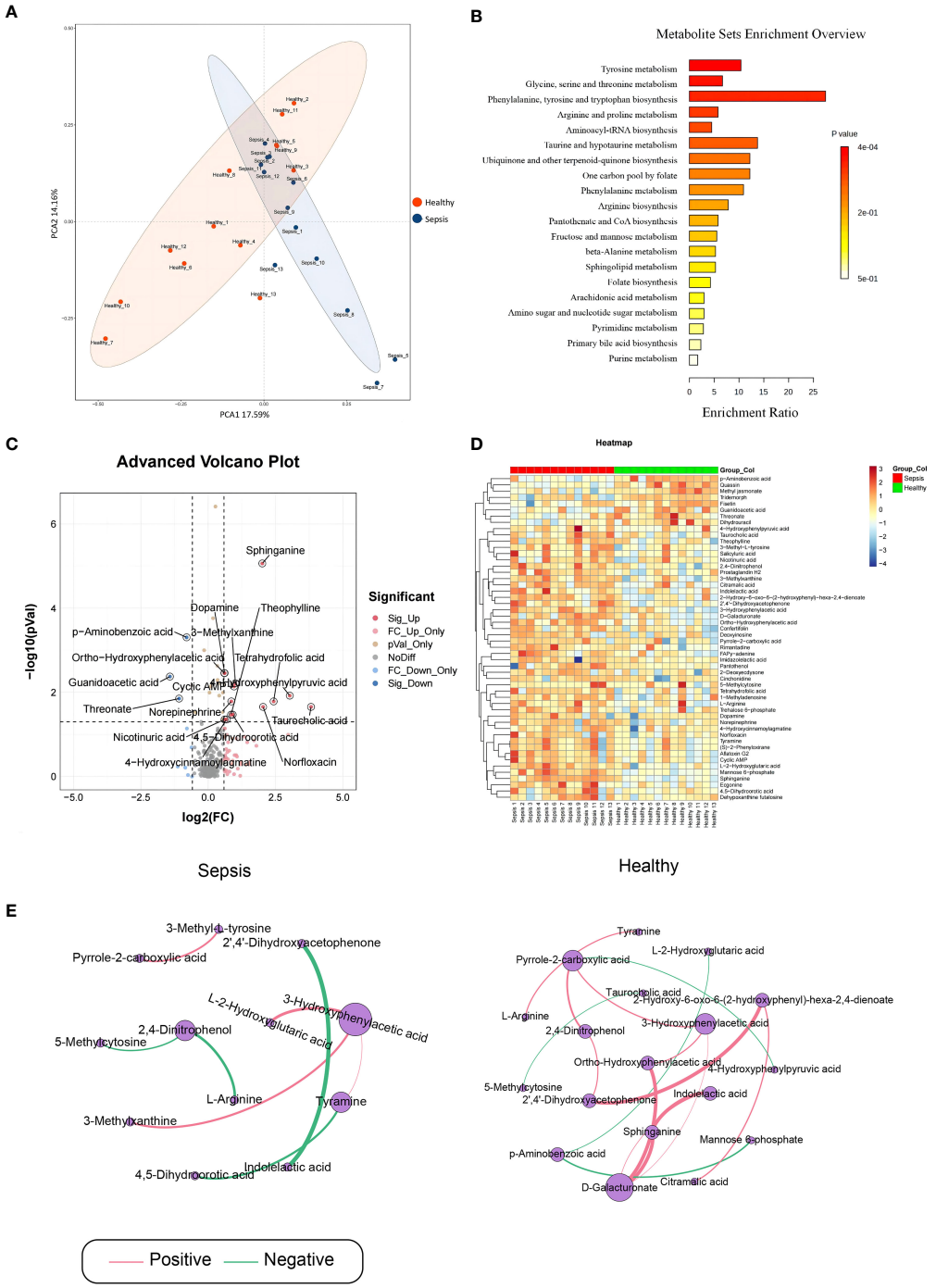

FIGURE 1
Analysis of sepsis-related serum metabolites. Serum samples were obtained from septic patients and healthy individuals. **(A)** Principal Component Analysis **(B)** The metabolite sets enrichment analysis **(C)** Volcano plot for differentially regulated metabolites between control and sepsis groups. **(D)** Heat map of differentially regulated metabolites. **(E)** significant pearson correlation.

3.3 Analysis of the correlation between the expression of metabolites and the severity of sepsis

To investigate the relationship between metabolites (Sphinganine and Mannose 6-phosphate) and the severity of sepsis, we analyzed the relative expression levels of metabolites in the healthy group and sepsis group, as well as the correlation between metabolites and Acute Physiology and Chronic Health Evaluation-II(APACHE-II) score, PCT (μg/L), white blood cell (WBC)×10^9/L, CRP(mg/L), and Interleukin-6(IL-6) (pg/ml). Relative expression of sphinganine in healthy group and sepsis group (Figure 3A). Furthermore, the expression of sphinganine and APACHE-II(R=0.69, P<0.001), PCT (R=0.81, P<0.001), CRP(R=0.65, P<0.001), IL-6(R=0.64, P<0.001),

