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Infections in the intensive care unit, volume II

Edited by
Zhongheng Zhang, George Dimopoulos and Yuetian Yu

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Infections in the intensive care unit, volume II

Topic editors

Zhongheng Zhang — Department of Emergency Medicine, Sir Run Run Shaw Hospital, China

George Dimopoulos — EVGENIDIO Hospital National and Kapodistrian University of Athens, Greece

Yuetian Yu — Shanghai Jiao Tong University, China

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EDITED AND REVIEWED BY
Ata Murat Kaynar,
University of Pittsburgh, United States

*CORRESPONDENCE
Yuetian Yu
✉ fishyyt@sina.com
Zhongheng Zhang
✉ zh_zhang1984@zju.edu.cn

†These authors have contributed equally to this work

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Editorial: Infections in the intensive care unit, volume II

Lihui Wang[1†], Bin Lin[2,3†], Zhongheng Zhang[4,5*] and Yuetian Yu[1,2*]

[1]Department of Critical Care Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [2]Key Laboratory of Intelligent Pharmacy and Individualized Therapy of Huzhou, Huzhou, China, [3]Department of Pharmacy, Changxing People's Hospital, Second Affiliated Hospital of Zhejiang University School of Medicine, Huzhou, China, [4]Department of Emergency Medicine, Sir Run-Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, [5]Provincial Key Laboratory of Precise Diagnosis and Treatment of Abdominal Infection, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

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Editorial on the Research Topic
Infections in the intensive care unit, volume II

Infection is one of the most serious challenges in the intensive care unit (ICU). According to data from a global multi-center cross-sectional study, almost half of the critically ill patients are admitted to ICU with infectious diseases. Furthermore, others may have ICU-acquired infection (IAI) because of invasive procedures, immunosuppression, or even inappropriate antibiotics treatment (1). Antimicrobial resistance is another problem that we have to face. It may lead to a shortage of antimicrobial agent selection and a substantial attributable mortality. Thus, applying the antimicrobial stewardship program (ASP) and infection control program (ICP) is much more essential, and the joint effort involving multi-disciplinary treatment (MDT) is needed (2).

Therefore, we have compiled this Research Topic on infection in the ICU, aiming for an in-depth discussion in the relevant fields. We are also pleased to receive over 50 submissions, of which 16 articles have been successfully published after revisions. This Research Topic mainly focuses on the following areas.

Epidemiological trends of drug-resistant pathogens in the ICU

Global epidemiological surveys indicate that 51% of critically ill patients admitted to the ICU have concomitant infectious diseases, among which pneumonia account for 63.5%, abdominal infections for 19.6%, and bloodstream infections for 15.1%. The pathogens causing these infections are primarily non-fermenting Gram-negative bacteria, with antibiotic-resistant non-fermenters accounting for approximately 20%. Fungal infections make up 16.4% (1). The misuse of antibiotics has led to an increase in antibacterial and antifungal resistance, making the demand for new antibiotics extremely urgent.

During the COVID-19 pandemic, the abuse of antibiotics has further increased bacterial and fungal resistance. According to a special report from the CDC in 2022, in the first year of the pandemic, the detection rate of Carbapenem-resistant *Acinetobacter* increased by 78%, the detection rate of Carbapenem-resistant *Enterobacterales* rose by 35%, and Antifungal-resistant Candida increased by 26%. Most of these pathogens originated

from adult ICU patients, resulting in a significant economic burden on healthcare (3). Therefore, based on factors such as resistance, therapeutic drug selectivity, and mortality rates, the World Health Organization (WHO) updated the priority lists for fungi and bacteria in 2022 and 2024, respectively, providing a basis for graded and tiered management of infectious diseases in different countries and regions.

The optimal timing for the initiation of antimicrobial therapy and the evolution of related concepts

Any disease, if detected and treated early, will lead to better outcomes. The same is true for sepsis. For every one-hour delay in initiating antimicrobial therapy, the mortality rate of sepsis patients increases by 7.6% (4). Therefore, we recommend "Hit hard and hit early" at the outset. The guidelines for the surviving sepsis campaign (SSC) also indicate that the bundled for sepsis should be shortened from 6 h to 3 h, or even 1 h (4). However, in the current era of resistant bacteria, we should reflect on our empirical antimicrobial prescriptions. Are we sufficiently accurate in diagnosing sepsis? Do these patients truly require broad-spectrum antimicrobial treatment? If we optimize empirical antimicrobial prescriptions, can we improve the outcomes while also reducing the incidence of acute kidney injury (AKI) or clostridium difficile infections (CDI)?

In the routine diagnostic and therapeutic work in the ICU, we typically prescribe empirical antibiotics for critically patients in the following situations: (1) Host factors, clinical signs, and laboratory tests are all consistent with the guidelines for infectious diseases; (2) High-risk hosts are in a high-risk stage of infection, such as patients who have undergone allogeneic stem cell transplantation (HSCT) and develop fever 1 week after leaving the transplant unit; (3) Clinicians have rich experience but lack laboratory support, for example, 1 month after kidney transplantation, a patient develops fever, dry cough, and chest CT indicates diffuse exudation in both lungs, which may lead to the initiation of empirical treatment with trimethoprim-sulfamethoxazole for pneumocystisjirovecii pneumonia; (4) Concerns about potentially missing rapidly progressing lethal infectious diseases may prompt the initiation of antimicrobial therapy, which is something we should particularly reflect on during the COVID-19 pandemic.

Currently, the initiation of antibiotics has shifted from "when to start" to "when not to start." Antibiotic treatment should not be initiated if the patient does not have an infectious disease. Another new concept is "watching and waiting," which means that when the patient's vital signs are relatively stable and there is significant difficulty in differentiating sepsis, a waiting period of 3 h can be allowed for the identification of pathogens and resistance phenotypes based on rapid diagnostic test (RDT). If the pathogen detection results are still unavailable after 3 h but the patient's vital signs remain stable, the waiting time can be extended to 6 h (4). However, for patients with septic shock, due to a mortality rate close to 50%, it is still recommended to initiate broad-spectrum antibiotics within 1 h according to the bundle (5). Two points need to be emphasized during this process: (1) Assess potential pathogens and resistance based on the epidemiological situation of resistant bacteria; (2) Clearly understand the reasons for using RDT, striving for accurate interpretation of test reports.

Selection of initial antibiotic therapy and optimize antibiotic dosing

Delayed initiation of antibiotic treatment may lead to poor prognosis in patients with severe infections. Similarly, even if antibiotic treatment is started promptly, if the pathogens causing the infection are not adequately covered, the prognosis for the patient remains poor (6). Therefore, it is necessary to infer the possible pathogens and their resistance phenotypes that could cause infection in patients based on epidemiological data. This requires not only referencing local epidemiological data on pathogen infections but also integrating the infection epidemiological data from the specific locality and department (7–9). In addition, it is necessary to further understand the patient's recent history of antibiotic use, the history of colonization by drug-resistant bacteria, whether they have undergone past decolonization treatments, and, if conditions permit, to make a comprehensive judgment based on the changes in the sensitivity breakpoints of drug-resistant bacteria (10, 11).

For immunocompromised hosts (ICHs) commonly found in the ICU, it is essential to assess their underlying diseases to determine the type of immune dysfunction and provide targeted coverage for core pathogens. If empirical treatment is ineffective, the antimicrobial spectrum should be further expanded to include common pathogens (12). Additionally, a large amount of clinical data can be generated daily in the ICU, which can be used to establish clinical prediction models. However, this should not be viewed as a "decision-making tool" but rather as an "exclusion tool" to rule out whether the patient has an infectious disease or an infection caused by resistant bacteria, thereby minimizing the unreasonable use of empirical broad-spectrum antibiotics (13).

As ICU practitioners deepen their understanding of infectious diseases and as microbial testing technologies continue to develop, the success rate of treating severely infected patients is increasing. However, there are still some patients whom we cannot cure, primarily due to the following five reasons: (1) Insufficient drainage of the infection site; (2) Presence of mixed infections where antibiotics do not fully cover the pathogens; (3) Induction of resistance during antibiotic use; (4) Excessively low host immune function levels; (5) Pharmacokinetic and pharmacodynamic (PK/PD) parameters of antibiotics failing to meet standards, which is increasingly being focused on. The pathophysiological changes in patients with severe infections in the ICU are extremely complex and dynamic. Factors such as apparent volume distribution (Vd), serum albumin levels, and the impact of extracorporeal life support devices all require continuous dynamic assessment. It is also encouraged to adjust doses precisely based on Bayesian models, leveraging big data, artificial intelligence (AI), and model-informed precision dosing (MIPD) to achieve individualized and precise treatment (14).

De-escalation strategy and duration of antibiotics treatment

Long-term use of broad-spectrum antibiotics can affect the stability of the host's internal microecology, while also increasing the burden on the liver and kidney of patients, potentially leading to the development of antibiotic resistance. Therefore, it is necessary to encourage the early implementation of a de-escalation strategy for antibiotics, which includes using monotherapy instead of combination therapy, employing narrow-spectrum antibiotics instead of broad-spectrum ones, and discontinuing the use of antimicrobial agents that do not target the causative pathogens of infections (15). However, in the ICU, nearly 45% of cases of sepsis remain culture-negative, making the accurate implementation of de-escalation strategies quite challenging. When the culture results for suspected sepsis patients are negative, it is essential to reassess the accuracy of the sepsis diagnosis, while also relying on non-culture methods such as molecular biology testing to identify the pathogens responsible for the infection, and to timely adjust the antimicrobial treatment regimen (16, 17).

The timing for discontinuing effective antibiotic treatment regimens has long been a contentious issue. Previously, there was a strong advocacy for completing the full course to prevent the recurrence of infectious diseases. However, there is now a greater emphasis on dynamically assessing the benefits and risks of antibiotics, suggesting that antibiotics should be discontinued as early as possible once therapeutic effects are achieved, in order to minimize potential side effects. Over the past 25 years, at least 45 randomized controlled trials (RCTs) have compared the efficacy of short vs. long courses of treatment for infectious diseases, including community-acquired pneumonia, intra-abdominal infections, cellulitis (18). Most studies indicate that there is no increase in the recurrence or mortality rates of diseases when comparing short courses to long courses. Consequently, the 2021 guidelines from the SSC recommend short courses for non-immunocompromised hosts with well-drained infections who respond clinically well to treatment, including pneumonia, bacterial blood stream infection, intra-abdominal infections, and urinary tract infections. No recommendations for short courses currently exist for conditions such as tuberculosis, osteomyelitis, and invasive fungal infections (IFI).

Summary of the Research Topic

In this Research Topic, "*Infections in the Intensive Care Unit, Volume II*," we have assembled a collection of articles that delve into the complexities of managing infections within the ICU. The contributions in this volume encompass a wide array of topics, from the application of novel diagnostic tools to the challenges of treating multidrug-resistant bacteria. Each article provides a comprehensive analysis of the current state of knowledge, offering insights into the latest strategies for diagnosing and treating infections in critically ill patients. The articles also highlight the importance of antimicrobial stewardship and the role of precision medicine in improving patient outcomes. Looking ahead, the prospects for research in ICU infections are promising, with a focus on leveraging technology and data to enhance diagnostic accuracy and treatment efficacy. The integration of artificial intelligence and machine learning into the analysis of clinical data holds the potential to significantly improve our ability to predict patient outcomes and personalize treatment plans. Furthermore, as new antimicrobial agents are developed, research will play a crucial role in determining their safety and efficacy in real-world settings.

The future also holds the promise of a more concerted effort in antimicrobial stewardship, aiming to preserve the effectiveness of existing antibiotics and slow the emergence of resistance. This will require collaborative research efforts across disciplines, combining the expertise of clinicians, microbiologists, pharmacists, and public health officials. We hope to provide more information related to infections in the ICU in the volume III, and we also hope that the next Research Topic will receive more support from everyone.

Author contributions

LW: Writing – original draft, Writing – review & editing. BL: Writing – original draft, Writing – review & editing. ZZ: Writing – original draft, Writing – review & editing. YY: Writing – original draft.

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Conflict of interest

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EDITED BY
Diamantis Plachouras,
European Centre for Disease Prevention and Control (ECDC), Sweden

REVIEWED BY
Charles-Hervé Vacheron,
Hospices Civils de Lyon, France
Abdel-Hameed Al-Mistarehi,
Johns Hopkins Medicine, United States

*CORRESPONDENCE
Vasco Ricoca Peixoto
✉ vrf.peixoto@ensp.unl.pt

†Deceased

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Difference in determinants of ICU admission and death among COVID-19 hospitalized patients in two epidemic waves in Portugal: possible impact of healthcare burden and hospital bed occupancy on clinical management and outcomes, March–December 2020

Vasco Ricoca Peixoto[1]*, André Vieira[1], Pedro Aguiar[1], Carlos Carvalho[2], Daniel Thomas[3], Paulo Sousa[1], Carla Nunes[1†] and Alexandre Abrantes[1]

[1]NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, NOVA University Lisbon, Lisbon, Portugal, [2]Unit for Multidisciplinary Research in Biomedicine (UMIB), School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal, [3]Communicable Disease Surveillance Centre, Public Health Wales, Cardiff, United Kingdom

Aim: Identify factors associated with COVID-19 intensive care unit (ICU) admission and death among hospitalized cases in Portugal, and variations from the first to the second wave in Portugal, March–December 2020.

Introduction: Determinants of ICU admission and death for COVID-19 need further understanding and may change over time. We used hospital discharge data (ICD-10 diagnosis-related groups) to identify factors associated with COVID-19 outcomes in two epidemic periods with different hospital burdens to inform policy and practice.

Methods: We conducted a retrospective cohort study including all hospitalized cases of laboratory-confirmed COVID-19 in the Portuguese NHS hospitals, discharged from March to December 2020. We calculated sex, age, comorbidities, attack rates by period, and calculated adjusted relative risks (aRR) for the outcomes of admission to ICU and death, using Poisson regressions. We tested effect modification between two distinct pandemic periods (March–September/October–December) with lower and higher hospital burden, in other determinants.

Results: Of 18,105 COVID-19 hospitalized cases, 10.22% were admitted to the ICU and 20.28% died in hospital before discharge. Being aged 60–69years (when compared with those aged 0–49) was the strongest independent risk factor for ICU admission (aRR 1.91, 95%CI 1.62–2.26). Unlike ICU admission, risk of death increased continuously with age and in the presence of specific comorbidities. Overall, the probability of ICU admission was reduced in the second period but the risk of death did not change. Risk factors for ICU admission and death differed by epidemic period. Testing interactions, in the period with high hospital burden, those aged 80–89, women, and those with specific comorbidities had a significantly lower aRR for ICU admission. Risk of death increased in the second period for those with dementia and diabetes.

Discussion and conclusions: The probability of ICU admission was reduced in the second period. Different patient profiles were identified for ICU and deaths among COVID-19-hospitalized patients in different pandemic periods with lower and higher hospital burden, possibly implying changes in clinical practice, priority setting, or clinical presentation that should be further investigated and discussed considering impacts of higher burden on services in health outcomes, to inform preparedness, healthcare workforce planning, and pandemic prevention measures.

KEYWORDS

COVID-19, health outcomes, risk factors, death, intensive care unit, hospital bed occupancy rate, healthcare burden, patient to physician ratio

Introduction

Early studies of clinical results of COVID-19 in patients in China (1), Italy (2, 3), and the United States of America (4) have described risk factors for poorer clinical outcomes, including age, sex, and comorbidities. Identifying these determinants while adjusting for confounding factors can help inform clinical risk stratification and implementation of public health measures and improve epidemiological scenarios and forecasts on needed healthcare resources.

Several studies have focused on risk factors for ICU admission and death among hospitalized patients with COVID-19 (5).

Although hospitalized patients are only a fraction of the total cases in the general population, they provide quality data on comorbidities and outcomes and may give important information on clinical course and clinical management in different epidemic periods.

Advanced age, male sex, obesity, immunosuppression, and diabetes have been previously identified as risk factors for ICU admission (6, 7). Asthma and COPD were not identified as risk factors for ICU admission and death related to SARS-CoV2 infection (8).

Independent factors associated with in-hospital mortality included older age groups, generally above 50, being male, immunosuppression, renal disease, chronic lung disease, cardiovascular disease, neurologic disorders, diabetes, and dementia (9, 10). Identifying these features among patients in routine clinical practice may improve COVID-19 management (11). However, various studies on risk factors for severe outcomes of COVID-19 included small series of patients, mostly in single hospital centers (12–14).

One of the largest initial studies in hospital patients showed higher risk of death for patients with increased age, cardiac, pulmonary, and kidney disease, as well as malignancy, dementia, and obesity (15). The OpenSAFELY Collaborative study (16), in the United Kingdom, which included 17 million adult COVID-19 patients, added the findings that living in a more socio-economically deprived community was a relevant risk factors for death by COVID-19 and reinforced that older age, male sex, and having various other prior medical conditions were also relevant. High-quality data on possible risk factors for poorer outcomes among hospitalized COVID-19 patients is needed to inform clinical management, public health policy, and preparedness.

However, there is little research (5) on how risk factors may change over time in different pandemic periods considering healthcare burden. This may have implications related to clinical management and preventive intervention priorities, resource allocation, and healthcare supply. Various studies have shown increased risks of more severe outcomes in periods with higher hospital burden, higher number of patients per healthcare worker, or higher hospital occupancy rate (17–20).

We aim to further understand COVID-19 risk factors for two outcomes, namely ICU admission and death in hospitalized cases in Portugal, while comparing two epidemic periods with different healthcare burden, bed occupancy, and healthcare worker to patient ratio to generate hypotheses on its causes to inform future research, policy, and practice regarding peak pandemic and hospital burden periods, health workforce planning, and pandemic and seasonal preventive measures.

Methods

Study design and data sources

A retrospective cohort study including all hospitalized COVID-19 cases in Portuguese National Health Services (NHS) hospitals from March to December 2020 was conducted to identify factors associated with ICU admission and death in two different periods with different healthcare burdens.

Data sources

All hospitalization diagnoses in NHS Hospitals are coded by trained medical doctors from clinical registries using the ICD-10 coding system. These ICD-10 codes are sequenced according to ICD-10 guidelines, considering specific COVID-19 guidelines for ICD-10 coding (21). Variables such as sex, age in years, and outcomes (which include death and transfer to ICU) are recorded as well as dates of admission and of outcome. A database with all admissions with COVID-19 U07.1 as a diagnosis was shared with the National School of Public Health by the Health Ministry services under a protocol for anonymized data sharing with academia during the COVID-19 pandemic.

Case definition

A confirmed case of COVID-19 is anyone with a positive RT-PCR result for SARS-CoV-2 RNA in nasopharyngeal and/or oropharyngeal

specimens regardless of clinical or epidemiological criteria. Included cases had a U07.1-COVID-19 diagnosis. This code does not include asymptomatic COVID-19, in the context of screening or acquired in the hospital.

Outcomes

We considered two primary outcomes for all patients admitted to hospital with a COVID-19 diagnosis: admission to ICU and death during hospitalization.

Exposures

We included in the analysis sex (Female/Male), age (recoded into "under 50," "over 89," and 10-year bands between 50 and 89 years of age), and comorbidities as independent variables/exposures. Dementia was categorized using the Charlston (22) comorbidity index. Mental/behavioral disease and pregnancy were categorized considering the corresponding ICD-10 category. Other ICD-10 codes were categorized considering Elixhauser (23) comorbidity index categories (24) if there were more than 100 observations in the category. We used these comorbidities index categories to avoid arbitrary inclusion of ICD-10 codes in categories of relevant comorbidities. We present adjusted models for those categories. We defined two Periods: since the first reported case in Portugal (2 March) to September 30; and from 1 October to 16 December. These correspond, respectively, to the first wave and summer period (low incidence and hospitalizations) and the second COVID-19 wave in Portugal (high incidence of cases hospitalizations, and ICU admission and high hospital and ICU bed occupancy rate; Figure 1).

Statistical analysis

We calculated attack rates (proportion of each outcome by stratum) by period. We calculated adjusted relative risk for ICU admission and death, by age group, sex, relevant comorbidities, and period, and tested interaction between the Period and other variables using robust Poisson regressions. To produce the final model with interaction terms between the period and the other variables we also tested for statistical significance of interaction terms. After that we created a model that included all interaction terms that were statistically significant when added individually to the fully adjusted model. Finally, we conducted backward elimination of non-significant interaction terms.

Statistical analysis was conducted in Stata (version 14, StataCorp, College Station, Texas, United States). All analyses used 95% CI and considered a p-value < 0.05 as statistically significant.

Ethical considerations

Anonymized data were shared with the National School of Public Health-NOVA University of Lisbon by services of the Ministry of Health under a protocol for COVID-19 research.

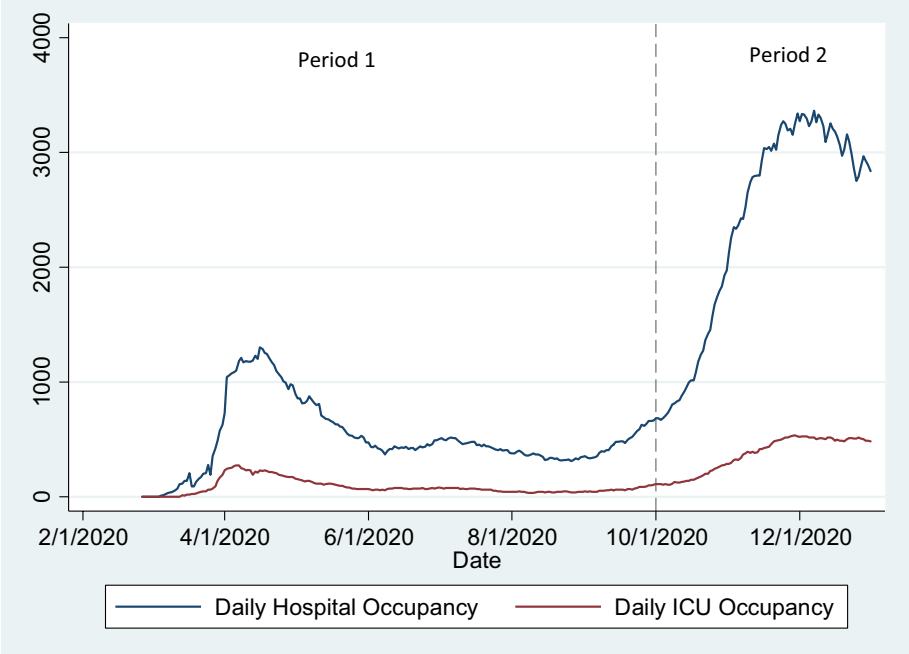

FIGURE 1
Number of hospital (general ward) and ICU occupied beds from 2 March to 31 December. Period 1 and 2 are visually represented. March–December 2020, Portugal.

TABLE 1 Attack rates (proportion by stratum) for the outcome ICU and death in period 1, and period 2, Portugal, March–December 2020 (n=18,105).

	(unite cells)						Death					
	Period 1 (March–September2020)			Period 2 (October–December 2020)			Period 1 (March–September2020)			Period 2 (October–December 2020)		
Exposure	Total	ICU	%	Total	ICU	%	Total	Death	%	Total	Death	%
Sex												
Female	4,440	387	**8.72**	4,153	208	**5.01**	4,440	815	**18.36**	4,153	887	**21.36**
Male	4,736	737	**15.56**	4,776	520	**10.89**	4,736	942	**19.89**	4,776	1,027	**21.50**
Age												
0–49	1933	180	**9.31**	1,255	96	**7.65**	1933	35	**1.81**	1,255	20	**1.59**
50–59	1,058	183	**17.30**	1,040	127	**12.21**	1,058	64	**6.05**	1,040	57	**5.48**
60–69	1,457	300	**20.59**	1,572	233	**14.82**	1,457	173	**11.87**	1,572	176	**11.20**
70–79	1722	298	**17.31**	1983	195	**9.83**	1722	393	**22.82**	1983	430	**21.68**
80–89	2,136	148	**6.93**	2,278	75	**3.29**	2,136	710	**33.24**	2,278	845	**37.09**
≥90	870	15	**1.72**	801	2	**0.25**	870	382	**43.91**	801	386	**48.19**
Comorbidities												
Malignant neoplasm	795	88	**11.07**	609	33	**5.42**	795	288	**36.23**	609	237	**38.92**
Metastatic cancer	91	5	**5.49**	55	0	**0.00**	91	44	**48.35**	55	29	**52.73**
Mental illness	2,138	356	**16.65**	1977	186	**9.41**	2,138	419	**19.60**	1977	393	**19.88**
Pregnancy	367	7	**1.91**	265	5	**1.89**	367	1	**0.27**	265	3	**1.13**
Cardiac disease (heart failure)	1,607	174	**10.83**	1,515	101	**6.67**	1,607	548	**34.10**	1,515	534	**35.25**
Chronic kidney disease (renal failure)	1,521	162	**10.65**	1,326	61	**4.60**	1,521	446	**29.32**	1,326	447	**33.71**
Chronic pulmonary disease	666	112	**16.82**	768	59	**7.68**	666	173	**25.98**	768	201	**26.17**
Asthma	309	45	**14.56**	339	41	**12.09**	309	36	**11.65**	339	34	**10.03**
Chronic liver disease	661	156	**23.60**	624	78	**12.50**	661	136	**20.57**	624	149	**23.88**
Obesity	1811	362	**19.99**	2,130	247	**11.60**	1811	312	**17.23**	2,130	414	**19.44**
Dementia	1,387	40	**2.88**	1,203	12	**1.00**	1,387	496	**35.76**	1,203	524	**43.56**
Hypertension	2,588	300	**11.59**	2,649	220	**8.31**	2,588	678	**26.20**	2,649	754	**28.46**
Diabetes	2069	279	**13.48**	2,191	178	**8.12**	2069	471	**22.76**	2,191	573	**26.15**
Neurologic disease	561	77	**13.73**	509	23	**4.52**	561	175	**31.19**	509	177	**34.77**
Total	9,176	1,124	**12.25**	8,929	728	**8.15**	9,176	1757	**19.15**	8,929	1914	**21.44**

Results

Of 18,105 hospitalized cases of COVID-19, 1.852 were admitted to the ICU (10.22%) and 3.671 died in hospital before discharge (20.28%).

Attack rates for ICU admission where higher for those aged 60–69 and progressively lower after that age. Attack rates for death increase continuously with age. Proportion of hospitalized patients who were admitted to the ICU was higher in the first period in most patient characteristics (Table 1).

Determinants of ICU admission

In multivariable analysis, there was an increase in probability of overall admission to ICU in age groups 50–59 and 60–69, and then decreasing above that age. The comorbidities with higher adjusted RR for admission to ICU were Obesity, Chronic Liver Disease, Mental Disease, and Asthma. Malignant neoplasms, Chronic Kidney Disease (CKD), and dementia reduced the probability of ICU admission. The risk of ICU admission was significantly lower in Period 2 (Table 2). When testing interactions with the Period, in the second period there was a significant increase in the adjusted risk of ICU admission for men, and a reduction for those aged 80–89 and those with metastatic cancer, CKD, pulmonary disease, and neurologic disease (Figure 2).

Risk factors for death

We observed that there was large, continuous increase of risk of death with age, unlike what was seen with ICU admissions.

In multivariable analysis, there was a continuous increase of risk of death with increasing age. Comorbidities that increase the risk of death were Malignant Neoplasms, Metastatic cancer, Cardiac Disease, CKD, Chronic Liver Disease, Dementia, and Neurologic Disease.

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TABLE 2 Fully adjusted model for the outcome of the ICU admission including significant interactions between the Period and other variables, Portugal, March–December 2020 (*n*=18,105).

ICU admission	aRR	*p*-value	[95%conf. interval]
Sex			
Female			Ref
Male	1.43	<0.001	[1.27–1.60]
Age			
0–49			Ref
50–59	1.47	<0.001	[1.26–1.72]
60–60	1.80	<0.001	[1.56–2.07]
70–79	1.57	<0.001	[1.35–1.82]
80–89	0.86	0.138	[0.70–1.05]
≥90	0.18	<0.001	[0.11–0.29]
Comorbidities			
Malignant neoplasms	0.77	0.004	[0.65–0.92]
Metastatic cancer	0.44	0.067	[0.18–1.06]
Mental/behavioral disease	1.16	0.001	[1.06–1.28]
Pregnancy	0.27	<0.001	[0.15–0.47]
Cardiac disease (heart failure)	1.09	0.233	[0.95–1.26]
Chronic kidney disease (renal failure)	0.85	0.047	[0.72–1.00]
Chronic pulmonary disease	1.25	0.012	[1.05–1.49]
Asthma	1.23	0.035	[1.02–1.50]
Chronic liver disease	1.42	<0.001	[1.25–1.60]
Obesity	1.52	<0.001	[1.38–1.66]
Dementia	0.28	<0.001	[0.21–0.37]
Hypertension	0.92	0.317	[0.78–1.08]
Diabetes	1.07	0.408	[0.91–1.25]
Neurologic disorders	1.15	0.181	[0.94–1.42]
Period			
Period2	0.60	<0.001	[0.51–0.71]
Effect modification by the period			
Male*Period2	1.24	0.026	[1.03–1.51]
80–89*Period2	0.75	0.047	[0.56–1.00]
Metastatic*Period2	0.00	<0.001	[0.00–0.00]
CKD (Renal failure)*Period2	0.72	0.032	[0.53–0.97]
Pulmonary*Period2	0.70	0.022	[0.52–0.95]
Neurologic*Period2	0.55	0.009	[0.35–0.86]

Pregnancy, Asthma, and Diabetes reduced the risk of death, although diabetes had only a mild reduction. Adjusted risk of death did not change significantly in the second period although death rate was higher in the second period.

When testing interaction terms between the period and other variables for the outcome death, only the interaction of the second period with diabetes and with Dementia were statistically significant. They remained significant in a fully adjusted model with all previously significant interaction terms (Figure 3; Table 3).

Discussion

We identified different adjusted risk factors for ICU admission and death in two different periods with different hospital burden, considering age, sex, and comorbidities. Obesity and Respiratory disease were significant risk factors for ICU admission but not for death. Risk of ICU increased with age but, unlike for death, risk of ICU admission stopped increasing after 70–79 years of age. Risk of ICU and death for age, sex, and various comorbidities changed in the second period of higher hospital burden. Testing effect modification by the period, in the period with high hospital burden those aged 80–89, women, and those with specific comorbidities had a significantly lower aRR for ICU admission. Risk of death increased in the second period for those with dementia and diabetes.

These findings identify knowledge gaps regarding ICU admission and clinical practice in periods of higher hospital burden. Changes may be due to the impact of healthcare burden in clinical management and ICU admission threshold, and variation in severity profile of patients admitted to hospital with COVID-19.

By observing variation in risk for different exposures in different periods we hypothesize that there may be changes in clinical care or severity profile of hospitalized patients in different time periods that can affect the probability of being admitted to an ICU and alter risk of death in some patients. In the face of strained resources and high bed occupancy rate, ICU admission may deprioritize patients with lower expected benefit from ICU admission. This may justify why patients aged 80–89 were less likely to be admitted to the ICU in the second period as well as those with relevant comorbidities. Patients over 90 and those with dementia did not see a difference in ICU admission probability in the two periods, possibly because they already had low admission rates due to low expected benefit, or because they die without ICU admission criteria or by causes and in clinical contexts that may not constitute ICU admission criteria. Similarly, increased risk of death for patients with dementia and diabetes may be due to changes in management related to hospital overload, implying that these patients may be of higher vulnerability when admitted to hospital in context of high hospital burden. A higher baseline severity or clinical vulnerability of patients admitted to hospital in the second period due to various factors may also contribute to the observed increase in risk of death for specific patient profiles.

Age remains the most relevant risk factor after adjustment. In our study, some comorbidities were weak risk factors or not associated with increased risk of death, such as chronic pulmonary disease, obesity, and diabetes. This may be partly because categorization using Elixhauser comorbidity index may include less severe disease or eventually because some patients in those categories may be admitted with less severe profile in a precautionary approach. In the adjusted model, obesity and respiratory disease were not significant risk factors for death but were strong risk factors for ICU admissions among comorbidities. These findings may imply lower thresholds for admission to the ICU for these patients but not intrinsically higher severity, although this must be considered with caution since obesity and respiratory disease have been risk factors for death in other studies (25, 26). In our study it is possible that Elixhauser chronic pulmonary disease and diabetes categories

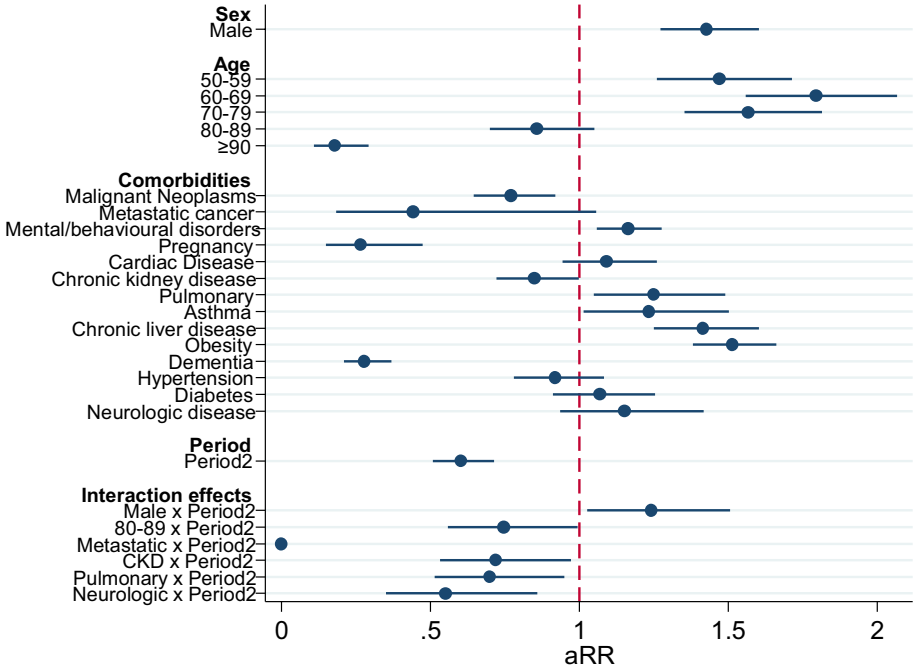

FIGURE 2
Forest plot representing the aRR of the adjusted model for the outcome ICU admission, including significant interactions between Period and other variables, Portugal, March–December 2020 (*n*=18,105).

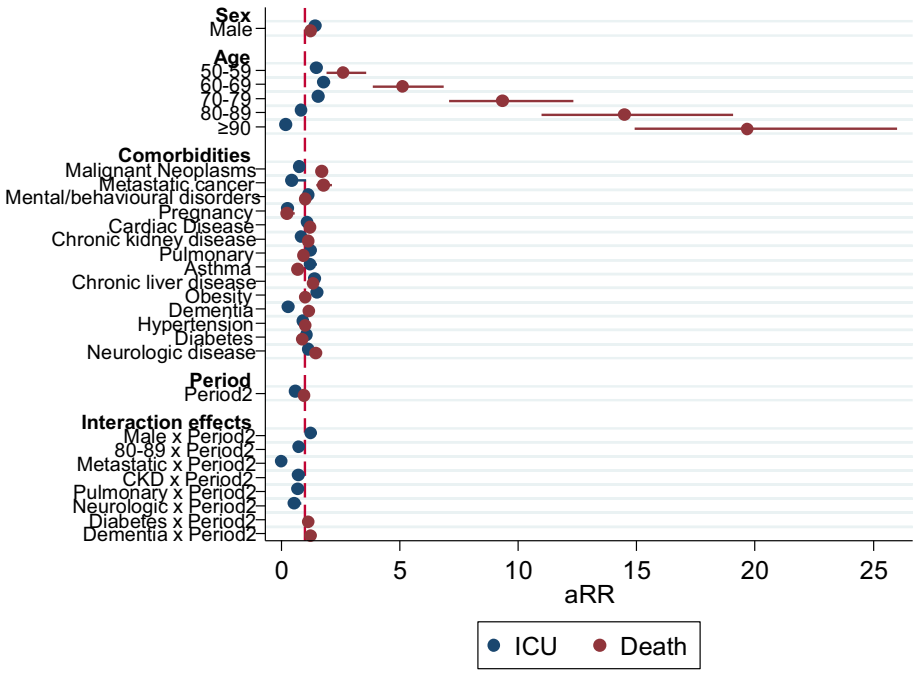

FIGURE 3
Forest plot representing the aRR of the adjusted model for the outcome death, including significant interactions between Period and other variables, Portugal, March–December 2020 (*n*=18,105).

includes less severe disease. Specifically, diabetes includes complicated and uncomplicated subcategories. In the first period it is possible that respiratory disease had a lower admission threshold because of a precautionary approach since in the second period there was a large reduction in adjusted risk for ICU admission in those with respiratory disease. However, other comorbidities such as cardiac disease and chronic kidney disease did not increase risk of ICU admission but increased the risk of death, which raises further

TABLE 3 Fully adjusted model for the outcome death including interactions between Period and other variables, Portugal, March–December 2020 (n=18,105).

Death	aRR	p-value	[95% conf. interval]
Sex			
Female			Ref
Male	1.24	<0.001	[1.18–1.32]
Age			
0–49			Ref
50–59	2.62	<0.001	[1.90–3.59]
60–60	5.15	<0.001	[3.86–6.86]
70–79	9.35	<0.001	[7.09–12.34]
80–89	14.49	<0.001	[11.00–19.08]
≥90	19.69	<0.001	[14.91–25.99]
Comorbidities			
Malignant neoplasms	1.71	<0.001	[1.57–1.85]
Metastatic cancer	1.78	<0.001	[1.47–2.15]
Mental/behavioral disease	1.02	0.58	[0.95–1.09]
Pregnancy	0.23	<0.001	[0.09–0.58]
Cardiac disease (heart failure)	1.24	<0.001	[1.15–1.34]
Chronic kidney disease (renal failure)	1.16	<0.001	[1.09–1.24]
Chronic pulmonary disease	0.94	0.18	[0.86–1.03]
Asthma	0.69	<0.001	[0.56–0.85]
Chronic liver disease	1.36	<0.001	[1.23–1.51]
Obesity	1.03	0.42	[0.96–1.10]
Dementia	1.17	<0.001	[1.07–1.28]
Hypertension	1.02	0.58	[0.94–1.11]
Diabetes	0.89	0.03	[0.81–0,99]
Neurologic disease	1.46	<0.001	[1.34–1.60]
Period			
Period2	0.98	0.59	[0.91–1.05]
Effect modification by the period			
Diabetes*Period2	1.16	0.02	[1.03–1.30]
Dementia*Period2	1.26	0.00	[1.12–1.41]

research questions related to patient ICU admission practices and outcomes.

We found that Neurologic Disease, Cancer, CKD, cardiac disease, liver disease, and dementia were important risk factors for death. Interestingly, the effect of dementia was modified by the period of analysis and the risk of death was even higher in the second period. This may be due to lower threshold for hospital admission of cases with dementia due to increase in hospital occupancy and due to changes in clinical management of cases outside of the hospital and of admitted cases that may have increased risk of death. It is also possible that disease severity in this group increased during the second period among cases in specific settings such as nursing homes in outbreak contexts; this could be related to variant severity profile, higher infectious dose exposure, or other non-observed factors related to pre-hospital and hospital clinical management.

Conversely, Cardiac Disease, CKD, malignant neoplasms, metastatic disease, and dementia had no association or a negative association with ICU admission but all increased the risk of death.

The risk of ICU admission decreases in older ages, as found in other Portuguese cohort studies of all confirmed cases in the first COVID-19 wave in Portugal (27). That reduction was larger during the second period (October–December). It is possible that some older patients may die without meeting criteria for ICU admission or eventually, many who end up meeting those criteria may die before they can be admitted. Furthermore, they may be no expected clinical benefit and very low recovery expectations if admitted to the ICU. Debate has been ongoing on this topic considering the challenges and ethics of admitting patients of a very advanced age to ICU, patient and family wishes, and therapeutic futility (28–32). Portuguese guidelines considered the existence of comorbidities for hospital admission but the criteria for admission to the ICU include mainly clinical severity criteria (33) which could impact the estimates for comorbidities for the two outcomes.

Combined, these findings suggest that there are differences in ICU admission thresholds for different patient characteristics or different expected benefits from admission, in different periods with higher and lower hospital and bed occupancy rates. This should remain relevant even if severity profile at admission changed for specific patient profiles. Few studies describe outcomes of ICU admission for different patient profiles with COVID-19 and continuous research in this area remains necessary.

This study has strengths and limitations. We used information extracted from the national electronic records of patients' hospitalizations that uses ICD-10 codes for comorbidities (coded by trained medical doctors after discharge, from clinical notes, through a standardized procedure in accordance with ICD-10 coding guidelines) (21). This assures good quality of comorbidity and outcome data.

By using Elixhauser (34) comorbidity Index categories, we intend to reduce arbitrariness in categorizing ICD-10 comorbidities by relying on a validated comorbidity index used to predict risk of severe outcomes. This may also facilitate replication of studies. A recent study compared Charlston Comorbidity Index categories and Elixhauser categories and found similar comorbidity risks for death (35). However, as previously described, using Elixhauser categories may include a range of clinical entities and clinical severities in some groups, that may include milder comorbidities, possibly underestimating risk for more specific comorbidities within those categories.