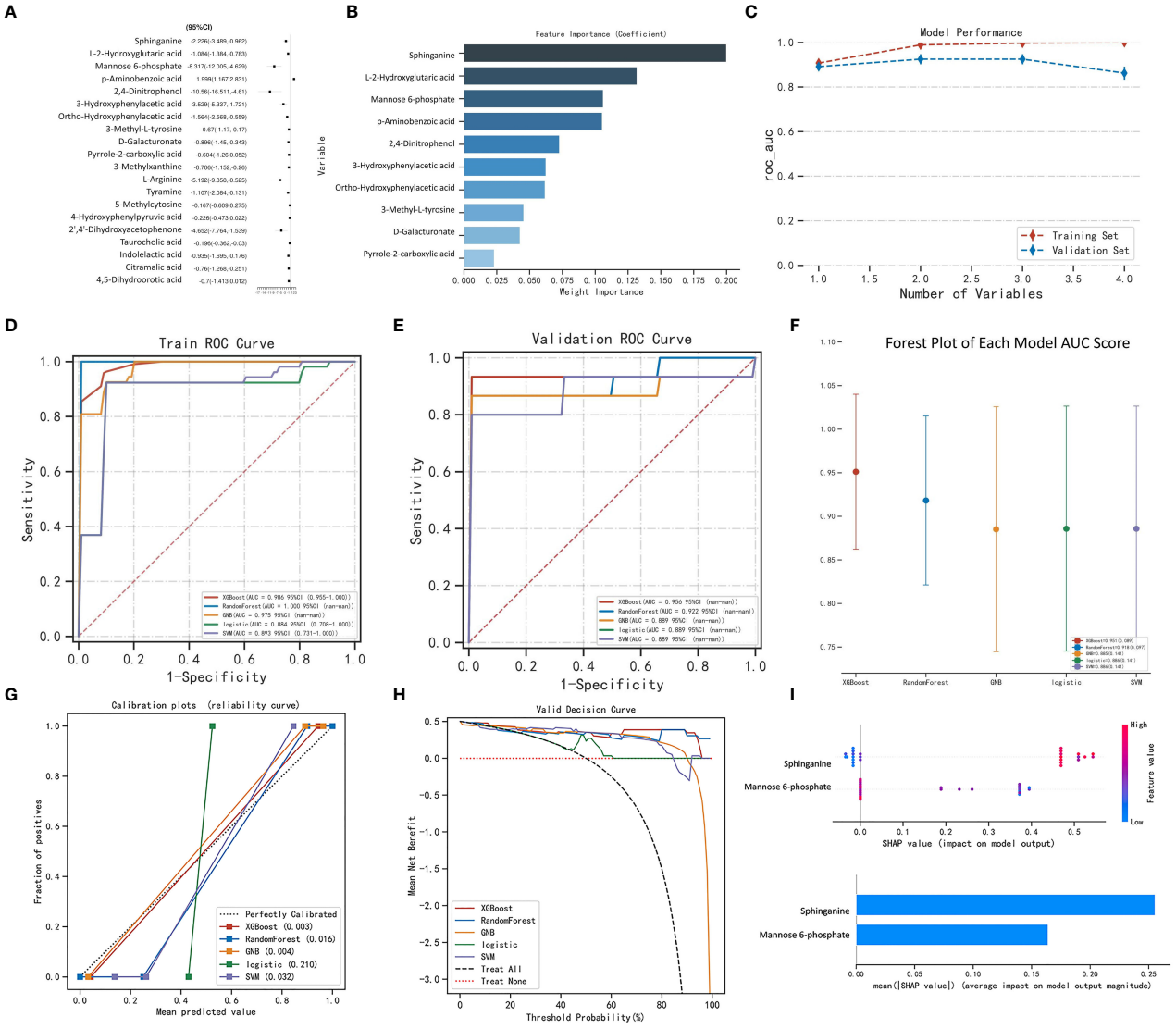

FIGURE 2
Machine learning model performance. Linear regression analysis. **(B)** XGBoost model: feature importance. **(C)** Model performance. Receiver-operating characteristic curves for 5 machine learning models. The XGBoost model achieved a larger (better) AUROC compared with the other models: **(D)** Train ROC curve, **(E)** Validation ROC curve. **(F)** Forest plot of the AUC Score of the 5 models. **(G)** Calibration plots of 5 models. The XGBoost achieved lower (better) Brier scores compared with the other models. **(H)** Decision curve analysis for machine learning models. **(I)** SHAP analysis was performed on the XGBoost model to visually represent the importance of each feature. Each feature is represented by a color that corresponds to the variable's value, with red indicating a larger value and blue indicating a smaller value. This analysis provides insight into the relationship between each feature and its importance in the model. **(A)**.

TABLE 1 Performance metrics for five models in the training dataset.

Model	AUC (SD)	Accuracy (SD)	Sensitivity (SD)	Specificity (SD)	PPV (SD)	NPV (SD)	F1 score (SD)	Kappa (SD)
XGBoost	0.986 (0.006)	0.885 (0.023)	0.907 (0.081)	0.964 (0.045)	0.960 (0.049)	0.838 (0.063)	0.928 (0.023)	0.771 (0.044)
RandomForest	1.000 (0.000)	0.942 (0.019)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	0.897 (0.032)	1.000 (0.000)	0.885 (0.037)
GNB	0.975 (0.019)	0.885 (0.037)	0.964 (0.073)	0.905 (0.086)	0.910 (0.078)	0.875 (0.054)	0.931 (0.039)	0.771 (0.074)
logistic	0.884 (0.037)	0.875 (0.022)	0.924 (0.038)	0.924 (0.038)	0.918 (0.041)	0.842 (0.034)	0.920 (0.023)	0.750 (0.044)
SVM	0.893 (0.037)	0.875 (0.022)	0.924 (0.038)	0.924 (0.038)	0.918 (0.041)	0.842 (0.034)	0.920 (0.023)	0.750 (0.044)

PPV, Positive Predictive Value; NPV, Negative predictive value; XGBoost, eXtreme Gradient Boosting; SVM, support vector machines; SD, Standard Deviation.

TABLE 2 Performance metrics for five models in the validation dataset.

Model	AUC (SD)	Accuracy (SD)	Sensitivity (SD)	Specificity (SD)	PPV (SD)	NPV (SD)	F1 score (SD)	Kappa (SD)
XGBoost	0.956 (0.089)	0.887 (0.157)	0.933 (0.133)	1.000 (0.000)	1.000 (0.000)	0.850 (0.200)	0.960 (0.080)	0.790 (0.283)
RandomForest	0.922 (0.097)	0.887 (0.157)	0.867 (0.163)	1.000 (0.000)	1.000 (0.000)	0.850 (0.200)	0.920 (0.098)	0.790 (0.283)
GNB:Gaussian Naive Bayes;	0.889 (0.141)	0.860 (0.196)	0.867 (0.163)	1.000 (0.000)	0.900 (0.200)	0.833 (0.211)	0.874 (0.170)	0.723 (0.391)
logistic	0.889 (0.141)	0.893 (0.137)	0.933 (0.133)	0.933 (0.133)	0.933 (0.133)	0.867 (0.163)	0.920 (0.098)	0.790 (0.273)
SVM	0.889 (0.141)	0.893 (0.137)	0.933 (0.133)	0.933 (0.133)	0.933 (0.133)	0.867 (0.163)	0.920 (0.098)	0.790 (0.273)

PPV, Positive Predictive Value; NPV, Negative predictive value; XGBoost, eXtreme Gradient Boosting; SVM, support vector machines; SD, Standard Deviation.

WBC(R=0.73, P<0.001), showed a strong positive correlation (Figures 3B–F). Relative expression of Mannose 6-phosphate in healthy group and sepsis group (Figure 3G). Furthermore, the expression of sphinganine and APACHE-II(R=0.80, P<0.001), CRP (R=0.63, P<0.001), IL-6(R=0.93, P<0.001), PCT(R=0.77, P<0.001), WBC(R=0.75, P<0.001), showed a strong positive correlation (Figures 3H–L).

3.4 Serum metabolite sphinganine alleviates LPS-induced intestinal epithelial cell injury *in vitro*

We employed the Caco-2 monolayer cell culture model to validate the mannose-6-phosphate- and sphinganine-mediated protection of the intestinal epithelium *in vitro*. Upon sphinganine treatment, LDH levels were significantly reduced (Figure 4A). Based on the metabolome database (HMDB), sphinganine (HMDB00296) is a phosphatidic acid molecule ($C_{18}H_{39}NO_2$) with a molecular weight of 301.5078Da (Figure 4B). To establish the optimal treatment concentration of sphinganine in Caco-2 cells, we performed the LDH release assay. Based on our results, the optimal dosage was 10μM sphinganine over a 48h period (Figure 4C). We also observed that the Occludin and ZO-1 contents were strongly enhanced in the LPS + sphinganine cohort, compared to the LPS cohort, thereby confirming the sphinganine-mediated protection of the colonic mucosal barrier from LPS-induced damage (Figures 4D, E).

3.5 Serum metabolite sphinganine alleviates sepsis-induced intestinal injury *in vivo*

To further explore the sphinganine-mediated protection of sepsis-induced intestinal injury, we established a sepsis animal model (Figure 5A). Based on our 0-24h observations, control mice exhibited a 100% survival rate (SR), whereas, sepsis mice exhibited a 15% SR at 24 h. Among the sepsis + sphinganine (10 mg/kg) mice, the SR was 22% at 24 h, whereas, in mice receiving increasing amounts of sphinganine (15 and 20 mg/kg) with sepsis, the SRs were 65.42% and 63.48%, respectively, at 24 h. Given these evidences, sphinganine at 15 mg/kg strongly diminished septic mice mortality in a dose-dependent manner (Figure 5B). In sepsis mouse, shortened colon length is a strong biomarker of colon inflammation severity. Relative to the sepsis mice, mice treated with sphinganine exhibited strongly enhanced colon length ($p < 0.05$) (Figure 5C), as well as markedly diminished D-lactic acid, IL-1β, and IL-6 contents ($p < 0.05$) (Figures 5D–F). Herein, we employed the Chiu pathological mucosal injury score to measure the extent of intestinal histological damage. In the control and sphinganine-treated mice, we observed normal epithelial cells without ulcers, a large percentage of goblet cells, a close arrangement of large intestinal glands, and a normal morphology of the colonic mucosa. In contrast, the sepsis mice exhibited ulcers in the colonic mucosa superficial layer, with complete disappearance of the mucosal tissue layer, and strong infiltration by inflammatory cells. Alternately, the sepsis + sphinganine mice showed no inflammatory cell infiltration, ulcers, or epithelial cell damage, and the quantity of goblet cells was vastly diminished. Based on the Chiu pathological scoring system, the sepsis + sphinganine mice had considerably less intestinal mucosa damage, compared to the sepsis mice (Figures 5G). In addition, the ZO-1 and Occludin protein expressions were enhanced among sepsis + sphinganine mice, relative to the sepsis mice. This indicates that sphinganine, indeed, protects the colon mucosa barrier from sepsis-induced damage. We further confirmed our findings using immunofluorescence immunostaining and qPCR (Figures 5H–J).