There are limitations in this study. Firstly, hospitalized patient characteristics at admission may have changed over time. This means that risk estimates for outcomes are subject to variation by this factor as previously discussed. For example, the first hospitalized patients in Portugal were hospitalized without severe disease for isolation purposes. However, this situation comprises a minimal number of cases in the first week of cases in Portugal and cannot produce relevant selection bias. However, in different periods, the clinical thresholds for general hospital admission may have varied differentially for different patient characteristics due to higher hospital burden and stretch of capacity. This could contribute to a more severe clinical profile at admission for specific comorbidities and age groups that could influence the change in risk estimates for ICU admission and death in the two analyzed periods.

Used data comprised all patients with a COVID-19 diagnosis registered in ICD-10 (21). It is possible that some patients were admitted for other conditions not related to COVID-19 infection. However, during this period of the pandemic, most hospitalized patients with COVID-19 were admitted due to COVID-19 symptoms, decompensation of chronic disease due to COVID-19 infection, or complications arising from the infection. Still, in theory, this could introduce bias in estimates of risk if COVID-19-infected patients with other reasons for admission had a systematically lower or higher risk of ICU admission or death. If patients with specific ages or comorbidities had more often been admitted for a condition that was not related to COVID-19, and systematically had lower risk of ICU admission or death, this would underestimate risk for those ages or comorbidities. However, in our study, COVID-19 (U07.1) was sequenced as the first diagnosis in 15,299 admissions, the second in 1,075, and the third in 744. These comprise approximately 95% of analyzed admissions, making the analysis robust for the purpose of exploring differences in risk factors in two periods for the outcomes of interest for COVID-19 patients.

ICD-10 coding guidelines only reccomends code (U07.1) COVID-19 as first diagnosis when COVID-19 meets the definition of principal diagnosis, when the reason for the encounter/admission is a respiratory manifestation of COVID-19. COVID-19 that is identified in screening or acquired in hospital, asymptomatic COVID-19, and probable COVID-19 are not assigned the (U07.1) COVID-19 code. A patient with sepsis, obstetrics, or transplant complications due to COVID-19 will be coded with those codes as first diagnosis. Decompensation of a relevant comorbidity atributed to COVID-19 will usually imply COVID-19 as one of the first diagnosis.

There are relevant comorbidities that we could not adjust for that have been previously found to be of relevance for the COVID-19 severity outcomes, such as economic deprivation (16) and minority ethnic groups (16, 36).

This is one of few studies comparing how risk changed for ICU admission and death among hospitalized COVID-19 patients in periods with high and low hospital burden. Further research is warranted to understand possible changes in clinical practice for older patients, women, and patients with specific comorbidities in periods of higher hospital burden to inform human resource planning and health practice and policy. Chances of survival in ICU should be further researched to help priority setting in increased burden contexts while aiming to increase capacity to guarantee access to all who may benefit from ICU and hospital admission in general. Further research is needed to understand how increase in hospital burden and bed occupancy may impact admissions and probability of ICU admission or death and what type of patients may have larger benefit from ICU admission. Personal and family considerations, as well as clinical judgment, are necessary to make decisions on a case-by-case approach but epidemiological sound data should contribute to these decisions.

Evidence that increased hospital burden has a negative impact on clinical management and outcomes for both COVID and non-COVID-conditions is relevant to inform control measures facing scenarios of increased hospital burden by COVID and other infectious respiratory diseases or due to reduced human resources. When facing increased burden, healthcare professionals will inevitably have to manage limited technical and human resources.

Conclusion

In this study, age was the strongest single risk factor for death among hospitalized patients. For ICU admission, after 60–69 years, the risk of ICU admissions starts reducing and becomes protective after 80 years old, but risk of death increases continuously with age. In the second analyzed period, probability of ICU admission was significantly lower, specially for older age groups, women, and those with specific comorbidities and the risk of death increased in the second period for those with dementia and diabetes. These findings may imply changes in clinical practice due to increased hospital burden or changes in clinical severity of hospitalized patients with specific characteristics, and should be further investigated. Further research on changes in determinants of admission, ICU admission, and death may improve understanding on how different severity profiles, increased hospital burden, or reduction of healthcare workforce and increased patient-staff ratio may affect clinical practice and outcomes to inform preparedness, healthcare workforce planning, prevention measures, and healthcare practice and policy.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: anonymized national hospital admissions database, shared with academia under a COVID-19 research protocol. Requests to access these datasets should be directed to geral@acss.min-saude.pt.

Author contributions

VR, AV, PA, AA, and CN: study design. VR and PA: analysis. VR: writing of first draft. VR, AV, PA, CC, DT, PS, CN, and AA: reviews and other significant contributions. All authors contributed to the article and approved the submitted version.

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In memoriam

In Memoriam of Professor Carla Nunes.

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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EDITED BY
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

REVIEWED BY
Lisa Kristina Dannenberg,
University Hospital of Düsseldorf, Germany
Eiji Kawamoto,
Mie University Hospital, Japan

*CORRESPONDENCE
Xiaofeng Liu
✉ liuxiaofeng3913@163.com

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Role of sphingosine 1-phosphate (S1P) in sepsis-associated intestinal injury

Gehui Sun[1,2], Bin Wang[1,2], Hongquan Zhu[2,3], Junming Ye[1,4] and Xiaofeng Liu[4,5]*

[1]Gannan Medical University, Ganzhou, Jiangxi, China, [2]The First Affiliated Hospital of Gannan Medical University, Ganzhou, Jiangxi, China, [3]Department of Critical Care Medicine, The First Affiliated Hospital of Gannan Medical University, Ganzhou, Jiangxi, China, [4]Suzhou Medical College of Soochow University, Suzhou, Jiangsu, China, [5]Department of Emergency, The First Affiliated Hospital of Gannan Medical University, Ganzhou, Jiangxi, China

Sphingosine-1-phosphate (S1P) is a widespread lipid signaling molecule that binds to five sphingosine-1-phosphate receptors (S1PRs) to regulate downstream signaling pathways. Sepsis can cause intestinal injury and intestinal injury can aggravate sepsis. Thus, intestinal injury and sepsis are mutually interdependent. S1P is more abundant in intestinal tissues as compared to other tissues, exerts anti-inflammatory effects, promotes immune cell trafficking, and protects the intestinal barrier. Despite the clinical importance of S1P in inflammation, with a very well-defined mechanism in inflammatory bowel disease, their role in sepsis-induced intestinal injury has been relatively unexplored. In addition to regulating lymphocyte exit, the S1P-S1PR pathway has been implicated in the gut microbiota, intestinal epithelial cells (IECs), and immune cells in the lamina propria. This review mainly elaborates on the physiological role of S1P in sepsis, focusing on intestinal injury. We introduce the generation and metabolism of S1P, emphasize the maintenance of intestinal barrier homeostasis in sepsis, and the protective effect of S1P in the intestine. We also review the link between sepsis-induced intestinal injury and S1P-S1PRs signaling, as well as the underlying mechanisms of action. Finally, we discuss how S1PRs affect intestinal function and become targets for future drug development to improve the translational capacity of preclinical studies to the clinic.

KEYWORDS

S1P, sepsis, S1PRs, signaling, intestinal epithelial barrier, immune

1. Introduction

Sepsis is a life-threatening organ dysfunction syndrome caused by a dysregulated host response to infection, Sepsis is clinically characterized by severe infection, systemic inflammatory response, and organ dysfunction, and is associated with high morbidity and mortality (1, 2). In ICU ward, clinicians usually use SOFA score to judge the prognosis of patients, the international Sepsis3.0 definition proposes that sepsis can be diagnosed when the patient reaches "infection and SOFA score $\geq$ 2" (3). An estimated 31.5 million cases of

sepsis and 19.4 million cases of severe sepsis have been reported worldwide. Sepsis accounts for potentially 5.3 million deaths annually and the mortality rate among patients with sepsis in ICU Ward can reach 41.9% (2, 4). Incidence of sepsis will continue to rise with the increasing ages of the populations and corresponding rise in the underlying diseases (5). Neglect in the disparities in sepsis cases in developing and middle-income countries and the lack of epidemiological data on sepsis in low- and middle-income countries can lead to underestimation of incidence of sepsis and associated mortality (2, 4). The intestine is the largest immune barrier organ in the human body and is the home for a microbiome composed of more than 100 trillion species of bacteria. The host and the bacteria are mutually beneficial and symbiotic, under normal physiological conditions, the intestinal environment maintains its balance. The intestinal barrier can protect the organism from intestinal microbes and toxins (6, 7). The earlier studies have indicated that the gut is the driving force for multiple organ dysfunction in critical illness (8). Intensive research on sepsis has demonstrated intestinal barrier dysfunction to be a common complication in sepsis (9). Intestinal injury is an early marker in the development of systemic inflammatory response syndrome and multiple organ failure (MOF) (10). Sepsis causes disturbance of the gastrointestinal microenvironment, causing an imbalance between the host and the gut microbiota, which further leads to compromised intestinal barrier function and reduced intestinal immunity (11, 12). Meanwhile, studies have shown that sepsis is associated with a higher mortality rate caused by gastrointestinal injury (13).

Sphingosine-1-phosphate (S1P) is a lipid substance that is widely present in human body fluids, tissues, and cells and is responsible for conveying intercellular information as a first messenger (14). During the pathogenic process of sepsis, S1P maintains the integrity of vascular endothelial cells (ECs), promotes lymphocyte circulation, reduces systemic inflammatory responses, as well as protects the integrity of the intestinal barrier (15, 16). With the development of drugs targeting S1P and agonists and antagonists of Sphingosine-1-phosphate receptors (S1PRs), several therapeutic strategies such as FTY720, an agonist of S1PRs, which improves systemic conditions and reduces local inflammation in the intestine of mice have been proposed for treating inflammatory bowel disease (17, 18). Currently, available clinical trials have demonstrated decreased plasma concentrations of S1P in patients with sepsis compared with normal individuals (19), and have also indicated that the S1P levels are further lowered with increasing severity of sepsis (20, 21). Recent studies have confirmed that S1P alleviates LPS-induced intestinal epithelial cell injury, maintains the colonic mucosal barrier, and prevents intestinal injury during sepsis (22). S1P binding to different S1PRs activates downstream signaling pathways involved in cell proliferation, migration, apoptosis, immune regulation, anti-inflammation, and information transmission processes, showing multiple effects (14, 23). Studies have shown that the intestine is an immune organ and S1P can attenuate sepsis intestinal injury by regulating immune responses (24). Therefore, understanding the role of S1P in the septic gut is of great significance for the treatment of sepsis and sepsis-associated intestinal injury (23, 25). In this review, we have summarized the recent research progress on protective effects of the epithelial barrier and potential therapeutic targets of S1P in sepsis-associated intestinal injury.

2. S1P generation and metabolism

Sphingosine 1-phosphate is synthesized either by a *de novo* pathway from serine and palmitoyl-CoA or produced by a the sphingomyelinase (SMase) pathway from the ubiquitous membrane lipid sphingomyelin (SM), ceramide (Cer), and sphingosine (Sph) are intermediates the sphingomyelinase in both pathways (26). Sph can be recovered by acylation, which is called "salvage pathway," leading to regeneration of Cer (27, 28). This is followed by hydrolysis of Cer to generate Sph, and finally Sph generates S1P following the action of sphingosine kinases (SphKs) in a process that occurs in the cell membrane and cytoplasm (29) (**Figure 1**, By Figdraw). Ultimately, S1P is either cleaved by S1P lyase or gets dephosphorylated to Sph by S1P phosphatase (30, 31). SMase pathway begins with sphingolipids (SLs), which are ubiquitous structural components in cell membranes, SLs metabolites affect cell apoptosis, cell growth and cell migration (32). Endogenous SLs play crucial roles at multiples stages in cell biological processes and human health (33). With the development of research, it has been found that bioactive SLs, e.g., SM, Sph, Cer, S1P, and ceramide-1-phosphate may derive from dietary SLs ingested through the diet (34). Dietary SLs are ingested and absorbed in the gastrointestinal tract, affecting immune activation status, contributing to pro-inflammatory and anti-inflammatory immune responses, and can be used by cells to regulate growth, differentiation, apoptosis, and other functions (15, 35). Some studies have shown that exogenous SLs may dampen both acute and chronic inflammatory responses in cell and animal models (36, 37). According to earlier studies conducted in the last century, consumption of SLs in the United States has been estimated to be 0.3–0.4 g/day (35). SM in milk has been shown to promote gut developmental maturation (36). Dietary SM is digested by intestinal alkaline SMase (alk-SMase) and neutral ceramidase (n-CDase), and finally hydrolyzed into Cer, choline phosphate, Sph and fatty acids in the small intestine (34). Sph can be completely absorbed into intestinal mucosal cells and transformed into S1P, which is transported to lymph circulation and blood circulation together with chyle particles (38). The metabolism of SLs is affected by the type of fatty acid in diet, vitamin B6, vitamin C, vitamin D, and vitamin K (34, 39).

Sphingosine 1-phosphate is mainly derived from red blood cells, lymphatic endothelial cells and platelets (40), and S1P concentration in blood and lymph fluid is much higher than that in lymphatic tissue, forming S1P gradient (29). Sph is converted to S1P in the intestinal mucosa, where SPHK1/2 and SPL are highly expressed, and where SPL content is also higher than in any other tissue (41, 42). S1P is expressed in epithelial cells of the small intestine and colon mucosa, but the level in the small intestine is more than 2-fold higher than that in the colonic mucosa (43). Due to the abundant blood flow, lymph and lymphocytes in the intestine, which can digest food, and the presence of a large number of intestinal microorganisms, studies have found that dietary SM is mainly destroyed into sphingosine and sphinganine in the jejunum through a large amount of alk-SMase and nCDase released by the intestinal epithelium and liver, which are rapidly absorbed and metabolized by intestinal mucosal cells to generate S1P (44). SL metabolites (including S1P) can also be produced

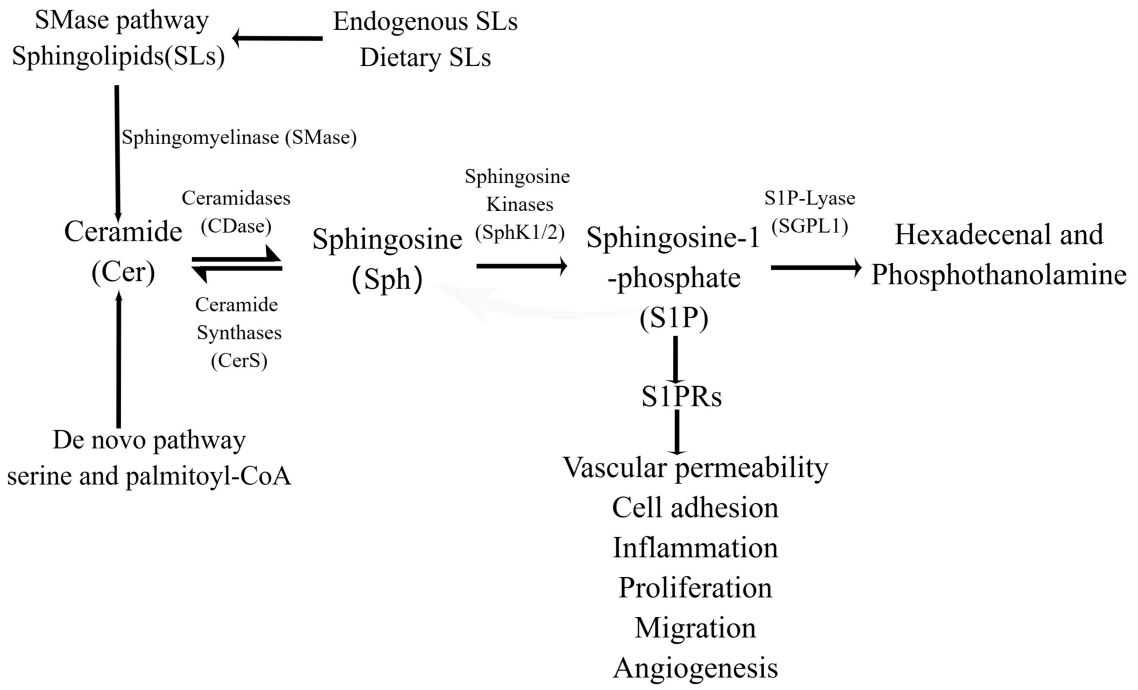

FIGURE 1
The production and function of S1P in the intestine. S1P is generated in the intestine through SMase pathway, *de novo* synthesis and rescue pathway, and the *de novo* synthesis pathway is the main one. In the SMase pathway, bioactive SLs are hydrolyzed to ceramides by SMases, and in the endoplasmic reticulum, serine and palmitoyl-coA are synthesized by the *de novo* pathway. Ceramide is transported to the Golgi apparatus to generate sphingosine under the action of ceramidase, S1P under the catalysis of intracellular SphK1/2, and hexadecenal and phosphoethanolamine under the action of SPL. In addition, sphingosine can also be acylated by ceramide synthetase to form ceramides in the ER, a process known as the "salvage pathway."

by gut microbes and directly affect host metabolic pathways, for example, *Bacteroides fragilis* (15, 45).

Sphingosine 1-phosphate is a bioactive sphingolipid that is present in high-density lipoprotein (HDL), and the main binding carrier is apolipoprotein M (apoM). In plasma, 60% of S1P is normally bound to apoM and 40% binds to albumin (46). S1P needs to bind to these carriers to be transported in the biological fluids (47). Moreover, S1P, being a small polar phospholipid, is transported through the lipid bilayer in a paracrine or autocrine manner, with the aid of the transporter ATP-binding cassette (ABC) transporters and spinster homolog 2 (SPNS2) (48).

Sphingolipids are metabolized in the intestine and are primarily found in the mucosal cells of the small intestine and colon, where their metabolites are absorbed and enter the blood circulation or lymph (39). S1P, which is widely found in the blood and lymph, is more abundant in the gut than in other tissues, and influences the immune status of the intestines (29, 43). The maximum concentration of S1P in circulation cells is found in erythrocytes, EC, immune cells, and platelets, as the activities of S1P lyase and phosphatase are weak within erythrocytes, which ensure that the high concentrations of S1P are maintained (49). Lymphatic ECs are the main source of S1P in the lymph (29). Multiple stimuli during sepsis regulate key enzymes, thereby affecting sphingolipid biosynthesis and metabolism, resulting in restricted S1P production (29). The finding in an earlier study, according to which the S1P levels in the blood of patients with sepsis were lower than normal, could be attributed to the fact that apoM levels are reduced in patients with sepsis and systemic inflammatory response syndrome; because of which S1P plasma levels are also lower (50). In an experiment on sepsis in humans and baboons, it was observed that S1P levels were lower in most septic groups, when compared to normal controls, less by almost 46% in severe septic shock, and a homogeneous distribution of S1P was observed between apoM and albumin. A significant correlation was observed between S1P decline and apoM during sepsis (20). Because hepatocytes express and secrete the majority of apoM and albumin, serum S1P levels were found to decrease sharply with advanced liver disease and predict early death in patients with chronic liver disease, and low S1P levels may significantly affect the progression of multiple organ dysfunction syndrome (MODS) (51). Makoto Kurano's experiment demonstrated that while knockout or knockdown of apoM aggravated lethality and organ damage in lipopolysaccharide (LPS) mice, apoM/S1P improved survival and protected organs by preventing apoptosis (52). While erythrocytes are the major source of S1P in plasma, during sepsis, they also contribute to iatrogenic blood loss, reduced plasma iron, suppressed erythropoietin production and shortened RBC lifespan as well as malnutrition (53), which in turn could account for the reduced S1P levels. Another important cause for reduced S1P levels in sepsis patients could be the fact that sepsis causes acute damage and dysfunction of the gastrointestinal tract, which results in insufficiency of nutritional sources for the patients (54), thereby affecting their nutritional status. However, the baboon model demonstrated no correlation between the number of erythrocytes and S1P. In contrast, platelet levels of S1P in humans closely correlate with those in the baboon sepsis (20).

Human megakaryocytes (MKs) release trillions of platelets into the circulation every day to maintain platelet levels. Studies have revealed that S1PR1 signaling continuously inhibits MKs growth, and when S1P metabolism is disrupted in the hematopoietic environment, S1PR2 signaling can further inhibit thrombosis. S1P generation is related to SphK1 activation (55).

Studies have shown that SphK1 is 15 times higher than SphK2 in normal human body, while the expression of SphK1 in platelets of sepsis patients is significantly reduced, while SphK2 has no significant change (56). The decreased activity of Sphk1 in cells will promote the production of platelets, and the metabolic pathway of S1P production is related to the disease of thrombocytopenia (55). In addition, recent studies have shown that platelets store S1P in the cytoplasm in the resting state, and after platelets are activated, this S1P pool is transported to the plasma membrane to regulate local cellular responses, in which the loss of the major facilitator superfamily transporter 2b (Mfsd2b) of S1P reduces platelet thrombosis (57, 58). In platelets, Sphk1 is responsible for S1P production, and Mfsd2b is responsible for S1P export. It has been shown that platelets release S1P to protect the heart during acute myocardial infarction (AMI), and antiplatelet strategy to preserve platelet S1P release during AMI is the most ideal strategy (59). After acute infection, the activation of coagulation and inflammation is the key cascade, leading to thromboinflammation and microthrombosis. The intestinal tract of septic mice shows strong inflammatory and thrombotic reactions (60), and severe imbalance of coagulation and anticoagulation can lead to disseminated intravascular coagulation (DIC) and death. DIC, as an extreme state of systemic coagulation activation, leads to excessive consumption of platelets due to continuous increase in coagulation activity after its appearance, and eventually forms thrombocytopenia (61). Thrombin has been considered as a potential therapeutic target for sepsis, and studying the thrombotic effects of Mfsd2b and S1P on platelets may be helpful for the treatment of the pathological process of sepsis in the future (62, 63).

It has been reported that SphK1 is 15-fold higher than SphK2 in normal humans, whereas in platelets from sepsis patients, SphK1 expression is significantly reduced with no change in SphK2 Recent studies indicate that platelets produce a large amount of S1P through *de novo* synthesis, which is released after platelet activation, thereby modulating local cellular responses (57). Thus, platelets provide S1P for a short time during sepsis, which may be associated with the activation of SphK1.

3. The five receptors for S1P

Sphingosine 1-phosphate, a metabolite of cell membrane sphingolipids, was reported to be a second messenger that mediates increases in intracellular calcium levels, which transmit signals by binding to S1PRs on the cell membrane surface. However, S1P was also unexpectedly found to function as an intercellular first messenger (64, 65). The five receptors for S1P are G protein-coupled receptor subtypes, and all immune cells express the unique characteristics of S1PRs, which are involved in adaptive immune cell trafficking, angiodevelopment, and homeostasis (14). For T-cells, S1PR1 signals tonically reduce T-cell chemotactic sensitivity to chemokines and thereby limit homing of blood and spleen T-cells to secondary lymphoid tissues (66).

Sphingosine-1-phosphate receptors are differentially expressed in various tissues. S1PR1 is located in most organs, and is expressed at high levels during embryonic development, vascular production and maturation, bone remodeling, and in the immune system (67). S1PR2 plays important roles in inhibiting apoptosis and proliferation, actin remodeling, and development of the cardiovascular, visual and auditory systems (23). Studies have shown that S1PR2 is highly expressed in intestinal epithelial cells (IECs) and promotes their proliferation (68). S1PR3 is found in most organ tissues, and current research focuses on cardiovascular issues, sepsis, cardiac conduction, stroke, and cancer metabolism (69). S1PR4 is mainly expressed in immune organs, and is involved in megakaryocyte differentiation and platelet development (70), and S1PR5 is expressed at high levels in spleen, skin, lung, and brain, plays a role in myelination, and is involved in nervous system expression (71, 72). The expression of S1PRs in intestinal endothelial cells is low, but a variety of S1PR-targeted drugs have high affinity for S1PR1 and S1PR5. Ozanimod, for example, has been approved for the first time in the United States, European Union and other countries for the treatment of moderate to severe active ulcerative colitis in adults, the main mechanism is to bind to S1PR1 and S1PR5 receptors. Internalization of S1PR1 reduces the ability of lymphocytes to secrete from lymphoid tissues (73). In addition, gut microbes affect gut and brain pathophysiology through the microbiome-gut-brain axis (MGBA). The gut microbiome of patients with sepsis may contribute to the pathogenesis of sepsis-associated encephalopathy (SAE). The study of septic intestinal injury will also help researchers decipher SAE (74).

The S1P-S1PR signaling system contributes to the regulation of complex inflammatory processes by influencing lymphocyte trafficking and maintenance of vascular integrity. thereby S1PR1-3 is mainly expressed by ECs, and S1PR5 is expressed only by blood-brain-barrier cells (16). Under inflammatory conditions, S1PR1-3 are widely distributed, with the highest expression levels in the cardiovascular and immune systems while S1PR4 and S1PR5 are relatively less expressed (75). The downstream signaling of these receptors is very complex because they are expressed differently in ECs and are coupled to multiple G proteins. For example, S1P in the blood circulation binds to G protein-coupled receptors on the cell membrane of intestinal ECs (**Figure 2**, By Figdraw). After coupling with the G αi/o, S1P-S1PR1 activates the downstream Phosphoinositide 3-kinase (PI3K), phospholipase C (PLC), and Rac families, leading to Rac-dependent cytoskeletal rearrangements and cell matrix contact. It also affects the adherens junction assembly, and barrier integrity, thereby enhancing the EC barrier (71, 76). However, S1PR2 and S1PR3 bind to G αi/o, G αq, and G $\alpha_{12/13}$, which combined with G αq activate PLC and increase the intracellular calcium concentration, leading to vasoconstriction, decreased vascular permeability, and weakened barrier function. Both S1PR2 and S1PR3, upon binding with G $\alpha_{12/13}$, activate the Rho family to promote the transition of ECs to a contractile phenotype, and attenuate inter-endothelial junctions. Both pathways disrupt the capillary endothelial barrier by increasing vascular permeability. Interestingly, the two pathways mentioned above have the exactly opposite effect of combining G αi/o production (71, 77). S1PR4 and S1PR5 bind to the G $\alpha_{i/o}$, G $\alpha_{12/13}$ protein pathway, causing weakening of the EC barrier. The

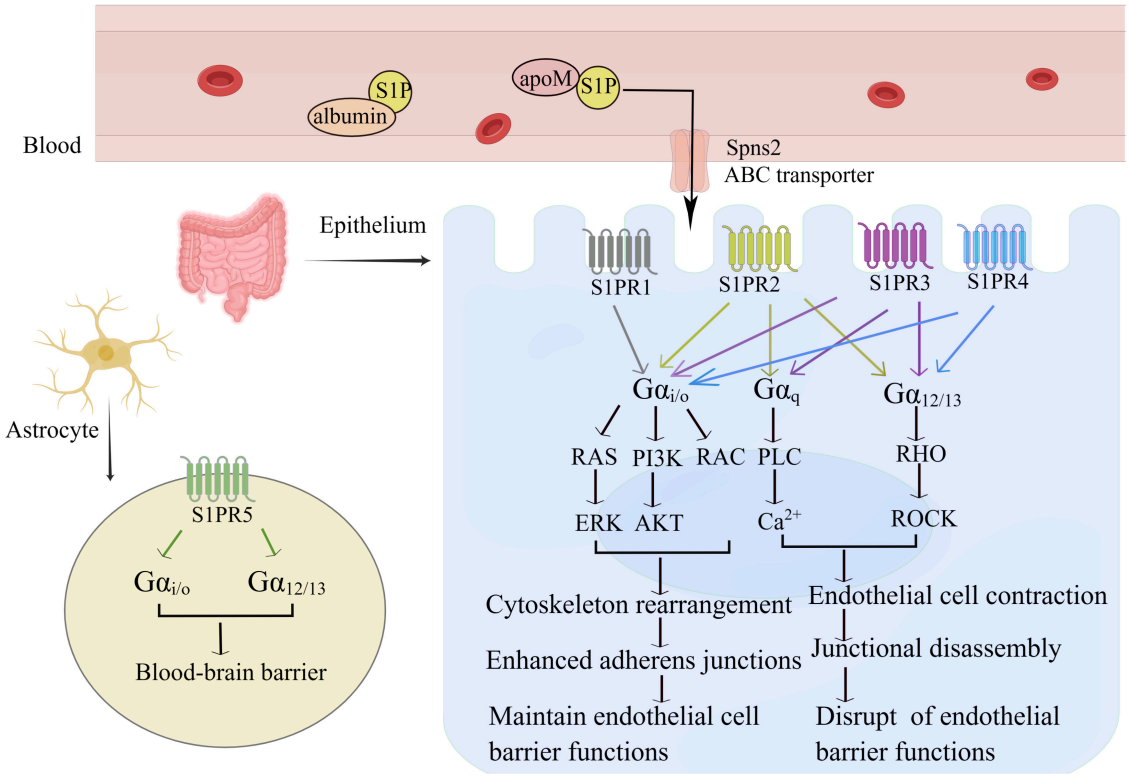

FIGURE 2
S1P maintains the intestinal intraepithelial EC barrier. S1P binds to five receptors and transmits different intracellular signals depending on the G protein-coupled G subunit and the expression pattern of each receptor in the cell type. S1PR1 is mainly coupled to G αi/o and promotes vascular stability via cytoskeleton rearrangement adherens junctions, and limits plasma and leukocyte extravasation; S1PR2 and S1PR3 are involved in G αi/o, G αq and G α12/13 pathways, S1PR4 connects G αi/o and G α12/13 pathways, interacts with Rho, leading to a diverse network of signals. S1PR2-4 can cause the contraction of vascular ECs, increase vascular permeability, and weaken endothelial barrier function. S1PR5 is mainly expressed in astrocytes and enhances the blood-brain barrier.

differential but overlapping expression patterns of the five receptors form the molecular basis for the different S1P functions.

4. The protective effect of S1P in sepsis

4.1. S1P protects the EC barrier in sepsis

The vascular surface is covered by a layer of ECs, which are responsible for the flow of water and solutes between the blood and the interstitium. The cells attached to the basement membrane and connected to the extracellular matrix (ECM) by focal adhesion (FA) (78). Adjacent ECs are connected by the three types of interendothelial junctions, namely, adherens junctions (AJ), tight junctions (TJ), and gap junctions (GJ) (79). Alterations in cell-cell junction expression leads to changes in EC barrier permeability. Sepsis is usually marked by increased vascular permeability resulting from a disrupted EC barrier due to changes in interendothelial junctions leading to leakage of water and proteins, resulting in tissue and organ damage (80). S1P promotes EC migration, reduces vascular permeability in sepsis, and plays an important role in maintaining EC barrier integrity (75, 81). The earlier studies have shown that S1P promotes cell migration during vascular development in zebrafish (82) and recent studies have demonstrated that S1P-S1PR1 signaling promotes cellular AJ by altering actin rearrangements in ECs, which results in the expression of molecules such as vascular endothelial cadherin (16). Liu et al. (83) hypothesized that activation of S1PR1 by S1P has a proangiogenic effect through experiments in mice in which S1PR1 knockout mice developed embryonic hemorrhage leading to intrauterine death, which was supported by studies demonstrating reduced vascular permeability after administration of exogenous S1P (84). The severity of the impairment of endothelial function in sepsis increases with a decrease in S1P concentration in the plasma (85). S1P participates in the sepsis disease process by maintaining the vascular endothelial barrier (16, 75).

In addition, S1P has been shown to have contractile effects in arteries of a variety of animals (86), angiotensin II (AngII) treatment leads to hypertension (BP) associated with increased plasma S1P and circulating T cell counts, and SphK2 activity is critical for AngII-induced lymphocyte trafficking. Moreover, the dysregulation of SphK2 expression is related to the thrombotic inflammatory phenotype of microvessels and the functional changes of small resistance arteries, leading to the development of hypertension (87). Ang II infusion can cause S1P to convert PRR into soluble PRR (sPRR) in the cells, which eventually promotes the increase of blood pressure through various reactions. However, the S1P inhibitor PF429242 could not achieve the pressor effect

by blocking the release of sPRR and the transformation of Ang I. The above results indicate that S1P and its signaling axis play an important role in blood pressure regulation (88, 89).

4.2. S1P promotes multiple immune cell trafficking in sepsis

Sepsis process is divided into two immune phases, including an initial immune activation phase, followed by an immunosuppressive phase that culminates in immune cell death (90). Cells of both the innate and adaptive immune systems play key roles in the host response to sepsis (91). In the early stage of sepsis, necrotic tissues and microorganisms release damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), leading to rapid activation of pattern recognition receptors (PRRs), including Toll-like receptors expressed by cells of the innate immune system (92), macrophages, dendritic cells. Neutrophils are stimulated to undergo hyper proliferation and clear the outbreak of infection from the body as soon as possible. Innate immune activation is followed by activation of the adaptive immune system, leading to T cell receptor activation of T helper and cytotoxic T cells, with cell differentiation and proliferation, resulting in a specific adaptive immune response. In addition, immune cells produce and release large amounts of inflammatory mediators, such as IL-1β, IL-2, IL-6, TNF-α, and chemokines, as well as cytokines acting on the ECs to increase vascular permeability. During sepsis, local inflammation gives way to a systemic response, leading to a widespread infection resulting in an inflammatory syndrome (91). Extensive vasodilation leads to hypoperfusion and tissue hypoxia, causing disseminated intravascular coagulation and MODS (93).

Sphingosine 1-phosphate is considered a circulating marker that signals immune cells to help them find blood and lymphatic vessels, and signals the ECs to stabilize the vasculature (94). S1P is involved in T cell migration and promotes the development, differentiation, trafficking, and other processes of B cells, NK cells, and neutrophils. S1P is distributed at various sites in varying concentrations, and in immune responses S1P follows a concentration gradient, transporting T cells from lymph node egress (low concentration) into lymph fluid (high concentration), and ultimately through the blood into the infected tissues, thereby defending against foreign pathogen invasion (24). As early as 2004, it has been demonstrated that S1PR1-deficient mice lack T cells in the peripheral blood and are unable to deliver mature T cells to the blood (95). S1P-S1PR1 signaling was subsequently found to regulate T cell migration and was found to be involved in the regulation of B cells, NK cells, and also the development and differentiation of leukocytes, antigen-presenting cells, and other immune responses (96). When lymphocytes are stimulated by inflammation, which temporarily shuts down the lymphoid organ egress of lymphocytes, the activation marker CD69 binds S1PR1 and promotes S1P internalization to exert anti-inflammatory effects. Also, the presence of an S1P concentration gradient *in vivo* enables the T cells to stay in the lymph nodes for an extended period of time, and allows them to remain at the inflammatory site, and does not affect transcription, translation, and modification (24, 97). Most immune cells express S1PR1, S1PR2 activates Rho and inhibits migration, confines cells to germinal centers, and regulates B cell migration and positioning (98), S1PR5 regulates NK cell egress and NK cell localization in LNs, favoring bacterial clearance (99), whereas the other receptors are restricted to the expression pattern of immune cell subsets. Moreover, a recent study has demonstrated that S1PR5 inhibits the formation of Tissue-resident memory T (TRM) cells and blocking S1P signaling in TRM is beneficial for the treatment of chronic inflammation (100). S1P induces chemotaxis of immature cells and regulates cytokine release in mature human dendritic cells (DCs) to generate Th2 immune responses (101).

4.3. S1P pathway suppresses sepsis-induced inflammatory factor storm

SphKs-S1P-S1PRs signaling suppresses sepsis-induced cytokine storm and provides a new potential therapeutic target for sepsis treatment (102, 103). Inflammatory factor storm is a major cause of mortality in patients suffering from severe sepsis. The mechanism involves an over activation of immune cells, causing release of a large amount of inflammatory factor in the cells, which strongly attack the infected cells to cause MODS. Prevention of the inflammatory factor storm has always been a critical aspect in the field of sepsis research (104). S1PR1 agonist CYM5442 markedly suppressed the exaggerated inflammatory response upon H1N1 influenza challenge even in the early H1N1 influenza phase, providing *in vivo* protection with reduced mortality (103). Several studies have demonstrated reversal of COVID-19 complications and improved survival by modulation of SphKs and S1P-S1PR pathways (103, 105) in the recent outbreak of COVID-19 pandemic, also characterized by an inflammatory storm, hyperinflammation and septic shock (106, 107). A recent report proposed that the lower the S1P level in the blood, the more severe was the condition of the COVID-19 patient (108). S1P exerts opposite effects by binding to different receptor, and when S1P binds to S1PR1, it inhibits TNF-α responses, to produce anti-inflammatory effects (109).

5. S1p signal pathways and barrier homeostasis in the gut

5.1. The intestinal barrier in intestinal injury in sepsis

The intestinal barrier function consists of three main lines of defense. Firstly, the biological barrier, which consists of the normal intestinal flora (gut microbiota). The microbiota not only inhibits the growth of potentially pathogenic bacteria, but also exerts important metabolic, immune, and gut protective functions such as synthesizing energy substances required by the intestinal epithelium to produce PAMP for promoting gut immune regulation and interacting with the immune system (110). Secondly, a mechanical barrier, consisting of IECs and capillary ECs, with an intact intestinal mucosal epithelium and TJs between

cells, which restricts the passage of ions, molecules, and cells through the paracellular space (9). The epithelial cells are not only involved in the direct defense against microorganisms, but also send signals to the mucosal immune system by producing cytokines and chemokines (111). Thirdly, the chemical barrier consisting of gastric acid, bile, trypsin, lysozyme, and intestinal fluid in the GI tract, as well as several organic acids produced by the gut flora (112). Fourthly, the immune barrier, composed of gut associated lymphoid tissue (GALT), T cells, B cells, innate lymphoid cells, and macrophages and dendritic cells in the lamina propria, which interacts with the microbiota to combat the invasion of foreign substances from the gut (113).

The epithelium, immune system, and microbiome are essential for maintaining homeostasis in the gut (114). Any irregularities in these can cause dysregulation of the immune system, interruption of intestinal epithelial homeostasis, uncontrolled bacterial colonization, and epithelial barrier dysfunction, leading to intestinal damage (10, 115). Under the stress state of severe infection and inflammatory response, the protective function of the intestinal mucosal barrier is weakened, and then the intestinal bacterial translocation occurs. Endotoxin, bacteria and antibody mediators continue to enter the blood and lymph, leading to the release of a variety of inflammatory mediators, inducing the production of a variety of cytokines, activating the inflammatory cascade and activating the acquired immune system. As a result, the body cannot effectively regulate the regulation of inflammation and immune response, which has become the key factor of MODS in sepsis patients (116, 117). Septic shock is characterized by endothelial glycocalyx breakdown and endothelial damage, leading to fluid extravasation, organ failure, and death. Several studies have pointed out that endothelial damage plays a critical role in sepsis-induced organ failure (118). Therefore, alteration of intercellular junction proteins leading to the disruption of intestinal membrane permeability is a critical factor in sepsis pathogenesis and can serve as an early marker in the development of MOF.

5.2. S1P maintains the intestinal barrier in septic intestinal injuries

Intestinal function mainly includes food digestion and absorption, material exchange and metabolism, preventing bacterial translocation, and maintaining an intact intestinal mucosal barrier, which is important with regards to sepsis (119). SM regulates the proliferation and migration of intestinal mucosal epithelium by participating in immune activation and participating in pro- and anti-inflammatory responses (15). The sphingolipids in food are digested and absorbed in the intestine to generate Sph and S1P, SLs and their metabolites alleviate inflammation by altering the intestinal microenvironment, protecting the intestinal barrier, and activating anti-inflammatory pathways (120–122).

Inflammatory stress in sepsis can cause intestinal epithelial apoptosis, intestinal injury and translocation of bacterial toxins, which can lead to intestinal organ dysfunction (123). Studies have found S1P to be a barrier enhancing molecule, in sepsis intestinal injury. S1P mainly operates through one of the following three mechanisms (**Figure 3**, By Figdraw): (1) promotes intercellular junctions strengthens the barrier of the intestinal ECs; (2) involved in the survival, differentiation, migration, and proliferation of a variety of immune cells in the gut; (3) reduces damage caused by the gut microbiome, maintains the barrier function of the intestinal epithelium thus alleviating sepsis-associated intestinal injury, thereby improving sepsis survival (16, 19, 118, 124). The mechanism of intestinal injury is mainly hemodynamic. Cellular alterations, and bacterial toxins cause intestinal edema and intestinal dysfunction by disrupting normal intercellular junctions, reducing vascular endothelial cadherin and TJ and increasing vascular permeability (13). S1P acts on the receptors S1PR1-3 present on the ECs to activate downstream signaling pathways, reduces the production of reactive oxygen species (ROS), nitric oxide (NO), and hydrogen sulfide (H2S), and reduces vascular permeability to maintain the EC barrier (125, 126). It is important to note that at the level of AJ, S1PR1 is an important vascular barrier protective mechanism (127). In addition, the binding of S1P-S1PRs has been reported to induce NO production and inhibit ROS production thereby exerting vascular protection effects with the participation of HDL (128). Vascular leakage is a feature of severe sepsis and SIRS, that can lead to a distributive S1P can preserve endothelial function, induce tight junction formation, and prevent vascular leakage (129). In a clinical trial, it was found that the plasma concentrations of syndecan-1 (SYN-1) and VE-cadherin (endothelial cell junctions) in septic shock patients significantly increased within 7 days, patients with more severe diseases have higher concentrations of SYN-1 and VE-cadherin in their plasma, while lower levels of S1P, demonstrate a close correlation between S1P and endothelial damage in patients with septic shock (118). In addition, studies have shown that the lower the S1P level, the higher the SOFA score, and the more severe the condition, S1P levels can predict the mortality rate of sepsis (56). Transgenic mice with low plasma S1P levels are more susceptible to lipopolysaccharide (LPS) infection as compared to wild-type mice. FTY720 or S1P treatment can inhibit vascular leakage and inflammatory responses in mice or rabbits subjected to LPS treatment (75).