3 Discussion

Herein, serum samples were collected from healthy individuals and septic patients for metabolome analysis. Based on our results, the relative gene expression of p-Aminobenzoic acid, Methyl jasmonate, Tridemorph, Fisetin, Guanidoacetic acid, Threonate, Dihydrouracil, and 4-Hydroxyphenylpyruvic acid were markedly enhanced among healthy individuals, relative to septic patients, thereby suggesting that the early stage of sepsis likely involves metabolite dysregulation. The XGBOOST algorithm was utilized to screen the variable sets. The results revealed the metabolites mannose-6-phosphate and sphinganine as the optimal sepsis-related variables. A diagnostic model was established by five machine learning methods, namely XGBoost, RandomForest, GNB, logistic, and SVM, finally we found that the XGBoost model has the most stable performance. Pearson analysis reinforced the expression of Sphinganine, Mannose 6-phosphate were positively associated with the APACHE-II, PCT, WBC, CRP,

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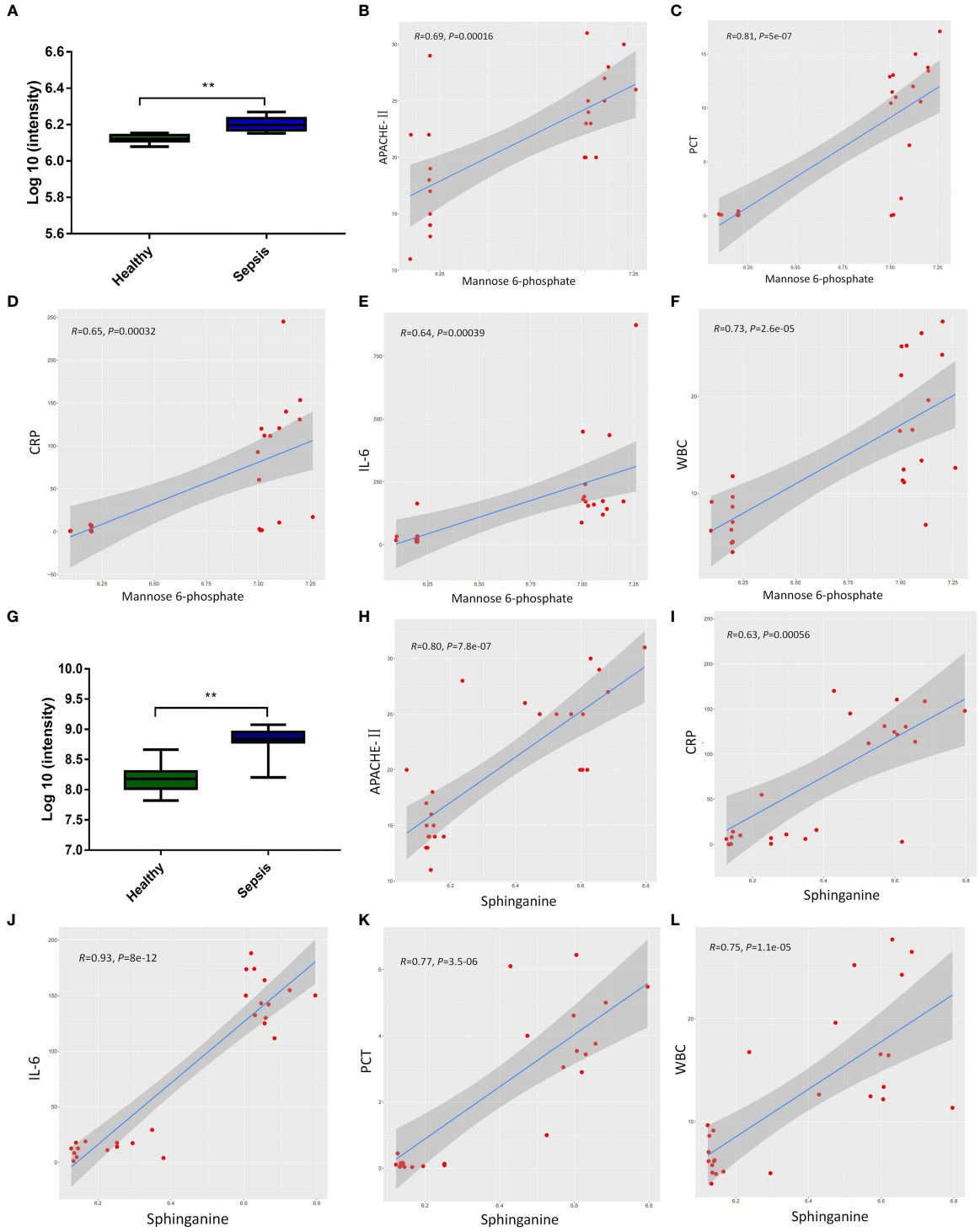

FIGURE 3
The correlation between the expression of metabolites and the severity of sepsis. **(A)** Relative expression of sphinganine in healthy group and sepsis group. **(B-F)** Pearson correlation of the expression of sphinganine and APACHE-II(R=0.69, *P*<0.001), PCT(R=0.81, P<0.001), CRP(R=0.65, P<0.001), IL-6(R=0.64, P<0.001), WBC(R=0.73, P<0.001). **(G)** Relative expression of Mannose 6-phosphate in healthy group and sepsis group. **(H-L)** Pearson correlation of the expression of mannose-6-phosphate and and APACHE-II(*R*=0.80, *P*<0.001), CRP(*R*=0.63, *P*<0.001), IL-6(*R*=0.93, *P*<0.001), PCT (*R*=0.77, *P*<0.001), WBC(*R*=0.75, *P*<0.001). *P* values 0.05 (*) or *P* values 0.01 (**) was regarded as significant.

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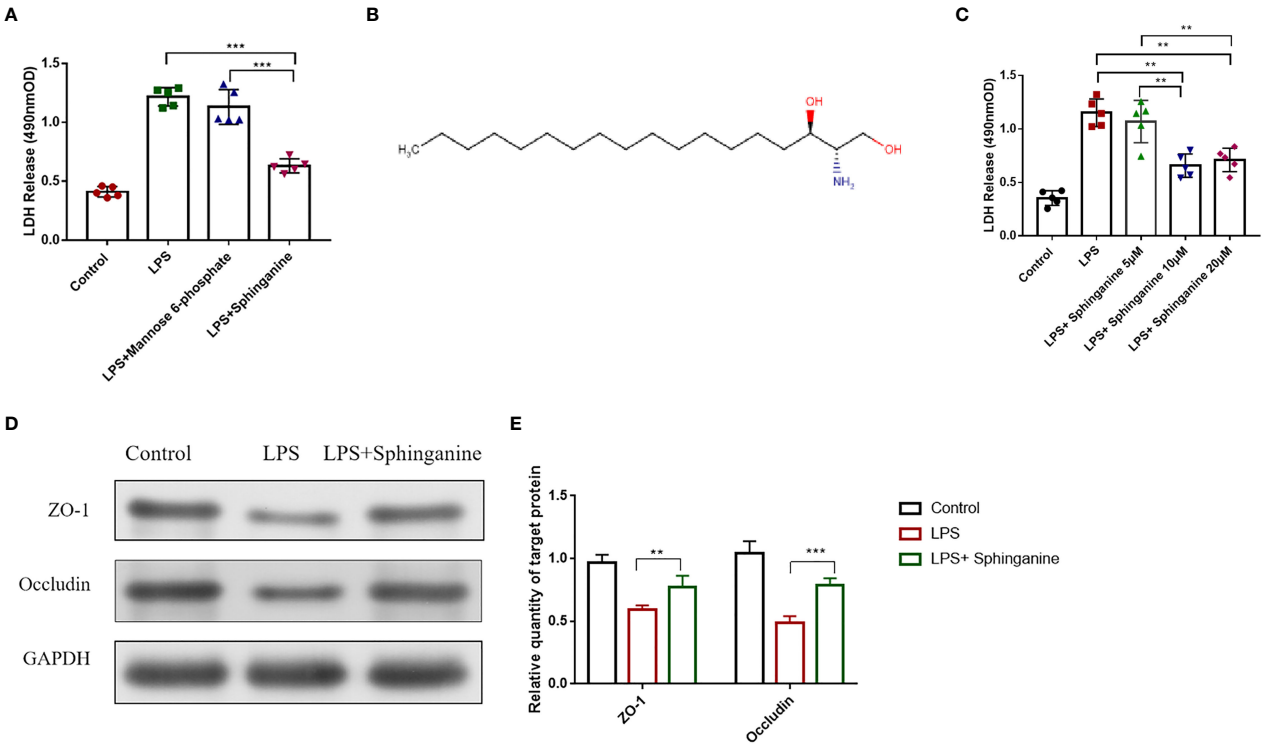