Sphingosine 1-phosphate concentrations in tissues increase in the early stage of the inflammatory response and inhibit the entry of T cells into lymphocytes in intestinal tissues, S1P levels in tissues reduce during the resolution of inflammation (130). S1P-S1PRs signaling has multiple effects, and S1PR1 inhibits the trafficking of T lymphocytes to inflamed tissues (96), thus alleviating intestinal injury. S1PR1 is also involved in and promotes macrophage recruitment, apoptosis and anti-inflammatory responses and dendritic cell trafficking (131). S1PR2 enhances the phagocytosis of macrophages and regulates mononuclear cell migration by reducing IFN- γ induced intestinal mucosal permeability damage (132). However, S1PR3 causes increased mortality in sepsis patients by delaying the maturation of phagosomes in macrophages (133). S1PR4 regulates monocyte and neutrophil recruitment, and S1PR5 is expressed by dendritic cells and natural killer cells, accelerating the withdrawal of natural killer cells from bone marrow and monocyte transport (134). Research has found that the alkaloid berberine, found in plants is associated with the metabolism of S1P and the regulation of intestinal immune response and inflammation related pathology; S1P regulates intracellular calcium levels, cell movement, proliferation, and apoptosis to maintain gastrointestinal immune homeostasis (15). In addition, nuclear peroxisome proliferator-activated-receptor-γ (PPAR-γ) is widely expressed in immune cells and ECs, and its main function is

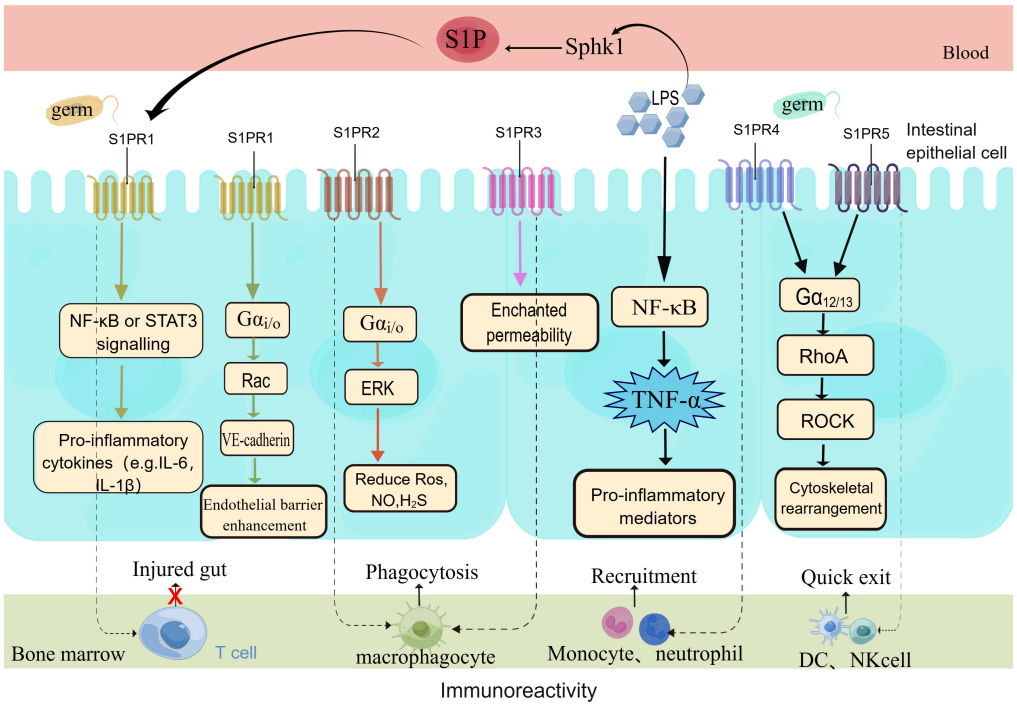

FIGURE 3
S1P protects the intestinal barrier through multiple means. S1P protects the intestinal barrier in multiple ways. Under the stimulation of pathogens, the body rapidly activates the inflammatory pathway and produces a large number of inflammatory factors. The body's immune response is unbalanced in sepsis, according to the different signaling pathways of S1PRs, barrier protection is exerted to restore body homeostasis. Endothelial cells mainly express the S1PR1-3 receptor signal, with the highest expression of S1PR1, which plays a major role in barrier protection. At the same time, the five receptors are involved in the migration of a variety of immune cells (T cells, B cells, macrophages, neutrophils, DC, NK cells.

to sense intracellular nutrient concentration and regulate the expression of the gene involved in maintaining metabolism and immune homeostasis (135). PPAR-γ can weaken the inflammatory response by enhancing the anti-inflammatory effect of dietary SM (120). PPAR- γ is expressed by $CD4^+$T cells, but a selective S1P1 receptor modulator significantly reduces $CD4^+$T cells on lymphocyte subsets in healthy humans (136). Studies have indicated that increased expression of PPAR-γ reduces ROS levels and inhibits the TXNIP/NLRP3 signaling pathway, thereby reducing pyroptosis and liver dysfunction during sepsis (137).

Sepsis seriously affects the composition of the intestinal microbiota, leading to organ failure (138). The intestinal microbiome plays an important role in intestinal injury. Bacterial toxins are released from the infection site into the intestinal tract. Bacterial translocation alters the intestinal microenvironment, and the imbalance in intestinal microbiome flora aggravates intestinal injury (8, 138). Previous research has demonstrated that the activation of SphKs-S1P-S1PR signaling in intestinal inflammation can be correlated to the imbalance in intestinal microbiota, and the corresponding signaling pathway can defend against bacterial toxins and protect the intestinal mucosal barrier (139). At the same time, the gut microbiota is crucial for the development and maturation of the immune system, and the host's immune response can be induced by the imbalance of the gut microbiota. Detection of PAMPs innate immune cells through pattern recognition receptors constitutes the first stage of host response. The endotoxin (LPS), and berberine, which is a commonly used drug for treating intestinal infections, can enhance the expression of S1P and subsequently the downstream signaling pathways, reducing LPS-induced intestinal injury (140).

5.3. S1P signaling pathway in the intestine

The gut plays a central role in the progression of sepsis (8). Sphingolipid signaling, particularly the signal transduction between tumor necrosis factor alpha (TNF-α) and NF-κB involved in S1P is considered as one of the mediators promoting gastrointestinal inflammation (141, 142). S1PR, which is expressed in all gastrointestinal tissues, activates classic inflammation related transcription factors. TNF-α is a major pro-inflammatory cytokine that activates the downstream target SphKs-S1P axis, which is the most commonly studied mechanism of intestinal inflammatory diseases. The TNF- α downstream pathway is the target for the treatment of inflammation related intestinal injury (143, 144). TNF-α signaling is a characteristic to proinflammatory cytokines in innate immunity, which induces the expression of inflammatory genes, and directly drives the inflammatory response to induce cell death (145). Research indicates that S1P affects the generation of lymphatic vessels by promoting the secretion of TNF-α and IL-1β through the NF-κB signaling pathway mediated by S1PR1 (146). Berberine can enhance S1P expression by regulating ApoM-S1P and inhibiting the NF-κB pathway, thereby protecting the damaged intestinal vascular barrier (140). The reason why S1PR-targeted modulators can specifically treat inflammatory bowel disease which

is one of the several autoimmune diseases is mainly associated with the TNF-α pathway (147). In addition, the experimental results indicate that the MHC-II expression is inhibited by the S1P-S1PR2 axis in IECs through extracellular signal-regulated kinase activity, promoting $CD4^{+}$T cell proliferation and protecting intestinal barrier function (148). The S1PR agonist FTY720 reduces vascular leakage and thus maintains the integrity of the barrier, and exerts a protective effect on the inflammatory cascade response caused by mesenteric stress (149). At the same time, a direct relationship was found to exist between gut Th17 cells induced by microbiota and peripheral injury, and the mechanism is that TNF T cells and Th17 cells rely on S1PR1 for excretion from intestinal lymphatic tissue (150).

6. S1P can be an intervention target for gastrointestinal injury in sepsis

A growing number of studies have demonstrated that S1P plays an important role in the vasculature, inflammatory response, immune response, and maintenance of the intestinal mucosal barrier, and is considered a potential therapeutic biomarker for sepsis. This might provide a new direction for the development of targeted therapeutics for sepsis by targeting the S1P-S1PR signaling mechanism (**Table 1**) (19, 126). Four S1PR modulators (fingolimod, siponimod, ozanimod, and ponesimod) have regulatory approval for multiple sclerosis. Targeting S1PRs for the treatment of inflammatory diseases has shown successful results in clinical trials, and they are mainly indicated for treating inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease (151). The immunomodulator FTY720 is a structural analogue of S1P and acts in its phosphorylated isoform as an unselective agonist on S1PR1 and S1PR3-5 and a selective functional antagonist on S1PR1 (152). Fingolimod (FTY720), the most investigated modulator, is efficacious on all subtypes except S1PR2. It inhibits S1PR1 expression after activation of S1PR1 on lymphocytes and limits the egress of lymphocytes from lymphoid tissues, thus reducing lymphocytic infiltration and reducing the extent of damage, and ameliorating local inflammation in the intestine in murine colitis (17, 18). FTY720 improved sepsis incidence and reduced sepsis-induced hypothermia and weight loss. Lymphopenia leads to the accumulation of lymphocytes in peripheral lymph nodes and reduces the bacterial load in the liver, and proinflammatory factors such as plasma IL-6, TNF-α, MCP-1, and IL-10 are significantly reduced (153). In acute lung injury, sepsis-associated encephalopathy and cardiac function injury, FTY720 can be seen to show a protective phenotype in experimental sepsis by regulating vascular and immune functions (154–156). In the latest DSS-induced colitis model study, it was confirmed that FTY720 improved abnormal immune response by capturing T cells and inhibiting the polarization of M1 macrophages in mice with colitis, and this effect has not been clarified in sepsis (157). Previous studies reported that S1P regulates peritoneal B cell trafficking and subsequent intestinal IgA production, and FTY720 significantly reduced the production of natural gut secretory IgA derived from peritoneal B cells, demonstrating the critical role of S1P in intestinal cellular immunity (158). This study found that there is the production of Th17 proinflammatory cells during intestinal inflammation, and FTY720 directly inhibited the differentiation of Th17 cells *in vitro*, while increasing Treg differentiation from naive CD4 + T cells and inhibiting IL-23-mediated activation of STAT4, NF-kB and AKT. In addition, it also inhibited Dectin-1 expression in mature and immature monocyte-derived dendritic cells, which in turn inhibited curdlan-mediated production of IL-23p19, p40, IL-6, and IL-1β cytokines (159). Lymphopenia (T and B cells) induced by FTY720 did not negatively affect mortality from sepsis during challenge with severe abdominal sepsis (160).

The discovery of the S1PR3 receptor mediating bradycardia in mice prompted the search for modulators devoid of S1PR3 signaling (161). The published study demonstrating that the Sphingosine 1-phosphate receptor modulator, siponimod, does not just ameliorate the inflammatory aspect but also the degenerative aspect of secondary progressive MS (162).

Ozanimod, which attenuates immune responses by reducing circulating lymphocytes, has been shown to reduce inflammation and disease severity in animal models of colitis (163). Phase III trials are currently being conducted to test its clinical efficacy in ulcerative colitis, clinical trials on Ozanimod intervention in COVID-19 are also ongoing (25, 164, 165), Furthermore, under conditions of non-interference with protective immunity, targeting specific T lymphocyte subsets of autoimmune disease by ozanimod improves the disease situation (166). Recently, ponesimod another receptor modulator has been highlighted for its safety and efficacy by the continuous research on autoimmune diseases, which specifically activate signal transduction through the S1PR1 pathway (167). Ponesimod can prevent inflammation due to autoimmune reactions and can also cross the blood-brain barrier hindering synaptic neurodegeneration, thus exerting neuroprotective behavior (168, 169).

Since S1P exhibits multiple protective effects in septic intestinal injury, the fatality rate associated with septic intestinal injury can be reduced by elevating S1P levels or using S1PRs modulators (19). With regards to the experimental observation of lowered S1P levels in the plasma of patients with sepsis, some studies have demonstrated that intravenous injection of exogenous S1P into mice caused a transient increase in plasma S1P levels. But this exogenous S1P was cleared out within 15–30 min, because of the short biological half-life of S1P. It was also observed that the S1P degradation was related to the cellular location since intracellular S1P was degraded by SPL, while the extracellular degradation was not related to SPL (170). Treatment of sepsis by direct injection of S1P is not yet feasible, and increasing S1P levels in plasma may either enhance S1P generation by SphKs, or reduce S1P degradation (19). It is important to explore the mechanism through which S1P functions through the immune response for the treatment of sepsis (24). Based on current clinical data on S1PRs modulators, they are thought to reduce inflammation in immune-mediated diseases mainly by blocking lymphocyte egress from lymph nodes to the bloodstream (151, 171). To date, S1PR modulators have made great progress in the treatment of autoimmune intestinal inflammatory diseases, and some molecules have already been approved for use with proven safety and efficacy (169), however, the efficacy of these agents in treating sepsis-associated intestinal injury requires further investigation (23). In addition, the present study found inter-individual functional differences between SphKs and S1PRs, for example, SphK2 inhibits the production of anti-inflammatory

TABLE 1 S1P modulators under development or in clinical testing.

Drug name	Target	Mechanisms	Disease	Influence	Current trial phase	Status
Fingolimod (FTY720)	S1PR1, 3, 4, 5	Functional "antagonist" and "agonist"	RRMS	Restrict lymphocyte egress from the lymph nodes; Reduce inflammatory cell infiltration of the central nervous system.	Marketed	
			Transplant	Decrease circulating alloantigen-reactive T cells without permanently destroying these cells	Phase 3	Completed
			Stroke	Agonist: Mitigate microvascular dysfunction; Agonist: Protect Blood-Brain-Barrier Functions; Agonist: Inhibit of autophagic pathways Agonist: Anti-inflammatory; Antagonist: Lymphopenia	Phase 2	Completed
			CIPD	Reduction in circulating memory T and B cells and increase in regulatory B cells.	Phase 3	Completed
			PPMS	Receptor internalization and degradation; Inhibit lymphocyte migration from lymph nodes	Phase 3	Terminated
			ALS	Decrease circulating lymphocytes	Phase 2	Completed
			Asthma	Reduce antigen-induced airway inflammation; Hyperresponsiveness	Phase 2	Completed
			Sepsis	Maintain the vascular endothelial barrier; Reduce secretion of pro-inflammatory cytokine	NO	
Siponimod	S1PR1, 5	Functional "S1PR1 antagonist" and "S1PR5 agonist"	SPMS	Reduce lymphocyte egress from lymph nodes; Inhibit lymphocyte migration from the periphery to the central nervous system	FDA approved	
			RRMS		Phase 2	Completed
			Active dermatomyositis	Participate in immunity	Phase 2	Terminated
			Hemorrhagic stroke	Anti-inflammatory and Neuroprotective	Phase 2	Completed
			Hepatic impairment	Siponimod were well tolerated with different levels of hepatic impairment	Phase 1	Completed
Ozanimod	S1PR1, 5	Functional "antagonist"	RMS	Induce lymphopenia by preventing lymphocyte egress from lymph nodes	FDA approved	
			UC	Induce peripheral lymphocyte sequestration and reduce circulating lymphocyte counts in the gastrointestinal tract Antagonist: Reduce clinical and histopathological severity of experimental colitis in animal models of human ulcerative colitis and Crohn's disease	Phase 3	Recruiting
			Crohn		Phase 3	Recruiting
			COVID-19	Inhibit the inflammatory pathway	Phase 2	Terminated

(Continued)

TABLE 1 (Continued)

Drug name	Target	Mechanisms	Disease	Influence	Current trial phase	Status
Ponesimod	S1PR1	Functional "S1PR1 agonist"	MS	Internalization of the receptors and inhibit the egress of lymphocytes from lymph nodes; Reduce the number of lymphocytes in peripheral blood; Prevent lymphocytes circulation to sites of inflammation	FDA approved	
			Psoriasis		Phase 2	Completed
ABC294640	SphK2	Functional "SphK2 antagonist"	Cancer and Solid Tumors	Inhibit the growth of tumor cells by blocking some of the enzymes needed for cell growth	Phase 2	Completed
Safingol	SphK1	Functional "SphK1 antagonist"	Locally advanced or metastatic solid tumors	Inhibit the growth of tumor cells by blocking the enzymes necessary for cell growth and by stopping blood flow to the tumor.	Phase 1	Completed
Cerenimod	S1PR1	Functional "S1PR1 agonist"	SLE	Internalization of the receptors and induce a long-lasting inhibition of the egress of lymphocytes from lymphoid organs	Phase 2	Completed

RRMS, relapsing remitting multiple sclerosis; CIPD, chronic inflammatory demyelinating polyradiculoneuropathy; PPMS, primary progressive multiple sclerosis; ALS, amyotrophic lateral sclerosis; SPMS, secondary progressive multiple sclerosis; RMS, relapsing multiple sclerosis; UC, ulcerative colitis; SLE, systemic lupus erythematosus.

factor IFN-β, in contrast to action exerted by SphK1 (102) and S1PR1 exerts anti-inflammatory effects, whereas S1PR2 accelerates the induction of TNF-α activation, which promotes inflammatory responses (172, 173). Therefore, there is a continuous effort to develop new drugs that target the function of different kinases with receptor subtypes, with the aim of providing new therapeutic targets for sepsis-associated intestinal injury (23).

7. Conclusion and perspectives

Sphingosine 1-phosphate as one of the EC barrier reinforcing molecules plays an important role in maintaining barrier integrity. In the pathogenic process of sepsis, S1P reduces the case fatality rate associated with sepsis by suppressing the storm of the inflammatory factor. The gut, as the prime factor in MODS, is often attacked by sepsis bacteria with toxins, causing sepsis-associated intestinal injury and dysfunction. S1P binds to different S1PRs to play different roles, for example, the S1P-S1PR1 pathway regulates multiple immune responses and decreases inflammatory responses and is an effective target for the treatment of sepsis. In intestinal injury, S1PR1 alleviates the inflammatory storm and is involved in diverse immune cell migration. The complex regulatory network of S1P signaling, metabolism, and trafficking have made it difficult to translate the present findings translated into clinical applications. Understanding the mechanisms by which intestinal inflammation, immune responses, and S1P interact could help in the development of novel targets with an aim to reduce the morbidity and mortality associated with intestinal injury in sepsis. In the future we need to explore the signaling pathways of S1P on individual S1PRs in the gut under sepsis conditions and observe the mechanism by which the gut microbiota regulates S1P levels. Whether S1PR modulators can be effective targets for intestinal injury in sepsis requires further study. Because of the short biological half-life of S1P, the current available experimental evidence does not support the direct injection of S1P. The currently developed treatment modalities involving receptor modulators show good stability versus effectiveness. Developing highly selective modulators of S1PRs are the future research directions. Finally, the mechanisms of S1P signaling could provide a high-value regimen superior to traditional therapy for combating sepsis-associated intestinal injury, providing a new avenue for exploring the pathogenesis of the disease and implementing individualized therapeutic strategies.

Author contributions

GS: Writing—original draft. BW: Writing—original draft. HZ: Writing—review and editing. JY: Writing—review and editing. XL: Writing—review and editing.

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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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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Takehiko Oami,
Chiba University, Japan
Martin Helán,
St. Anne's University Hospital, Czechia

*CORRESPONDENCE
Weidong Jin
✉ jwd202110@163.com
Kunchen Wei
✉ w1501966523@outlook.com

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Association between corticosteroid use and 28-day mortality in septic shock patients with gram-negative bacterial infection: a retrospective study

Yi Dong[1,2], Gang Heng[1], Jianxin Zhang[1], Yanbing Shen[1], Zhen Lan[1], Kunchen Wei[3]* and Weidong Jin[1,2]*

[1]Department of General Surgery, General Hospital of Central Theater Command, Wuhan, China, [2]The First School of Clinical Medicine, Southern Medical University, Guangzhou, China, [3]Department of Respiratory and Critical Care Medicine, Changzheng Hospital, Navy Medical University, Shanghai, China

Purpose: Although corticosteroids are recommended in the 2021 Surviving Sepsis Campaign (SSC) guidelines, evidence with respect to their effects on short-term mortality remains conflicting. We conducted this study to identify whether corticosteroids alter 28-day mortality in septic shock patients with gram-negative bacterial infection.

Materials and methods: A total of 621 patients with septic shock and gram-negative bacterial culture results were identified from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database. Propensity score matching (PSM) was performed, and Kaplan–Meier survival curve analyses with log-rank tests were used to determine the relationship between corticosteroid use and the risk of 28-day mortality. Subgroup analyses were conducted to assess whether the conclusions were stable and reliable.

Results: Corticosteroid administration was associated with increased 28-day mortality in septic shock patients with gram-negative bacterial infection (log-rank test $P = 0.028$). The incidence of Stage 2 or 3 AKI and the rate of hospital mortality were higher among patients who received corticosteroids. The incidence of Stage 2 or 3 AKI in the early period significantly mediated the relationship between corticosteroid use and 28-day mortality [$P = 0.046$ for the average causal mediation effect (ACME)]. Interaction tests indicated that the effect of corticosteroid use was maintained in patients with a neutrophil-to-lymphocyte ratio (NLR) of <20 (P-value for interaction $= 0.027$).

Conclusion: Systemic corticosteroid use could be harmful in septic shock patients with gram-negative bacterial infection, especially in patients with relatively low NLR.

KEYWORDS

corticosteroids, gram-negative bacterial infection, septic shock, acute kidney injury, immunosuppression

Introduction

The latest definition of sepsis, as revised in 2021, has directed more attention to the overwhelming host response caused by microbial invasion (1, 2), inspiring clinicians to propose more individualized interventions. Septic shock is a notably life-threatening disorder, with ICU mortality approaching 40% (3, 4), in which most pathogenic organisms reported are bacteria, accounting for over 80% of all sepsis-causing organisms (5, 6). Although the incidence of gram-positive bacteria has shown an upward trend over the past decades, gram-negative bacteria remain the predominant pathogen, with higher ICU mortality compared to gram-positive bacterial infection in sepsis patients (7, 8).

The heterogeneity of clinical characteristics in patients with septic shock results not only from the diversity of susceptible populations but also from the differences in pathophysiology induced by various microorganisms (9). It is acknowledged that endotoxins produced by gram-negative bacteria play a pivotal role in the progression of sepsis, including the release of proinflammatory cytokines, immune suppression, and complement activation (10). Moreover, corticosteroids are regarded as one of the available adjuvant therapies for septic shock and are recommended weakly in septic shock patients with ongoing vasopressor supplementation according to the 2021 Surviving Sepsis Campaign (SSC) guidelines (1). Large-scale randomized trials published in 2018 showed that low-dose corticosteroids brought about benefits in terms of shock reversal and duration of mechanical ventilation support (11, 12). The incidence of the need for mechanical ventilation has also been shown to be reduced with corticosteroid therapy (13). However, these benefits did not contribute to a significant improvement in short-term mortality. The outcomes of previous studies may have been influenced by internal factors such as the immunity and inflammatory status of septic shock patients (14). Since neutrophils are essential for clearance of pathogenic organisms, studies have indicated that monocyte and neutrophil function could be inhibited with endotoxin stimulation (15). Simultaneously, glucocorticoids are also responsible for the reduction of neutrophil activation and migration (16). We therefore speculated that, in the context of endotoxin-induced immunosuppression, the use of corticosteroids may result in more pronounced impairment of immune function and severe rebound of inflammatory factors. Failure to use corticosteroids appropriately when faced with a lipopolysaccharide (LPS) challenge could lead to poorer clinical outcomes.

We initiated our study based on clinical information from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database to investigate the association between systemic corticosteroid treatment and 28-day mortality in septic shock patients with gram-negative bacterial infection.

Materials and methods

Database

We enrolled patients from a retrospectively collected medical database known as the Medical Information Mart for Intensive Care IV (MIMIC-IV version 2.0) (17), which contains detailed medical records of over 40,000 patients who were admitted to the ICU at the Beth Israel Deaconess Medical Center (Boston, USA) between 2008 and 2019. Author Heng has completed the Collaborative Institutional Training Initiative examination (certification number 39516115) and obtained permission to access the database.

Study population

Based on the definition of Sepsis 3.0, we selected adult patients who obtained a total sequential organ failure assessment (SOFA) score of ≥2 points as a consequence of bacterial infection, hyperlactatemia (>2 mmol/L), and who required vasopressors and volume resuscitation to reverse hypotension during the first 24 h of ICU admission. Suspected infection was determined with the combination of body fluid culture records and the timing of commencement of antibiotics (18). Identification of pathogenic organisms was based on the culture results obtained during the period of suspected infection.

The exclusion criteria were as follows: (1) patients aged <18 years old, with an ICU stay of <24 h in duration, or not being admitted to the ICU for the first time; (2) diagnosis, according to the International Classification of Diseases 9th Edition (ICD-9) and ICD-10 codes, with other types of shock (hypovolemic shock, cardiogenic shock, traumatic shock, anaphylactic shock, or postoperative shock) or with adrenal hypofunction, indicating corticosteroid use; (3) fungal infection, viral infection, or gram-positive bacterial infection according to pathogenic organism culture records taken within 72 h before or after the time of suspected infection; and (4) corticosteroid therapy that started over 48 h after the onset of septic shock, non-systemic use of corticosteroids, or etomidate treatment during the period of ongoing septic shock, due to its adrenal-suppressant effects (19).

Data collection

In our study, we collected clinical information including demographic characteristics (age, sex, and weight), comorbidities (diabetes, hypertension, chronic pulmonary disease, malignant disease, and rheumatic disease), SOFA score, Simplified Acute Physiology Score II (SAPS II) score, laboratory values (neutrophils, lymphocytes, platelets, hemoglobin, creatinine, BUN, and lactate), infection sites, pathogenic organisms, and measures of treatment in the first 24 h (equivalent norepinephrine dose, antibiotic use, fluid received, mechanical ventilation support, and renal replacement therapy). Hydrocortisone-equivalent doses were further calculated (0.75 mg dexamethasone = 4.0 mg methylprednisolone = 20 mg hydrocortisone) (20). Data extraction from the MIMIC IV databases was executed using MYSQL version 13.1, Navicat Premium 15.

Primary and secondary outcomes

The primary outcome was 28-day mortality following the first day of admission to the ICU. Secondary endpoints included hospital mortality; length of hospital stay; duration of vasopressor use; use of antibiotics; invasive ventilation support; and incidence of stage 2 and 3 acute kidney injury (AKI) within 72 h after the first 24 h of ICU admission. Evaluation of AKI was based on the KIDGO criteria (21).

Statistical analysis

Baseline characteristics are expressed as median and interquartile range (IQR), or mean and standard deviation (SD) for continuous variables; skewness and kurtosis normality tests were used to evaluate the distribution of data; and categorical variables are presented as frequency and percentage. To compare septic shock patients with corticosteroid use and patients without corticosteroid use, we used the *t*-test or the Mann–Whitney test for continuous variables, and the chi-squared (χ^2) test for categorical variables. With respect to abnormal values, the winsor2 command in the Stata software package was used with a threshold of 1–99%. Missing data were imputed using the missForest method only if the portion missing was <20%; otherwise, the variable in question was abandoned (22).

Propensity score matching (PSM) was conducted using a logistic regression model to eliminate the imbalance of baseline characteristics between patients with corticosteroid use and patients without corticosteroid use; this model was constructed with all possible confounders. Nearest neighbor matching with 1:2 PSM was performed, and the standard variation of propensity score (PS) over 0.02 was considered unmatched (23). Standardized mean difference (SMD) was used to estimate the balance of baseline characteristics after PSM. Once potential bias was controlled, Kaplan–Meier survival curve analyses and log-rank tests were used to examine the difference in survival between patients with and without corticosteroid use. Secondary endpoints were compared using the chi-squared (χ^2) test or Wilcoxon rank-sum test as appropriate.

Using the "mediation" package in R, we conducted a causal mediation analysis to evaluate whether the occurrence of Stage 2 or 3 AKI mediated the primary outcome following the administration of corticosteroids (24). The model was adjusted for covariates including age, sex, weight, comorbidities, SOFA score, laboratory values (neutrophils, lymphocytes, hemoglobin, platelets, creatinine, lactate, and BUN), and treatments given during the initial 24 h (renal replacement therapy, total fluids via IV). Based on 1,000 bootstrap resamples, the mediation analysis was tested, and the proportion of the intermediary effect, with the 95% confidence interval (CI), was obtained. The corresponding directed acyclic graph (DAG) and effect decomposition plot were generated to illustrate the average causal mediation effect (ACME), average direct effect (ADE), and total effect of the mediator model.

Subgroups were stratified by median value of SOFA score, neutrophil-to-lymphocyte ratio (NLR), and arterial blood gas lactate level. Stratified and interaction analyses were performed to investigate the relationship between corticosteroid use and 28-day mortality in separate subgroups using the study cohort, and interaction tests were additionally performed by incorporating an interaction term into each model. The statistical analyses mentioned above were conducted using the Stata/MP software package (version 16.0), and a bilateral $p < 0.05$ was considered statistically significant.

Results

The study flowchart is presented in Figure 1. After exclusion of 33 patients who received etomidate therapy or did not receive corticosteroids at the onset of septic shock, a total of 621 septic shock patients from the MIMIC-IV database with gram-negative bacterial infection were included. Baseline characteristics of the study cohort are described in Table 1. Among the cohort, 110 patients received corticosteroid therapy; the equivalent corticosteroid dose was 187 mg (IQR: 135, 217 mg), and the median duration of corticosteroid therapy was 3 days (IQR: 2, 5 days). Compared to patients who did not receive corticosteroids, those who received systemic corticosteroids had a significantly higher incidence of malignant disease and rheumatic disease; had higher lactate, SAPS II scores, and SOFA scores; and underwent more radical management. The 28-day mortality rate was 44% in septic shock patients with corticosteroid use and 23% in those without corticosteroid use ($P < 0.001$).

Corticosteroid use and outcomes

Baseline characteristics were well balanced after PSM between 90 patients with corticosteroid use and 123 patients without corticosteroid use; these are presented in Supplementary Table 2. There were cases in which patients were not matched to two untreated subjects, although 1:2 propensity score matching was allowed. However, we minimized the sample loss. With baseline variables being comparable, the Kaplan-Meier survival curves and log-rank test (presented in Figure 2) showed that patients with gram-negative bacterial infection exhibited decreased survival after corticosteroid administration (log-rank test $P = 0.028$).

Secondary outcomes were compared in the propensity-matched cohorts and are displayed in Table 2. There was no significant difference in the length of vasopressor use, duration of invasive ventilation support, duration of use of antibiotics, or length of hospital stay. However, we observed that the proportion of patients who developed Stage 2 or 3 AKI was higher in the corticosteroid use group ($P = 0.003$). Additionally, hospital mortality was also higher in patients with corticosteroid use (40% *vs.* 25%, $P = 0.022$).

As shown in Figures 3A, B, the estimated point for ACME was 2.4% (95% CI: 0.3%−4%; $P = 0.018$), and the occurrence of Stage 2 or 3 AKI in the early period mediated the relationship between corticosteroid use and 28-day mortality,

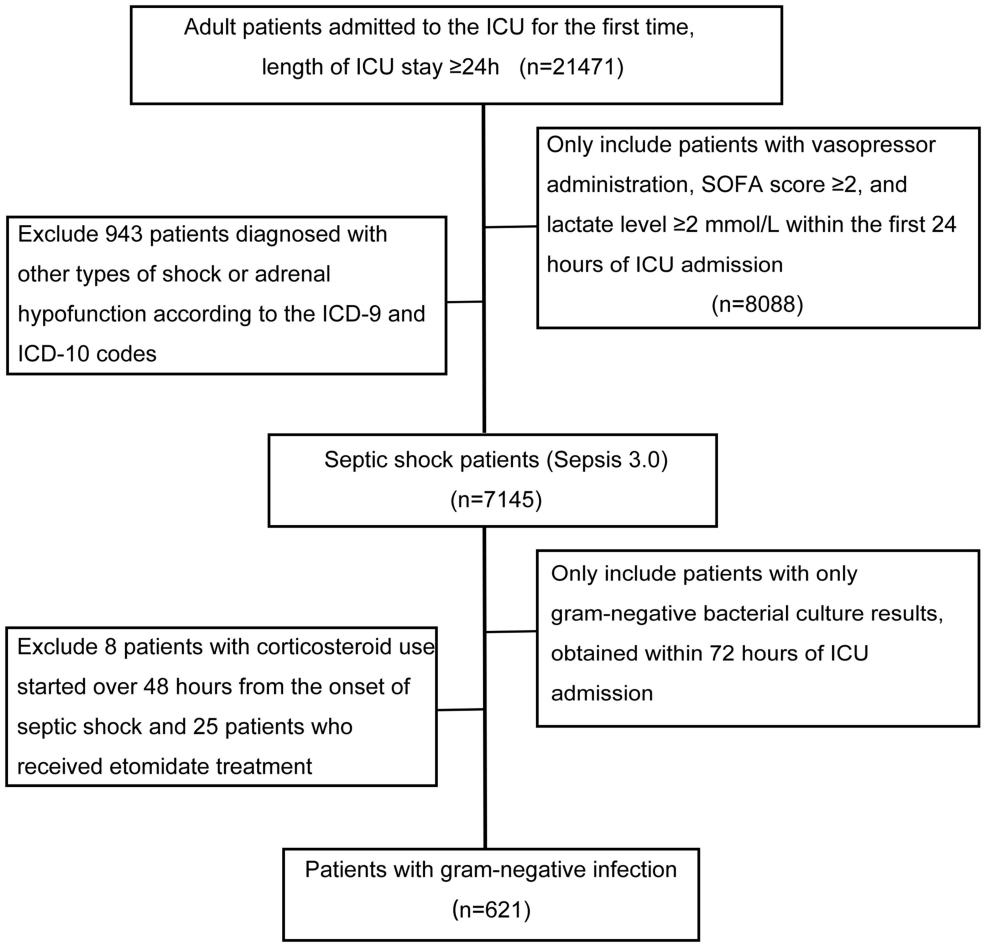

FIGURE 1
Flowchart illustrating the selection of patients. ICU, intensive care unit. Other types of shock included hypovolemic shock, cardiogenic shock, traumatic shock, anaphylactic shock, and postoperative shock. Adrenal hypofunction included secondary malignant and benign neoplasm of the adrenal glands, unspecified adrenal hypofunction, and other specified disorders of the adrenal glands.

with a mediation proportion of 21% (95% CI:1.9%−81%; $P = 0.046$).

Subgroup analyses

As illustrated in Figure 4, multivariate logistic regression analysis revealed the association between corticosteroid use and 28-day mortality in each specified group. Corticosteroid administration was independently associated with increased 28-day mortality in septic shock patients with gram-negative bacterial infection (OR = 1.75; 95% CI, 1.05–2.91; $P = 0.029$). This was further verified in the subset of patients with positive blood culture (OR = 3.51; 95% CI, 1.48–8.32; $P = 0.004$). While interaction test results indicated that the detrimental effect of corticosteroid use was attenuated in patients with NLR over 20, the effect was maintained in those with NLR <20 (P for interaction = 0.027). When patients were grouped according to the type of corticosteroid used, we found that hydrocortisone use was an independent risk factor in patients with gram-negative septic shock (OR = 1.84; 95% CI, 1.03–3.29; $P = 0.038$).

Discussion

We performed a propensity score analysis to balance the baseline characteristics, given that corticosteroids were prone to be administered to patients with more severe illnesses in our study. Subgroup analyses were further applied in different specified groups to determine the stability and heterogeneity of the present results. Ultimately, our study demonstrated that corticosteroid use was an independent risk factor in septic shock patients with gram-negative bacterial infection, and a significant increase in 28-day mortality was observed. An interaction test indicated that corticosteroid use was particularly unfavorable for patients with relatively low levels of NLR.

Corticosteroid use in septic shock has been a hot topic of research, but conflicting results have been obtained over multiple

TABLE 1 Baseline characteristics and outcomes of 621 septic shock patients with gram-negative infection.

Characteristics	Overall	No corticosteroid use ($n = 511$)	Corticosteroid use ($n = 110$)	p value
Age>65	379 (61%)	418 (61%)	65 (59%)	0.674
Sex (male)	329 (53%)	356 (52%)	65 (59%)	0.157
Weight (kg)	81.4 ± 23.1	80.6 ± 22.8	85.3 ± 24.4	0.051
Comorbidities				
Diabetes, *n* (%)	186 (30%)	206 (30%)	31 (28%)	0.714
Hypertension, *n* (%)	366 (59%)	404 (59%)	65 (59%)	0.971
Chronic pulmonary disease, *n* (%)	180 (29%)	192 (28%)	37 (34%)	0.254
Malignant disease, *n* (%)	118 (19%)	116 (17%)	30 (27%)	**0.013**
Rheumatic disease, *n* (%)	25 (4%)	21 (3%)	10 (9%)	**0.002**
Charlson Comorbidity Index	5.5 ± 2.9	5.4 ± 2.9	6.1 ± 2.8	**0.015**
Laboratory values				
Neutrophils, 10^9/L	13.7 ± 9.3	14 ± 9.1	12.3 ± 10	0.078
Lymphocytes, 10^9/L	0.9 ± 0.8	1 ± 0.8	0.9 ± 0.9	0.357
Hemoglobin, g/L	10.4 ± 2.1	10.4 ± 2.1	10.3 ± 2.2	0.835
Platelets, 10^9/L	191 ± 108	194 ± 106	171 ± 110	0.038
Creatinine, mg/dL	1.7 ± 1.3	1.7 ± 1.3	1.9 ± 1.2	0.113
BUN, mg/dL	33.4 ± 23.3	32 ± 23	36 ± 24	0.083
Lactate, mmol/L	3.9 ± 3.2	3.4 ± 2.7	6 ± 4.6	**<0.001**
Severity of illness				
SAPS II score	48 ± 16	46 ± 15	54 ± 17	**<0.001**
SOFA score	8 ± 4	8 ± 3	10 ± 4	**<0.001**
Infection site, *n* (%)				0.331
Pulmonary	173 (28%)	145 (28%)	28 (25%)	
Urinary	210 (34%)	179 (35%)	31 (28%)	
Abdominal	43 (7%)	33 (6%)	10 (10%)	
Blood	123 (20%)	95 (19%)	28 (25%)	
Other	72 (12%)	59 (12%)	13 (12%)	
Pathogen, *n* (%)				0.342
Escherichia coli	240 (39%)	202 (39%)	38 (34%)	
Klebsiella	76 (12%)	64 (12%)	12 (11%)	
Pseudomonas	54 (9%)	40 (8%)	14 (13%)	
Other	251 (40%)	205 (41%)	46 (42%)	
Treatment in first 24 h				
Equivalent norepinephrine dose (μg/kg/min)	0.18 ± 0.16	0.16 ± 0.13	0.27 ± 0.21	<0.001
Total fluids via IV (ml)	5,838 ± 3,986	5,295 ± 3,404	8,365 ± 5,322	**<0.001**
Antibiotics, *n* (%)	590 (95%)	644 (94%)	109 (99%)	**0.023**
Renal replacement therapy, *n* (%)	37 (6%)	27 (4%)	21 (19%)	**<0.001**
Mechanical ventilation, *n* (%)	379 (61%)	397 (58%)	84 (76%)	**<0.001**
Duration of corticosteroid use (days)	-	-	3 (2, 5)	-
Daily hydrocortisone-equivalent dose (mg/day)	-	-	187 (135,217)	-

(Continued)

TABLE 1 (Continued)

Characteristics	Overall	No corticosteroid use ($n = 511$)	Corticosteroid use ($n = 110$)	*p* value
Outcomes				
Length of ICU stay (days)	4 (2, 8)	4 (2, 8)	5 (2, 9)	0.835
Length of hospital stay (days)	10 (6, 17)	10 (6, 17)	10 (6, 18)	0.953
Hospital mortality, *n* (%)	147 (24%)	97 (18%)	50 (45%)	**<0.001**
28-day mortality, *n* (%)	168 (27%)	119 (23%)	49 (44%)	**<0.001**

SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; BUN, blood urea nitrogen; SOFA, score was calculated within the first 24 h of ICU admission.

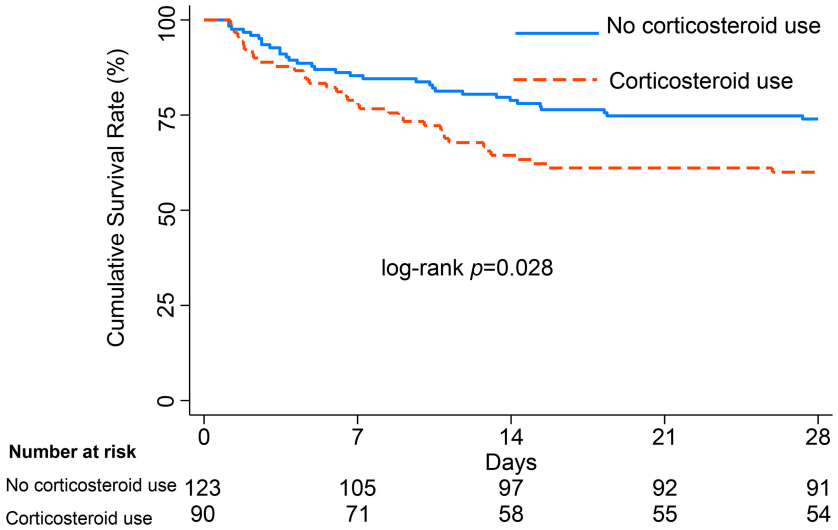

FIGURE 2
Survival curves from ICU admission to 28 days, with log-rank test, among patients with gram-negative bacterial infection after PSM.