FIGURE 4
Sphinganine alleviates sepsis-induced intestinal injury *in vitro*. **(A)** Effect of a 24 h treatment with mannose-6-phosphate (10μm) and sphinganine (10μm) on Caco-2 cell cytotoxicity, as evidenced by the LDH assay. **(B)** Chemical structure of sphinganine. **(C)** Outcome of 24 h treatments with varying sphinganine concentrations on Caco-2 cell cytotoxicity, as evidenced by the LDH assay. **(D)** ZO-1 and occludin protein expressions, as assessed by Western blot analysis. **(E)** Statistical plot of gray value of ZO-1 and Occludin were detected by Western blot. *P* values 0.05 (*) or 0.01 (**) was regarded as significant.

and IL-6. Moreover, we explored the physiological roles the aforementioned metabolites. We demonstrated that sphinganine strongly diminished the LDH content in LPS-treated Caco-2 cells. In addition, using both *in vitro* and *in vivo* examination, we revealed that sphinganine strongly protects against sepsis-induced intestinal barrier injury.

Sepsis is a systemic inflammatory response that leads to systemic inflammation and multi-organ failure (14, 15). Despite years of research and clinical trials, there is still no reliable therapy targeting the dysregulated and inflammatory response that characterizes sepsis. The current manual assessment of sepsis using screening tools, such as the (Sequential Organ Failure Assessment)SOFA score for ICU patients, can be complicated due to the number of clinical signs measured, and may also lack sufficient sensitivity (16). On contrast, automated decision support systems based on artificial intelligence (AI) and machine learning, which utilize electronic health record (EHR) data, have shown a marked improvement in adherence to treatment protocols in ICUs (17). Herein, we employed the XGBOOST algorithm to screen the variable sets and the XGBoost model has the most stable performance in constructing the machine learning methods of sepsis. Machine learning (ML) models are often considered to be a "black box" in which data goes in and decisions come out, but the processes that occur between input and output are not transparent. In this study, we employed the SHAP value to interpret our XGBoost model, the result revealed that Sphinganine and Mannose 6-phosphate were the primary factors that contributed to the XGBoost model. Previous study has reported the ML models showed a good prognostic prediction ability in septic patients requiring ICU readmission (18). Another study reported that the study aimed to develop a high-performance machine learning sepsis prediction algorithm based on routinely collected intensive care unit data, designed to be implemented in European intensive care units, the result showed that the algorithm uses 4 hours of input and can identify patients with high risk of developing sepsis, with high performance (area under the receiver operating characteristics curve 0.90; area under the precision-recall curve 0.62) for predictions up to 3 hours before sepsis onset (19).

Following sepsis, there is a rise in intestinal permeability, which can lead to the translocation of intestinal bacteria and endotoxins. This process can exacerbate the sepsis and worsen the overall condition of the individual (20, 21). Hence, it is crucial to develop effective measures of sepsis-induced intestinal barrier injury prevention and treatment (22, 23). Currently, the treatment of intestinal injury involves several approaches such as protopathy, anti-infective therapy, immune regulation, and organ support and protection. However, despite these efforts, the effectiveness of the treatment remains limited and the mortality rate remains high (24). Ultimately, finding new strategies to treat intestinal injury will be critical for improving outcomes for patients with sepsis.

With advancements in metabolomics, there is a substantial increase in functional metabolites exploration and discovery (25,

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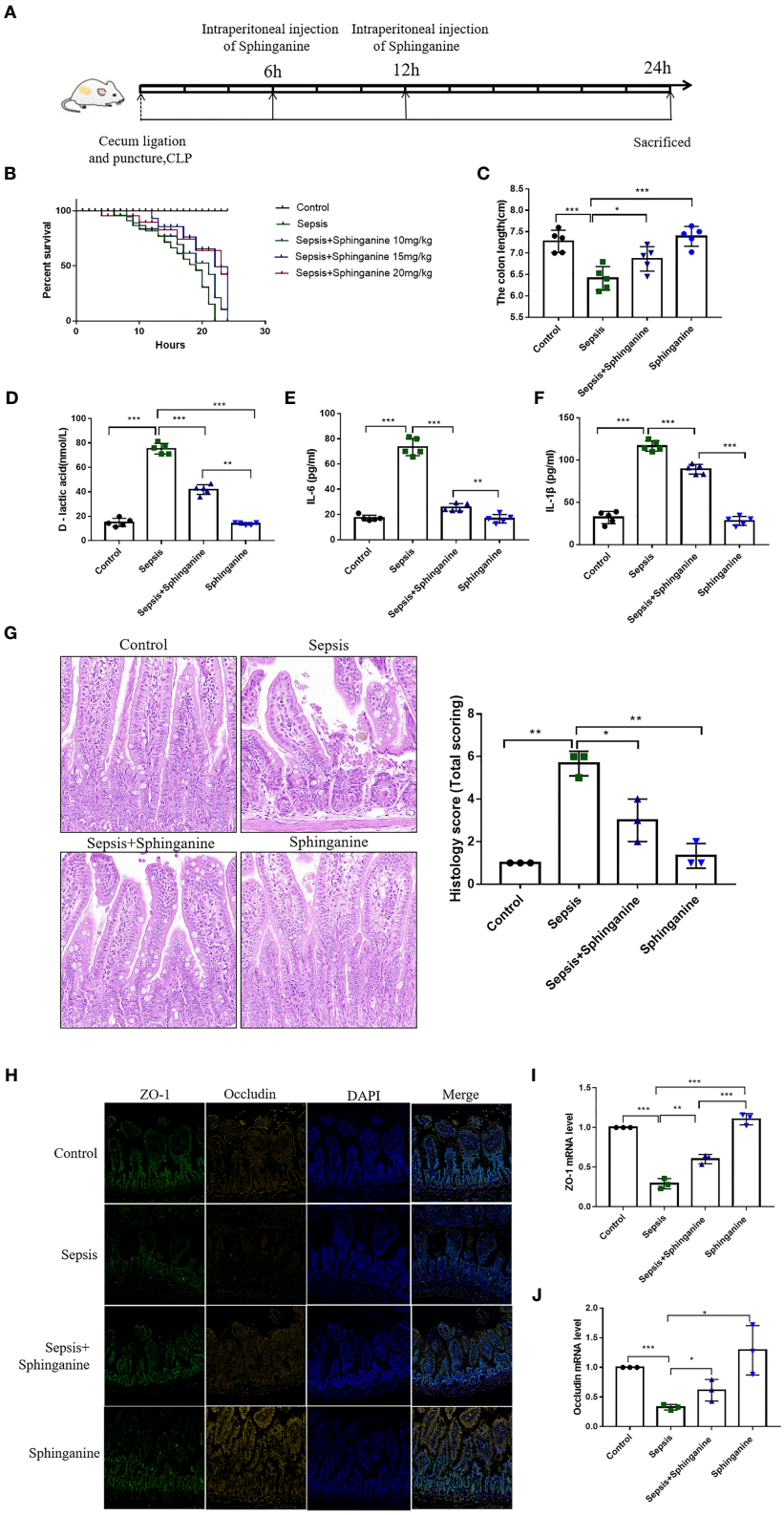

FIGURE 5
Sphinganine alleviates sepsis-induced intestinal injury *in vivo*. **(A)** Design of animal experiment. **(B)** Effect of varying sphinganine concentrations on the SR of sepsis mice. **(C)** Colon length. Serum levels of **(D)** D-lactic acid, **(E)** IL-6, and **(F)** IL-1β. **(G)** Colon tissues stained with HE and histopathological scores analysis from slides. **(H)** Immunofluorescent staining of intestinal TJ proteins, namely, ZO-1 and occludin (scale bar, 50um). **(I, J)** Intestinal ZO-1 and occludin gene expression analysis *via* qPCR. Mann-Whitney U test was employed for comparison. *P* values 0.05 (*), 0.01 (**) or 0.001 (***) was regarded as significant.