TABLE 2 Comparison of outcomes between patients with and without corticosteroid use after PSM.

	No corticosteroid use ($n = 123$)	Corticosteroid use ($n = 90$)	*p* value
Developed Stage 2 or 3 AKI, *n* (%)	72 (58%)	77 (78%)	**0.003**
Length of vasopressor use (days)	3 (2, 5)	3 (2, 5)	0.875
Duration of invasive ventilation support (days)	3 (1, 8)	3 (2, 7)	0.575
Length of antibiotic use (days)	10 (6, 16)	9 (6, 14)	0.106
Length of hospital stay (days)	13 (7, 20)	10 (6, 17)	0.091
In-hospital mortality (%)	31 (25%)	36 (40%)	**0.022**

In accordance with the KIDGO 2012 criteria, we evaluated AKI stage based on the standard of urine output and serum creatinine.

decades. Annane et al. (25) found that a low dose of corticosteroids was associated with improved 28-day mortality since 2002, and a meta-analysis published in 2004 revealed an identical result (26). However, no significant effect on the rate of death at 28 days was observed in the Corticosteroid Therapy of Septic Shock (CORTICUS) study (27). Annane et al. subsequently conducted the Activated Protein C and Corticosteroids for Human Septic Shock (APROCCHSS) trial to investigate whether corticosteroids improve clinical outcomes, and inspiring results published in 2018 demonstrated an absolute difference in 90-day mortality. However, similar to Venkatesh's study (11), a beneficial effect on 28-day mortality was not observed based on that research. With the latest randomized trials included, a recent study discovered that short-term mortality was unaffected when septic shock patients were treated with low-dose corticosteroids (28). Despite the administration of a daily low dose of 187 mg (IQR: 135,217 mg) of corticosteroids, our study demonstrated a harmful effect. With the recognition of this and the updated definition of Sepsis 3.0,

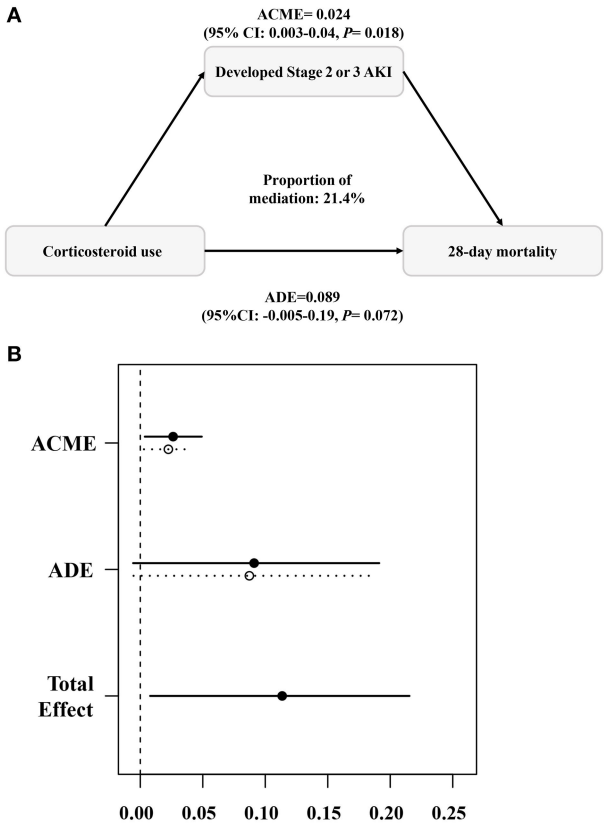

FIGURE 3
Causal mediation analysis **(A, B)**. The occurrence of Stage 2 or 3 AKI mediated the relationship between corticosteroid use and 28-day mortality. ACME, average causal mediation effect; ADE, average direct effect. The mediation analysis was completed using the "mediation" package in R with 1,000 simulations.

we believe this factor is non-negligible for the optimization of patient selection. As Antcliffe et al. (29) showed, Sepsis Response Signature (SRS) endotypes were essential in determining the degree of immunosuppression when corticosteroids were administered, which altered the effect of corticosteroids in patients with septic shock. In addition, more consideration should be given to the timing of corticosteroid use in septic shock patients.

Septic shock is characterized by the overzealous production of pro-inflammatory cytokines, immunosuppression, and hemodynamic instability. Hence, it is rational to mediate the response of inflammation and immunity and improve the effect of vasoactive agents by using corticosteroids (30). Corticosteroids are expected to suppress the occurrence of uncontrolled systemic inflammatory cytokine storm by inhibiting AP1 and NF-κB activity (31, 32). Although it may be intractable to fully handle the balance between pathogen eradication and excessive inflammation, the immunoregulatory properties of corticosteroids may also bring about side effects during the management of septic shock. As reported, LPS is associated with immunosuppression, which includes decreased production of TNF and lymphocyte proliferation (33, 34). Venet et al. (15) found that the function of monocytes and neutrophils is inhibited by regulatory T cells after LPS challenge. Futhermore, endotoxin tolerance is also accompanied by a reduction in pro-inflammatory cytokines (35–37). Beyond this, the NLR is regarded as an indicator of systemic inflammation and a predictor of prognosis in sepsis (38, 39). We hypothesized that patients with relatively low NLR also had milder levels of inflammatory factor release and that suppressed immune cell function may be a predominant unfavorable factor. Consequently, we speculated that the immunosuppression could be aggravated after corticosteroid use. More seriously, insufficient clearance of pathogenic bacteria may increase the rate of mortality.

After reducing the confounding bias between the two groups via PSM, we found that the incidence of Stage 2 or 3 AKI was significantly higher in patients receiving corticosteroids. Further analysis revealed that the occurrence of severe AKI mediated the relationship between corticosteroid use and 28-day mortality. Sepsis-associated AKI is a complication that contributes to poor prognosis and high mortality (40). A previous study identified the fact that steroids may induce sensitization to tubular necrosis by ferroptosis, resulting in AKI (41). However, the underlying mechanism of this life-threatening organ dysfunction can be more complicated. The rebound of pro-inflammatory cytokine storm attack after corticosteroid use could additionally be the cause of renal hypoperfusion (42).

Although the conclusions of this study may be limited to the current data and need to be confirmed by mechanistic and clinical research with larger samples, based on the latest guidelines for sepsis, the present study at least suggests an urgent need to focus on the populations for which corticosteroids are indicated, rather than just the method of application, in order to improve therapeutic precision, ultimately leading to patient benefit.

The limitations of the present study are largely due to its retrospective nature. First, only patients treated with relatively low doses of corticosteroids were included, but there was variation in dosage, route of administration, and type of drug (75% hydrocortisone, 9% dexamethasone, 7% hydrocortisone plus fludrocortisone, and others). Furthermore, a relatively small proportion of patients with abdominal infections were included in this study; to some extent, this results in reduced representativeness of the results. Third, unmeasured confounders such as resuscitation strategies and the motivation to use corticosteroids in this study could have affected our findings.

Conclusion

In conclusion, our study demonstrated that systemic use of corticosteroids in septic shock patients with gram-negative bacterial infections and lower NLR may be harmful, and that severe AKI may mediate this harm. Clinicians need to exercise caution in the use of corticosteroids in septic shock patients with LPS challenges, especially in patients with relatively low NLR. The findings indicate that more measures need to be taken for comprehensive evaluation of inflammatory and immune status, as well as the severity of disease, in order to achieve more appropriate application of corticosteroids.

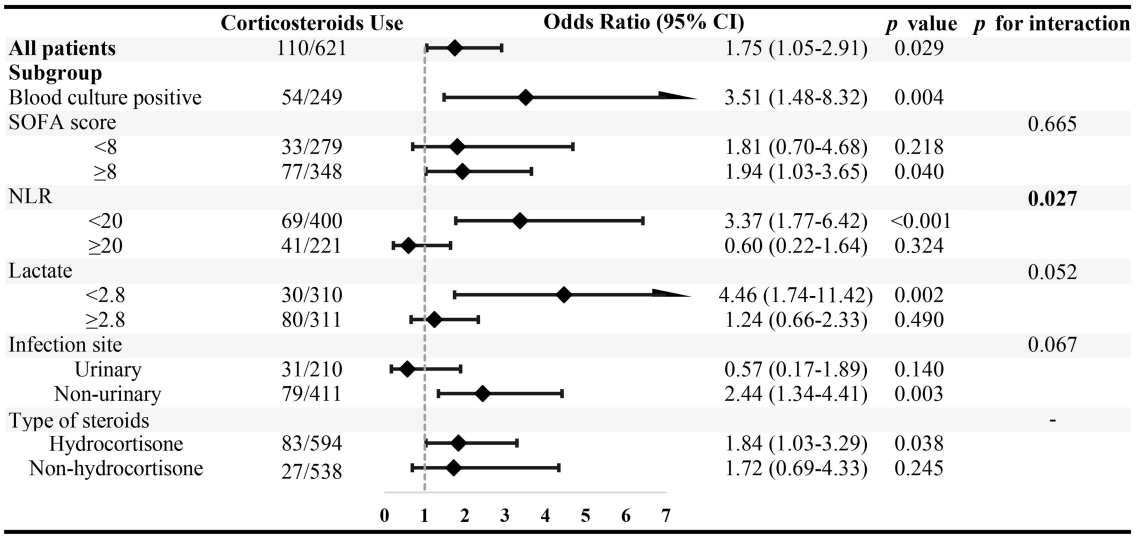

FIGURE 4
The association between corticosteroid use and 28-day mortality in specified subgroups. Subgroups were stratified by the median value of SOFA score, neutrophil–to-lymphocyte ratio (NLR), and arterial blood gas lactate level.

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: https://doi.org/10.13026/7vcr-e114.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

YD: Project administration, Writing—original draft, Data curation, Investigation, Software, Validation, Writing—review & editing. GH: Data curation, Resources, Software, Writing – original draft. JZ: Writing—original draft, Resources, Conceptualization, Methodology. YS: Writing—original draft, Supervision, Visualization. ZL: Supervision, Visualization, Writing—original draft. KW: Writing—original draft, Funding acquisition, Validation. WJ: Conceptualization, Funding acquisition, Writing—original draft, Project administration, Supervision.

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

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Supplementary material

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EDITED BY
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

REVIEWED BY
Andrea Glotta,
Istituto Cardiocentro Ticino, Switzerland
Filippo Binda,
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy

*CORRESPONDENCE
Mohamed H. Eid
✉ Mohamed.eid@plymouth.ac.uk;
✉ dr.mhmie1@mans.edu.eg

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Suction circuit flushing with chlorhexidine decreases ventilator-associated pneumonia: a quasi-experimental study

Mohamed H. Eid[1,2]*, Monica – Marilena Ţânţu[3], Jos M. Latour[1,4], Mohammed Ahmed Sultan[5] and Nahed Attia Kandeel[2]

[1]School of Nursing and Midwifery, Faculty of Health, University of Plymouth, Plymouth, United Kingdom, [2]Critical Care and Emergency Nursing Department, Faculty of Nursing, Mansoura University, Mansoura, Egypt, [3]Medical Assistance and Physical Therapy Department, Faculty of Science, Physical Education and Informatics, University of Piteşti, Piteşti, Romania, [4]Faculty of Nursing, Fudan University, Shanghai, China, [5]Anaesthesia and Intensive Care Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Background: Endotracheal suctioning of mechanically ventilated patients differs across the world. In many low and middle-income countries, endotracheal suctioning is often performed with a sterile suctioning catheter that is used for 12 h or during the length of one nursing shift. The effect of flushing multiple used endotracheal suction system with chlorhexidine after suctioning to reduce ventilator associated pneumonia (VAP) remains unclear.

Aim: The aim of the study is to assess the effectiveness of flushing multiple-used open endotracheal suction catheters and suctioning system with chlorhexidine gluconate 0.2% to reduce VAP in mechanically ventilated patients in a resource-limited Intensive Care Unit (ICU).

Methods: Due to the difficulty of blinding the intervention for nurses who perform endo-tracheal suction procedures, we adopted a quasi-experimental method with a randomized controlled trial design. A sample of 136 ICU patients were allocated to the intervention (n = 68) or control group (n = 68) between May and November 2020. The intervention was flushing the multiple-used suction catheter and suction system with 40ml chlorhexidine gluconate 0.2% and in the control group we used normal saline to flush the catheter and suction system. The primary outcome was incidence of VAP and the cost of the flushing solutions was the secondary outcome measure.

Results: Patients in the intervention group had a lower incidence of VAP compared to patients in the control group; 15 (22.1%) vs 29 (42.6%), p = 0.01. The incidence of late-onset VAP was 26.2% in the intervention group and 49% in the control group (p = 0.026) and the early-onset VAP was 13.2% in the intervention group and 25% in the control group (p = 0.081). Chlorhexidine gluconate 0.2% reduced the cost of suction system flushing (median: 78.4 vs 300 EGP, $p < 0.001$).

Conclusion: Using chlorhexidine gluconate 0.2% to flush multiple-used suctioning catheters after every endo-tracheal suction procedure might reduce the incidence of VAP in mechanically ventilated patients. Chlorhexidine gluconate 0.2% can be a cost-effective solution for flushing the suction circuit. Nurses

working in resource-limited ICUs and using suctioning catheters multiple times might consider using chlorhexidine gluconate 0.2% instead of normal saline or distilled water when flushing the suction system.

Clinical trial registration: ClinicalTrials.gov, identifier NCT05206721.

KEYWORDS

airway management, chlorhexidine, endotracheal suctioning, intensive care units, ventilator-associated pneumonia, global health, green ICU

1 Introduction

In low and middle-income countries, open endotracheal suction catheter is used multiple times to perform suctioning due to limited resources (1, 2). Currently, there is limited evidence for using a new suction catheter for each suction pass, acknowledged in a review article of endotracheal suction procedures in pediatric populations (3). Additionally, the latest artificial airway suctioning practice guidelines published by the American Association for Respiratory Care in 2022 did not mention any recommendations regarding suction catheter changing frequency (4). The guidelines adopted a study conducted in 2001 which showed that reusing an open tracheal suctioning catheter is safe and cost effective (5). Therefore, the current evidence of reusing suctioning catheters remains unclear, which rationalize the reason why some resource limited intensive care units (ICUs) using the catheter multiple times during a 12-h shift, and possibly explain the high ventilator associated pneumonia (VAP) incidence in these ICUs (1, 2).

Ventilator associated pneumonia is defined as pneumonia that develops 48 h or more after the initiation of mechanical ventilation in Intensive Care Unit (ICU) patients (6). The impact of VAP is recognized in the ICU as a main challenge because it prolongs the duration of mechanical ventilation, increases ICU length-of-stay and healthcare costs (7). The incidence of VAP has been reported to affect 5%–40% of patients receiving invasive mechanical ventilation for more than 2 days (8). The estimated attributable mortality of VAP is approximately 10%, with higher mortality rates among patients in surgical ICU (8).

Ventilator associated pneumonia is classified into two types according to the onset-time of pneumonia. Early-onset VAP occurs within the first 4 days of mechanical ventilation, whereas late-onset VAP occurs five or more days after mechanical ventilation initiation (9). Early-onset VAP is usually caused by community acquired pathogens, whereas late-onset VAP involves hospital flora (10). The most prevalent pathogens causing 80% of hospital acquired pneumonia are *Staphylococcus aureus*, *Klebsiella* species and *Pseudomonas aeruginosa* (11). Therefore, VAP is considered a hospital acquired condition and needs ongoing attention by ICU staff to prevent it.

Chlorhexidine is a well-known, widely used low-cost product, and is used as an antiseptic and disinfectant to kill microorganisms to reduce hospital-acquired infections spread in ICUs (12). It has been proposed as one of the five interventions in the care bundle to prevent VAP, daily oral care with chlorhexidine (13). Some studies have shown that intraoral application of chlorhexidine reduces VAP occurrence in mechanically ventilated patients (14–17). A trial reported that oral decontamination with 2% chlorhexidine concentration is more effective compared with 0.2% concentration in VAP prevention and oropharyngeal colonization reduction (18). We used 0.2% concentration to conduct this study because of its availability in the study setting. However, chlorhexidine is reported to be associated with some side effects including stinging, burning sensation of the tongue, reversible discoloration of the teeth and tongue, and transient disturbances of taste (19). Some studies recommended the exclusion of chlorhexidine from oral hygiene because of its side effects (20, 21). Our intervention used chlorhexidine gluconate 0.2% for flushing the suction system without patient's integration to avoid any side effects and/or resistance.

Ventilator-dependent patients are at risk of increased secretions because of being sedated, and the presence of mechanical ventilator adjuncts which prevent spontaneous clearance of secretions (22). Therefore, endotracheal suctioning to clear secretions has been standard practice in the care of mechanically ventilated patients (23). It is recognized that the inner surface of artificial airways becomes colonized with biofilms containing pathogenic organisms and passing the suction catheter through it can dislodge these biofilms leading to inoculation of pathogenic organisms into the lungs, causing VAP (24). Therefore, Suctioning is a sterile procedure that is performed only when the patient needs without a routine schedule (25).

Suction catheter is used to remove tracheal secretions and may be either open or closed tracheal suctioning system (26). Some studies showed that there is no difference between using the open or the closed tracheal suctioning system method on the incidence of VAP or mortality rate (26–28). We used the open tracheal suctioning system to conduct this study as the closed system is not available in the study setting. Closed suction circuit catheters can be used multiple times, while the open tracheal suctioning catheter should be single use only (2, 3).

We decided to conduct this study for four reasons: (1) The endotracheal suction system might act as a good medium for the proliferation and colonization of pathogenic bacteria which can then migrate to patient's lung during suctioning procedure causing VAP, which necessitate its cleaning and disinfection. (2) We have not identified in the literature any study investigating

Abbreviations: VAP, ventilator associated pneumonia; ICU, intensive care unit; MCPIS, Modified Clinical Pulmonary Infection Score; MGCS, Modified Glasgow Coma Score.

the effect of flushing this system with chlorhexidine gluconate 0.2% and whether it has an impact on VAP. (3) In low-middle income countries, many hospitals with resource limitations are still using one sterile suctioning catheter for 12 h or during a shift instead of single use, and the incidence of VAP is still high in these countries (1, 29). (4) Investigating whether chlorhexidine gluconate 0.2% will disinfect the multiple-used catheter to mimic the sterile single-used effect. Therefore, the aim of this study was to investigate the effect of flushing the open tracheal suctioning system with multiple-used catheters using chlorhexidine gluconate 0.2% on the occurrence of VAP among mechanically ventilated patients in limited resource intensive care units. We hypothesized that suction circuit flushing with chlorhexidine might reduce the incidence of VAP in mechanically ventilated patients compared to flushing with normal saline.

2 Materials and methods

A quasi-experimental design was adopted to conduct this study. Recruitment was between May and November 2020. During the study period, the recruitment was temporarily stopped for 3 months due to COVID-19 pressures. Ethical approval was obtained from the Local Research Ethical Committee (Ref. No. 245/2020). The study protocol was registered at ClinicalTrials.gov with the number NCT05206721.

2.1 Setting

This study was conducted at three ICUs located in one university hospital in Egypt. The three ICUs were a surgical, neurological and trauma respectively. Each ICU has 10 beds and provides care to mechanically ventilated patients. These units are well equipped with advanced technology required for high quality ICU care. The total admissions of the three ICUs are around 650 patients annually. The nurse-patient ratio in these units is 1 nurse to 2 patients. Standard VAP prophylaxis measures in these settings follow the ventilator bundle checklist published by Institute for Healthcare Improvement [IHI], 2012 including elevating head of the bed between 30 and 45 degrees, daily sedation vacation and readiness to weaning assessment, peptic ulcers disease prophylaxis, and deep venous thrombosis prophylaxis (30).

2.2 Participants

Patients admitted to the ICUs who were intubated within the previous 24 h and were expected to receive mechanical ventilation for more than 48 h were recruited into the study. The researchers used their clinical expertise and the patient ICU admission diagnoses to evaluate enrollment of the patients. Excluded were: (1) patients diagnosed with pneumonia at the time of admission and/or having a Modified Clinical Pulmonary Infection Score (MCPIS) of 5 or greater (31); (2) patients who had contraindications to suctioning (i.e., severe hemoptysis, increased intracranial pressure, and cerebrospinal fluid leaks); (3) patients with pulmonary edema, acute respiratory distress syndrome and atelectasis because of their pulmonary infiltrate disease pathophysiology; (4) patients known to be allergic to chlorhexidine.

2.3 Sample size and randomization

Based upon power analysis, the sample size was calculated using the free online software https://www.sphanalytics.com/sph-analytics-dss-research/and the values were set at 5% α error (95.0% significance) and 20.0% β error (80.0% power of the study). The sample size needed for the study was 136 patients. The patient admission number was used to randomly assign the patient to the intervention or control group. Patients with even admission numbers were assigned to the intervention group and the odd admission numbers to the control group (68 in each group).

2.4 Recruitment

Recruitment was performed by the Principal Investigator (PI). An initial assessment was performed on the first day for all mechanically ventilated patients using the MCPIS to confirm that they were free from pneumonia and exclusion criteria. Informed consent was obtained from the patients' families (next of kin) who were informed about the aim, procedure, benefits, and risks of the study. The voluntariness nature of participation and the right to withdraw at any time without responsibility were also emphasized to them. Confidentiality of the participants' personal information was maintained throughout the study procedure. Dropout patients before the 3rd day of admission were excluded and replaced by new cases to reach the sample target of 136 patients.

2.5 Intervention, standard care and procedures

The intervention group received chlorhexidine gluconate 0.2% as the flushing solution for the open tracheal suction system. The control group received normal saline as the flushing solution.

The PI did not develop a suctioning performance checklist according to standard guidelines or even ask staff nurses to modify their current suctioning practices neither in the study group nor in the control group. This was to ensure that VAP incidence was related to the use of chlorhexidine gluconate 0.2% as the flushing solution for the suction system effect and not a modified suctioning technique performed by the ICU nurses. Indication for suctioning in the study settings includes presence of secretions in patient's chest and ausculation of crackles or wheezing patient's chest.

2.5.1 Intervention

The appropriate volume of chlorhexidine gluconate 0.2% solution required for effective flushing was investigated by the PI. The test of the total milliliter (ml) required chlorhexidine gluconate 0.2% for flushing the 1-meter open tracheal suction system with a catheter size of 16 Fr was 40ml. The details of the test are presented in **Supplementary material I**. The intervention as performed in four steps. Step I: Although allergic reactions to chlorhexidine are relatively rare, a sensitivity test was performed

for the intervention group patients using chlorhexidine irrigation solution (0.1% concentration) for performing intradermal allergy test. Step II: Routine suctioning technique (following the hospital's protocol) was performed on patients by nursing staff. Step III: The responsible nurse poured 40 ml chlorhexidine gluconate 0.2% solution from the chlorhexidine gluconate 0.2% bottle into a sterile container. Step IV: After suctioning the patient's secretions, the nurse inserted the suction catheter into the 40 ml chlorhexidine gluconate 0.2% filled container and flushed the entire suction circuit with chlorhexidine. Before inserting the reused catheter, the nurse needed to do first 5 s of "dry suctioning" to make sure that there are no chlorhexidine gluconate 0.2% droplets in the catheter, to avoid chlorhexidine instillation into participants lungs.

2.5.2 Standard care

The same procedure above was performed from step II to IV using normal saline (according to our standard protocol) instead of chlorhexidine gluconate 0.2% for flushing the open tracheal suction system; Routine suctioning technique (following the hospital's protocol) was performed on patients by nursing staff. The responsible nurse poured 40 ml saline 0.9% from the normal saline bottle into a sterile container. After suctioning the patient's secretions, the nurse inserted the suction catheter into the 40 ml saline filled container and flushed the entire suction circuit with normal saline. Critical care nurses performed hand washing, used sterile disposable gloves, kept the endotracheal suctioning catheter inside its plastic sheath after each suctioning procedure during the 12-h shift in both study groups.

2.6 Outcome measures

The occurrence of VAP was the primary outcome measure, and the cost of the flushing solutions was the secondary outcome measure. Regarding the primary outcome, the MCPIS was used to diagnose VAP and it was calculated on day three for early-onset VAP and on day six for late-onset VAP. The minimum score was 0, and the maximum score was 10. Patients who obtained scores above 5 were diagnosed with pneumonia, and those who scored below 5 were considered free of pneumonia. Patients who obtained a score of 5 (borderline) with hemodynamic stability, the PI re-evaluated these patients after 2 days (day eight). For hemodynamically unstable patients, the PI ordered a sputum culture (microbiological confirmation) on day six to verify their score. Patients with negative culture results obtained 0 points upon their score (MCPIS = 5) and were considered free of VAP, whereas those with positive culture obtained 2 more points upon their score (MCPIS = 7) and were considered to have VAP. Regarding the secondary outcome, the cost for each patient was calculated. the total number of required chlorhexidine gluconate 0.2% and saline bottles consumed by each patient in the intervention or control group was multiplied by their commercial price.

2.7 Data collection

Two data collection tools were used. The first tool, *data assessment tool,* was developed by the PI after reviewing relevant literature (16, 26, 32). The tool consists of three parts. The first part was the patients' socio-demographics and health data, including age, gender, occupation, smoking habits, and health-relevant data such as date and reason of ICU admission, medical diagnosis, past medical history, ICU length-of-stay, and the modified Glasgow Coma Scale (MGCS) (32). The second part included mechanical ventilator modalities data, including the mechanical ventilation initiation date, artificial airway used, endotracheal tube size, mechanical ventilation mode and duration. The third part included the endotracheal suctioning data such as size of suction catheter, type of catheter connector, and duration of the total suctioning procedure (**Supplementary material II**).

The second data collection tool was the *VAP diagnostic criteria sheet* adopted from Singh et al. (31) and was used to assess the patients for clinical diagnosis of VAP. It includes the MCPIS based on five clinical assessments; each variable is worth 0–2 points including the patient's body temperature, number of white blood cells, purulence and quantity of tracheal secretions (i.e., rare secretions, abundant, and purulent abundant secretions), oxygenation (calculated as PaO2 divided by the fraction of inspired oxygen [FiO2]), and chest radiography findings (no infiltrates, diffused infiltrates and localized infiltrates). The points for each variable of the MCPIS were summed, yielding a total score varied from 0 to 10 for data analysis (**Supplementary material II**).

The two data collection tools were tested before starting the data collection. The content validity of data assessment tool was assessed by seven experts in ICU nursing and medicine. The VAP diagnostic criteria sheet was adopted and has been extensively used in many studies. The data assessment tool and the VAP diagnostic criteria sheet were tested on 10% of the total sample (14 patients) to evaluate the tools' clarity, feasibility, and applicability. Participants in the pilot study were excluded from the main study sample.

2.8 Statistical analysis

The obtained data were coded, computed, and statistically analyzed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA). Data were presented as frequency and percentages (categorical variables) and mean, standard deviation (continuous variables). Distribution of data was assessed using the Shapiro–Wilk test and visual observation of histogram. Chi-square ($\chi 2$) was used for comparing categorical variables and was replaced with Fisher's exact test or Monte Carlo exact test if the expected value of any cell was less than 5. Student's *t*-test was used for comparing continuous variables. The median was used as a central tendency measure for continuous quantitative variables that were not normally distributed. The Mann–Whitney U-test (Z) was used to compare the two groups. The difference was considered significant at $p \leq 0.05$.

3 Results

3.1 Patient's sociodemographic and health-relevant data

In our study, 136 patients were enrolled of whom 68 patients were assigned to the intervention group. Patients lost in follow-up

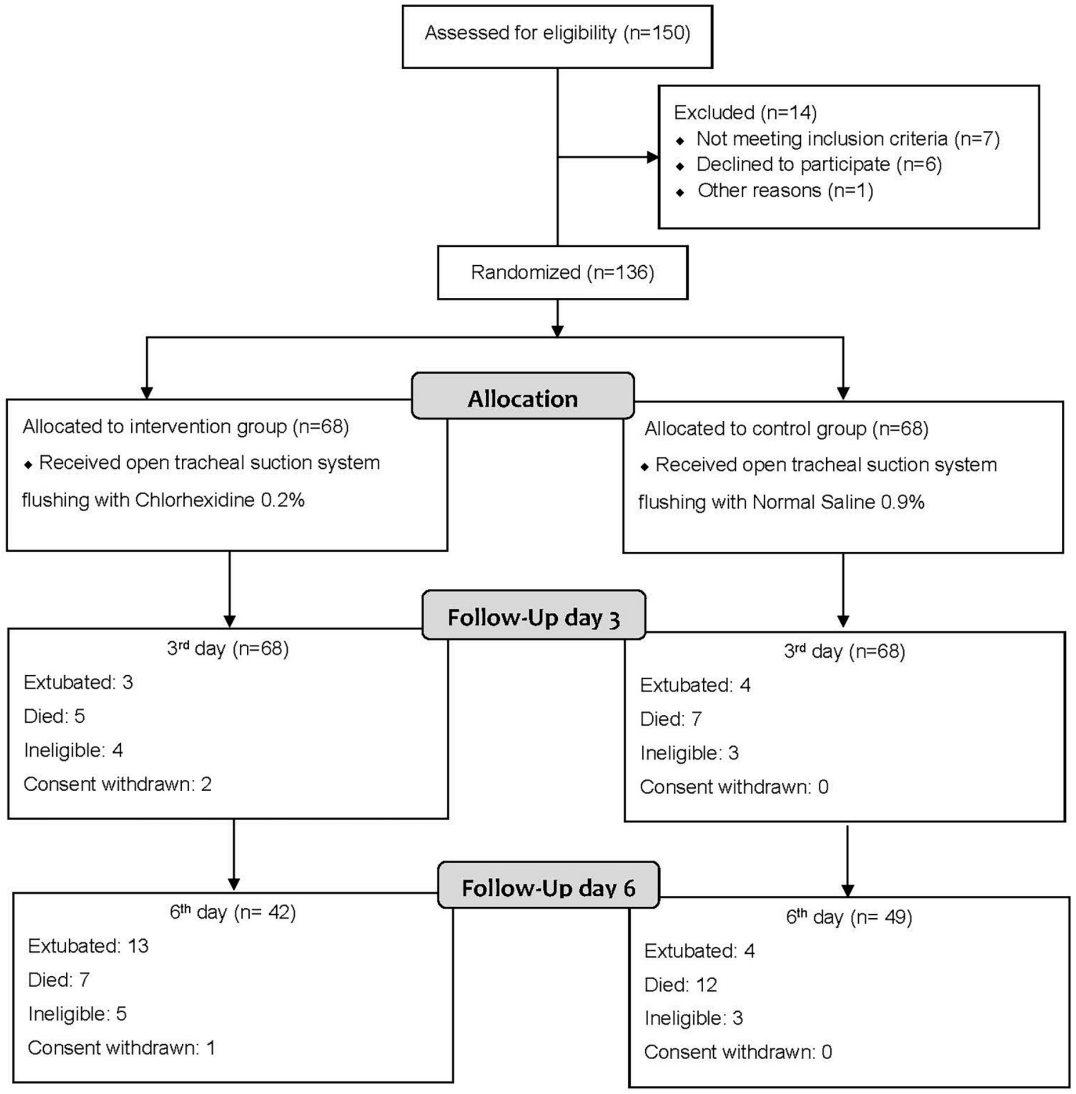

FIGURE 1
Flow diagram of study participants. All dropped out patients before the 3rd day have been excluded and replaced by new cases to fulfill our sample of 136 patients (68 in each group). While dropped out patients after the 3rd day and before day 6th were not replaced and have been evaluated for 3rd day early-onset VAP occurrence only.

after the 3rd day and before day 6 were not replaced and have been evaluated for the early-onset VAP only (Figure 1).

There were no differences in age, severity of disease, underlying diseases between both groups, the majority of the study participants were male, and most patients were admitted for neurological disorders or trauma (Table 1). It was observed that the majority of patients had an ICU length-of-stay of more than 7 days. A statistically significant difference was observed in the mean modified GCS score between the two study groups on day 4 and 5 (Table 1).

3.2 Ventilator modalities and endotracheal suctioning data

Most patients in the study groups were intubated via an endotracheal tube with a tube size of 7–7.5 mm and were on assisted modes of mechanical ventilation (Table 2). The duration of ventilation for most patients in both groups was $\geq$ 7 days. Concerning endotracheal suctioning data, no differences were observed between both groups (Table 2). We have not compared the fequency and duration of suctioning for each patient as it's an individualized care procedure depends on presence of secretions for each participant.

3.3 VAP incidence

The incidence of VAP among patients in the intervention group was lower than in the control group (22.1% vs 42.6%, p = 0.010). The in-depth focus of this incidence showed no statistically significant difference on the third day for early-onset VAP. However, statistically significant difference between both

TABLE 1 Sociodemographic and health relevant data of the study groups.

Variables	Intervention group	Control group	*p*-value
	n (%)	*n* (%)	
Age in years			
Mean (SD)	50.99 (20.61)	49.25 (17.23)	0.595
Gender			
• Male	49 (72.1%)	50 (73.5%)	0.847
• Female	19 (27.9%)	18 (26.5%)	
Smoking habit			
• Yes	18 (26.5%)	24 (35.3%)	0.265
• No	50 (73.5%)	44 (64.7%)	
Reason for ICU admission			
• Neurological disorders	40 (58.8%)	36 (52.9%)	0.116[1]
• Multiple trauma injuries	26 (38.2%)	22 (32.4%)	
• Cardiac disorders	0 (0%)	4 (5.8%)	
• ENT	1 (1.5%)	1 (1.5%)	
• Toxicology	1 (1.5%)	5 (7.4%)	
Past medical history			
• Yes	44 (64.7%)	42 (61.8%)	0.722
• No	24 (35.3%)	26 (38.2%)	
• Diabetes mellitus	26 (38.2%)	35 (52.9%)	0.121
• Hypertension	34 (50%)	32 (47.1%)	0.731
• Ischemic heart disease	18 (26.5%)	18 (26.5%)	–
• Renal failure	10 (14.7%)	8 (11.8%)	0.613
• Hepatic impairment	6 (8.8%)	4 (5.9%)	0.511
• Others	4 (5.9%)	3 (4.4%)	1.00
Length of ICU stay (days)			
• 3–4 days	15 (22%)	11 (16.1%)	0.445[1]
• 5–6 days	16 (23.5%)	22 (32.4%)	
• ≥7 days	37 (54.5%)	35 (51.5%)	
Average MGCS [mean (SD)]			
• Day 1	68 [7.09 (1.86)]	68 [7.32 (1.52)]	0.147
• Day 2	68 [6.54 (1.67)]	68 [6.92 (1.37)]	0.147
• Day 3	68 [6.22 (1.59)]	68 [6.73 (1.62)]	0.064
• Day 4	66 [5.91 (1.82)]	64 [6.78 (1.80)]	0.007*
• Day 5	48 [6.15 (1.67)]	55 [6.82 (1.78)]	0.052*
• Day 6	42 [6.090 (1.85)]	49 [6.38 (1.83)]	0.337

Ear, Nose, Throat diseases (ENT), Modified Glasgow Coma Score (MGCS). Data are presented as numbers (n), frequency (%), Mean and standard deviation [Mean (SD)]. *P* by Chi-Square test (χ^2), [1]Monte Carlo Exact Probability (MEP), *refers to significance if *P*-value $\leq$ 0.05. Others past medical history included (Asthma, Cancer, Epilepsy, and Stroke). It must also be clarified that after the 3rd day, we witnessed a daily drop out in the number of patients in the study groups due to patient's recovery, MV weaning, unexpected ineligibility, sudden discharge/transfer, or death.

groups on day 6 for late-onset VAP incidence was observed (Table 3).

3.4 Economic cost

Statistically significant differences regarding the average cost of flushing solutions were observed between the two groups, with a significant cheaper commercial cost for chlorhexidine gluconate 0.2% compared to normal saline (Table 3).

4 Discussion

The aim of our study was to test a new intervention in endotracheal suction management in mechanically ventilated ICU

TABLE 2 Ventilator modalities and endotracheal suctioning data of the study groups.

Variables	Intervention group	Control group	*p*-value
	n (%)	*n* (%)	
Artificial airway used			
• Endotracheal tube	67 (98.5%)	66 (97.1%)	1.00
• Tracheostomy tube	1 (1.5%)	2 (2.9%)	
Intubation process			
• Urgent	66 (97.1%)	65 (95.6%)	1.00[1]
• Elective	2 (2.9%)	3 (4.4%)	
ETT size (mm)			
• 5–5.5	1 (1.5%)	1 (1.5%)	0.071[1]
• 6–6.5	0 (0.00%)	4 (5.8%)	
• 7–7.5	49 (72.1%)	38 (55.9%)	
• 8–8.5	18 (26.4%)	25 (36.8%)	
Mode of ventilation			
• Controlled	5 (7.4%)	9 (13.2%)	0.126
• Assisted	60 (88.2%)	51 (75.0%)	
• Spontaneous	3 (4.4%)	8 (11.8%)	
Duration of MV			
• 3–4 days	21 (30.9%)	13 (19.1%)	0.184
• 5–6 days	13 (19.1%)	20 (29.4%)	
• ≥7 days	34 (50.0%)	35 (51.5%)	
Suction catheter size (Fr)			
• ≤10	1 (1.5%)	1 (1.5%)	0.278[1]
• 12	14 (20.6%)	17 (25.0%)	
• 14	46 (67.6%)	36 (52.9%)	
• 16	7 (10.3%)	14 (20.6%)	
Type of SC connector			
• Standard connector	42 (61.8%)	40 (58.8%)	0.861[1]
• Thumb control connector	19 (27.9%)	19 (27.9%)	
• Fingertip control connector	7 (10.3%)	9 (13.3%)	
Duration of total suction time			
• <30 s	8 (11.8%)	12 (17.7%)	0.298[1]
• 30–60 s	33 (48.5%)	37 (54.4%)	
• >1 min	27 (39.7%)	19 (27.9%)	

Endotracheal Tube (ETT), Mechanical Ventilation (MV), Millimeter (mm), Suction Catheter (SC), French gauge (Fr). Data are presented as numbers (n) and frequency (%), *P* by Chi-Square test (χ^2), [1] Monte Carlo Exact Probability (MEP).

patients. The intervention was to use chlorhexidine gluconate 0.2% in flushing the tracheal suction system, including the multiple use of a sterile suctioning catheter, compared to normal saline. We investigated the effect of this intervention on VAP incidence at day 3 and day 6 of ICU admission. The main findings of our study indicated that VAP incidence was reduced in the intervention group. Also, we identified that the cost of the chlorhexidine gluconate 0.2% was less than the standard care group. Also, we have not identified any adverse reactions in airways of study participants in the intervention group using chlorhexidine gluconate 0.2% during or after conducting the study.

To our knowledge and reviewing the current evidence, we have not identified any study describing a similar intervention. Therefore, we believe that this is the first study testing chlorhexidine gluconate 0.2% for flushing the suction system. Subsequently, rather than other studies comparing routine, our discussion is based on the analysis of the study results. Hopefully, our findings will shed light on a new knowledge gap, guiding further research for a more in-depth understanding of endotracheal suction procedures in resource-limited ICU.

TABLE 3 Main study findings of the study groups.

Variables	Intervention group	Control group	*p*-value
	n (%)	*n* (%)	
Total VAP			
• Not VAP	53 (77.9%)	39 (57.4%)	0.010*
• VAP	15 (22.1%)	29 (42.6%)	
Third day early-onset VAP			
• Not VAP	59 (86.8%)	51 (75.0%)	0.081
• E-VAP	9 (13.2%)	17 (25.0%)	
Sixth day late-onset VAP			
• Not VAP	31 (73.8%)	25 (51.0%)	0.026*
• L-VAP	11 (26.2%)	24 (49.0%)	
MCPIS [Mean (SD)]			
• Day 1	68 [1.32 (1.11)]	68 [1.54 (1.15)]	0.258
• Day 3	68 [3.38 (1.39)]	68 [4.31 (1.20)]	0.001*
• Day 6	42 [4.24 (1.51)]	49 [5.04 (1.64)]	0.018*
• Day 8	2 [5.50 (2.12)]	5 [6.40 (2.70)]	0.696
Cost* **			
• Mean (SD)	74.79 (30.14)	307.65 (115.49)	$p < 0.001$**
• Min–max	39.20 – 147.0	120.0 – 560.0	
• Median	78.4	300.0	

Ventilator associated pneumonia (VAP), Early-onset ventilator associated pneumonia (E-VAP), Late-onset ventilator associated pneumonia (L-VAP). Data are presented as numbers (n), frequency (%), Mean and standard deviation [Mean (SD)]. *P* by Chi-Square test (χ^2), *refers to significance if *P*-value $\leq$ 0.05, **refers to high significance if *P*-value less than 0.001. ***Cost refers to Egyptian Pounds (EGP).

Neurological disorders were the most common medical diagnosis among the study groups followed by multiple trauma injuries. This could be attributed to the fact that data were collected from surgical ICUs, which provide care to patients with surgical problems, neurological disorders, and those with multiple trauma injuries. In developing countries, neurological disorders contributed to 92 million disability-adjusted life-years in 2015 and were projected to 103 million in 2030 worldwide (33). In Egypt, neurological disorders are the leading cause of death accounting for 14.9% of total deaths (34).

The ICU length-of-stay in more than half of our study participants was more than 7 days without statistical differences. A prospective observational study, involving patients from 27 ICUs in nine European countries, reported that VAP resulted in 6% higher mortality, longer ICU length-of-stay (10 and 12 days), and increased duration of mechanical ventilation (35). Furthermore, systematic reviews with meta-analysis of randomized controlled trials revealed that VAP occurrence increased the ICU length-of-stay and duration of mechanical ventilation (36, 37). This might explain the reason why most of our study population with a confirmed VAP had an ICU length-of-stay of more than 7 days.

Concerning the average MGCS, statistically significant differences were noted between the two groups on days 4 and 5. Patients who developed VAP had a lower MGCS compared with non-VAP patients. Other investigations reported similar findings between patients who developed VAP and the non-VAP group regarding MGCS (38, 39).

According to our findings, it was noted that the majority of patients were intubated via an endotracheal tube sized 7–7.5 mm. Endotracheal intubation is a critical and life-saving intervention for airway management in ICUs (40). In an emergency, it is the first step to maintain a patent airway, allow adequate positive ventilation, and facilitate suctioning (41, 42). The standard adult tube size is 7.5–8 mm (24). Smaller tubes impede the clearance of secretions and create increased airflow resistance when weaning from the ventilator and might contribute to the development of VAP.

More than half of the participants underwent a suctioning procedure using the standard type of suction catheter connector. This is because it is the most widely known connector type in Egypt. In addition, we observed that it is the most preferable connector type for being not tedious, as suctioning initiation/stop can be controlled using one hand and the suction catheter insertion into the endotracheal tube is performed with the other hand. Moreover, slightly more than half of the participants consumed between 30 and 60 s as a total duration time for suctioning procedure. This is the usual time for suctioning procedure by the nursing staff in the study settings. However, it is widely recognized that suctioning duration should be limited to minimize adverse events. Additionally, it is recommended by American association of respiratory care, 2010 (43) that the duration of the suctioning event should be limited to less than 15 s. This highlights the need for training programs on suctioning for critical care nurses in the study settings.

The incidence of VAP among the patients in the intervention group was 22.1%, whereas the incidence among the control

group patients was 42.6%. This indicates that using chlorhexidine gluconate 0.2% as a flushing solution might contribute to the reduction of VAP in ICU patients. These findings support other studies that reported the effectiveness of oral care or bed bathing using chlorhexidine solutions in reducing VAP incidence (12, 14, 17). One study conducted in Egypt investigated the effect of oral care with chlorhexidine on VAP incidence reported reduced VAP incidence (16). This might support our intervention to be applied with the care bundle to reduce the incidence of VAP.

The VAP bundle has been implemented in many ICUs, along with teamwork, and communication strategies (30). There is a level III evidence that successful implementation of care bundle results in decreased VAP rates (44). In Egypt, studies showed that implementation of VAP bundle in adult and neonatal ICU resulted in decreasing the incidence of VAP (45, 46). However, the bundle is not widely implemented in ICUs in Egypt. Therefore, we designed a new easy intervention to be implemented that might contribute to the reduction of VAP incidence as well as the costs of the flushing solution.

Based upon the day 3 early-onset VAP incidence in our study, no significant difference was noted between the study groups. This aligns with an Egyptian study that investigated the effects of oral care with chlorhexidine on VAP incidence and reported no statistically significant difference between the two groups regarding early-onset VAP incidence (16). However, our study demonstrated that on day 6 more patients in the control group were diagnosed with late-onset VAP, which was also confirmed in the Moustafa et al. study (16) reporting a significant difference between their study groups on day 6.

Finally, the average cost of chlorhexidine gluconate 0.2% used among the patients in the intervention group and the average cost (commercial price) of normal saline used among control group patients showed that chlorhexidine gluconate 0.2% suction system flushing intervention significantly decreased the cost of patient care.

Nurses' performance and limited resources in many low and middle-income countries has direct contribution to the development of VAP (47). These countries need to adjust their own bundles with low-cost and high level of evidence variables adapted to the limited resources. Therefore, our intervention is a novel technique that has no harm and good impact on reducing the occurrence of VAP.

4.1 Limitations and recommendations

Our study warrants mentioning some limitations. Although our study used three ICU sites, these were all in one single hospital, which limits the generalizability of our study findings. Also, the sample size can be considered small and more large scale and multi-center studies are needed to confirm the effect of our intervention. Moreover, the VAP bundle and international suctioning guidelines were not rigorously implemented in the study settings. Another limitation is the blinding of the intervention. In a follow-up study we aim to blind the two solutions by giving the nurses the same shape container with different solution to limit the blinding. Also, the lack of uniformity in the training and gesture of bronchial breathing by nurses is another limitation to this study. Due to limited resources, the incidence of VAP was assessed using the MCPIS however the alveolar bronchoscopy is the accurate test for detecting VAP (diagnosis with BAL $> 10^4$ CFU/ml). The study recruitment was temporarily stopped for 3 months due to COVID-19 pressures, and we are unsure this has affected the quality of the data collection. The duration of mechanical ventilation and the length of stay in the ICU should be reported using mean and standard deviation. Recording the frequency of suctioning procedures for each patient is necessary. A final limitation is the effect of the use of chlorhexidine on bacterial species. We did not perform additional lab testing to collect a sample from the suctioning collecting jar to identify bacterial species in the circuit after chlorhexidine flushing.

5 Conclusion

The findings of this study contribute to the evidence-based practice related to the care of ventilator-dependent patients. Endotracheal suction system flushing with chlorhexidine gluconate 0.2% might reduce the VAP incidence in ICU and reduce the cost of flushing solutions. Future large-scale studies on different patient populations and in different settings are also required to obtain solid evidence to support this approach and cover that significant gap of knowledge.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Ethics statement

The studies involving humans were approved by the Research Ethics Committee of Faculty of Nursing, Mansoura University, Egypt. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MHE and NAK contributed to the design of the study. MHE, MAS, and NAK contributed to the data collection. MHE, MMṬ, and JML contributed to the data analysis and interpretation of data. MHE and JML drafted the first manuscript. MMṬ, MAS, and NAK provided revisions. All authors equally contributed, read, 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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1295277/full#supplementary-material

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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
M. Jahangir Alam,
University of Houston, United States
Aref Shariati,
Iran University of Medical Sciences, Iran

*CORRESPONDENCE
Yiming Li
✉ lym-fly@whu.edu.cn
Zhiyong Peng
✉ zn001590@whu.edu.cn

†These authors have contributed equally to this work and share first authorship

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Clostridioides difficile infection after extracorporeal membrane oxygenation support for acute myocardial infarction: a case report

Yanan Hu[1,2†], Chang Hu[1,2†], Jun Jiang[1,2], Jing Zhang[1,2], Yiming Li[1,2*] and Zhiyong Peng[1,2,3*]

[1]Department of Critical Care Medicine, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, China, [2]Clinical Research Center of Hubei Critical Care Medicine, Wuhan, Hubei, China, [3]Center of Critical Care Nephrology, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

Introduction: Restored cardiopulmonary function is efficiently achieved by utilizing extracorporeal membrane oxygenation (ECMO). Nevertheless, the incidence of *Clostridioides difficile* infection (CDI) associated with ECMO is relatively uncommon.

Case presentation: In this report, we present the case of a 59-year-old male with severe chest pain due to acute myocardial infarction, subsequently necessitating ECMO support. During the first day of hospitalization, pulmonary infections were observed, and piperacillin-tazobactam was prescribed for 7 days at low dosages. However, the patient developed severe diarrhea 4 days later. After ruling out common pathogens, we suspected the occurrence of CDI and performed genetic testing for *C. difficile* toxin, confirming our diagnosis. The prescription of vancomycin resulted in slight improvement, while fecal microbiota transplantation (FMT) proved to be more effective.

Conclusion: In this case, temporary application of ECMO was applied, and the anti-infective treatment relied on the use of antibiotics at short-term, low-dose, and low CDI risk. Hence, the occurrence of CDI was considered an uncommon event, which may serve as a reference for future cases.

KEYWORDS

***Clostridioides difficile*, *Clostridioides difficile* infection, fecal microbiota transplantation, extracorporeal membrane oxygenation, case report**

Introduction

Clostridioides difficile infection (CDI) is a type of bacterial infection that primarily affects intestines. It arises from the disruption of the normal intestinal flora, which facilitates the colonization of *C. difficile* (1). Although CDI was traditionally considered a hospital-acquired infection, recent studies have indicated a significant proportion of community residents affected by *C. difficile* (2). Statistics show that approximately half a

million individuals in the United States were infected with *C. difficile*, leading to a substantial annual cost of $4.8–6.3 billion for in-hospital management (3, 4). Several patient-related risk factors contribute to the risk of CDI, with antibiotics exposure being the most significant risk factor. The use of various antibiotics, particularly cephalosporins, clindamycin, fluoroquinolones, and carbapenems, is associated with the development of CDI. The risk of infection also increases with the number and duration of antibiotic applications. Furthermore, CDI is closely associated with advanced age, hospitalization, cancer, chronic kidney diseases, and the usage of immunosuppressants or gastrointestinal interventions like surgeries, nasal feeding, proton pump inhibitor (PPI), histamine-2 receptor antagonist (H_2RA) (1, 5). The manifestation of CDI varies widely, ranging from asymptomatic carriage and mild to moderate diarrhea, to severe cases of fulminant colitis that can be fatal (6). Intensive Care Unit (ICU) patients, due to their critical and severe condition, often require concurrent antibiotics, nasogastric tube intubation, and intensive care, placing them at a higher risk of CDI. Therefore, it is crucial to emphasize universal prevention measures and carefully consider CDI as a potential cause when evaluating suspected symptoms.

Extracorporeal membrane oxygenation (ECMO) is a form of extracorporeal life support (ECLS) used for addressing circulatory or respiratory failure in patients. This technique utilizes a mechanical device that provides either short- or long-term extracorporeal life support. The most frequent conditions that warrant ECMO initiation are acute respiratory distress syndrome (ARDS) and cardiogenic shock (7). Despite its frequent utilization in the ICU, ECMO is a highly invasive procedure associated with multiple complications, including large vessel tears, hemorrhage, hemolysis, and infection. It is crucial to acknowledge that patients undergoing ECMO are typically critically ill, malnourished, or immunocompromised, which markedly increases the risk of infection (8). Stefano Biffi et al. (9) reported that lower respiratory tract infection was the most frequent type of infection in adults undergoing ECMO, with bloodstream infections, urinary tract infections, surgical site infections, and intestinal infections being less frequent. Additionally, Wang's study (10) indicated that gram-negative pathogens, including *Acinetobacter baumannii* and *Klebsiella pneumoniae*, were the primary pathogens, while fungal pathogens like *Candida albicans* and *Candida glabrata* were also prevalent. In contrast, gram-positive pathogens were rarely observed. Based on clinical experience with ECMO, the incidence of *Clostridioides difficile* infection following ECMO support is uncommon among ICU patients. It is suspected that surgical interventions and intensive care associated with ECMO may promote the translocation of normal intestinal microbiota and increase the likelihood of *C. difficile* colonization.

Herein, this study reports on a patient suffered acute myocardial infarction and received ECMO treatment, who subsequently developed *C. difficile* infection. The clinical data will provide valuable insights for preventing and treating similar cases in the future.

Case presentation

The patient was a 59-year-old male with a history of diabetes and hypertension. On November 9, 2021, at 4 pm, he experienced sudden chest pain and tightness without any apparent triggers, leading to his immediate transfer to the local hospital's emergency department. An electrocardiogram (ECG) was performed, revealing an acute inferior myocardial infarction and a third-degree atrioventricular block. The patient was then received Alteplase at the standard dose. Shortly after, a follow-up ECG showed slight ST-segment resolution, but the patient had already entered a state of shock. To receive coronary stent implantation, he was immediately transferred to a higher-level hospital. However, during the surgery, the patient's blood pressure and pulse oxygen saturation remained unstable. Temporary measures were taken, including the administration of norepinephrine (NE) at a rate of 160 ug/min, dopamine (DA) at 10 ug/min, and epinephrine at 20 ug/min. The patient was then admitted to the ICU at Zhongnan Hospital of Wuhan University, where he received tracheal intubations and an intra-aortic balloon pump (IABP). On the November 11 (Day 1), Veno-Arterial ECMO (VA-ECMO) support was provided to avoid deterioration.

The patient's physical examination revealed body temperature of 36.5°C, heart rate of 72 bpm, SpO_2 of 86%, respiration rate of 16 bpm while receiving endotracheal intubation and ventilator support using pressure control mode, with FiO_2 set at 100%, frequency (f) set at 16 bmp, positive end-expiratory pressure (PEEP) at 12 cmH_2O, and blood pressure reading of 144/99 mmHg (with NE injection at 80 ug/min). The patient's bilateral pupil diameter was approximately 2.0 mm, with no icteric sclera and retained light reflexes. Cardiac sounds were low and unremarkable, and no other significant physical findings were noted. The laboratory results showed a white blood cell (WBC) count of 11.13×10^9/L, C-reactive protein (CRP) level of 171.2 mg/L, procalcitonin (PCT) level of 46.17 ng/mL, brain natriuretic peptide (BNP) level of 334.8 pg./mL, creatine kinase (CK) level > 4,267 u/L, creatine kinase-MB (CK-MB) level of 823 u/L, myoglobin level > 1200.0 ng/mL, and high-sensitivity troponin I level > 50000.0 pg./mL. The arterial blood gas analysis reflected a pH of 7.21, PaO_2 of 57.49 mmHg, $PaCO_2$ of 50.55 mmHg, K^+ of 6.04 mmol/L, Na^+ of 138.39 mmol/L, $HCO3^-$ of 19.8 mmol, and Lac of 4.28 mmol/L. Regarding the patient's VA-ECMO settings, the speed was set at 7000 rpm/min, blood flow velocity at 3.3 L/min, gas flow velocity at 2 L/min, and fraction of inspired oxygen at 60%.

The patient initially suffered from acute myocardial infarction, despite attempts with medication and emergency surgery, these treatments proved ineffective. Therefore, VA-ECMO was promptly initiated to delay heart failure and provide additional time for cardiac function recovery. Fortunately, after 5 days of ECMO support, the patient's general condition and heart function returned to normal, as indicated by stable vital signs and cardiac ultrasound (CUS) results. VA-ECMO was discontinued on the sixth day of hospitalization. Table 1 provides a record of the ECMO operating parameters and changes in left ventricular ejection fraction (LVEF). In addition to the primary condition, routine blood tests on day 1 showed abnormal indices: WBC: 11.1×10^9/L, neutrophil percentage (N%): 83.3%, procalcitonin (PCT): 46.2 ng/mL, and interleukin-6 (IL-6): 660 pg./mL, along with a low-grade fever of 37.5°C. These findings suggested the occurrence of an infection, which was confirmed by chest X-ray and computed tomography (CT) showing pulmonary infections. As a result, piperacillin-tazobactam (iv. 4.5 g

q8h) was prescribed for temporary control. Although clinical signs of infection were evident, specific bacteria were not detected in the sputum culture, blood culture, or bronchoalveolar lavage fluid (BLF). The infection was gradually improved with the prescribed medication (Figure 1).

On Day 12, the patient's condition deteriorated since he developed diarrhea. The diarrhea was characterized by yellow, water-like stools and the patient defected seven times that day, amounting to approximately 1,100 mL in total. The following day, the patient experienced eight bowel movements, with the volume increasing to 1,320 mL. Despite the prescription of antidiarrheal medications, including berberine and montmorillonite powder, the patient's condition did not improve. Bacterial culture, biochemical identification, and susceptibility tests were performed on the fecal sample, but they did not yield any useful information. Considering the patient's medical history of antibiotic administration during the first week of hospitalization, *Clostridioides difficile* was suspected as the likely cause of the persistent diarrhea. Genetic testing, using a real-time polymerase chain reaction assay for *C. difficile* toxin, confirmed that the patient tested positive for toxin B. Coupled with a WBC count exceeding 15×10^9/L and creatinine (Cr) above 1.5 mg/dL, the patient was diagnosed with a severe CDI.

According to the ACG Clinical Guidelines (11), the patient was initially treated with vancomycin at a dosage of 125 mg every 6 h via a nasogastric tube. As a result, the patient's stool frequency decreased to 3 times per day on Day 15, and then stabilized at 5–6 times per day with partially formed stool for the subsequent 4 days. Despite some improvement in diarrhea after 7 days of vancomycin treatment, the patient's stool consistency did not fully return to a healthy state. Therefore, fecal microbiota transplantation (FMT) was proposed to prevent the progression of intestinal infections. Fecal samples were obtained from the Second Hospital Affiliated with Nanjing Medical University and were stored at a temperature of −20°C after being transported under low temperature conditions. The FMT infusion was administered on Day 20.

Before the operation, the samples were thawed in 37°C water for 1 h, and the patient was assisted to sit upright with intravenous metoclopramide (10 mg). Then, a nasal jejunal feeding tube was pre-placed, and the sample was infused through the tube. The whole procedure was completed within 3–5 min, and normal saline (5 mL)

TABLE 1 Detailed parameters of ECMO and the change in LVEF.

Date	ECMO parameters				LVEF (%)
	Speed (rpm/min)	Blood flow velocity (L/min)	Gas flow velocity (L/min)	FiO_2 (%)	
Day 1	7,000	3.3	2	60	42
Day 2	7,300	3.2	3	80	25
Day 3	7,300	3.6	2	60	28
Day 4	5,900	3.1	2	60	32
Day 5	5,600	2	1	50	42
Day 6	Removed ECMO				55
Day 7	Removed IABP				54
Day 8	Took off NE				52

ECMO, extracorporeal membrane oxygenation; LVEF, left ventricular ejection fraction; IABP, intra-aortic balloon pump; NE, norepinephrine.

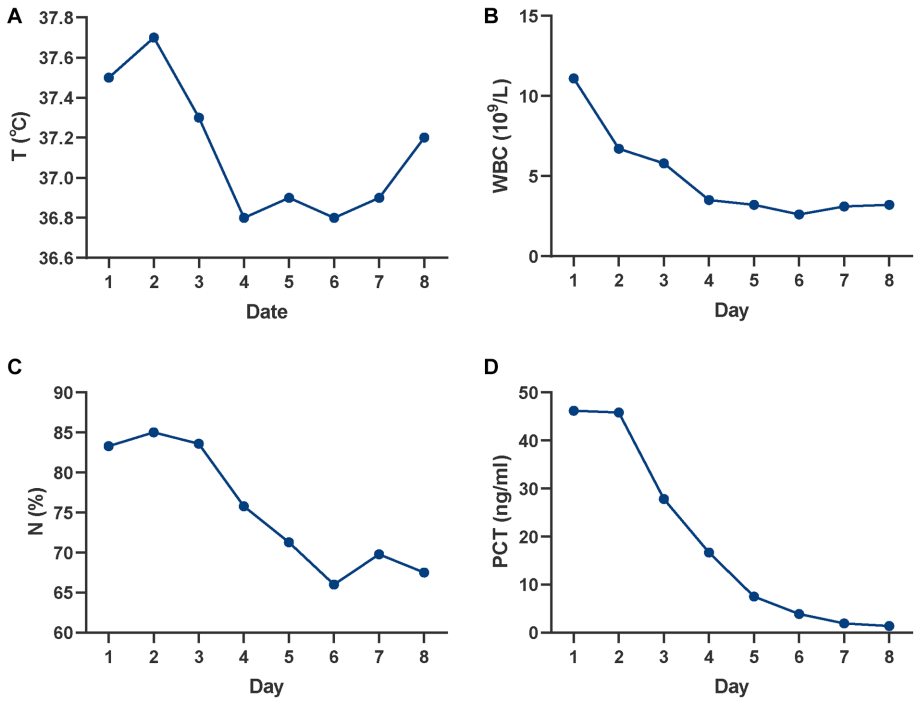

FIGURE 1
Changes in infectious indicators over time. **(A)** Changes in T; **(B)** Changes in WBC; **(C)** Changes in N%; **(D)** Changes in PCT. T, temperature; WBC, white blood cell; N%, neutrophil percentage; PCT, procalcitonin.

was used to flush the tube. The effect of the FMT was immediate, resulting in a significant reduction in stool frequency (3 times per day with a total volume of 330 mL on Day 21). In the following week, the patient experienced approximately 2–4 bowel movements per day, with a lower volume than before. Furthermore, genetic tests for *C. difficile* toxin conducted on both Day 24 and Day 28 yielded negative results, indicating a good outcome of FMT therapy for CDI. Detailed data on the development of CDI are presented in Table 2.

TABLE 2 Changes in fecal condition and relevant treatments.

Date	Fecal properties	Frequency (d^{-1})	Volume (ml)	Treatments
Day12	yellow, loose	7	1,100	Berberinum and Montmorillonite
Day13	yellow, loose	8	1,320	diagnosis of CDI
Day14	yellow, loose	6	1800	vancomycin 125 mg q6 h
Day15	yellow, loose	3	260	vancomycin 125 mg q6 h
Day16	yellow, loose	5	850	vancomycin 125 mg q6 h
Day17	yellow, loose	6	290	vancomycin 125 mg q6 h
Day18	yellow, partly formed	5	460	vancomycin 125 mg q6 h
Day19	yellow, partly formed	6	550	vancomycin 125 mg q6 h
Day20	yellow, partly formed	8	550	FMT
Day21	yellow, partly formed	4	330	–
Day22	yellow, formed	2	50	–
Day23	yellow, formed	4	240	–
Day24	yellow, formed	6	450	retest: tcd B (−)
Day28	yellow, formed	2	300	retest: tcd B (−)

CDI, *Clostridioides difficile* infection; FMT, fecal microbiota transplantation; tcd B, *Clostridioides difficile* toxin B.

After 28 days of ICU treatment, the patient regained consciousness, exhibited a good mood and demonstrated normal cardiac function, and regular bowel movements without any complaints of infections or intestinal discomfort. Comprehensive assessments led to the patient's transfer from ICU back to the ward, where close monitoring was continued during the early phase of recovery. The whole process is illustrated in Figure 2.

Discussion

ECMO is a crucial intervention for replacing cardiac and pulmonary functions in the short term. VA-ECMO is primarily used to manage heart failure, including cardiogenic shock following myocardial infarction, extracorporeal cardiopulmonary resuscitation (ECPR), and fulminant myocarditis (7). In this case study, the patient was admitted to the hospital due to acute myocardial infarction and initially received empiric treatment with thrombolytic drugs and IABP. However, the effectiveness of the treatment was limited, and the patient's symptoms worsened. Consequently, ECMO was employed to provide more consummate support. Nonetheless, the duration of ECMO application increased, thereby increasing the likelihood of bacterial infection (12). Thus far, no reports have been published regarding *Clostridioides difficile* infection in patients undergoing ECMO support.

CDI is a leading cause of infectious diarrhea in medical institutions, with increasing incidence in the ICU (13, 14). This condition occurs from an imbalance in the normal gut flora, primarily due to the abuse of broad-spectrum antibacterial drugs and other contributing factors (15). Disturbances in the gut microbiota may lead to proliferation of *C. difficile* from both endogenous and exogenous sources. A meta-analysis comparing various antibiotic classes and their association with hospital-acquired CDI found that third-generation cephalosporins had the highest odds, followed by clindamycin, second-generation cephalosporins, and fourth-generation cephalosporins (16). In this report, the patient demonstrated infectious characteristics from the time of admission, including fever, increased leukocyte count, procalcitonin, neutrophils, and a diagnosis of pulmonary inflammation based on imaging. Piperacillin-tazobactam was used, resulting in the normalization of infection indicators, and antibiotics were discontinued after 7 days. Interestingly, numerous

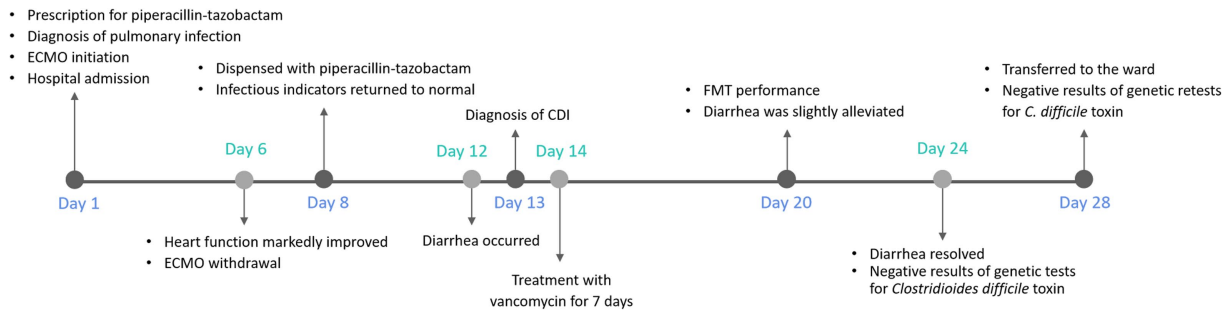

FIGURE 2
Diagnosis and treatment timeline of the patient. The timeline shows the major clinical events during the patient's hospitalization. ECMO, extracorporeal membrane oxygenation; CDI, *Clostridioides difficile* infection; FMT, fecal microbiota transplantation.

studies have shown a higher risk increase for CDI associated with broad-spectrum antimicrobials, such as cefepime and meropenem than with piperacillin-tazobactam (17–19). Kundrapu (20) explains that piperacillin-tazobactam has the potentially to combat the colonization of *C. difficile*, unlike cefepime and other cephalosporins, which supports a widely accepted hypothesis. Although the patient receiving antibiotics with a lower CDI risk and at a lower dosage for a shorter duration, it did not alter the patient's CDI status. Several possible reasons were analyzed, including: (1) the spores of *C. difficile* were widely distributed in hospital settings, (2) the patient's cardiogenic shock and 5-day ECMO support, rendering him more susceptible to multi-drug-resistant bacteria, like *C. difficile*, (3) the invasive facilities used in the ICU, such as mechanical ventilation and nasogastric tubes, which were major risk factors for CDI, (4) the disruption of gut flora balance by piperacillin-tazobactam as an antibiotic, leading to the colonization of various opportunistic pathogens, and (5) the patient's concurrent conditions of hypoxic–ischemic encephalopathy and acute kidney injury during hospitalization, contributing to a decline in overall immune function.

The diagnosis of CDI necessitates a thorough evaluation of clinical symptoms and confirmatory tests. First, CDI should be suspected when patients present with acute diarrhea, defined as loose stools occurring three or more times in a 24-h period. The severity of symptoms can vary, with mild cases characterized by watery diarrhea and mild abdominal cramping or tenderness. Severe cases can present with extremely frequent voiding (10–15 times/day), intense abdominal pain, nausea, fever, and potentially life-threatening complications such as toxic megacolon, sepsis, and multiorgan failure. Second, the diagnosis of CDI requires positive genetic evidence or colonoscopic confirmation of pseudomembranous colitis (6, 21–23). According to the ACG clinical guidelines (11), a WBC count greater than 15×10^9/L and a serum Cr level greater than 1.5 mg/dL classify the patient as having severe CDI.

Regarding the treatment of CDI, it is crucial to evaluate the discontinuation of antibiotic therapy for all patients diagnosed with CDI, unless it hampers the recovery of other conditions. Vancomycin is the preferred treatment for mild to severe CDI, with evidence supporting the use of fidaxomicin as an alternative. Metronidazole is suggested solely for the first episode of mild or moderate CDI if vancomycin or fidaxomicin are not available (24, 25). In this report, the patient was diagnosed with severe CDI and received vancomycin, which proved ineffective. The lack of effectiveness against the infection may be attributed to common antibiotic resistance.

CDI is rooted in gut-induced dysbiosis, leading to extensive research on microbial therapies. FMT is an advanced approach that restores the complete spectrum of microorganisms constituting normal colonic flora by transferring the colonic microbiome from a healthy individual. When standard medical therapy for severe CDI is ineffective, FMT should be considered a treatment option (26, 27). Serious adverse events associated with FMT are rare, and gastrointestinal symptoms such as nausea, belching, or bloating are the most commonly reported issues, particularly when FMT is administered through the upper gastrointestinal route (28, 29). The risk of infection transmission, such as enteropathogenic *Escherichia coli* (EPEC) or Shiga toxin-producing *Escherichia coli* (STEC), can be mitigated through careful donor selection and screening. Nevertheless, the acceptability of this application method to patients and the safety of transportation during the coronavirus disease 2019 (COVID-19) pandemic should be taken into account (30–32). In this report, FMT was used to treat CDI after unsatisfactory outcomes with vancomycin, resulting in significant declines in stool frequency and volume, as well as the absence of the toxin B gene detected in two subsequent tests conducted a few days later.

The prevention strategies for CDI hold paramount importance, with the primary approach being the restriction of antibiotic use, particularly high-risk antibiotics. In case where elderly individuals (aged 65 years or older), patients with a history of healthcare exposure or other inevitable risk factors for CDI, it is crucial to minimize the duration of antibiotic administrations as much as possible (33). Second, decontamination of hospital environments is imperative to prevent the transmission of CDI, as the spores of *C. difficile* are largely resistant and actively released from infected patients (34). Alongside ensuring high-quality disinfection and sterilization in hospitals, particular attention should be given to hand hygiene. Healthcare personnel in close proximity to patients must adhere to recommended procedures for thorough hand disinfection, using running water (35). It is worth noting that, at present, there is insufficient evidence to recommend any probiotics for the primary or secondary prevention of CDI in most patients (11).

Conclusion

In the ICU, ECMO stands as an easily accessible medical advancement. However, it is important to acknowledge that patients requiring ECMO support often suffer from critical and emergent illnesses, which increase their likelihood of antibiotic use, complications, and immune system impairment. Under these conditions, the intestinal flora becomes particularly vulnerable to dysregulation, potentially leading to the development of CDI. Therefore, clinicians should prioritize the preservation and enhancement of gastrointestinal function, recognizing the paramount significance of implementing all reasonable precautions and treatments to positively influence therapy, prognosis, and healthcare costs. Both healthcare professionals and patients share a common goal of efficiently combatting the risk of CDI.

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.

Ethics statement

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

Author contributions

YH: Conceptualization, Investigation, Writing – original draft. CH: Methodology, Supervision, Writing – original draft. JJ: Project administration, Writing – original draft. JZ: Resources. YL: Formal analysis, Supervision, Writing – review & editing. ZP: Funding acquisition, Resources, Writing – review & editing.

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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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Glossary

ECMO	extracorporeal membrane oxygenation
CDI	*Clostridioides difficile* infection
PPI	proton pump inhibitor
H_2RA	histamine-2 receptor antagonist
ICU	Intensive Care Unit
ECLS	extracorporeal life support
ARDS	acute respiratory distress syndrome
ECG	electrocardiogram
NE	norepinephrine
DA	dopamine
IABP	intra-aortic balloon pump
VA-ECMO	Veno-Arterial ECMO
PEEP	positive end-expiratory pressure
WBC	white blood cell
CRP	reactive protein
PCT	procalcitonin
BNP	brain natriuretic peptide
CK	creatine kinase
CK-MB	creatine kinase-MB
CUS	cardiac ultrasound
LVEF	left ventricular ejection fraction
N%	neutrophil percentage
PCT	procalcitonin
IL-6	interleukin-6
CT	computed tomography
BLF	bronchoalveolar lavage fluid
Cr	creatinine
FMT	fecal microbiota transplantation
ECPR	extracorporeal cardiopulmonary resuscitation
EPEC	enteropathogenic *Escherichia coli*
STEC	Shiga toxin-producing *Escherichia coli*
COVID-19	the coronavirus disease 2019

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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Rudy Tedja,
Renown Health, United States
António Machado,
Universidad San Francisco de Quito, Ecuador

*CORRESPONDENCE
Emili Masferrer
✉ emilimasferreriniubo@gmail.com

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Randomized controlled trial on healthy volunteers of pharmacokinetic and antimicrobial activity of a novel hydrogel-containing chlorhexidine dressing to prevent catheter-related bloodstream infection

Emili Masferrer[1]*, Laura Riera-Rodríguez[1], Víctor Farré-Alins[2], Sandra Vilà de Muga[2], Francisco-Javier Arroyo-Muñoz[3] and María-Dolores González-Caro[3]

[1]Department of Dermatology, Hospital Universitari Mútua Terrassa, Terrassa, Spain, [2]Iberhospitex, S.A., Barcelona, Spain, [3]Intensive Care Unit, Hospital Universitario Virgen Macarena, Sevilla, Spain

Introduction: Catheter-related blood stream infection (CRBSI) is one of the most relevant complications associated to the use of intravascular catheters. In this context, chlorhexidine gluconate (CHG) releasing dressings have been developed to reduce the catheter colonization rate and the risk of infection. The aim of this study is to analyze the release rate of CHG and the antimicrobial activity of a novel CHG-releasing dressing, Oper film® protect CHG, and to compare these parameters to those of the dressing Tegaderm™ CHG in healthy volunteers.

Methods: The study was performed in a cohort of 25 healthy volunteers. Two commercially available chlorhexidine-containing dressings were evaluated and compared in this study, Oper film® protect CHG and Tegaderm™ CHG. The release of CHG and the antimicrobial capacity was determined for one week.

Results: HPLC analysis revealed that both dressings have an equivalent CHG release to the skin 2 days (Oper film® protect CHG, 321 μg/cm^2; Tegaderm™ CHG, 279 μg/cm^2) and 7 days (Oper film® protect CHG, 456 μg/cm^2; Tegaderm™ CHG, 381 μg/cm^2) after the placement of the products in the non-disinfected back of the subjects. On the other hand, Oper film® protect CHG and Tegaderm™ CHG similarly reduced colony forming units (CFU) in cultures obtained from the skin under the CHG-containing hydrogel compared to control cultures at both 2 days (control, 3.34 $\log_{10}$ cfu/cm^2; Oper film® protect CHG, 0.64 $\log_{10}$ cfu/cm^2; Tegaderm™ CHG, 0.7 $\log_{10}$ cfu/cm^2) and 7 days (control, 3.95 $\log_{10}$ cfu/cm^2; Oper film® protect CHG, 0.11 $\log_{10}$ cfu/cm^2; Tegaderm™ CHG, 1 $\log_{10}$ cfu/cm^2).

Discussion: Data confirm that the recent commercially available dressing Oper film® protect CHG maintains the release of CHG and the antimicrobial activity during at least 7 days, and possesses equivalent drug release and antimicrobial action to Tegaderm™ CHG.

KEYWORDS

dressings, chlorhexidine, hydrogel, catheter-related bloodstream infection, antimicrobial activity

1 Introduction

The use of intravascular (IV) catheters is associated with the risk to develop catheter-related blood stream infection (CRBSI). CRBSI is a serious medical problem linked to an increase of morbidity, mortality, length of hospital stays and healthcare costs (1, 2). In Europe, 1,247 Intensive Care Units (ICUs) of 15 countries were studied during the period 2008–2012. The incidence of primary bacteriaemia in patients with a catheter inserted for more than 48 h was 3.5%. During the same period, it was estimated that 4,505 deaths were a direct consequence of bacteriaemia; furthermore, it was related with an increase of the length stay in ICU of 1.26 million of days (3). In a similar cohort study, it was detected an increase of mortality in patients admitted at ICU that suffered CRBSI (4). Additionally, Zimlichlman et al. analyzed the costs of the most frequent nosocomial infections in USA, being CRBSI the one that had a higher impact in the sanitary system (5).

It has been described that biofilms are the predominant mode of growth in nearly all bacterial species, and they are linked to the occurrence of nosocomial infections arising from catheter insertions. Gram-positive and negative bacteria and yeasts are the main CRBSI-related microorganisms, being *Staphylococcus* spp., *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. epidermidis* the most common species reported in the bibliography (6).

Recently, hospitals have made considerable efforts to reduce CRBSI. However, despite the improvements, this type of IV catheters-related complication is still a hospital problem. Data from 2019 show a rate between 0.5 and 5.5 CRBSI per 1,000 catheter days in European ICUs (7). As a considerable number of CRBSI could be prevented (8), current attempts are focused on the development of preventive strategies. For this reason, chlorhexidine-containing dressings have emerged as a promising tool to prevent CRBSI.

Chlorhexidine gluconate (CHG) is an antiseptic drug with a broad spectrum of antimicrobial activity. CHG is lipophilic and positively charged, properties that allow the interaction of the drug with lipopolysaccharides and phospholipids of the bacterial cell wall or the outer membrane. At low concentrations, this contact damages the cell wall, enabling the leakage of low molecular weight components and inhibiting enzymes related to the cytoplasmatic membrane. At high concentrations, CHG penetrates the cell and generates severe intracellular damage that leads to cell death (9).

The incorporation of CHG into catheter dressings decreases the microbial burden on the skin and the catheter colonization by microorganisms could be reduced. It is effective against the most common bacteria that generate CRBSI as well as less frequent pathogens (the most common microorganisms isolated in CRBSI are >30% coagulase-negative *Staphylococcus*, 22% *S. aureus*, 8% *Enterecoccus* and 8% *Candida*) (10).

Moreover, CHG-impregnated dressings have a low risk for the development of antimicrobial resistance since CHG is applied topically, has non-specific mechanisms of actions and is not susceptible to efflux pumps (10). Two studies did not find an association between CHG dressings and CHG resistance (11, 12). Other studies found an increased average resistance to CHG in some bacterial species in *in vitro* assays, although after several decades the variation was low. However, the clinical relevance of these results is very limited since CHG concentrations used in clinical practice are far superior to the minimal inhibitory concentration for any analyzed microbes (9, 13).

Indeed, the updated clinical guidelines strongly recommend the use of chlorhexidine-impregnated dressings to reduce CRBSI (5, 7). Previously, the main recommendations were focused on: (i) education and training of healthcare personnel who manipulate catheters highlighting hand hygiene; (ii) use of maximal sterile barrier precautions during central venous catheter (CVC) insertion; and (iii) use of >0.5% chlorhexidine skin preparation with alcohol for antisepsis. The scientific evidence generated in the last years has allowed to include in the clinical guidelines the recommendation to use chlorhexidine-impregnated dressings. Specifically, there are three types of commercially available CHG dressings: CHG-impregnated sponge rings, CHG-containing hydrogel pad dressings and dressings with CHG integrated in the adhesive.

Numerous clinical trials have been published assessing CHG dressings performance and safety. The most recent meta-analysis includes 20 studies (18 of them controlled clinical trials) (2). General results show that CHG dressings reduce the risk of CRBSI by 33%, obtaining strongest evidence for adults with short-term CVCs. In contrast, there is a notable risk of contact dermatitis in neonates and pediatric population and lack of evidence of usefulness in these groups. In fact, contact dermatitis was the most common adverse event reported in studies (14, 15). Safdar et al. detected 1.2% of CRBSI in patients receiving CHG dressings compared with 2.3% in patients receiving conventional dressings (14). This study found a significant decreased risk of CRBSI in adult patients admitted in ICUs while no reduction was found in pediatric population. Similarly, another meta-analysis analyzing 12 clinical trials indicate that CHG dressings are useful tools for the prophylaxis of CRBSI (16).

Nevertheless, there are various points to be addressed in the knowledge of CHG dressings. The most important questions are to clarify which groups of patients could obtain a direct benefit from the use of CHG dressings and determine if there are differences among the three types of commercially available CHG dressings (CHG-impregnated sponge rings, CHG-containing hydrogel pad dressings and dressings with CHG integrated in the adhesive) regarding effectivity. Another crucial parameter is the delivery of CHG to the skin and the capacity of absorption of the drug. Some studies have evaluated its absorption in aqueous solutions containing 2% CHG. In an *in vitro* model, topical application showed poor penetration of CHG into the skin (17). In patients, the investigations have focused on newborns and neonates, in which trace amounts of CHG were detected after treating the skins with aqueous solutions

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(18). The recent advances in the medical field have led to examine the antimicrobial capacity of CHG dressings. A significant reduction of microorganisms was detected along the first week after the placement of the dressing in healthy volunteers (19); however, the quantification of the release pattern of CHG from dressings to the skin has not still been analyzed.

Considering the importance of CHG dressings in the prevention of CRBSI, the aim of this study is to compare the pharmacokinetics and the antimicrobial activity of the novel dressing Oper film® protect CHG to the gold standard product, Tegaderm™ CHG.

2 Methods

2.1 Materials

Two commercially available chlorhexidine-containing dressings were evaluated and compared in this study: Oper film® protect CHG (Iberhospitex, S.A.) and Tegaderm™ CHG (3 M). Both devices are self-adhesive transparent polyurethane dressings that incorporate a hydrogel that contain 2% CHG pads. These products are indicated to be used in patients eligible for IV catheter placement.

2.2 Study population

A total of 25 healthy volunteers were included in the study. Demographic data are shown in Table 1. Subjects were ≥ 18 of age and provided written informed consent. Exclusion criteria were (i) to have incompatibilities with the participation in the study (i.e., to be participating in other clinical trial); (ii) to be allergic/hypersensitive to polyurethane, acrylic adhesive, CHG or any other component of the dressings; (iii) to be pregnant or breastfeeding.

The protocol, conducted in accordance with the GCP standards (CPMP/ICH/135/95) and the current legislation, was approved by the Ethics Committee of Fundació Assitencial Mútua Terrassa. The study was performed at the facilities of Hospital Universitari Mútua Terrassa.

TABLE 1 Demographic data of the patient cohort.

Number of patients	25
Number of dressings	
Oper film® protect CHG	50
Tegaderm™ CHG	50
Mean age	40 ± 12.5 years
Amount of hair	
Absent	23
Some	2
Body sweat	
Absent	28
Some	6
Dryness of the skin	
Absent	18
Some	7

2.3 Treatment

Each subject received four dressings on their back. The back, which did not receive disinfection treatment, was divided in two middles (left and right), and each middle contained one Oper film® protect CHG and one Tegaderm™ CHG. The dressings were aleatory allocated in the above or below area (see Figure 1).