26). For instance, bile acids, which are critical for immune regulation, were also shown to regulate the balance between TH17 and Treg cells using receptors like the farnesoid x (FXR) and G-protein coupled bile acid receptors (TGR5) (27, 28). Similarly, 3-indolepropionic acid, a derivative of gut microbiota tryptophan metabolism, also serves as an anti-inflammatory agent which protects the intestinal barrier integrity (29). In this study, we demonstrated that sphinganine protected against sepsis-induced intestinal barrier injury. Prior investigations revealed that sphinganine is a synthetic bioactive sphingolipid that inhibits C. glabrata and C. albicans development (30). This is the first report to demonstrate a protective role of sphinganine in the intestines. However, further research is needed to fully understand the mechanism by which sphinganine protects against intestinal damage.

In conclusion, our analysis of serum metabolites revealed that sepsis causes a strong dysregulation in serum metabolites. Based on our ML findings, serum metabolites not only have a good value in sepsis diagnosis, but also possess a protective value against sepsis-induced intestinal barrier injury. Our findings highlightedthe potential diagnostic value of the ML, and also provided new insight into enhanced therapy and/or preventative measures against sepsis. However, the number of patients in the sample is relatively small, and large sampling sizes are needed to comprehensively assess the diagnostic value of metabolites for sepsis.

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 below: MTBLS7878 (Metabolights).

Ethics statement

The studies involving human participants were reviewed and approved by the Shanghai Fifth People's Hospital. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Animal Center of East China Normal University of Shanghai.

Author contributions

TJ conceived and designed the project. ZW wrote the manuscript. YQ provided data analysis support. FW contributed to clinical sample collection. BZ supervised the study. 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.

Publisher's note

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Predictive value of cell population data with Sysmex XN-series hematology analyzer for culture-proven bacteremia

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Background: Cell population data (CPD) parameters related to neutrophils, such as fluorescent light intensity (NE-SFL) and fluorescent light distribution width index (NE-WY), have emerged as potential biomarkers for sepsis. However, the diagnostic implication in acute bacterial infection remains unclear. This study assessed the diagnostic value of NE-WY and NE-SFL for bacteremia in patients with acute bacterial infections, and those associations with other sepsis biomarkers.

Methods: Patients with acute bacterial infections were enrolled in this prospective observational cohort study. For all patients, a blood sample, with at least two sets of blood cultures, were collected at the onset of infection. Microbiological evaluation included examination of the blood bacterial load using PCR. CPD was assessed using Automated Hematology analyzer Sysmex series XN-2000. Serum levels of procalcitonin (PCT), interleukin-6 (IL-6), presepsin, and CRP were also assessed.

Results: Of 93 patients with acute bacterial infection, 24 developed culture-proven bacteremia and 69 did not. NE-SFL and NE-WY were significantly higher in patients with bacteremia than in those without bacteremia ($p<0.005$, respectively), and were significantly correlated with the bacterial load determined by PCR ($r=0.384$ and $r=0.374$, $p<0.005$, respectively). To assess the diagnostic value for bacteremia, receiver operating characteristic curve analysis was used. NE-SFL and NE-WY showed an area under the curve of 0.685 and 0.708, respectively, while those of PCT, IL-6, presepsin, and CRP were 0.744, 0.778, 0.685, and 0.528, respectively. Correlation analysis showed that the levels of NE-WY and NE-SFL were strongly correlated with PCT and IL-6 levels.

Conclusion: This study demonstrated that NE-WY and NE-SFL could predict bacteremia in a manner that may be different from that of other indicators. These findings suggest there are potential benefits of NE-WY/NE-SFL in predicting severe bacterial infections.

KEYWORDS

cell population data, bacteremia, sepsis, XN-series, procalcitonin, presepsin, interleukin-6

Introduction

Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to infection. It is a major problem in hospitalized patients worldwide, with high mortality (1). To prevent the progression of sepsis into septic shock or multiple organ failure, early and rapid diagnosis and management are crucial (2). Positive blood culture is still considered the gold standard for diagnosis and detection of bacterial sepsis. However, blood culture has several disadvantages, including long turnaround times and low sensitivity (3, 4). Recently, several serum (or plasma) biomarkers have been proposed for the timely diagnosis and prognostications of patients with sepsis, including C-reactive protein (CRP), procalcitonin (PCT), presepsin, and interleukin 6 (IL-6). However, these sepsis biomarkers also have several disadvantages for routine assessment in septic patients, such as insufficient diagnostic performance with lower specificity, inability to detect causative pathogens, and high costs (3, 4).

Cell population data (CPD) obtained using hematology analyzers has recently attracted attention as a new method for early detection of sepsis, which has enabled the expansion of information available from the complete blood count (5). The Sysmex hematology analyzers such as XN-series can detect the activation of neutrophils, lymphocytes, and monocytes in real time, and in an accurate and reproducible manner. It is based on fluorescent-flow cytometry, using blood-cell membrane surfactant reagents, and fluorescence dyes specific for staining nucleic acids and proteins (6, 7).

Among CPD generated by Sysmex XN analyzers, fluorescent light intensity of the neutrophil area (NE-SFL) and/or fluorescent light distribution width index of the neutrophil area (NE-WY) have been reported as potential biomarkers for sepsis or bacteremia. A few studies have investigated the diagnostic utility of NE-SFL and NE-WY for predicting bacteremia (8–11). Park et al. (8) reported that NE-SFL and NE-WY showed high AUC of 0.909 and 0.905, respectively, for the detection of culture-proven sepsis in their study cohort, which consisted of 130 sepsis patients and 280 normal controls. Lemkus et al. (11) reported that NE-SFL and NE-WY showed high AUC of 0.84 and 0.78 for the detection of culture-proven bacteremia in their study cohort, which consisted of 23 patients with bacteremia and 13 healthy controls. These studies demonstrated the high predictive potential of NE-SFL/NE-WY for detection of bacteremia, when compared with the healthy control. However, it remains unclear whether NE-SFL/NE-WY could predict the presence of bacteremia among patients with acute bacterial infections.

In the present study, we evaluated the accuracy and usefulness of CPD, NE-SFL and NE-WY, as biomarkers for culture-proven bacteremia in hospitalized patients, in comparison with the other commercialized sepsis biomarkers in Japan, including CRP, PCT, presepsin, and IL-6. Furthermore, we measured the bacterial load in the blood using polymerase chain reaction (PCR) to determine whether NE-SFL/NE-WY is truly affected by the presence of bacteria in the blood.

The primary objectives of this study were to assess the diagnostic value and clinical utility of NE-SFL/NE-WY for bacteremia in hospitalized patients who developed acute infection. The secondary objective was to assess the association between NE-SFL/NE-WY and commercialized sepsis biomarkers.

Methods

Study design

This prospective observational study was primarily designed to investigate the clinical utility of CPD obtained using Automated Hematology analyzer Sysmex series XN-2000 (Sysmex XN-2000; Sysmex Corporation, Japan) and was approved by the Ethics Committee of Toyama University Hospital (Approval No.29–152) in accordance with the tenets of the Helsinki Declaration. We recruited all consecutive patients who developed acute infections at the Toyama University Hospital between July 1, 2017, and January 31, 2018. Informed consent was obtained from all the patients.

Study participants and protocol

The inclusion criteria were as follows: (1) men or women aged ≥18 years (2) patients who developed acute infection, clinically diagnosed as bacterial in origin, and (3) culture examinations (at least two sets of blood cultures) submitted before antimicrobial therapy.