Two days after the placement, the two dressings of one of the middles (one Oper film® protect CHG and one Tegaderm™ CHG) were detached and a skin smear from the area covered by the hydrogel pad was immediately taken. Moreover, in order to collect a negative control, a smear from a skin area that had not been in contact with any component of the dressing was collected. All the dressings were stored for subsequent analysis of the quantity of CHG delivered by the hydrogel pad. Seven days after the placement, the two other dressings (one Oper film® protect CHG and one Tegaderm™ CHG) were detached. The same steps described in the previous paragraph were followed.

The subjects could not shower their back during the seven days they wore the dressings. In the case of the appearance of any adverse event, it had to be followed up until it was completely resolved.

2.4 Microbial burden

The skin under the hydrogel was rubbed in circles with a swab moistened with sterile sodium chloride. The swab was placed in a tube with stuart transport medium (Deltalab, ref. 300291) and stored at 4°C; subsequently, it was expanded on blood agar plates (Scharlab, ref. 064-PA0004) and incubated at 37°C for 48 h. After two days of incubation, a photograph of each plate was taken to count the number of colony-forming units (CFU) grown on the agar. All the area under the hydrogel, that is, all the skin surface that was in contact with CHG, was rubbed with the swab. Thus, the surface indicated in Figure 2 includes the area of the skin that was under the containing-CHG hydrogel. The CFU were manually counted in each culture plate.

2.5 CHG quantification

Liquid chromatography was performed on a high-performance liquid chromatography (HPLC) unit from Agilent Technologies 1,200 series. Injections (10 µL) were made on XBridge® C18 column (25 mm × 4.6 mm × 5 µm) from Waters. The column temperature was maintained at 30°C and injector sample racks at 12°C. The flow rate was 1.0 mL/min. The mobile phase was a mix of A: distilled water and acetonitrile (80:20) containing 0.1% TFA and B: distilled water and acetonitrile (10:90) containing 0.1% v/v TFA. The analytical method was validated with respect to parameters such as linearity, range, precision, accuracy, selectivity, and robustness. Chlorhexidine was quantified using an UV–VIS detector. The HPLC method was validated against a Reference Standard from the European Pharmacopoeia (Sigma-Aldrich, ref. PHR1294).

2.6 Skin irritation

The erythema degree was evaluated in the skin that was in contact with the chlorhexidine-containing hydrogel. A categorical

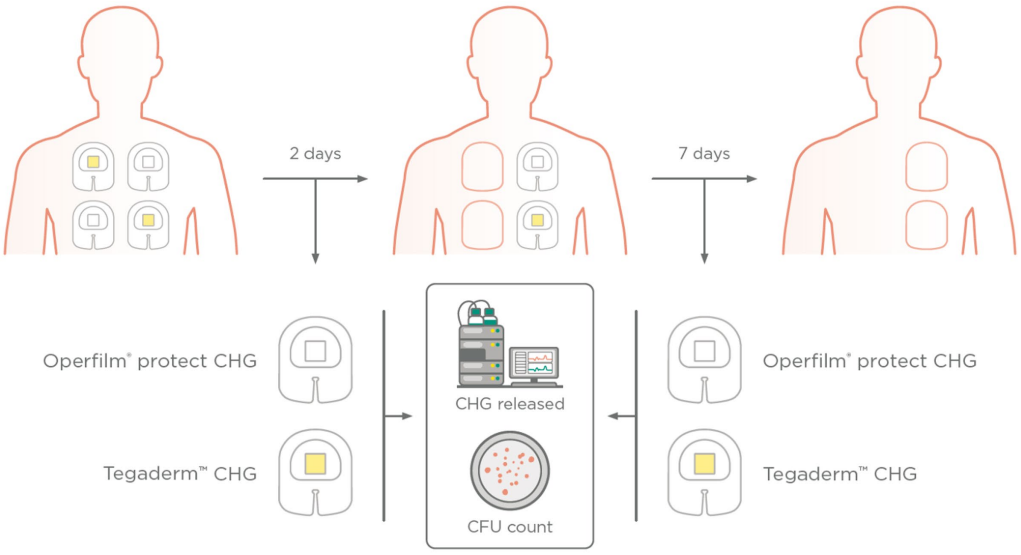

FIGURE 1
Scheme of the placement of Tegaderm™ CHG and Oper film® protect CHG in the back of the healthy volunteers. The dressings were detached 2 and 7 days after placement. The release of CHG was calculated by HPLC and skin cultures were performed to count the colony forming units (CFU).

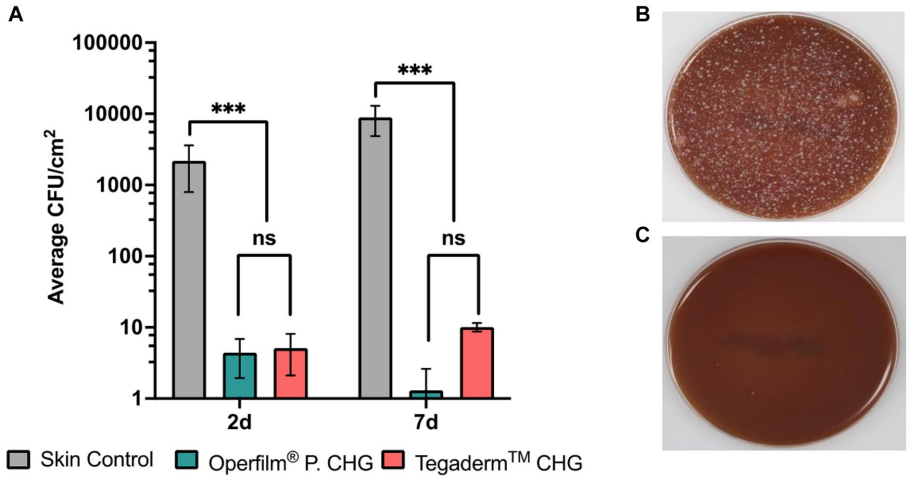

FIGURE 2
Effect of Oper film® protect CHG and Tegaderm™ CHG on the growth inhibition of microorganisms. **(A)** Colony forming Units (CFU) per square centimeter in skin controls and areas under CHG pads of Tegaderm™ CHG and Oper film® protect CHG. Mean ± SEM ($n = 25$ per group). Groups are compared using Mann–Whitney U non-parametric test; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns $p > 0.05$. **(B)** representative picture of control skin culture and **(C)** representative picture of a culture from skin treated with Oper film® protect CHG or Tegaderm™ CHG.

classification of erythema was elaborated, classifying its degree in absent, mild, moderate or severe. Erythema was monitored on day 2 and day 7 after the placement of the dressing.

2.7 Statistical analysis

Data is presented as mean ± SEM. Since data did not follow normal distribution, Kruskal-Wallis and Mann–Whitney U non-parametric tests were used to determine differences between groups for CHG release (Figure 3) and microbial count (Figure 2). Fisher's exact test was used for erythema detection (Table 2). A $p < 0.05$ was considered statistically significant. All statistical analysis and graphical representation were performed using GraphPad Prism.

3 Results

All the 25 volunteers finalized the study. Considering that each subject was applied with 4 dressings (2 Oper film® protect CHG and 2 Tegaderm™ CHG), a total of 100 dressings were used.

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The quantity of CHG that remained in the hydrogel was measured by HPLC. Thus, the absorption of the drug was inferred from these values. Two days after the placement of the dressings, Oper film® protect CHG (321 μg/cm^2) released an equivalent amount of CHG compared to Tegaderm™ CHG (279 μg/cm^2). Similar results were obtained at 7 days (Oper film® protect CHG, 456 μg/cm^2; Tegaderm™ CHG, 381 μg/cm^2) since no significant differences were detected in this pharmacokinetic parameter between the two products (Figure 3). The detected CHG values could be affected by a range of factors such as sweating, the type of skin or the type of patient. These characteristics could notably influence the absolute quantified values; however, the impact in the relative differences between groups or temporal points would be limited.

The count of CFU revealed that both Oper film® protect CHG and Tegaderm™ CHG dramatically reduced the microbial burden at 2 days as well as at 7 days after the placement of the dressings. Moreover, the differences in the decrease of CFU between the two dressings were not significant at neither time point (Figure 2A). These results are shown in detail in Table 2. No group of dressings had a count that exceeded 1 $\log_{10}$ cfu/cm^2. Pictures shown at Figures 2B,C demonstrate an equivalent 2/3-fold log reduction in CFU count produced by the two products. The skin of the patients was not disinfected, thus the microorganisms' levels of the skin remained intact. Therefore, the non-disinfection of the skin would be the worst situation in which these dressings would be used, and the results demonstrate that under these conditions Oper film® protect CHG possesses a strong antimicrobial activity.

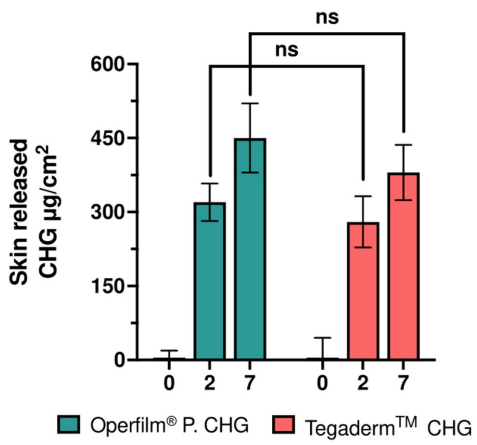

FIGURE 3
Pharmacokinetics of CHG dressings. CHG delivered to the skin of healthy volunteers at 2 and 7 days after the placement. Mean ± SEM (n = 25 per group). Groups are compared using Kruskal-Wallis non-parametric test; ***p < 0.001, **p < 0.01, *p < 0.05, ns p > 0.05.

Erythema degree was evaluated in the skin surface that was in contact with the chlorhexidine-containing hydrogel to assess if one week of permanent contact with the drug leads to irritation. Table 2 lists the number and percentages of subjects in each grade of erythema at both clinical visits. At day 2, only 1 volunteer treated with Oper film® protect CHG and 3 volunteers that received Tegaderm™ CHG had low erythema detection. In the second and last visit, low erythema was observed in 4 subjects of each group. No statistical differences were detected neither at 2 days (p = 0.6) nor at 7 days (p > 0.99). These results confirm the good tolerability of released CHG by these dressings to adult human skin since, after 1 week of permanent contact, 84% of the cohort did not suffer erythema; furthermore, the affected volunteers had a low skin reaction. Moderate or severe erythema was not detected in any subject.

4 Discussion

In this study, we have demonstrated that the novel dressing Oper film® protect CHG possesses equivalent CHG release pattern and antimicrobial activity to Tegaderm™ CHG. Furthermore, the results determine that Oper film® protect CHG maintains the antimicrobial action during at least 7 days (Figure 2), which is the maximum period of time the dressing is indicated to be in contact with the skin. Other studies have analyzed the antimicrobial capacity of gels and sponges containing CHG in healthy volunteers during the first week after application; specifically, these investigations studied 3 time points, 1 day, 4 days, and 7 days post-placement of the dressing (19, 20). The results obtained in these studies are similar to the values we show at Figure 2 and confirm the efficiency of the dressings along the week (19, 20).

TABLE 2 Data of $\log_{10}$ calculations of cfu/cm^2 and erythema degree assessment at both time points for each dressing.

	2 days			7 days		
	Control	Oper film® protect CHG	Tegaderm™ CHG	Control	Oper film® protect CHG	Tegaderm™ CHG
n	25	25	25	25	25	25
Mean $\log_{10}$ cfu/cm^2	3.34	0.64	0.7	3.95	0.11	1
Standard deviation ($\log_{10}$ cfu/cm^2)	3.84	1.1	1.18	4.3	0.8	0.83
Erythema detection						
Absent	-	24 (96)	22 (88)	-	21 (84)	21 (84)
Low	-	1 (4)	3 (12)	-	4 (16)	4 (16)
Moderate	-	0	0	-	0	0
Severe	-	0	0	-	0	0

Baseline log counts detected between 3 and 3.5 $\log_{10}$ cfu/cm^2 (19, 20); in our case, we have detected between 3.3 and 3.9 $\log_{10}$ cfu/cm^2 (Figure 2). Bashir et al. demonstrated that both CHG-containing gel (Tegaderm™ CHG) and disk (Biopatch™ CHG) significantly reduced the microbial count, and similar performance were seen between both types of dressings (19). However, in this study antisepsis was applied using a commercially available skin solution that contained 2% CHG in 70% isopropyl alcohol, which substantially decrease the number of CFU when dressings are placed. In the present work, we showed the results obtained in non-disinfected skin prior to dressing application. We have observed a dramatic and equivalent decline in CFU counts for both hydrogel-based dressings and at both time points, reaching values between 0 and 1 log10 cfu/cm^2 (Figure 2; Table 2). Although this is the worst situation in which the dressings could be used, the inhibition capacity is very similar to the obtained with disinfected skin (19), finding that demonstrates the strong antimicrobial capacity of Oper film® protect CHG.

The antimicrobial capacity of dressings that integrate CHG in the adhesive have also been studied in healthy volunteers (20). The results indicate a lower antimicrobial activity of this type of dressings compared to CHG-containing hydrogel dressings. This study shows around 2–2.5 $\log_{10}$ cfu/cm^2 at three time points (1, 4, and 7 days after the placement) while we have obtained values between 0 and 1 for both dressing in all the measurements (Table 2). Hence, these results indicate that, at least in healthy skin, not all the dressings that contain CHG have the same performance.

The good antimicrobial activity is related with the release pattern of CHG. Nonetheless, the delivery of CHG from the dressing into the skin have been poorly investigated. One *in vitro* study evaluated the kinetics of delivery of CHG-containing sponges, which detected an increasing CHG concentration in saline medium (21). Here, we show the quantification of the drug in two hydrogel-based dressings. The delivery of CHG is progressively increased throughout the week, which demonstrates that the hydrogel enables a sustained and continued release of CHG. In addition, the amount of CHG transferred to the skin is equivalent between both Oper film® protect CHG and Tegaderm™ CHG (Figure 3). These results are aligned and correlate with the inhibition of microorganisms shown in Figure 2. Therefore, a prolonged and sustained release of CHG into the skin for one week affords a powerful antimicrobial response during all this time.

This continued and prolonged inhibition of microbial growth is the cause of the reduction of CRBSI incidence by CHG-containing dressings, a clinical benefit extensively reported in the literature. Three meta-analyses have concluded that the use of CHG dressings provide significant reduction of the risk of catheter colonization and CRBSI in adult patients with central venous catheters compared to traditional dressings used to protect the insertion site (2, 14, 16). Besides dressings, CHG has also been used in the coating of CVCs as an antibiofilm agent due to the inherent ability of several bacterial and fungi to form biofilms that enable them to evade the host immune response (6, 22).

Contact dermatitis is the most common adverse effect reported in clinical trials (14). As it is known that CHG is the causal agent of this adverse event (2, 23), it was explored the dermal reaction in the area under the hydrogel, which is the part of the dressing that contains and delivers the drug.

No statistical differences in skin irritation were detected between dressing groups, albeit Tegaderm™ CHG may have a slightly higher tendency to irritate. Some scientific publications have detected medical adhesive related skin injury (MARSI) associated to the use of Tegaderm™ CHG (8, 10). Adhesion is mainly based on a balance between mechanical damage to the skin and the ability to avoid dressing detachment, and this equilibrium and clinical benefit must be clinically stablished.

We did not detect any major skin reaction, and only low erythema degree was observed in few patients (Table 2). Although the drug was continuously released during one week, only 16% of volunteers experienced low erythema, which confirms the good safety profile of CHG-containing hydrogel dressings while maintaining the microbial inhibition properties (Table 2).

Currently, there are three main types of CHG dressings commercially available: CHG-impregnated sponge rings, CHG-containing hydrogel pad dressings and dressings with CHG integrated in the adhesive (11, 12, 14). Specifically, hydrogel pads present some advantages compared to sponge rings, such as Biopatch™ CHG, due to improved visibility of the insertion point of the catheter and homogenous CHG release (15). Moreover, dressing disruptions are less frequent in gel dressings, probably explained by the difficulty (24). Even though it is not confirmed, dressing disruption could be a risk factor for CRBSI and thus should be prevented. On the other hand, the antimicrobial capacity of dressings that contain CHG in the adhesive seems to be inferior (20), and is necessary to provide evidence to clarify if this type of dressings reduce CRBSI to a comparable level to gel-based dressings.

Another important factor is the pH of the skin. The acid pH of the skin, that ranges from 4.1 to 5.8, is a key component to maintain a healthy skin since acidic environments inhibit microorganisms' growth and avoid bacterial colonization (16). The unique formulation of Oper film® protect CHG buffers skin pH and consequently limits the potential infection development. This technical attribute may provide an additional advantage in preventing CRBSI.

5 Conclusion

The novel dressing Oper film® protect CHG possesses equivalent CHG release pattern and antimicrobial activity to Tegaderm™ CHG, the gold standard product among CHG dressings. Furthermore, the results of this study determine that Oper film® protect CHG maintains the release of CHG and the antimicrobial action during at least 7 days, which is the maximum period of time the dressing is indicated to be in contact with the skin. Moreover, the improved visibility of the insertion point and the management of the surrounding pH provided by Oper film® protect CHG afford an interesting new tool to prevent CRBSI infections.

The study has some limitations. Firstly, it is monocentric, and the sample size is small. This issue was tried to be solved using 4 dressings in each patient to increase the number of units included in the research. Secondly, the cohort is composed by healthy volunteers with a relatively homogenous age. Next clinical studies should be focused on the analysis of microorganism proliferation in the skin

of patients admitted to ICUs or hospital specialized services to specifically determine the decline in the rate of CRBSI infections that presents Oper film® protect CHG when used according to its intended purpose.

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 humans were approved by Fundació Assistencial Mutua Terrassa. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

EM: Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. LR-R: Methodology, Writing – review & editing. VF-A: Conceptualization, Writing – original draft, Writing – review & editing. SV: Conceptualization, Project administration, Writing – review & editing. F-JA-M: Writing – review & editing. M-DG-C: Writing – review & editing.

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Conflict of interest

VF-A and SV were employed by Iberhospitex, S.A.

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

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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Matthew Geriak,
Sharp Grossmont Hospital, United States
Tahereh Navidifar,
Shoushtar Faculty of Medical Sciences, Iran

*CORRESPONDENCE
Wang Deng
✉ dengwang@hospital.cqmu.edu.cn

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Omadacycline for the treatment of severe pneumonia caused by *Chlamydia psittaci* complicated with acute respiratory distress syndrome during the COVID-19 pandemic

Dao-Xin Wang[1,2], Ling-Xi Xiao[1,2], Xin-Yu Deng[1,2] and Wang Deng[1,2]*

[1]Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital of Chongqing Medical University, Chongqing, China, [2]Chongqing Medical Research Center for Respiratory and Critical Care Medicine, Chongqing, China

Introduction: *Chlamydia psittaci* infection in humans is a rare cause that mainly present as community-acquired pneumonia. Severe *Chlamydia psittaci* pneumonia can lead to acute respiratory distress syndrome (ARDS), septic shock, or multiple organ dysfunction with a mortality rate of 15%–20% before accurate diagnosis and targeted treatment. Metagenomic next-generation sequencing (mNGS) has an advantage in achieving early diagnosis. In the study, omadacycline implementation was described to provide a better understanding of effectiveness in severe psittacosis pneumonia with ARDS.

Methods: Sixteen patients with severe psittacosis pneumonia with ARDS were selected between September 2021 and October 2022. They were diagnosed using mNGS and treated with omadacycline. Retrospective analysis of clinical manifestations, laboratory data, disease progression, diagnostic tool, treatment, and prognosis was summarized.

Results: Common symptoms included fever, dyspnea, and cough. All patients developed ARDS, accompanied by septic shock (43.7%) and pulmonary embolism (43.7%). Laboratory data showed normal leucocytes, increased creatine kinase isoenzyme, and decreased albumin with liver dysfunction in most patients. All patients had increased neutrophils, C-reactive protein, procalcitonin, and D-dimer with decreased lymphocytes. Airspace consolidation, ground glass opacity, and pleural effusion were found on chest CT. mNGS results were obtained in 24–48 h to identify the diagnosis of Chlamydia psittacosis. All patients received mechanical ventilation with omadacycline treatment. Fourteen patients experienced complete recovery, while the other two patients died from multidrug-resistant bacterial infection and renal failure.

Conclusion: mNGS has a significant value in the diagnosis of *Chlamydia psittaci* infection. Timely treatment of omadacycline can improve prognosis and provide a promising new option for the treatment of severe *Chlamydia psittaci* pneumonia with ARDS.

KEYWORDS

ARDS, *Chlamydia psittaci*, mNGS, omadacycline, severe pneumonia

Introduction

Chlamydia psittaci is an intracellular parasitic pathogen transmitted from the inhalation of aerosols from contaminated bird substances to humans, which is common in adults and rare in children (1). Psittacosis is an infectious zoonosis caused by *Chlamydia psittaci* from poultry, wild birds, and some other animals (2). *Chlamydia psittaci* pneumonia accounts for less than 5% of community-acquired pneumonia (CAP) in hospitals (3). *Chlamydia psittaci* was considered the common pathogens for severe CAP in China (4). The severity of pneumonia ranges from atypical flu-like symptoms to fatal conditions with acute respiratory distress syndrome (ARDS) and/or multiple organ dysfunction (5–7). Few cases of clinically diagnosed *Chlamydia psittaci* pneumonia with ARDS have been reported or may even be underestimated probably due to atypical clinical presentation, low sensitivity of routine laboratory tests, and low awareness of the disease (8, 9).

The typical manifestation may include high fever, dry cough, headache, myalgia, chills, and gastrointestinal symptoms. In severe conditions, symptoms can progress to respiratory failure, endocarditis, jaundice, and neurological complications. In addition to the symptoms and an exposure history, the diagnosis of *Chlamydia psittaci* requires a laboratory examination meeting any one of the three criteria: (1) isolation of *Chlamydia psittaci* from respiratory secretions; (2) IgM antibody against *Chlamydia psittaci* titer of 1:16 or higher by micro-immunofluorescence (MIF) detection; (3) a 4-fold or greater increase in antibody titer between serum samples collected 2 weeks apart, using a complement fixation test or MIF (10). Specific diagnostic tests such as polymerase chain reaction (PCR) and pathogenic culture are only available in some specialized microbiology laboratories. Therefore, the limitation of diagnosis raises a great concern (11, 12).

Metagenomic next-generation sequencing (mNGS) has been widely used to detect different types of potential microorganisms including viral, bacterial, fungal, or parasitic pathogens (13). The application of mNGS technology provides a helpful method for the diagnosis of infectious diseases using a culture-independent approach.

Most importantly, mNGS has the ability to diagnose atypical pathogens within 48–36 h that usually takes a long time in traditional culture methodologies, which leads to a favorable clinical outcome based on early diagnosis and more effective antibiotic therapy (14). For critically ill patients, mNGS has recently been highlighted as the most promising tool for the accurate diagnosis of rare pathogenic infections, particularly for severe pneumonia. Recent studies have shown that the mNGS provides high specificity and sensitivity for species-level identification with a less level in false-positive and false-negative results compared with traditional methods (14, 15).

Omadacycline is a novel tetracycline for the treatment of CAP approved by the United States Food and Drug Administration in 2018 (16). There is still a lack of clinical data on omadacycline treatment for severe *Chlamydia psittaci* pneumonia with ARDS. In the study, severe CAP caused by *Chlamydia psittaci* with ARDS was summarized for the clinical characteristics, laboratory data, treatments, and outcomes. Furthermore, we evaluate the contribution of omadacycline in the treatment of severe psittacosis pneumonia.

Patients and methods

Study design

We conducted a retrospective case review of 16 patients with severe CAP caused by *Chlamydia psittaci* admitted to the Second Affiliated Hospital of Chongqing Medical University between September 2021 and October 2022 when COVID-19 pandemic was supposed to spread in China. All cases were diagnosed with ARDS based on clinical manifestations, laboratory data, and mNGS. For each case, clinical characteristics, laboratory examination, imaging, diagnosis, treatment, outcomes, and follow-up data were recorded from the electronic medical system. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University. All data were anonymized prior to analysis.

Diagnostic criteria for severe psittacosis pneumonia with ARDS

The diagnosis of severe psittacosis pneumonia with ARDS should be considered as the following criteria: (1) meet the criteria for severe CAP (17); (2) identify specific fragment DNA of *Chlamydia psittaci* by mNGS; (3) fulfill the Berlin definition of ARDS (18); (4) have negative results for other causative organisms by routine pathogen tests with bronchoalveolar lavage fluid (BALF), sputum, and blood.

The criteria for severe CAP include one major criterion or more than three minor criteria. Major criteria contain septic shock with the need for vasopressors and respiratory failure requiring mechanical ventilation. Minor criteria contain respiratory rate ≥30 breaths/min, PaO_2/FiO_2 ratio ≤250, multilobar infiltrates, confusion/disorientation, uremia (blood urea nitrogen level ≥20 mg/dL), leukopenia (white blood cell count <4,000 cells/μL), thrombocytopenia (platelet count <100,000/μL), hypothermia (core temperature <36°C), and hypotension requiring aggressive fluid resuscitation.

The severity of ARDS is based on the degree of hypoxemia: mild (200 mm Hg <PaO_2/FIO_2 ≤300 mm Hg), moderate (100 mm Hg <PaO_2/FIO_2 ≤200 mm Hg), and severe (PaO_2/FIO_2 ≤100 mm Hg).

mNGS detection method

1) Sample processing and DNA extraction: clinical samples (1.5–3 mL BALF or 3–4 mL blood) were collected according to standard procedures. Samples were treated with enzymes at 4°C for liquefaction within 24 to 48 h. Then the samples were transferred to new microcentrifuge tubes and broken by vortex mixer with glass beads on a horizontal platform vigorously agitated at 1,600 × g for 30 min. DNA was extracted from a 0.3 mL sample using the TIANamp Micro DNA Kit (Tiangen Biotech, China). The extracted DNA samples were used for the construction of DNA libraries.
2) Library preparation and sequencing: an Agilent 2100 Bioanalyzer was used as quality control of DNA libraries to analyze the length of the inserted fragments. The construction of DNA libraries were performed using transposase-mediated methods (Vision Medicals, China) and assessed by a Qsep1

biofragment analyzer before sequencing. Qualified DNA libraries were loaded into the sequencing chip and performed on the NextSeq 550Dx sequencing platform (Illumina, San Diego, CA).

3) Data analysis: high-quality data was obtained by removing low-quality reads and short reads (length <40 bp), followed by subtraction of human host sequences mapped to the human reference genome (hg38 and YH sequences) using Burrows–Wheeler Aligner software. The remaining data by eliminating low-complexity reads were compared in the special microbial database. The classification reference data were downloaded and optimized from an open database such as NCBI, China National GenBank Database or EMBL. Data of suspected pathogenic microorganisms with the numbers of coverage rate, strictly mapped reads and depth were then analyzed and produced according to the Microbial Genome Database. The final result is exported and interpreted with microbiology and clinical background. The diagnosis was determined based on the clinical manifestations, imaging, pathogens and other essential laboratory tests.

Results

Patients' characteristics

Sixteen patients with severe *Chlamydia psittaci* pneumonia complicated with ARDS were enrolled. Among them, eleven were men with five women. Their median age was 65 (range 39–76) years. Nine patients (56.2%) had underlying diseases, such as hypertension, diabetes, hepatitis B, or chronic obstructive pulmonary disease (COPD). Six patients had a smoking history. All patients (100%) had a history of exposure or close contact with parrots, pigeons, or poultry. Eight of them raised parrots, pigeons, chickens, or ducks at home for years as a definite exposure history. The rest had a contact history such as live poultry market visits or live poultry for cooking. All patients were diagnosed with *Chlamydia psittaci* detected by mNGS (Table 1). The detection of novel coronavirus disease 2019 (COVID-19) by reverse transcription PCR using nasal swabs in all patients was negative. Other respiratory pathogens for CAP such as influenza virus, respiratory syncytial virus, adenovirus, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* were negative by pathogenic detection at admission.

Clinical symptoms

Fever (100%), dyspnea (87.5%), and cough (68.7%) were the most common symptoms in patients. All patients had a recurrent fever higher than 39°C. Eleven patients had cough without apparent expectoration. Six patients had chill (37.5%) and headache (37.5%). Seven patients had myalgia (43.7%). Fourteen patients developed respiratory failure due to progressive dyspnea. All patients presented with ARDS (mild 25%, moderate 37.5%, and severe 37.5%) with seven patients developing septic shock (43.7%) during hospitalization. The average of acute physiology and chronic health evaluation and sequential organ failure assessment scores were 17 (range 8–28) and 5.8 (range 2–12), respectively (Tables 1, 2). The physical examination of the patients was non-specific including increased respiratory rate, enhanced tactile vocal fremitus, and moist rales on the affected lung.

Laboratory examinations

The average of white blood cell (WBC) count in all patients was 8.70×10^9/L. Three patients presented an elevated WBC count (18.7%), whereas thirteen patients presented a normal WBC count on admission. All patients had increased neutrophils, procalcitonin (PCT), and C-reactive protein (CRP) with a decreased percentage of lymphocytes. Patients had a mean neutrophil percentage of 84.3%, an average PCT level of 3.42 ng/mL, and an average lymphocyte percentage of 6.15%, respectively. Fourteen patients (87.5%) had an increase in creatine kinase isoenzyme (CK-MB) with the highest level of 14,722 U/L. Most patients had hepatic dysfunction, and among them, eleven patients (68.7%) had increased alanine aminotransferase (ALT) and fifteen patients (93.7%) had increased aspartate aminotransferase (AST). A decreased albumin was presented in fifteen patients (93.7%) with an average level of 29.1 g/L. All patients had an increased level of plasma D-dimer. In addition, one patient had an elevated level of creatinine (Table 3). Seven patients (43.7%) were diagnosed with pulmonary embolism (PE) by pulmonary angiography.

Imaging examination

All patients had inflammatory lesions on the chest computed tomography (CT) examination on admission. Consolidation with air bronchogram or ground-glass opacity (75%) can be observed bilaterally in the lungs of most patients. Three patients had lesions in the upper lobe of the lung without infiltrates in other lobes. Moreover, pleural effusion was also found in one patient. After treatment, the lung lesions gradually disappeared with no residual fibrosis (Figures 1, 2).

mNGS results

Bronchoalveolar lavage was conducted on all patients by bronchoscopy. Samples of BALF or sputum were collected for mNGS detection. BALF collection can reflect the pathogenic infection of the lungs due to extraction from the bronchi and the low possibility of sample contamination. The results of mNGS showed that *Chlamydia psittaci* identified in all patients with also some other microorganisms at the same time (Table 4). The sensitivity and detection rate of *Chlamydia psittaci* were low because of its intracellular growth and small extracellular release into BALF, sputum, or blood. Pathogenic bacteria such as *Streptococcus oralis*, *Neisseria mucosa*, *Prevotella intermedia*, and *Streptococcus anginosus* should be considered as colonization or contamination due to fewer detection.

The coverage of mNGS in some patients is shown in Figure 3. Forty-five specific *Chlamydia psittaci* sequences covered 0.45% of the total *Chlamydia psittaci* genome were detected by mNGS in the BALF sample of patient 12. Twenty-six specific *Chlamydia psittaci* sequences covered 0.21% of the total *Chlamydia psittaci* genome were detected by mNGS in the BALF sample of patient 13. Eleven specific *Chlamydia*

TABLE 1 Basic characteristics of patients with severe psittacosis pneumonia.

Patient No.	Gender	Age (y)	Exposure contact	Underlying disease	Smoking history	Confirmed method	Severity of ARDS
1	Male	75	Raised pigeon	Hypertension	Yes	mNGS	Severe
2	Male	68	Parrot contact	Diabetes, hepatitis B	Yes	mNGS	Moderate
3	Female	67	Raised parrot	Hypertension, diabetes	No	mNGS	Moderate
4	Female	39	Raised parrot	None	No	mNGS	Moderate
5	Male	74	Ducks contact	Hepatitis B	No	mNGS	Mild
6	Male	61	Parrot contact	None	No	mNGS	Mild
7	Female	48	Raised chickens and ducks	None	No	mNGS	Mild
8	Male	69	Parrot contact	COPD	Yes	mNGS	Severe
9	Male	73	Parrot contact	Hypertension	Yes	mNGS	Moderate
10	Female	67	Pigeon contact	None	No	mNGS	Mild
11	Male	65	Raised parrot	Hypertension	No	mNGS	Severe
12	Male	71	Raised parrot	Hypertension	Yes	mNGS	Severe
13	Female	59	Raised pigeon	None	No	mNGS	Severe
14	Male	70	Parrot contact	None	Yes	mNGS	Moderate
15	Male	58	Raised chickens	Diabetes	No	mNGS	Severe
16	Male	76	Parrot contact	None	No	mNGS	Moderate

COPD, chronic obstructive pulmonary disease; mNGS, metagenomic next generation sequencing.

TABLE 2 Clinical symptoms of patients with severe psittacosis pneumonia.

Patient No.	Fever	Cough	Chill	Headache	Myalgia	Dyspnea	Septic shock	P/F ratio (mm Hg)	APACHE II	SOFA
1	Yes	Yes	No	No	No	Yes	Yes	97	28	10
2	Yes	Yes	No	No	No	Yes	Yes	105	18	8
3	Yes	No	No	Yes	Yes	Yes	No	130	13	3
4	Yes	Yes	Yes	Yes	No	Yes	Yes	110	16	6
5	Yes	No	No	No	Yes	No	No	250	8	4
6	Yes	Yes	No	No	No	No	No	243	9	3
7	Yes	Yes	Yes	No	Yes	Yes	No	224	11	2
8	Yes	Yes	Yes	No	Yes	Yes	Yes	99	24	12
9	Yes	No	No	Yes	No	Yes	No	190	18	5
10	Yes	No	No	Yes	No	Yes	Yes	216	24	8
11	Yes	Yes	Yes	Yes	No	Yes	No	87	20	5
12	Yes	Yes	Yes	No	No	Yes	No	99	17	3
13	Yes	Yes	No	No	Yes	Yes	Yes	75	18	10
14	Yes	Yes	No	Yes	No	Yes	No	185	13	2
15	Yes	No	No	No	Yes	Yes	No	84	24	4
16	Yes	Yes	Yes	No	Yes	Yes	Yes	148	14	8

APACHE II; acute physiology, age and chronic health evaluation; P/F, PaO_2/FiO_2, SOFA; sequential organ failure assessment.

psittaci sequences covered 0.23% of the total *Chlamydia psittaci* genome were detected by mNGS in the BALF sample of patient 14. A total of 3,650 specific *Chlamydia psittaci* sequences covered 14.34% of the total *Chlamydia psittaci* genome were detected by mNGS in the BALF sample of patient 15. A total of 2,973 specific *Chlamydia psittaci* sequences covered 7.81% of the total *Chlamydia psittaci* genome were detected by mNGS in the BALF and sputum samples of patient 16 (Figure 3).

TABLE 3 Laboratory testing of patients with severe psittacosis pneumonia.

Patient No.	WBC (4–10 × 10^9/L)	N% (45–75%)	CRP (<10 mg/L)	PCT (0.02–0.05 ng/mL)	L% (20–50%)	CK-MB (0–25 U/L)	Albumin (35–50 g/L)	ALT (7–40 U/L)	AST (13–35 U/L)	Cre (40–130, μmol/L)	D-dimer (0–550, ng/mL)	PE	Chest CT
1	7.53	89.2	136.2	6.56	4.7	196	35.7	53	52	56.5	1548.3	No	Consolidation
2	25.98	95.3	155.73	8.56	2.9	125	27.3	19	58	92.8	2723.2	No	Consolidation
3	8.83	91.5	159.77	0.803	5.3	93	32.5	59	42	69.9	3879.3	Yes	Consolidation
4	6.72	85.6	>200	0.16	10.2	87.4	28.4	110	140	51.9	2805.8	No	Consolidation
5	10.14	92.3	141	1.16	3.3	14.6	34.1	33	113	59.9	685.1	No	Lobe lesion
6	11.17	97.1	>200	17.92	1.5	2.8	26.7	152	122	61.6	4086.4	Yes	Consolidation
7	5.64	91.8	>200	0.993	6.4	54	31	114	113	96.8	689	No	Lobe lesion
8	5.62	78.6	155.9	0.93	12	218.9	32.5	23	64	52.2	563.5	No	Consolidation
9	7.85	84	149.75	0.162	8.9	300	26.2	107	131	54.9	8263.9	Yes	Lobe lesion, Pleural effusion
10	6.55	97.1	>200	9.77	1.5	47	26.6	54	77	48.6	2,800	No	Consolidation
11	6.12	84.1	>200	3.7	10.1	14,722	34.7	155	429	77.8	2880.8	No	Consolidation
12	9.09	8.29	>200	0.799	4	321	27.9	32	55	52.2	3335.8	Yes	Consolidation
13	8.69	90.7	>200	0.52	6.5	31.0	19.3	13	32	60	1814	Yes	Consolidation
14	5.68	87.7	186.14	0.08	8.5	57	26.7	58	99	55.2	1990	No	Consolidation
15	4.94	91.6	>200	2.05	7.2	676	25.3	87	118	254.2	2278.1	Yes	Lobe lesion
16	5.29	92.1	127.67	0.627	5.5	1938	31	55	48	74.2	2717.8	Yes	Consolidation

WBC, white blood cell; N, neutrophils; L, lymphocyte; CRP, C-reactive protein; PCT, procalcitonin; CK-MB, creatine kinase isoenzyme; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cre, creatinine; PE, pulmonary embolism; CT, computed tomography.

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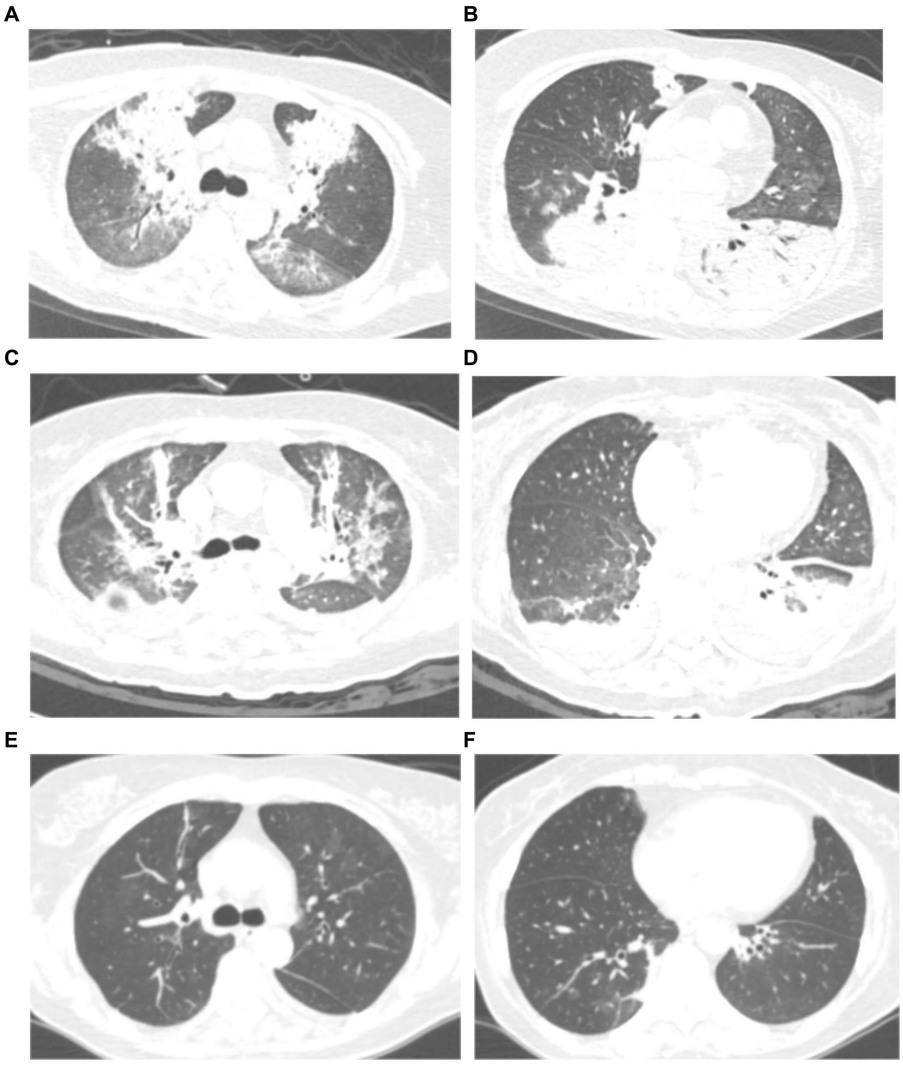

FIGURE 1
Chest computed tomography (CT) scan of a 59 years-old woman with severe psittacosis pneumonia (No. 13 patient). The initial CT scan (on admission) shows diffused consolidation with air bronchogram of both lungs **(A,B)**. Consolidation gradually decreased after targeted treatment in CT scan (12 days after admission) **(C,D)**. The consolidation completely disappeared on follow-up (28 days after admission) with lung recovery **(E,F)**.

Treatment process

After admission to the hospital, the patients were subjected to bronchoscopy and were collected BALF for mNGS test. Before the results of mNGS, all patients received empirical antibiotic therapy on admission, according to the CAP management guideline (17), including β-lactam/β-lactamase inhibitor and combinations of carbapenems or quinolones (Figure 4). Eight patients were initially receivedβ-lactam/β-lactamase inhibitor or carbapenems, respectively. Eleven patients were initially administered quinolones. The patients did not have sufficient efficacy with the empirical antibiotic therapy due to their clinical conditions not improved or even gradually deteriorated, leading to progression of ARDS. After the confirmation of *Chlamydia psittaci* infection by mNGS, antibiotic therapy was changed to omadacycline for all patients (Table 5). Omadacycline was recommended for the treatment of severe psittacosis pneumonia for at least 2 weeks (7). In addition, some patients not only had psittacosis pneumonia but were also infected with other pathogens through mNGS testing. For these patients, the corresponding antibiotics were applied accordingly (Table 5). It is worth mentioning that patients with severe illness were treated with carbapenems or polymyxin B. Since one patient (No. 1) continued to have multidrug-resistant *Acinetobacter baumannii*, polymyxin B was administered (Table 5).

All patients who developed ARDS received mechanical ventilation (MV), including 10 patients with invasive MV and 6 patients with non-invasive MV. Lung-protective ventilation strategy with low tidal volume, high PEEP, sedation, and analgesia was performed. Four patients received high-flow nasal cannula oxygen therapy after the patients' condition improved with mechanical ventilation. Seven patients (PaO_2/FIO_2 ratio <150 mmHg) were performed prone position ventilation with an average of 4.6 days as recommended previously (19). During the treatment, 4 patients received continuous renal replacement therapy (CRRT) because of acute renal injury, of which one patient had diabetes for years.

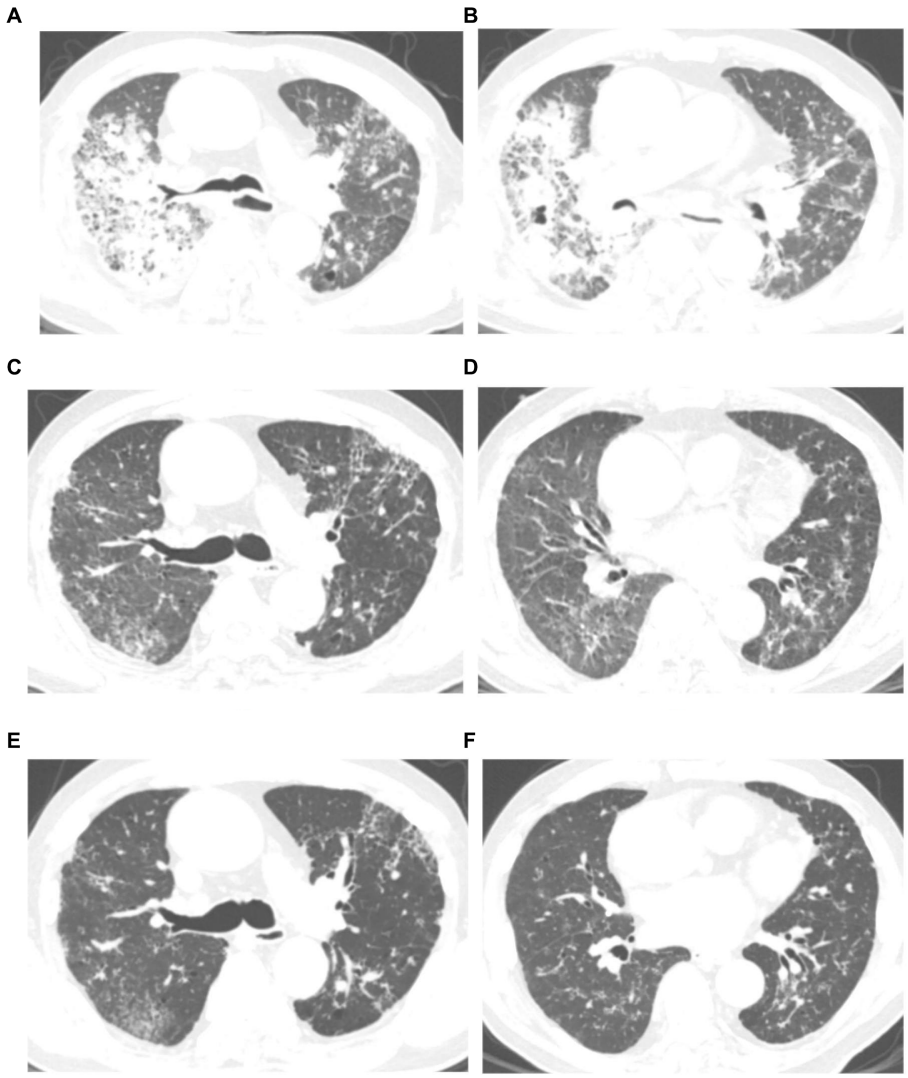

FIGURE 2
Chest computed tomography (CT) scan of a 76 years-old man with severe psittacosis pneumonia (No. 16 patient). The initial CT scan (on admission) shows diffused consolidation with ground-glass opacity of the right lung **(A,B)**. Consolidation gradually decreased after targeted treatment in CT scan (14 days after admission) **(C,D)**. The consolidation completely disappeared on follow-up (28 days after admission) with lung recovery **(E,F)**.

Outcomes

After omadacycline treatment, the clinical symptoms including fever, cough, headache, and dyspnea gradually improved. Inflammatory indicators such as WBC, neutrophils percentage, CRP, PCT, and PaO_2/FiO_2 ratio were dropped to near normal (Table 5). In addition, CK-MB, ALT, AST, and D-dimer were returned to normal. Two patients (No. 1and 8) were eventually died (Table 5). The cause of death was possibly attributed to the secondary infection of multidrug-resistant *Acinetobacter baumannii* of patient No. 1 and severe infection of *Chlamydia psittaci* with uncontrolled inflammation of patient No. 8, which contributed to the aggravation of septic shock and renal failure requiring continuous renal replacement therapy. In addition, a longer time from the onset to diagnosis of the two patients may lead to delay in treatment with poor outcome compared with other patients. In surviving patients, consolidations on chest CT were absorbed with full recovery of discharge from the hospital.

The treatment duration of all patients is presented in Table 6. The mean time from the onset of the illness to diagnosis was 6.6 days (range 2–13 days). The median time from illness to respiratory failure was 6.6 days (range 3–15 days). The longest duration of MV was 15 days, and the shortest duration of MV was 4 days. The mean time from illness to ICU stay was 7.8 days (range 5–15 days). The longest duration from the illness to hospital stay was 16 days.

Discussion

In this study, we conducted a retrospective review of 16 patients diagnosed with *Chlamydia psittaci* infection by the application of mNGS, manifesting as severe pneumonia with ARDS. Human

TABLE 4 mNGS results of patients with severe psittacosis pneumonia.

Patient No.	Sample type	Data vol (No. of reads)	Detected pathogen(s) (No. of species-specific reads)
1	BALF sputum	24,274,859	*Chlamydia psittaci* (292), *Streptococcus oralis* (2), *Klebsiella pneumoniae* (1)
2	BALF	12,003,511	*Chlamydia psittaci* (115), *Candida albicans* (19)
3	BALF	30,398,231	*Chlamydia psittaci* (373)
4	BALF	28,215,284	*Chlamydia psittaci* (221), *Human alphaherpesvirus* 1 (2)
5	BALF	34,919,252	*Chlamydia psittaci* (69), *Candida albicans* (9)
6	BALF	18,194,616	*Chlamydia psittaci* (28)
7	BALF	20,545,068	*Chlamydia psittaci* (34), *Human alphaherpesvirus* 1 (3)
8	BALF sputum	27,924,632	*Chlamydia psittaci* (1297), *Prevotella intermedia* (23), *Neisseria mucosa* (5)
9	BALF	27,043,045	*Chlamydia psittaci* (3477)
10	BALF	17,988,072	*Chlamydia psittaci* (150), *Human betaherpesvirus* (3)
11	BALF	19,495,853	*Chlamydia psittaci* (72), *Candida albicans* (2), *Staphylococcus epidermidis* (5)
12	BALF	35,447,319	*Chlamydia psittaci* (45)
13	BALF	28,514,379	*Chlamydia psittaci* (26), *Klebsiella pneumoniae* (9)
14	BALF	68,207,678	*Chlamydia psittaci* (11), *Human alphaherpesvirus* 4 (4)
15	BALF	14,842,044	*Chlamydia psittaci* (3650), *Klebsiella pneumoniae* (6), *Haemophilus influenzae* (30), *Human alphaherpesvirus* 4 (5), *Ureaplasma parvum* (4)
16	BALF sputum	39,570,000	*Chlamydia psittaci* (2973), *Streptococcus anginosus* (13)

BALF, bronchoalveolar lavage fluid.

infection of psittacosis was commonly known as inhalation of feather, feces, or corpses of birds or poultry, contact with their excreta, activities that involve cooking or pruning, and cleaning of contaminated cages (20, 21). Transmission from human to human by psittacosis is considered rare due to a few reports of psittacosis outbreaks in humans (22). Studies have reported that 90% of patients infected with *Chlamydia psittaci* had bird or poultry contact history with seropositivity rates of 13.3%, 38.9%, and 31.1% in chickens, ducks, and pigeons sold in Northwest China, respectively (23, 24). In the study, all patients had a direct or indirect exposure to parrots, pigeons, chickens, or ducks, which was considered the main risk factor for psittacosis. Additionally, 8 out of the 16 patients with underlying diseases had moderate to severe ARDS, suggesting that *Chlamydia psittaci* could infect human subjects as a critical illness condition.

The lungs are the most common sites of *Chlamydia psittaci* infection after entry through contaminated aerosols. Probably owing to the atypical clinical manifestations and relatively low awareness by physicians, the diagnosis of *Chlamydia psittaci* pneumonia is challenging and tends to be overlooked with the lack of routine diagnostic methods. Patients are usually treated for common CAP when the lack or omission of contact history collected in medical history. The misdiagnosis of *Chlamydia psittaci* pneumonia may also be increased due to the indistinguishable symptoms,familial aggregation and lack of medical resources under COVID-19. Furthermore, measurement of *Chlamydia psittaci* is not often available in the routine test. Thus, patients are mainly administered empirical antibiotics, which potentially leads to misdiagnosis and underestimation of the accurate incidence of *Chlamydia psittaci* pneumonia (25). The typical symptoms include fever, chill, cough, and myalgia (26, 27). In the study, almost all patients had fever, dyspnea, and cough. All patients had hypoxemia, resulting in ARDS during the process. Some patients had headache at the onset of disease. Headache is rarely reported in *Chlamydia psittaci* pneumonia. It has been reported that the cases infected with *Chlamydia psittaci* to the central nervous system had poor outcomes with the unclear mechanisms (28). Headache should draw an attention to the possible lung infection if it cannot be explained by neurological disease. The major manifestations of chest CT are consolidation, ground glass shadow, lobular distribution, and pleural effusion mainly located in the lower lobes of the lungs (29, 30). In the study, there was one patient coexisted with lobe lesions and pleural effusion, indicating the gradually development from the lung to pleural cavity. Furthermore, *Chlamydia psittaci* is associated with several extrapulmonary manifestations, including myocarditis, endocarditis, arthritis, hepatitis, encephalitis, and ocular adnexal lymphoma (31). Therefore, difficulty in differentiating *Chlamydia psittaci* pneumonia from other pathogens causing CAP based on the absence of characteristic clinical manifestations and images can lead to misdiagnosis and delay in treatment. This will indicate more information of *Chlamydia psittaci* pneumonia for us.

In the current study, the laboratory data of patients generally showed normal leucocytes, increased neutrophils, CRP, and PCT. *Chlamydia psittaci* has been reported to be more pathogenic than other Chlamydiales species, causing more severe inflammatory reactions (27). In another case, a significantly lower level of leucocytes was also reported (32). All patients had decreased lymphocyte counts, indicating immune dysfunction associated with psittacosis infection. Lymphocytopenia is a common symptom in patients with severe community-acquired infections by the destruction of the cytoplasmic components and apoptosis of lymphocytes with some atypical pathogens (33). However, the reason of lymphocytopenia caused by *Chlamydia psittaci* still needs further investigation. Most patients had an elevated level of CK-MB, ALT, and AST, suggesting multiple organ dysfunction such as

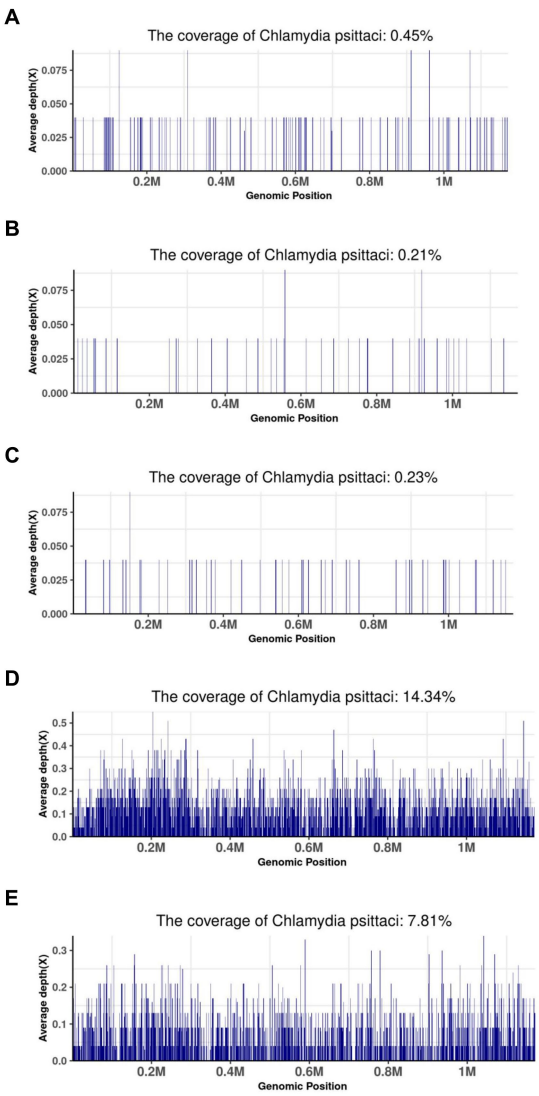

FIGURE 3
Metagenomic next-generation sequencing results of some patients. **(A)** BALF sample of patient 12; **(B)** BALF sample of patient 13; **(C)** BALF sample of patient 14; **(D)** BALF sample of patient 15; **(E)** BALF and sputum sample of patient 16.

myocardial damage and hepatic dysfunction by *Chlamydia psittaci* infection as previously reported (25). CK is considered a high-risk factor for severe *Chlamydia psittaci* pneumonia that is not rare in clinics (34). In addition, changes in liver enzymes are attributed to the entrance of the reticular endothelial cells of the liver by *Chlamydia psittaci*. When patients with fever, increased liver enzyme, or nervous system with an age of <60 years, the diagnosis of *Chlamydia psittaci* should be considered to avoid ARDS or septic shock at a later stage (35). Healthcare providers should be on high alert when patients present with these conditions in order to avoid severe sequelae. Furthermore, we also found a relatively high incidence of PE with an increased level of plasma D-dimer in severe *Chlamydia psittaci* pneumonia that had not been reported before. The mechanism of PE by psittacosis infection still remained unclear. Patients with PE received anticoagulant therapy due to the definite aggravation of hypoxemia in the condition of ARDS.

Laboratory testing for *Chlamydia psittaci* has drawn attention with the lack of ideal method tools. Routine sputum culture usually takes 5–7 days with a low sensitive rate of *Chlamydia psittaci* and hazardous for personnel in a P3 containment laboratory (36). The serological assay has a cross-reaction with other Chlamydia species and is appropriate for retrospective diagnosis and epidemiological investigation in acute and convalescent stages (37). PCR can help identify the pathogen in a more rapid, sensitive, and specific way during the acute phases of the infection (9). However, PCR for *Chlamydia psittaci* is unavailable in most hospitals in China. The advantage of mNGS is its quick and accurate detection of lower respiratory tract infections in a wide range of pathogens, including atypical bacterial pathogens, viruses, and fungi. In our hospital, the results of mNGS are available within 24–48 h, which is beneficial and important for accurate diagnosis and targeted treatment as early as possible when treating patients with severe *Chlamydia psittaci* pneumonia. In the current study, all patients were diagnosed and adjusted treatment through mNGS after no improvement with empirical antibiotic therapy. It is noteworthy that the diagnosis of severe CAP caused by *Chlamydia psittaci* with the application of mNGS presents a valuable method in timely therapy.

The sensitivity of *Chlamydia psittaci* is relatively low because of its intracellular bacterium and the small number of body fluids such as blood, sputum, and BALF by mNGS detection. Therefore, even with small amounts of DNA sequence, *Chlamydia psittaci* should be considered as the causative pathogen for pneumonia in the study. In addition, some patients had other pathogens detected by mNGS. Patients with underlying diseases or immunocompromised condition, bacteria colonization, or workflow quality may affect the interpretation of mNGS results. It is required the ability of clinicians to be strict enough for interpretation of mNGS results.

There is a higher rate of incidence of severe CAP caused by *Chlamydia psittaci*. The mortality rate was 15%–20% before the targeted antibiotics were used (38). Antibiotics, including tetracycline, macrolide, and quinolones, are recommended for *Chlamydia psittaci* pneumonia treatment (39). The duration of treatment should continue for at least 10–14 days to prevent relapse (40). Quinolones have been reported to be less effective compared with tetracyclines and macrolides in psittaci pneumonia (41). In our study, patients who received moxifloxacin/ levofloxacin treatment initially had poor response, covering the targeted pathogen prior to diagnosis, especially in severe conditions. The possible reason may be related to the low intracellular activity of quinolones in *Chlamydia psittaci* and the insensitivity of *Chlamydia psittaci* response to quinolones. Tetracycline is the first-line treatment recommended for *Chlamydia psittaci* pneumonia (42). Omadacycline, a third-generation tetracycline, has a structure of an alkylaminomethyl group that replaces the glycylamido group at the C-9 position of the D-ring derived from minocycline that is helpful for bacterial resistance (16, 43). Unlike other tetracyclines, omadacycline inhibits bacterial protein synthesis by only binding to the 30S ribosomal subunit and not binding to the 50S ribosomal subunit. As we know, doxycycline and minocycline are prone to drug-induced organic injury with liver metabolism (44). On the contrary, omadacycline has a higher concentration in the lung than the plasma and is eliminated mainly by feces which is not required for dose adjustment in the elderly and patients with hepatic and

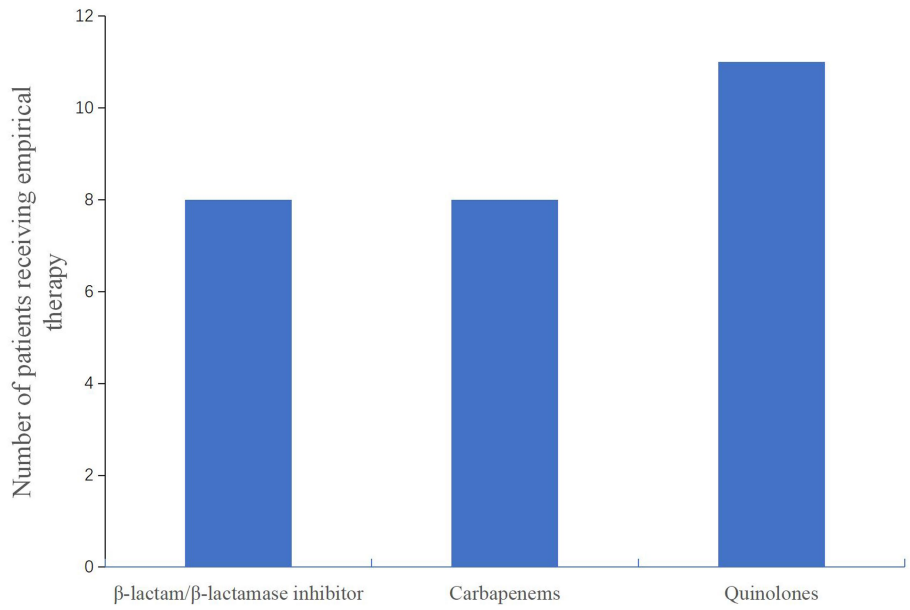

FIGURE 4
Number of patients receiving empirical antibiotic therapy on admission.

TABLE 5 Clinical outcomes of patients with severe psittacosis pneumonia after treatment.

Patient No.	WBC (4–10×10^9/L)	N% (45–75%)	CRP (<10mg/L)	PCT (0.02–0.05 ng/mL)	P/F ratio (mm Hg)	Initial antibiotic treatment	Adjustment of antibiotic	Outcome
1	4.24	74.4	41.87	3.25	84	Cefoperazone/tazobactam	Biapenem + omadacycline + polymyxin B	Death
2	6.32	68.6	2.52	0.049	324	Meropenem + moxifloxacin	Meropenem + omadacycline + caspofungin	Good
3	4.19	59.8	5.34	0.022	308	Piperacillin/tazobactam	Biapenem + omadacycline	Good
4	7.81	72.7	7.61	0.036	312	Cefoperazone/tazobactam	Biapenem + omadacycline	Good
5	6.33	73.8	9.96	0.052	345	Piperacillin/tazobactam	Omadacycline + caspofungin	Good
6	9.8	75	3.15	0.048	323	Meropenem + moxifloxacin	Omadacycline	Good
7	6.79	72.7	7.92	0.035	309	Cefminox + levofloxacin	Omadacycline	Good
8	7.56	73	3.93	5.044	58	Biapenem + moxifloxacin	Biapenem + omadacycline	Death
9	6.01	75	9.55	0.031	318	Piperacillin + moxifloxacin	Piperacillin + omadacycline	Good
10	5.24	65.4	4.58	0.038	363	Biapenem	Piperacillin/tazobactam + omadacycline	Good
11	7.84	74.7	4.13	0.011	306	Biapenem + moxifloxacin	Biapenem + omadacycline + caspofungin	Good
12	4.84	62.7	7.26	0.055	324	Imipenem + moxifloxacin	Imipenem + omadacycline	Good
13	9.04	70.1	5.43	0.049	332	Meropenem + moxifloxacin	Meropenem + omadacycline	Good
14	6.85	67.3	5.83	0.033	339	Piperacillin + levofloxacin	Piperacillin + omadacycline	Good
15	6.86	57.4	5.1	0.011	302	Biapenem + moxifloxacin	Biapenem + omadacycline	Good
16	5.28	72.2	9.32	0.023	341	Cefoperazone + levofloxacin	Cefoperazone + omadacycline	Good

WBC, white blood cell; N, neutrophils, L, lymphocyte; CRP, C-reactive protein; PCT, procalcitonin; P/F, PaO_2/FiO_2.

TABLE 6 Treatment duration of patients with severe psittacosis pneumonia.

Patient No.	Admission date (year-month-day)	Days from onset to diagnosis	Days from illness to respiratory failure	Mechanical ventilation duration (days)	Days from illness to ICU stay	Days from illness to hospital stay	Prone position duration (days)	Oxygen therapy method	CRRT
1	2022-10-19	13	15	15	15	15	7	MV	Yes
2	2022-01-20	2	3	7	5	14	4	MV	No
3	2022-09-17	7	14	6	7	16	3	MV/HFNC	No
4	2022-03-18	4	6	5	8	12	3	MV	No
5	2022-10-16	7	8	5	5	13	No	MV/HFNC	No
6	2022-09-23	6	5	6	6	12	No	MV	No
7	2022-10-30	6	4	4	7	11	No	MV	No
8	2022-12-21	10	7	12	12	12	6	MV	Yes
9	2022-11-01	4	10	4	7	10	No	MV	No
10	2022-10-06	5	6	5	9	15	No	MV	No
11	2022-09-19	5	4	7	8	10	4	MV/HFNC	Yes
12	2022-04-01	9	5	6	6	9	5	MV/HFNC	No
13	2022-03-31	8	7	8	7	11	5	MV	No
14	2022-04-12	5	8	5	8	12	No	MV	No
15	2022-04-08	7	15	6	10	15	5	MV	Yes
16	2022-03-15	8	5	5	5	10	No	MV	No

MV, mechanical ventilation; HFNC, high-flow nasal cannula oxygen therapy; CRRT, continuous renal replacement therapy.

renal impairment (45). In the study, most patients showed good efficacy and recovery after the administration of omadacycline. Severe *Chlamydia psittaci* pneumonia can lead to multiple organ failure. All patients had ARDS with septic shock, which need MV and organ support therapy. In the present study, patients with PaO_2/FIO_2 ratio <150 mmHg received prone position. Four patients with acute renal injury were treated with CRRT. Additionally, patients with septic shock and MV were susceptible to secondary infections, as illustrated by the patient who died from multiple drug-resistant bacterial infections. To the best of our knowledge, this is the first report of omadacycline for the treatment of severe *Chlamydia psittaci* pneumonia with ARDS in China.

This study had some limitations: (1) the number of the critically ill patients was relatively small and was insufficient to investigate the relevant data of psittacosis pneumonia; (2) there was a lack of verification with serologic tests in the current study because the requirements were unavailable in our hospital; (3) the retrospective nature of the study provided limited evidence of the therapeutic effect of omacycline. A prospective study with larger sample sizes is ongoing to confirm our findings.

Conclusion

Severe CAP caused by *Chlamydia psittaci* could lead to ARDS or sepsis or even multiple organ failure. mNGS is a valuable method for accurate diagnosis and targeted antibiotic treatment. mNGS can save time to diagnose and identify pathogens, especially important for those with poor response to empirical antibiotics. This study showed that omadacycline may be considered as a new option for the treatment of *Chlamydia psittaci* pneumonia with ARDS.

Data availability statement

The data presented in the study are deposited in the China National GeneBank DataBase (https://db.cngb.org/) accession number CNP0004281.

Ethics statement

The studies involving humans were approved by the Second Affiliated Hospital of Chongqing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because given the retrospective nature of the study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

WD proposed the idea of the study, participated in the design of the study, and revised the manuscript. D-XW drafted the manuscript. L-XX collected and analyzed clinical data. X-YD performed data interpretation. 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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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Xiangrong Zuo,
The First Affiliated Hospital of Nanjing Medical University, China
Bao-ping Tian,
Zhejiang University School of Medicine, China

*CORRESPONDENCE
Hanwei Chen
✉ docterwei@sina.com
Jinhua He
✉ hejinhua@pyhospital.com.cn

†These authors have contributed equally to this work

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Identification and validation of a novel glycolysis-related ceRNA network for sepsis-induced cardiomyopathy

Lulu Cheng[1,2†], Jiabin Liang[2†], Fangmei Xie[2], Zeping Han[2], Wenfeng Luo[2], Hanwei Chen[3*] and Jinhua He[2*]

[1]Postgraduate Cultivation Base of Guangzhou University of Chinese Medicine, Panyu Central Hospital, Guangzhou, China, [2]Central Laboratory, Guangzhou Panyu Central Hospital, Guangzhou, China, [3]Radiology Department of Panyu Health Management Center (Panyu Rehabilitation Hospital), Guangzhou, China

Purpose: Sepsis-induced cardiomyopathy (SIC) is a major life-threatening condition in critically infected patients. Early diagnosis and intervention are important to improve patient prognosis. Recognizing the pivotal involvement of the glycolytic pathway in SIC, this study aims to establish a glycolysis-related ceRNA network and explore novel diagnostic avenues.

Materials and methods: SIC-related datasets were carefully filtered from the GEO database. CytoHubba was used to identify differentially expressed genes (DEGs) associated with glycolysis. A predictive method was then used to construct an lncRNA-miRNA-mRNA network. Dual-luciferase reporter assays validated gene interactions, and the specificity of this ceRNA network was confirmed in peripheral blood mononuclear cells (PBMCs) from SIC patients. Logistic analysis was used to examine the correlation between the ceRNA network and SIC. Diagnostic potential was assessed using receiver operating characteristic (ROC) curves, and correlation analysis investigated any associations between gene expression and clinical indicators.

Results: IER3 was identified as glycolysis-related DEG in SIC, and a ceRNA network (SNHG17/miR-214-3p/IER3) was established by prediction. Dual luciferase reporter gene assay confirmed the presence of mutual binding between IER3, miR-214-3p and SNHG17. RT-qPCR verified the specific expression of this ceRNA network in SIC patients. Multivariate logistic analysis established the correlation between the ceRNA network and SIC. ROC analysis demonstrated its high diagnostic specificity (AUC > 0.8). Correlation analysis revealed a negative association between IER3 expression and oxygenation index in SIC patients ($p < 0.05$). Furthermore, miR-214-3p expression showed a negative correlation with NT-proBNP ($p < 0.05$).

Conclusion: In this study, we identified and validated a ceRNA network associated with glycolysis in SIC: SNHG17/miR-214-3p/IER3. This ceRNA network may play a critical role in the onset and development of SIC. This finding is important to further our understanding of the pathophysiological mechanisms underlying SIC and to explore potential diagnostic and therapeutic targets for SIC.

KEYWORDS

sepsis-induced cardiomyopathy, glycolysis, ceRNA network, bioinformatics analysis, IER3

1 Introduction

Sepsis is a common and complex medical condition, currently defined as an unbalanced response by the body to the invasion of harmful microorganisms, like bacteria, resulting in systemic inflammation and acute organ dysfunction (1). Among the myriad clinical consequences of sepsis, sepsis-induced cardiomyopathy (SIC) stands out prominently. It is characterized by ventricular dilatation, poor contractility, and decreased ejection fraction (2, 3). Unfortunately, this cardiac complication significantly contributes to the already high mortality rate associated with sepsis, leading to a considerably worse prognosis. Septic patients without SIC face a mortality rate of approximately 20%, while those with SIC experience a strikingly higher mortality rate ranging from 70 to 90% (4).

Recognizing the critical implications of SIC, early diagnosis becomes paramount for effective patient management. However, the existing clinical diagnostic methods for SIC, primarily relying on conventional indicators like transthoracic echocardiography, atrial natriuretic peptide, and cardiac troponin (5), lack the specificity required for prompt identification and treatment of SIC (6). Therefore, elucidating the molecular mechanisms underlying the occurrence and development of SIC is of utmost importance, offering promising targets for its prevention, diagnosis, and treatment.

The development of SIC is intricately associated with aberrations in myocardial cell metabolism. Glucose serves as the primary metabolic substrate for myocardial cells, primarily undergoing glycolysis and oxidative phosphorylation (OXPHOS) to produce ATP, thereby providing energy support for myocardial cells (7). In the normal heart, the majority of ATP is derived from mitochondrial OXPHOS, whereas glycolysis and lactate oxidation account for only 10–40% of ATP production (8, 9). However, in the context of sepsis, there is an imbalance in myocardial energy metabolism (10). Myocardial mitochondrial fatty acid oxidation is disrupted, leading to an accelerated rate of aerobic glycolysis, excessive glucose consumption, and accumulation of pyruvate in the myocardium (11). This worsens the heart's function in sepsis (12). In a word, glycolysis plays a significant role and may offer new avenues for early diagnosis and treatment of SIC.

The theory of Competing Endogenous RNAs (ceRNA) was initially proposed by Salmena et al. (13). This theory suggests that different RNA molecules can competitively bind to microRNAs (miRNA), thereby disrupting their inhibition of target genes and regulating gene expression (13). Long non-coding RNAs (lncRNAs) have been shown to act as ceRNAs by competitively sequestering miRNAs to modulate the expression of target genes (14). They play regulatory roles in various diseases, including sepsis (15) and SIC (16). Using bioinformatics techniques, we can identify hub DEGs in SIC and build specific ceRNA regulatory networks using high-throughput sequencing tools. This approach contributes to a deeper understanding of the pathogenic mechanisms of SIC and aids in the discovery of new methods for early prediction and diagnosis of SIC.

In order to explore specific genes related to glycolysis in SIC, we first screened for common DEGs in SIC datasets from the GEO database. Subsequently, we identified pivotal genes associated with glycolysis among these DEGs. Utilizing multiple databases, we predicted the miRNAs and lncRNAs targeted and constructed a ceRNA network related to glycolysis. To validate specific binding within the ceRNA network, dual-luciferase reporter gene assays were performed. In addition, we verified the specific expression of ceRNAs in SIC patients by RT-qPCR experiments using peripheral blood mononuclear cell (PBMC) samples. The accuracy of these ceRNAs in diagnosing SIC was evaluated using ROC curves. The flowchart representing the methodology of this study is shown in Figure 1.

DEGs: Differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein–protein interaction; RT-qPCR: Reverse transcription-quantitative real-time PCR.

2 Materials and methods

2.1 Bioinformatics analysis

2.1.1 Selection of microarray dataset

We selected 5 microarray datasets related to SIC from the GEO database (17),[1] as shown in Table 1. Among these, the GSE79962 series (including 20 human SIC samples and 11 non-SIC samples) and GSE44363 (including 4 mouse SIC samples and 4 non-SIC samples) were used as the training set. The GSE142615 series (comprising 4 mouse SIC samples and 4 non-SIC samples) contains both mRNA and lncRNA data and was used as the validation set.

2.1.2 Extraction of DEGs

To identify and analyze the DEGs between the SIC and control groups within the GEO datasets, we utilized the GEO2R online tool.[2] GEO2R is an online tool supplied by the GEO database that analyzes and visualizes GEO data using R programming, presenting results in a gene table sorted by importance (17). We set the criteria for DEGs selection as follows: $p < 0.05$ and $|\log FC| > 1$ to identify DEGs with significant expression differences. Subsequently, we used the online Venn tool (18)[3] to identify DEGs that were common to both datasets.

2.1.3 Functional enrichment analysis of DEGs and PPI analysis

We assessed the biological functions of the identified DEGs using the DAVID Bioinformatics Resources (19).[4] We generated the PPI network using the STRING database (20)[5] with a minimum required interaction score set to 0.4. The obtained PPI information was then imported into Cytoscape 3.7.1 software (21).[6] We used the cytoHubba plugin within Cytoscape to identify important DEGs as hub genes in the PPI network. The cytoHubba plugin employs various topological algorithms to predict and explore key nodes and subnetworks within a given network (22). Therefore, we applied the MCC algorithm and selected the top 50% ranked genes as hub genes for further analysis.

1 https://www.ncbi.nlm.nih.gov/geo/
2 http://www.ncbi.nlm.nih.gov/geo/geo2r/
3 https://jvenn.toulouse.inrae.fr/app/example.html
4 https://david.ncifcrf.gov/tools.jsp
5 https://string-db.org
6 https://cytoscape.org/

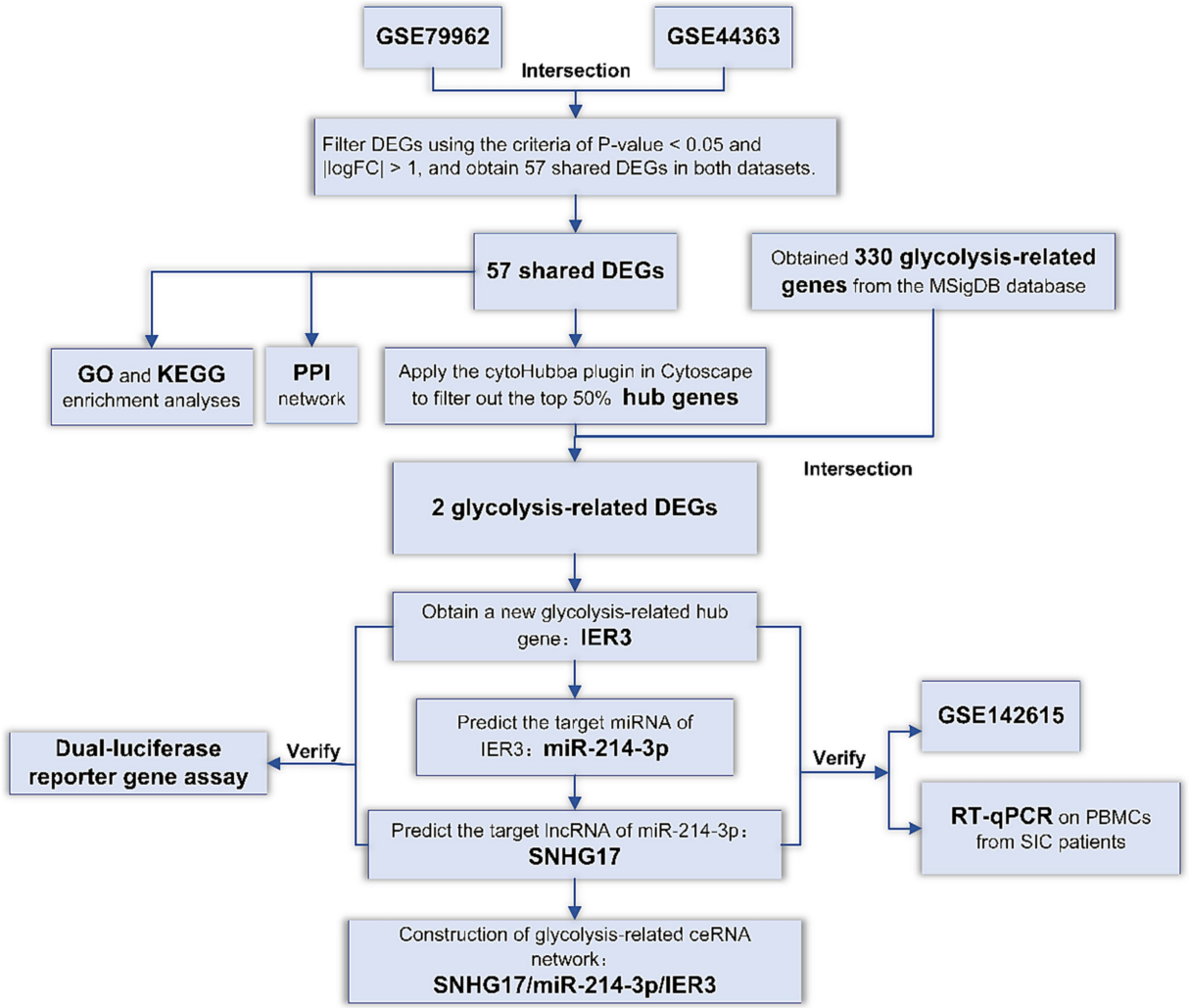

FIGURE 1
Flowchart of the study.

TABLE 1 Information on the datasets utilized in this study.

GEO number	Platform	Species	Source tissue	Sample (SIC/control)	Data	Attribute
GSE79962	GPL6244	Human	Heart	20/11	mRNA	Test set
GSE44363	GPL1261	Mice	Heart	4/4	mRNA	Test set
GSE142615	GPL27951	Mice	Heart	4/4	mRNA and lncRNA	Validation set

2.1.4 Selection of glycolysis-related hub genes

We first identified five gene sets related to glycolysis from MSigDB[7] (23): BIOCARTA_GLYCOLYSIS_PATHWAY,GO_GLYCOLYTIC_PROCESS, HALLMARK_GLYCOLYSIS, KEGG_GLYCOLYSIS_GLUCONEOGENESIS, and REACTOME_GLYCOLYSIS. After merging and removing duplicates from these five gene sets, we obtained a total of 330 glycolysis-related genes (Supplementary Table 1: S1). We then used Venn diagrams to identify the intersection between the glycolysis-related gene sets and the hub genes, leading to the selection of glycolysis-related hub genes. Finally, we conducted validation of the selected GRHGs using the dataset GSE142615.

2.1.5 Construction of the ceRNA network

We predicted miRNAs related to GRHGs using three online miRNA databases: TarBase (24),[8] starBase (25),[9] and miRWalk (26).[10]

7 https://www.gsea-msigdb.org/gsea/msigdb/

8 https://dianalab.e-ce.uth.gr/html/diana/web/index.php?r=tarbasev8

9 http://starbase.sysu.edu.cn/

10 http://mirwalk.umm.uni-heidelberg.de/

The intersection of miRNA predictions from these three databases was obtained using the Venn tool. Subsequently, we used RNA22 (27)[11] to predict lncRNAs that interact with the target miRNAs. We validated the predictions using dataset GSE142615 and selected a subset of lncRNAs with $|logFC| > 1$ from the validation results.

2.2 The dual-luciferase reporter gene assay

Through the TargetScan website[12] (28), the binding sites of IER3/miR-214-3p and miR-214-3p/SNHG17 were predicted. Wild-type and mutant PCR primers were designed accordingly. HEK293-T cells DNA was extracted as the template, and PCR was performed to amplify the 3'UTR sequences of IER3 and SNHG17. After amplification, the PCR products were subjected to enzymatic digestion, and the digested products were purified following the instructions of the Gel Extraction Kit (DONGSHENG BIOTECH, China). The purified digested products underwent ligation reactions and were separately introduced into *Escherichia coli* DH5α competent cells. Single colonies were picked for amplification, and plasmid extraction was carried out using the Plasmid Isolation Kit (DONGSHENG BIOTECH, China) following the instructions. The resulting plasmids were identified by enzymatic digestion and separated on a 1% agarose gel with ethidium bromide. Positive clones were confirmed and subsequent sequencing of the plasmids was performed.