Isolation of bacteria from at least one set of blood culture was defined as confirmed bacteremia (culture-proven bacteremia). If less virulent bacterial species, such as coagulase-negative staphylococci, *Bacillus*, *Corynebacterium*, or *Propionibacterium*, were identified after 48–72 h of incubation with only one bottle or one set of bottles, it was diagnosed as contamination, as described in the previous report (12, 13).

According to the Third International Consensus Definition for Sepsis and Septic Shock (Sepsis-3) (14), 'definite sepsis' was defined as an increase in Sequential Organ Failure Assessment (SOFA) score ≥ 2 at the onset of infection.

The exclusion criteria were as follows: (1) deniable acute infection, that is, acute exacerbation of collagen disease, tumor fever and (2) lack of sufficient clinical information, and (3) definite diagnosis of fungemia. The details of patient enrollment are shown in Figure 1.

Sample collection

Blood samples were collected from each participant at the onset of infection. We obtained data on selected CPD of neutrophils from the blood sample database of our central medical laboratory.

Control blood samples were obtained from healthy immunocompetent volunteers ($n = 37$) at Toyama University Hospital. The volunteers were hospital staff with no known underlying disease. Blood sampling was conducted under afebrile conditions, and serum was stored and utilized for CPD. Written informed consent was obtained from all the volunteers prior to the blood sampling.

Cell population data

CPD were obtained with Sysmex XN-2000 (Sysmex Corporation, Kobe, Japan), as previously described (8). Briefly, the leukocyte differential channel discriminates leukocytes, and the signals are plotted in a scattergram (WDF). The signals obtained from the three-axes after preincubation with unique surfactant reagents and

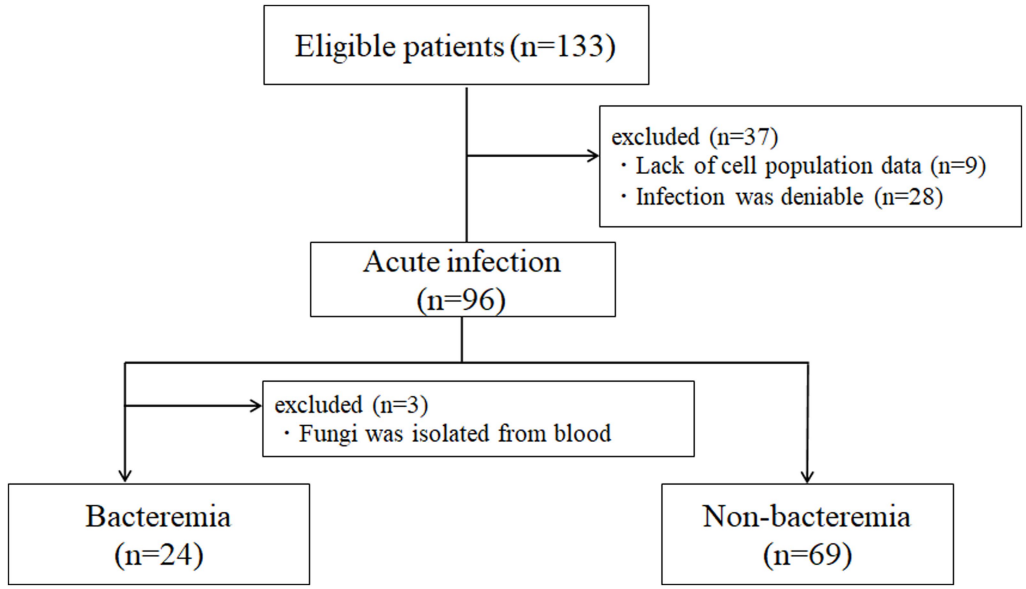

FIGURE 1
Flow chart showing patient selection.

fluorescence staining are analyzed and calculated according to the distribution width. The optical signals along the X-axis (side scatter) are proportional to the internal complexity; fluorescence along the Y-axis correlates with the nucleic acid content, while forward scatter (Z-axis) is related to cell size (5). The morphological and functional characteristics of the whole CPD panel measured using Sysmex XN-2000 are summarized in Supplementary Table S1.

Sepsis biomarkers

The serum levels of CRP, PCT, IL-6, and presepsin were measured as sepsis biomarkers, at the time of enrollment. Among the biomarkers, IL-6 and presepsin were measured by SRL, Inc. and LSI Medience Corporation, while the others were measured in the laboratory at our hospital, as routine examinations (using commercialized test reagents and automated analysis biochemistry system, Cobas ® 8,000; Roche Diagnostics K.K., Japan).

Quantitative PCR assay measuring bacterial load in blood

We measured bacterial load in the blood using a PCR assay, as described previously (15). Briefly, DNA was extracted from blood samples using a DNA extraction kit (QIAamp UCP Pathogen Mini Kit; Qiagen, Germany) according to the manufacturer's instructions. Bacterial universal primers designed to amplify the seven regions of the bacterial 16S ribosomal RNA gene (16S rDNA) (15) were used. Two step of amplification reactions were performed, and the threshold cycle values of amplification in the second PCR were analyzed using the Rotor-Gene Q software program. If no amplification was observed by the 35th cycle in secondary PCR, the sample was defined as containing no bacteria.

Statistical analysis

Background factors are expressed as median (interquartile range) or number (percentage). To evaluate the differences between the two groups, the Mann–Whitney test and Pearson's chi-squared test were used to compare continuous and nominal variables, respectively. Receiver operating characteristic (ROC) curves and the respective areas under the ROC curve (AUC) were generated using GraphPad Prism 9 software (GraphPad Software, San Diego, CA, United States). In each ROC analysis, the cutoff value for the detection of bacteremia was determined using the nearest point relative to the left corner of each ROC curve. The association between each pair of sepsis biomarkers or bacterial load was determined using Spearman's rho correlation coefficient. Statistical significance was set at $p < 0.05$. JMP Pro 16 ((SAS Institute, Cary, NC, United States)) and GraphPad Prism 9 (GraphPad Software) were used for statistical analysis.

Results

Study participants

Detailed information on patient enrollment is presented in Figure 1. Among the patients with bacteremia, three were excluded from further analysis as they were confirmed to have fungemia (two for Candida and one for Cryptococcus). One case in which *Bacillus cereus* was isolated from one of the sets of blood culture, after more than 48 h-incubation, was determined as contamination, and subsequently categorized as 'infection without bacteremia'. Finally, 24 patients with culture-proven bacteremia and 69 patients without bacteremia were included for further analysis.

TABLE 1 Clinical features and laboratory data of patients in the study.

	Total (*n*=93)	Bacteremia (*n*=24)	Non-bacteremia (*n*=69)	*p*-value
Age, years	70 [56–78]	68.5 [63–81]	70 [52–77]	0.062
Male / Female	58/35	14/10	44/25	0.636
Underlying disease				
Diabetes mellitus	26 (28)	4 (17)	22 (32)	0.153
Malignancy	29 (31)	11 (46)	18 (26)	0.087
Severity				
SOFA score	2 [1–4]	4 [2–7]	2 [0–3]	0.002
Sepsis (≥2 SOFA score)	56 (60)	20 (83)	36 (52)	0.007
Laboratory data				
White blood cell ($\times10^3$/μL)	10.6 [7–14]	10.5 [6–13]	10.6 [8–14]	0.235
Neutrophils ($\times10^3$/μL)	8.8 [6–12]	9.5 [4.8–12]	8.7 [6–12]	0.410
Platelets ($\times10^4$/μL)	20.6 [15–28]	17.3 [13–22]	22.6 [16–29]	0.005
CRP (mg/dL)	7.1 [1.6–15]	8.4 [1.3–13]	6.7 [1.6–15]	0.268
Procalcitonin (ng/mL)	0.4 [0.1–1.6]	1.2 [0.3–21]	0.24 [1.0–1.7]	0.012
IL-6 (pg/mL)	204 [39–1,122]	1,122 [504–19,200]	116 [29–387]	0.012
Presepsin (pg/mL)	564 [366–1,200]	1,095 [550–1,590]	534 [338–867]	0.009
30-days mortality	0 (0)	0 (0)	0 (0)	—

Continuous variables are reported as median (interquartile range). Categorical variables are reported as number (percent). SOFA, sequential organ failure assessment; WBC, white blood cell; CRP, C-reactive protein; PCT, procalcitonin; IL-6, interleukin-6.

TABLE 2 Results of blood culture and Tm mapping in this study.

	Bacteremia (*n*=24)	Non-bacteremia (*n*=69)	*p*-value
Infected organ			
Lower respiratory tract	2 (8)	26 (38)	0.007
Urinary tract	7 (29)	13 (19)	0.289
Enteral/intra-peritoneal	3 (13)	8 (12)	0.906
Hepatobilliary/ pancreatic	5 (21)	4 (6)	0.032
Necrotizing fasciitis/ bone	2 (8)	6 (8)	0.956
Others	5 (21)	9 (13)	—
Focus unknown (bacteremia)	0 (0)	3 (4)	0.566
Blood bacterial load determined by PCR			
Positive (above detection limit)	16 (67%)	9 (13%)	<0.001
Copy/mL	525 [0–7,150]	0 [0–0]	0.011
Causative bacteria (cultured from blood)			
Eschelia coli + *Klebsiella* spp.	15 (63)	—	—
Staphylococcus aureus	3 (13)	—	—
Pseudomonas aeruginosa	1 (4)	—	—
Others	5 (21)	—	—

Continuous variables are reported as median (interquartile range). Categorical variables are reported as number (percent).

The clinical characteristics and laboratory findings of the 93 patients included in this study are summarized in Table 1. The SOFA scores were significantly different between patients with and without bacteremia, and 20 patients with bacteremia (20 of 24; 83%) and 36 patients without bacteremia (36 of 69; 52%) were determined according to the criteria of definite sepsis. The platelet count was significantly lower in patients with bacteremia. Whilst IL-6, PCT, and presepsin levels were significantly higher in patients with bacteremia than in those without bacteremia.

Microbiological findings

The results of bacterial load and causative bacteria identified from blood culture are summarized in Table 2. Urinary tract and

hepatobiliary tract/pancreatic infections were the most dominant organ-specific infections in patients with bacteremia, with a frequency of 50%. Bacterial load in the blood was detected by PCR in 16 patients (67%) with bacteremia and in 9 patients (13%) without bacteremia. Quantitative analysis showed that the bacterial load in the blood was significantly higher in patients with bacteremia than in those without bacteremia ($p = 0.011$). The most frequent causative bacteria for bacteremia were *Enterobacteriaceae* (15 cases; 63%), followed by *Staphylococcus* spp. (3 cases; 13%).

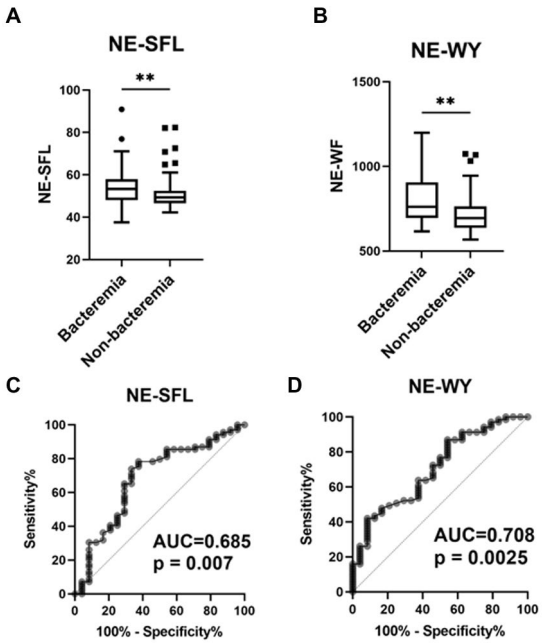

FIGURE 2
Value of the neutrophil parameters (NE-SFL and NE-WY) in patients who developed bacterial infection with or without bacteremia. NE-SFL **(A)** and NE-WY **(B)** in the patients with or without bacteremia. Data are presented as Tukey box-plots and individual values. $^{**}p<0.005$. ROC, AUCs of NE-SFL **(C)** and NE-WY **(D)** for detecting bacteremia.

Association of cell population data and bacteremia

Among the cell population data measured using Sysmex's automated hematology analyzer, NE-SFL and NE-WY were significantly higher in patients with bacteremia than in those without bacteremia (Figures 2A,B). There were no significant differences in CPD of lymphocytes and monocytes between patients with and without bacteremia (Supplementary Table S2).

Furthermore, ROC curves assessing these biomarkers for the diagnosis of bacteremia in acute infections were constructed and analyzed. The AUC of NE-SFL was 0.685 (sensitivity, 66.7%; specificity, 74.3%; cutoff value, 52.2), and that of NE-WY was 0.708 (sensitivity, 62.5%; specificity, 62.2%; cutoff value, 734) (Figures 2C,D). Among the sepsis biomarkers, IL-6 had the highest AUC (0.778), followed by PCT (0.744), presepsin (0.685), and CRP 0.528 (Supplementary Figure S1).

In an additional ROC curve analysis assessing the diagnostic value of these biomarkers for bacteremia in patients with definite sepsis (those with ≥2 SOFA score), the AUC of NE-SFL decreased to 0.632 ($p = 0.190$), while that of NE-WY increased to 0.744 ($p = 0.005$) (Supplementary Table S3).

As shown in Figures 3A,B, a similar positive correlation of NE-SFL and NE-WY with bacterial load was determined by PCR ($r = 0.384$ and 0.374, $p < 0.005$, respectively).

Correlations among immunoinflammatory biomarker levels

Among the tested sepsis biomarkers, IL-6 levels were most significantly correlated with NE-SFL ($r = 0.57$; $p < 0.001$) and NE-WY ($r = 0.68$; $p < 0.001$), followed by PCT ($r = 0.56$ and $r = 0.59$) and presepsin ($r = 0.30$ and $r = 0.38$, respectively) (Figure 4). However, leukocyte and neutrophil counts did not significantly correlate with NE-SLF or NE-WY.

In an additional analysis assessing the correlation between these biomarkers in definite sepsis patients, the correlation between NE-SFL/NE-WY and other parameters was similar to that in patients with acute infection (Supplementary Figure S2).

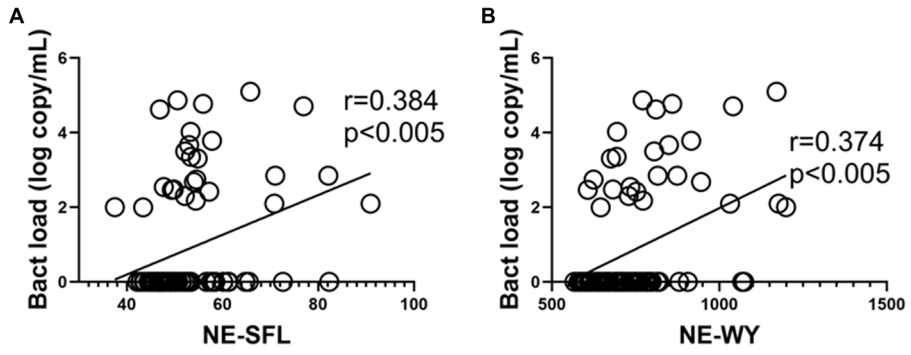

FIGURE 3
Correlations between the neutrophil parameters and bacterial load (determined by PCR) in patients who developed bacterial infection with or without bacteremia. Correlation between the bacterial load in blood and NE-SFL **(A)**/NE-WY **(B)**. Spearman correlation test was used, and Spearman correlation coefficient is shown. Corresponding logarithmic trendlines are shown.

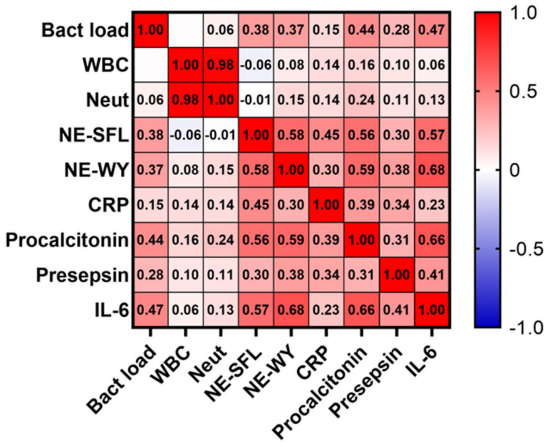

FIGURE 4
Correlation matrix of sepsis biomarkers and blood bacterial load in patients who developed acute bacterial infection. Results are presented as a correlation matrix. Spearman correlation coefficients are plotted. Cells were colored according to the strength and trend of correlations (shades of red=positive, shades of blue=negative correlations). *p<0.05. **p<0.001.

Discussion

This study demonstrated the significant diagnostic value of NE-SFL and NE-WY for detection of bacteremia in patients with acute infection. The definite correlation between blood bacterial load and NE-SFL/NE-WY, determined by PCR, demonstrate that these parameters are affected by initial bacterial invasion into the blood. Among the sepsis biomarkers, NE-SFL/NE-WY were strongly associated with PCT and IL-6, but not with presepsin or CRP. To the best of our knowledge, this study is the first to document an association between CPD and sepsis biomarkers, including IL-6 and presepsin in acute bacterial infection.

In this study, we assessed the diagnostic value of NE-SFL and NE-WY in patients who developed acute bacterial infections, with or without culture-proven bacteremia. The ROC curve for bacteremia in bacterial infection analyzed using NE-SFL and NE-WY revealed a relatively high AUC of 0.685 and 0.708, respectively. Together with the definite correlation between NE-SFL/NE-WY and bacterial load in the blood, we suggest that NE-SFL and NE-WY both have predictive value to detect bacteremia among patients with acute infection. Moreover, NE-WY showed a relatively strong correlation with PCT and IL-6 in patients with definite sepsis compared to NE-SFL, as shown in Supplementary Table S3. These differences may be partly due to the nature of NE-WY, which potentially reflects infection-related cell death rather than cell proliferation.

NE-SFL and NE-WY are postulated to reflect the immaturity or activation of neutrophils because high fluorescence intensity indicates a high RNA/DNA ratio in immature cells. NE-SFL reflects the increase in proportion of the amount of cellular DNA and RNA, while NE-WY reflects the degree of heterogeneity of the neutrophil population. In the early phase of bacteremia, the mobilization of juvenile leukocytes with high nucleic acid content increases in the peripheral blood, resulting in a high NE-SFL (16). Simultaneously, neutrophils undergo cell death through several mechanisms after responding to bacteria, including apoptosis, necrosis, and NETosis (17–19), which is reflected in the variety of nucleic acids, possibly resulting in high NE-WY values. Since severe bacterial infection induces cell death in addition to the proliferation of juvenile neutrophils, a stronger association with severity or sepsis markers might be observed with NE-WY than with NE-SFL.

In this study, we determined the quantitative bacterial load of patients using the PCR method (15), which is part of the 'melting temperature mapping (Tm mapping)' method (15). Tm mapping is a novel molecular genetic method for identifying a broad range of pathogenic bacteria using a real-time PCR-based system. In attempt to detect a wide range of bacteria, the Tm mapping method is designed with seven bacterial universal primer sets targeting bacterial conserved regions in 16S ribosomal RNA gene in a nested PCR assay to detect and identify bacterial isolates with high sensitivity and specificity; the Tm mapping method was able to detect 95.6% of culture-proven bacteremia in the analysis using 200 blood samples (15). Using this PCR method, we confirmed that the value of sepsis biomarkers, including NE-WY/NE-SFL, was significantly correlated with bacterial load in patients with acute bacterial infection. Interestingly, NE-WY strongly correlated with bacterial load in the blood of patients with definite sepsis, which might be induced by an increase in cell death or phagocytosis of bacteria.

Among different biomarkers which have been proposed to predict sepsis or bacteremia, the main attributes of successful and effective biomarkers are high sensitivity, specificity, possibility of bed-side monitoring, and financial accessibility (20). To date, the sepsis biomarkers that commercially available in Japan are CRP, PCT, presepsin and IL-6. Emerging evidence has suggested several other biomarkers as novel diagnostic tools in acute bacterial infections, such as lipopolysaccharide-binding protein (21), interleukins and cytokines other than IL-6 (e.g., IL-8, IL-10, TRAIL) (22, 23), surface markers of circulating leukocytes such as Cluster of Differentiation 64 (CD64) (24), and precursor of hormones such as mid-regional fragment pro-adrenomedullin (25). To establish more accurate and efficient diagnostic procedures, the combination of biomarkers and diagnostic methods has been investigated as a novel diagnostic approach in bacterial infection (26, 27).

In this study, IL-6 and PCT showed the highest predictive value for the presence of bacteria, whereas presepsin and CRP showed a relatively lower predictive value, than NE-WY/NE-SFL. Of note, the sepsis biomarkers IL-6, PCT, presepsin and CRP are released from various other non-neutrophil immune cells (22, 28). The significant association between NE-WY/NE-SFL, which reflect the immune response in neutrophils, and IL-6/PCT, suggests potential benefits in the use of a combination of these biomarkers in predicting severe bacterial infections.

Presepsin, the soluble fraction of cluster of differentiation 14 (CD14), is a sepsis biomarker that is released into circulation when monocytes are activated after binding with lipopolysaccharides (LPS) and LPS-binding proteins (29, 30). Recently, Park et al. (31) reported that presepsin showed a higher AUC than PCT (0.720 and 0.593; $p = 0.002$) for the prediction of 28-day mortality in 757 patients with bacterial infection, whereas the AUC of presepsin for detecting culture-proven bacteremia was lower than that of PCT (0.685 and 0.791; $p < 0.001$). As NE-SFL/NE-WY showed a higher AUC than presepsin, we consider presepsin to predict mortality rather than the presence of bacteremia in sepsis patients. The lack of fatal sepsis cases in this study may have also affected the diagnostic value of presepsin.

Based on these findings, we suggest the potential synergistic benefit and interaction between NE-WY/NE-SFL and conventional sepsis biomarkers. As NE-WY/NE-SFL determination incurs no additional costs other than routine examination by the automated differential, these CPD parameters have strong potential to be routine sepsis biomarkers that predict bacteremia or severe infection in acute bacterial infections.

This study had several limitations. First, the single-center observational study design may have resulted in selection bias. Second, the relatively small sample size, particularly regarding patients who developed culture-proven bacteremia, may limit the reproducibility of the results. However, because this study focused on the association between CPD, sepsis biomarkers, and bacterial load, we believe that these limitations did not have a major effect on our conclusions.

Conclusion

In this study, we demonstrated that NE-SFL and NE-WY measured using the Sysmex XN-2000 have a high diagnostic efficacy for prediction of bacteremia in acute bacterial infections. We also found that NE-SFL and NE-WY are strongly associated with the blood bacterial load, determined by PCR. In addition to the diagnostic value and substantial financial accessibility, the significant association with conventional sepsis biomarkers suggest potential benefits of NE-WY/NE-SFL in routine use in predicting severe bacterial infections. To further advance the early detection and understanding of bacteremia in acute bacterial infections, more investigation into the diagnostic value of CPD, particularly with NE-WY and NE-SFL is warranted.

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 Toyama University Hospital Ethics Committee (29–152). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YM and HN designed and interpreted the clinical data and experimental findings. YM, KN, and HN prepared the manuscript. YM with the assistance of TU, AM, YH, NK, and MK collected the clinical data and blood. YM and HN contributed to the analysis of the experimental and microbiological findings. YM and KN confirmed the accuracy of the statistical analysis. YM, HN, TU, AM, YH, NK, MK, YI, KN, YY, and IK contributed to the discussions throughout the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

NK and YI were employed by the company Sysmex Corporation. Author MK was employed by the company Subsidiary of Sysmex Corporation. The authors declare that this study received funding from Sysmex Corporation. The funder had the following involvement in the study: study design, analysis, interpretation of data.

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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1156889/full#supplementary-material

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