HEK293-T cells are widely used as a functional cell for producing adenovirus vectors, adeno-associated virus vectors and cellular biology research. The day before transfection, HEK293-T cells were seeded at a density of 2×10^4 cells per well in a 24-well plate using DMEM high-glucose medium (Gibico, United States) containing 10% FBS. On the day of transfection, when the cell confluence reached approximately 50–60%, each well was treated with 1 μL of cellfectin II Reagent (Invitrogen) diluted in OPTI-MEM medium (Gibico, United States). Subsequently, a mixture containing 20 μM miR-214-3p mimic or miR-214-3p inhibitor (Guangzhou RiboBio, China) and 0.5 μg of wild-type or mutant plasmid was added to each well. Negative control (NC) groups with empty plasmid and NC inhibitor were also set up with three replicates for each group. The medium was changed for new growth medium after 6 h of transfection, and the cells were then cultured for a further 48 h. After that, the cells were extracted, and a GloMax bioluminescence detector was used to quantify the activity of firefly luciferase and Renilla luciferase. The measurements were performed following the instructions provided in the Promega Dual-Luciferase System Kit (Promega, United States), and the values for firefly luciferase and Renilla luciferase activities were recorded.

2.3 Clinical sample collection and RT-qPCR

PBMC samples from SIC patients were collected from the Panyu Central Hospital in Guangzhou, China. This study strictly adhered to the Helsinki Declaration and relevant legal and regulatory requirements, and it was approved by the Ethics Committee of Panyu Central Hospital (Approval No: PYRC-2023-086). Informed consent was obtained from all participants before the study commenced. Inclusion criteria were as follows: (1) Patients diagnosed with sepsis according to the Sepsis-3 criteria upon admission (1); (2) Patients with sepsis exhibiting the following conditions: left ventricular ejection fraction (LVEF) ≤ 50% and elevated myocardial injury markers; (3) Inclusion within a time frame of no more than 7 days from the initial diagnosis of SIC. Exclusion criteria were as follows: (1) Age < 18 years; (2) Concomitant acute myocardial infarction or severe arrhythmias; (3) History of chronic heart failure or chronic renal insufficiency; (4) End-stage tumor or hematological malignancies.

Each participant collected a 5 mL venous blood sample, which was stored at 4°C for a short period of time. PBMC was extracted on the day of blood collection and RNA was extracted using the TRIzol method. Subsequently, RNA was reverse transcribed into cDNA. RNA samples for IER3 and SNHG17 determination were reverse transcribed using the Hifair® first-strand cDNA synthesis kit (Yeasen, China), while miR-214-3p RNA samples were processed using the miRNA 1st strand cDNA synthesis kit (Accurate Biotechnology, China). The miR-214-3p stem-loop primer sequence was: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATAC GACACTGCC. After reverse transcription, qPCR experiments were performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China). GAPDH was used as the internal reference gene for IER3 and SNHG17, and U6 served as the internal reference gene for miR-214-3p. The relative expression of target genes was analyzed using the 2-ΔΔCt method. Primer sequences are shown in Table 2, and all experiments were performed in triplicate.

2.4 Statistical analyses

Statistical analyses were conducted using SPSS 25 and GraphPad Prism 9.5.1 software, and all data are presented as mean ± standard deviation. When comparing data between two groups, paired *t*-tests were used for data that followed a normal distribution, and non-parametric tests were used for data that did not follow a normal distribution. ANOVA was used for comparisons of relative luciferase activity among different groups. Univariate and multifactorial logistic regression analyses were performed to determine the correlation between the identified ceRNAs and SIC. The receiver operating characteristic (ROC) curve and area under the curve (AUC) values are used to compare diagnostic accuracy. The correlation between the relative expression levels of each gene and clinical parameters was assessed using either Pearson's or Spearman's correlation coefficient. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Selection of SIC-related DEGs

Differential genes in datasets GSE79962 and GSE44363 were analyzed using GEO2R tool. All differential genes were visualized in volcano plots (Figures 2A,B), and the top 20 upregulated and downregulated genes were selected for generating a heatmap of differential genes (Figures 2C,D). A filter criterion with $p < 0.05$ and

11 https://cm.jefferson.edu/rna22/Precomputed/

12 http://www.Targetscan.org

TABLE 2 Primer sequence.

Genes	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
IER3	GCAGCCGCAGGGTTCTCTACC	CTCTTCAGCCATCAGGATCTGG
miR-214-3p	GGCACAGCAGGCACAGACA	AGTGCAGGGTCCGAGGTATT
SNHG17	TGGGATCTGGGTTTGCTGATATTTCT	GGTAGCCTCACTCTCCATTCTCTG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	AGAAGGCTGGGGCTCATTTG	GCAGGAGGCATTGCTGATGAT

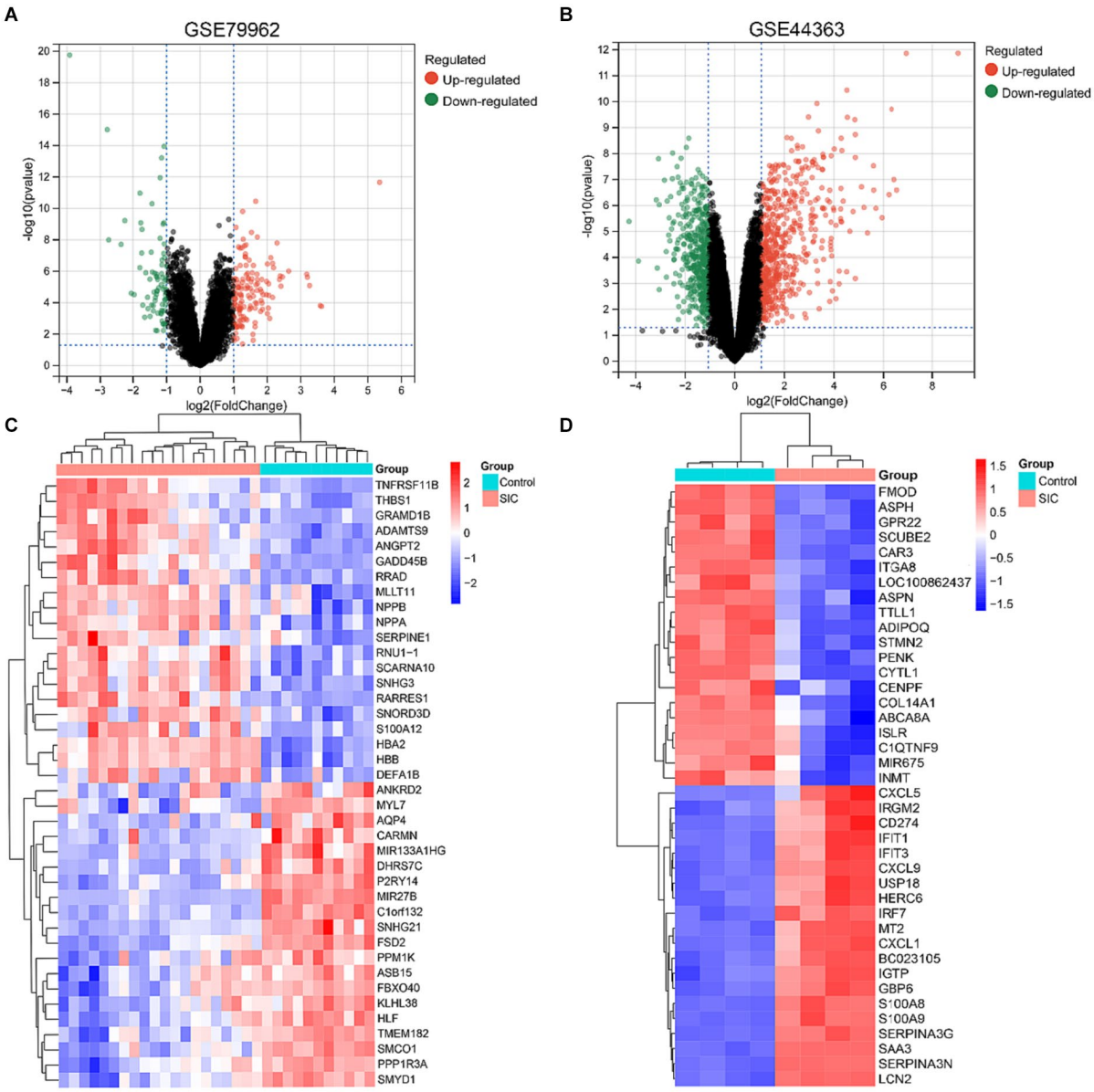

FIGURE 2
DEGs identified in GSE79962 and GSE44363. **(A)** Volcano plot of all DEGs in GSE79962. **(B)** Volcano plot of all DEGs in GSE44363. **(C)** Heatmap of the top 20 upregulated and downregulated DEGs in GSE79962. **(D)** Heatmap of the top 20 upregulated and downregulated DEGs in GSE44363.

|logFC| > 1 was applied, resulting in the selection of 222 significantly DEGs in the GSE79962 dataset, which included 141 upregulated and 81 downregulated genes. In the GSE44363 dataset, a total of 930 significant DEGs were identified using the same criteria, with 539 genes upregulated and 391 genes downregulated. The intersection of 2 datasets was used to obtain 57 common DEGs (Supplementary Table 1: S2).

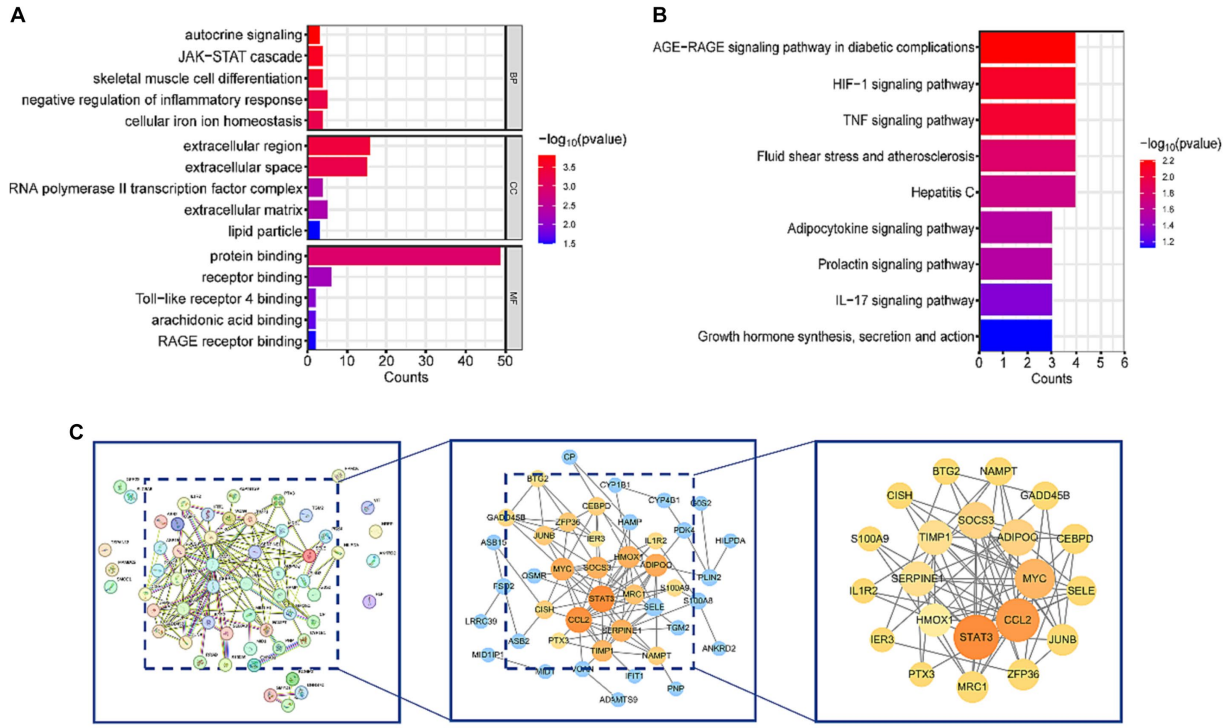

FIGURE 3
Functional enrichment analysis and PPI network of DEGs. **(A)** Results of GO analysis. **(B)** Results of KEGG analysis. **(C)** PPI network diagram of DEGs and hub genes. After removing isolated nodes, the orange and yellow nodes represent the top 50% of nodes ranked by the MCC algorithm. Nodes with larger diameters and darker colors indicate higher degrees in the PPI network.

3.2 Functional enrichment analysis and PPI network construction

DAVID database was used to perform KEGG and GO analyses on the 57 common DEGs. GO analysis revealed that DEGs were involved in several biological processes (BP), including autocrine signaling, JAK–STAT cascade, skeletal muscle cell differentiation, negative regulation of inflammatory response, and cellular iron ion homeostasis. The major molecular functions (MF) of DEGs included protein binding, receptor binding, Toll-like receptor 4 binding, arachidonic acid binding, and RAGE receptor binding. The cellular components (CC) mainly associated with DEGs were the extracellular region, extracellular space, and RNA polymerase II transcription factor complex (Figure 3A). KEGG enrichment analysis showed that DEGs were significantly enriched in pathways related to HIF-1, TNF, IL-17 signaling, AGE-RAGE signaling pathway in diabetic complications, and Fluid shear stress and atherosclerosis (Figure 3B). These pathways are closely associated with inflammatory responses. Detailed data are presented in Supplementary Table 1: S3, S4.

To gain a deeper understanding of the interactions among DEGs, we constructed a PPI network using STRING, resulting in a network with 57 nodes and 106 edges. Subsequently, this PPI network was imported into Cytoscape software, and isolated nodes were removed, resulting in a DEGs network with 43 nodes and 106 edges. o further identify the most specific DEGs within this network, we used the CytoHubba plugin for analysis and ranking. This plugin evaluates the importance of nodes in the PPI using 11 different node ranking methods (20). We selected the MCC method, which offers high sensitivity and specificity, and identified the top 50% of DEGs as hub genes (Figure 3C). The specific gene names are listed in Table 3.

3.3 Identification of glycolysis-related hub gene

A total of 330 glycolysis-related genes were obtained from the MSigDB database. Crossing these genes with the 57 DEGs and 21 hub genes led to the identification of two glycolysis-related DEGs, namely IER3 and STAT3 (Figure 4A). STAT3, as a well-known transcription factor, has been previously studied for its relevance to sepsis. IER3, a novel and specific gene discovered in this study, was selected as the glycolysis-related hub gene for further investigation. This gene showed significant upregulation in the SIC group in both GSE79962 and GSE44363 datasets. Validation was performed using the training dataset GSE142615, which confirmed that IER3 was also significantly upregulated in the SIC group of the GSE142615 dataset (Figure 4B).

3.4 ceRNA network construction

We predicted the miRNAs interacting with IER3 using the TarBase, starBase, and miRWalk databases (Supplementary Table 1: S5–S7). After intersecting the results from these three databases, we identified miR-214-3p (Figure 4C). Subsequently, we employed the RNA22 database to predict 3,748 lncRNAs that interact with miR-214-3p (Supplementary Table 1: S8). To validate the relevance of these lncRNAs

TABLE 3 Hub genes ranked in the top 50% by CytoHubba.

Gene name	Description
STAT3	Signal transducer and activator of transcription 3
CCL2	C-C motif chemokine ligand 2
SERPINE1	Serpin family E member 1
TIMP1	TIMP metallopeptidase inhibitor 1
ADIPOQ	Adiponectin, C1Q and collagen domain containing
HMOX1	Heme oxygenase 1
SELE	Selectin E
MYC	V-myc avian myelocytomatosis viral oncogene homolog
SOCS3	Suppressor of cytokine signaling 3
NAMPT	Nicotinamide phosphoribosyltransferase
MRC1	Mannose receptor, C type 1
JUNB	JunB proto-oncogene, AP-1 transcription factor subunit
CEBPD	CCAAT/enhancer binding protein delta
ZFP36	ZFP36 ring finger protein
CISH	Cytokine inducible SH2 containing protein
GADD45B	Growth arrest and DNA damage inducible beta
BTG2	BTG anti-proliferation factor 2
IL1R2	Interleukin 1 receptor type 2
S100A9	S100 calcium binding protein A9
PTX3	Pentraxin 3
IER3	Immediate early response 3

to SIC, we utilized the GSE142615 dataset, which includes 670 differentially expressed lncRNAs. By taking the intersection, we identified 2 lncRNAs for validation: SNHG17 and KCNQ1OT1. Among them, SNHG17 was upregulated in the SIC group (logFC = 1.22764595) (Figure 4D), while KCNQ1OT1 was downregulated in the SIC group (logFC = −1.72168747) (Figure 4E). According to the theory of ceRNA, when the expression of lncRNAs is upregulated in SIC, they can better serve as "sponges" that competitively bind with miRNAs. Therefore, we chose SNHG17 and constructed a ceRNA network related to glycolysis: SNHG17/miR-214-3p/IER3.

3.5 Validation of gene-specific binding through dual-luciferase activity

Potential binding sites between IER3 and miR-214-3p and miR-214-3p and SNHG17 were predicted using the TargetScan website. PCR primers were designed based on the 3'UTR sequences of IER3 and SNHG17, and both wild-type and mutant gene sequences were synthesized (Figure 5A). Successful amplification of the PCR products for IER3 and SNHG17 was confirmed by agarose gel electrophoresis (Figure 5B). After PCR product recovery, enzymatic digestion, ligation, and transformation, plasmids were extracted and identified as positive clones by enzymatic digestion (Figure 5C). Subsequent plasmid sequencing results revealed that the wild-type plasmids (IER3-3′UTR-wt, SNHG17-3′UTR-wt) had sequences identical to the reference sequence. In contrast, mutant plasmids (IER3-3′UTR-mut and SNHG17-3′UTR-mut) exhibited a mutation from CCTGCTG to TTGATGA in both cases (Figure 5D).

In the wild-type IER3 group, relative luciferase activity significantly decreased in the IER3-3′UTR-wt + miR-214-3p group compared to the empty plasmid NC group. Conversely, the IER3-3′UTR-wt + miR-214-3p inhibitor group showed a significant increase in relative luciferase activity compared to the NC inhibitor group. No significant change in relative luciferase activity was observed in the IER3-3′UTR-mut group, indicating that miR-214-3p can specifically bind to the 3′UTR target site of the IER3 gene, and there is only one binding site (Figure 5E). In the wild-type SNHG17 group, relative luciferase activity significantly decreased in the SNHG17-3′UTR-wt + miR-214-3p group compared to the empty plasmid NC group. Conversely, the SNHG17-3′UTR-wt + miR-214-3p inhibitor group showed a significant increase in relative luciferase activity compared to the NC inhibitor group. No significant change in relative luciferase activity was observed in the SNHG17-3′UTR-mut group, indicating that miR-214-3p can specifically bind to the 3′UTR target site of the SNHG17 gene, and there is only one binding site (Figure 5F). All the raw data is presented in Supplementary Table S2.

3.6 Validation of the ceRNA network by PBMC samples

In this study, a total of 20 patients meeting the criteria for SIC were included, and an additional 18 healthy individuals were recruited as the control group. Detailed clinical data for SIC patients can be found in Supplementary Table 3: S2. The expression of SNHG17/miR-214-3p/IER3 in PBMCs of SIC patients was detected using RT-qPCR (Supplementary Table 3: S1). The results showed that the expression of IER3 and SNHG17 was significantly upregulated in PBMC samples from SIC patients ($p < 0.05$) (Figures 6A,C), while miR-214-3p was downregulated ($p < 0.05$) (Figure 6B). Logistic regression analysis showed a significant correlation between IER3, miR-214-3p, SNHG17 expression and SIC (Table 4). ROC curve analysis showed that IER3 (AUC: 0.833), miR-214-3p (AUC: 0.778), and SNHG17 (AUC: 0.792) had good diagnostic capabilities (Figure 6D). Subsequently, we combined various indicators and predictive ability of the model again， the multivariate ROC analysis revealed that the combined model of IER3 + miR-214-3p + SNHG17 had the best diagnostic performance (AUC: 0.942), followed by the models combining two genes: IER3 + miR-214 (AUC: 0.914), IER3 + SNHG17 (AUC: 0.881), and miR-214-3p + SNHG17 (AUC: 0.892), all of which exhibited higher diagnostic capabilities than the single-gene models (Figure 6E). We used the Spearman correlation coefficient to examine the correlation between the relative expression levels of each gene and clinical indicators in patients. It was found that the relative expression level of IER3 was negatively correlated with the oxygenation index ($p < 0.05$), and the relative expression level of miR-214-3p was negatively correlated with NT-proBNP (N-terminal pro-brain natriuretic peptide) ($p < 0.05$) (Table 5).

4 Discussion

Sepsis is a disease characterized by a dysregulated host response to infection, resulting in multi-organ dysfunction (1), and it claims the lives of millions of people worldwide each year (29). SIC is a severe complication resulting from sepsis, often indicating a poorer prognosis

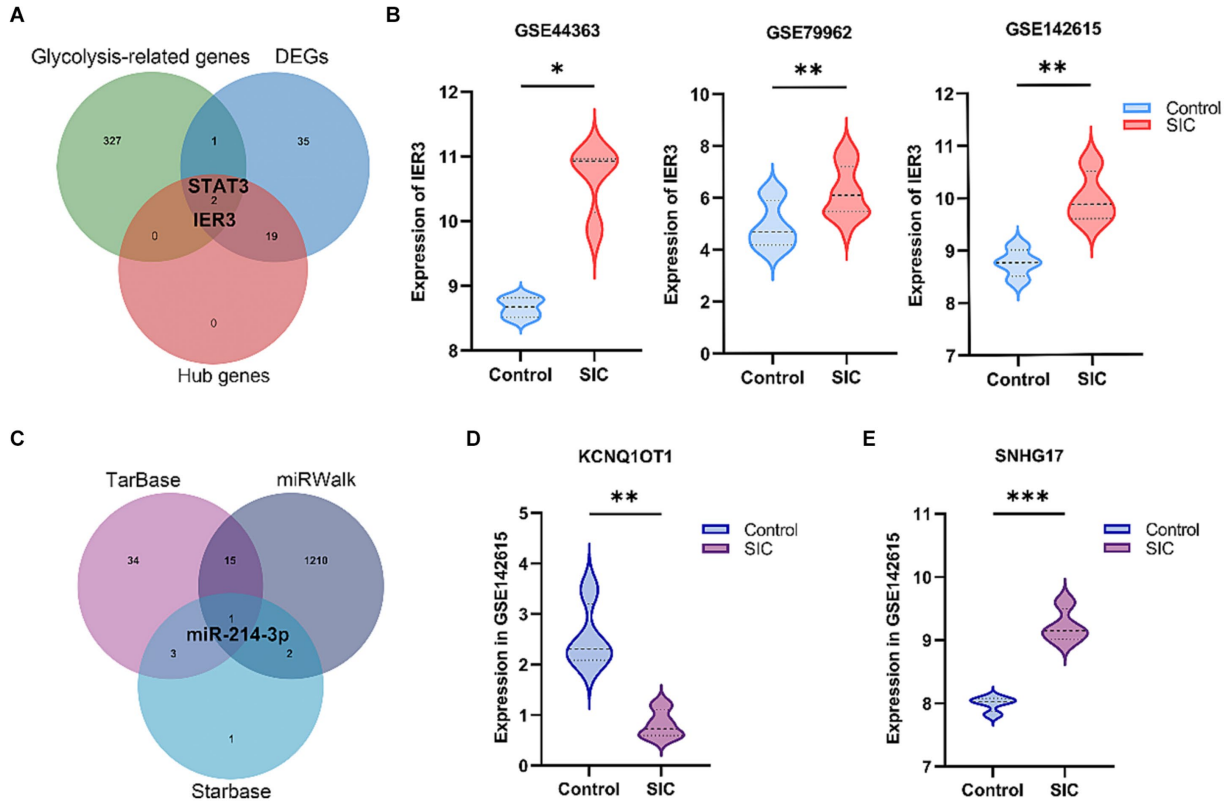

FIGURE 4
Selection of glycolysis-related DEGs and construction of ceRNA network. **(A)** Venn diagram results show the presence of 2 glycolysis-related hub genes (STAT3 and IER3). **(B)** Differential expression of IER3 in GSE79962, GSE44363, and GSE142615. **(C)** The common miRNA target identified in the 3 databases is miR-214-3p. **(D)** Expression of KCNQ1OT1 in GSE142615. **(E)** Expression of SNHG17 in GSE142615. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

and a higher mortality rate. Due to the urgency of diagnosing and treating SIC, there is a clinical need for highly specific diagnostic tools to promptly recognize the condition.

Currently, echocardiography and biomarkers of myocardial injury are the preferred modalities for clinical assessment, but they are not specific enough to diagnose SIC. A significant issue is that reduced afterload resulting from the distributive shock may pseudo-normalize a depressed EF (coupling between contractility and afterload) (30, 31). While echocardiographic parameters like diastolic function and right ventricular (RV) systolic function lack the same specificity as LVEF in diagnosing SIC, they often require exclusion in conjunction with other diagnostic methods (6). Novel parameters such as global longitudinal strain (GLS), myocardial performance index (MPI) currently lack reliability data in clinical applications (32). Therefore, exploring the pathophysiological mechanisms of SIC and identifying indicators that are more sensitive and specific will provide robust assistance in the diagnosis and treatment of SIC.

The pathogenesis of septic cardiomyopathy is complex, with metabolic changes playing a pivotal role (33). During sepsis, there is a shift in cellular metabolism from oxidative phosphorylation to glycolysis, a phenomenon known as the Warburg effect (34). Enhanced glycolysis can lead to rapid activation of immune cells, resulting in the release of numerous pro-inflammatory cytokines. In some cases, this process can trigger a "cytokine storm," further exacerbating organ dysfunction (4). Studies have shown that inhibiting glycolysis with 2-deoxyglucose (2-DG) significantly alleviates cardiac dysfunction and improves survival rates in septic mice. Additionally, this intervention enhances the expression of Sirt1 and Sirt3, which are associated with mitochondrial function protection in cardiac muscle, while suppressing the expression of apoptotic genes Bak and Bax, as well as JNK phosphorylation (12). These findings underscore the close relationship between glycolysis and SIC. However, the specific mechanisms by which glycolysis operates in the context of SIC remain to be fully elucidated, warranting further in-depth research.

With the development of genomics technology in recent years, sepsis diagnosis and treatment have benefited from the use of both gene sequencing and gene therapy (35, 36). The discovery of novel biomarkers through genomic sequencing techniques has provided new avenues for identifying diagnostic targets in diseases. Analyzing differential gene expression from datasets such as GEO and constructing ceRNA networks has emerged as a crucial approach in current research.

In this study, we used bioinformatics techniques to identify 2 hub genes related to glycolysis in SIC: IER3 and STAT3. STAT3, as a classical transcription factor, plays a crucial role in regulating various physiological pathways, including cell growth, differentiation, and apoptosis. Previous research has confirmed the pivotal role of STAT3 in LPS-induced myocardial dysfunction (37). IER3, also known as IEX-1, is a stress-inducible immediate-early gene It plays a role in influencing mitochondrial F1Fo ATPase activity, regulating mitochondrial reactive oxygen species balance, and participating in the modulation of mitochondrial oxidative phosphorylation and glycolysis (38). IER3 has a unique role in the

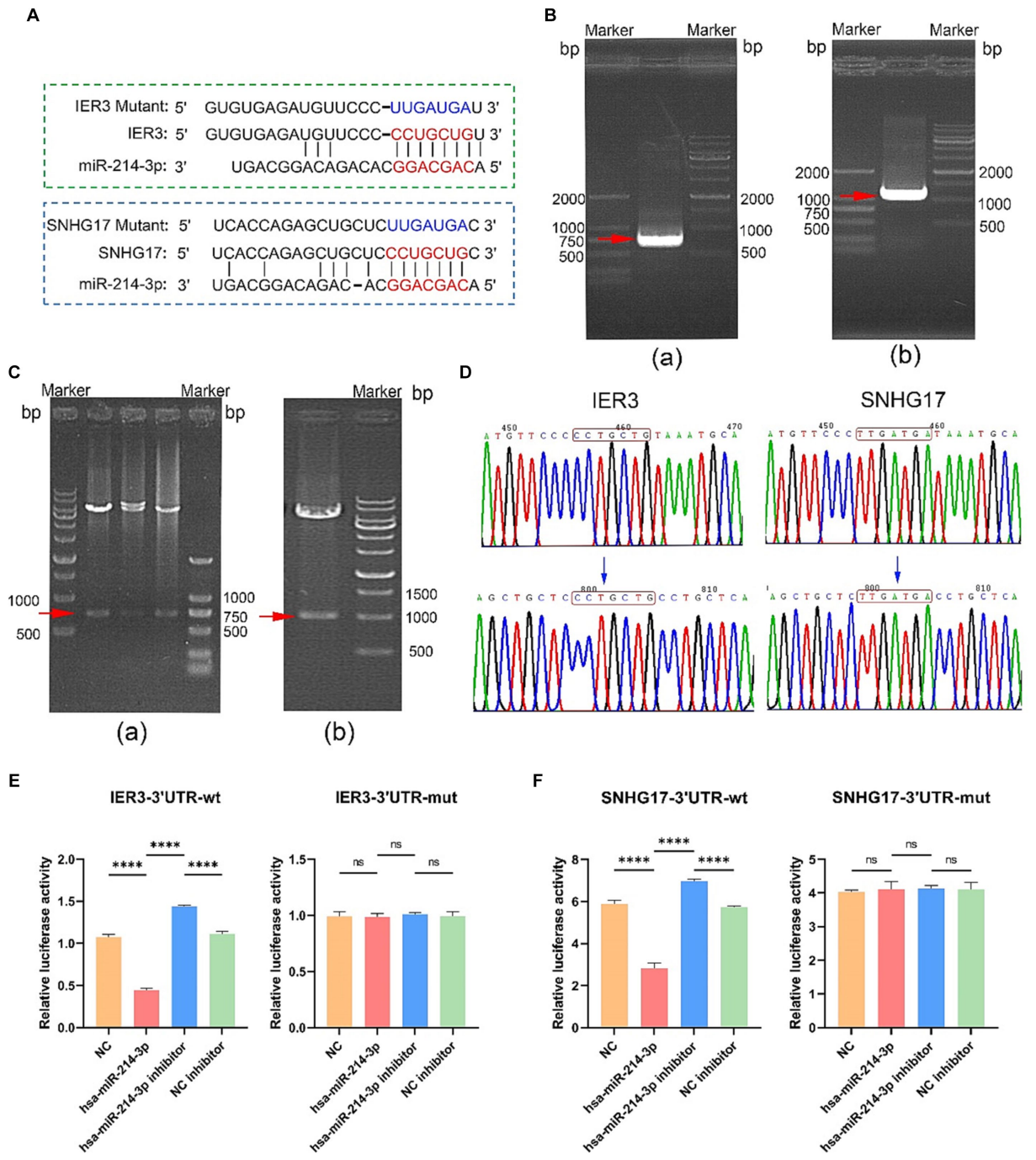

FIGURE 5
Dual-luciferase reporter gene assay. **(A)** Binding sites of miR-214-3p with IER3 and SNHG17, along with the sequences after mutation. **(B)** Amplification confirmation of PCR products. (a) IER3-3'UTR-wt PCR identification results (737bp); (b) SNHG17-3'UTR-wt PCR identification results (1086 bp). **(C)** Identification of plasmid enzyme digestion products. (a) IER3 plasmid enzyme digestion products (737bp); (b) Identification results of SNHG17 plasmid enzyme digestion products (1086bp). **(D)** Sequencing results of wild-type and mutant plasmids. The wild-type plasmids of IER3 and SNHG17 are consistent with the reference sequence, while the corresponding positions of mutant plasmids have been successfully mutated. **(E,F)** Dual-luciferase reporter gene assay results. ****$p < 0.0001$, ns: $p > 0.05$.

pathogenesis of cardiovascular and inflammatory diseases. Its expression is significantly upregulated in the myocardial tissues of mice subjected to pressure overload, and IER3 gene knockout may lead to hypertension and cardiac hypertrophy in mice (39). It can impact inflammatory responses by regulating pathways such as NF-κB and Nrf2 (40). In this study, we revealed a relationship between IER3 and SIC for the first time and gained new insights into the study of IER3.

Subsequently, We then predicted miRNAs targeting IER3 from multiple databases and identified miR-214-3p as one of the miRNAs targeting IER3. Previous research has suggested a potential link between miR-214-3p and the pathogenesis of SIC. Overexpression of miR-214-3p

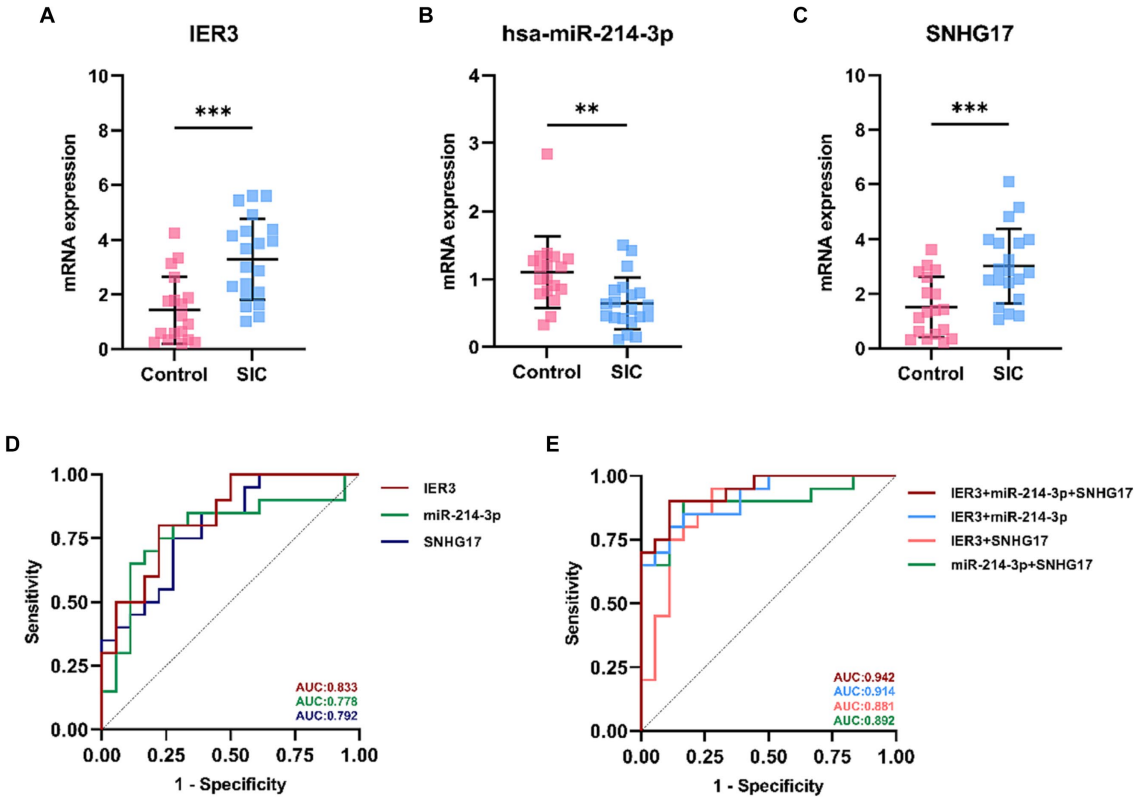

FIGURE 6
Expression and ROC curve of various genes in SIC patients. **(A)** Relative expression levels of IER3 in PBMCs of SIC patients. **(B)** Relative expression levels of miR-214-3p in PBMCs of SIC patients. **(C)** Relative expression levels of SNHG17 in PBMCs of SIC patients. **(D)** ROC curves assessing the individual diagnostic ability of IER3, miR-214-3p, and SNHG17 for SIC. **(E)** ROC curves assessing the combined diagnostic ability of IER3, miR-214-3p, and SNHG17 for SIC.**$p < 0.01$,***$p < 0.001$.

TABLE 4 Univariate and multivariate logistic analyses in SIC patients.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p* value	OR	95% CI	*p* value
IER3	2.352	1.314–4.208	0.004	2.329	1.042–5.203	0.039
miR-214-3p	0.062	0.08–0.497	0.009	0.02	0.001–0.423	0.012
SNHG17	2.738	1.367–5.483	0.009	2.8	1.055–7.428	0.039

CI, confidence interval; OR, odds ratio.

in septic mice models has been shown to alleviate myocardial dysfunction and damage. Additionally, it inhibits myocardial inflammation, and reduce autophagy (41). Upregulation of miR-214-3p has an inhibitory effect on myocardial cell apoptosis and injury in rats with myocardial ischemia/reperfusion injury (42). Conversely, its deficiency may exacerbate cardiac fibrosis (43). We then predicted lncRNAs targeted by miR-214-3p, among which SNHG17 was identified. Studies have indicated that SNHG17 is upregulated in ovarian cancer and acts as a molecular sponge for miR-214-3p, relieving miR-214-3p's inhibitory effect on the cell cycle regulator CDK6, thereby promoting the growth of ovarian cancer cells (44). SNHG17 is upregulated in various tumors and is closely associated with adverse prognosis and advanced clinical-pathological characteristics in cancer patients (45). However, its role in cardiovascular diseases has not been thoroughly investigated.

Based on the above research, we predicted and established a novel ceRNA network, SNHG17/miR-214-3p/IER3. To validate the authenticity of this network, we conducted a luciferase assay, confirming the specific binding relationships between IER3/miR-214-3p and SNHG17/miR-214-3p. The unique expression of IER3, miR-214-3p, and SNHG17 was validated by qPCR utilizing PBMC samples from clinical SIC patients. The ROC curves demonstrate that this ceRNA network possesses a strong diagnostic capability. Interestingly, we also observed correlations between IER3 and oxygenation index, as well as miR-214-3p and NT-proBNP.

It should be noted that in this study, a direct correlation between IER3-miR-214-3p-SNHG17 and LVEF was not observed. This finding is similar to previous research by Parker et al. (46), who reported that only about 50% of patients with septic shock had a reduced LVEF. Additionally, survivors had a lower LVEF on average compared to non-survivors (46). While a decreased LVEF is a clinical diagnostic criterion for SIC, it is important to note that LVEF values can be influenced by cardiac loading conditions and vary with individual

TABLE 5 Correlation of IER3, miR-214-3p, and SNHG17 with clinical parameters.

Clinical parameters	IER3		miR-214-3p		SNHG17	
	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value
Age	−0.044	0.853	0.269	0.251	−0.214	0.366
NT-proBNP	0.044	0.854	**−0.457***	**0.043**	0.3	0.199
Oxygenation- Index	**−0.468***	**0.037**	−0.036	0.879	0.115	0.629
LVEF	0.297	0.204	0.242	0.904	0.014	0.954
PCT	−0.259	0.269	−0.125	0.6	0.238	0.311
WBC	0.28	0.232	0.128	0.591	0.033	0.89
NEU	0.107	0.654	−0.137	0.565	0.104	0.663
Platelets	0.119	0.617	−0.165	0.487	0.164	0.489
Creatinine	0.02	0.932	−0.132	0.578	−0.076	0.75
Total bilirubin	0.156	0.512	−0.128	0.591	0.354	0.126

NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEF, Left ventricular ejection fraction; PCT, Procalcitonin; WBC, White blood cell count; NEU, Neutrophil count. *$p < 0.05$. Bold font indicates that this correlation coefficient *p* value is statistically different.

differences in filling pressures and cardiac afterload. Therefore, the specificity of LVEF values in diagnosing and prognosticating SIC is suboptimal. Although IER3-miR-214-3p-SNHG17 did not show a direct correlation with LVEF, its expression in SIC and the sensitivity of this diagnostic model still suggest the potential diagnostic value of this network. These findings are significant for understanding SIC's pathophysiology and identifying possible treatment options.

In this study, we have established a novel glycolysis-related ceRNA network, SNHG17/miR-214-3p/IER3, which has not been previously reported in current studies, and discovered the precise expression of IER3 in SIC for the first time. To increase the credibility of our results, we experimentally validated the specific binding of this ceRNA network through dual-luciferase reporter assays and confirmed the differential expression of SNHG17/miR-214-3p/IER3 in PBMCs using external datasets and human PBMC samples. Nevertheless, this study has several limitations. Firstly, the sample sizes from the selected datasets were restricted due to the scarcity of SIC samples in the GEO database. Additionally, the number of participants included in our PBMC validation was relatively small, requiring further clinical research to validate and broaden the applicability of our findings. Furthermore, considering the restricted specificity of LVEF in SIC diagnosis and prognosis, incorporating additional novel biomarkers like global longitudinal strain (GLS) and myocardial performance index (MPI) during the collection of clinical cases could be beneficial. Alternatively, applying stricter inclusion criteria, such as LVEF < 45% (47–49), for the combined diagnosis of SIC might enhance the accuracy and specificity of SIC diagnosis. In future studies, we will employ more rigorous diagnostic criteria for the collection of clinical cases and further validate the accuracy of this ceRNA network in diagnosing and prognosticating SIC.

6 Conclusion

We have identified IER3 as a novel target related to glycolysis in SIC and established a new ceRNA network: SNHG17/miR-214-3p/IER3. This ceRNA network may be closely associated with the development and occurrence of SIC. Despite certain limitations, this study opens up new avenues for a more profound understanding of the pathophysiological mechanisms of SIC and the development of more effective diagnostic tools. Future research will require more rigorous and extensive clinical studies to validate its diagnostic potential in clinical settings.

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.

Ethics statement

The studies involving humans were approved by The Ethics Committee of Panyu Central Hospital, Guangzhou, China. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LC: Conceptualization, Software, Validation, Visualization, Writing – original draft. JL: Validation, Writing – original draft. FX: Methodology, Validation, Writing – original draft. ZH: Methodology, Writing – original draft. WL: Methodology, Writing – original draft. HC: Funding acquisition, Project administration, Resources, Writing – review & editing. JH: Funding acquisition, Project administration, Resources, Writing – review & editing.

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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.2024.1343281/full#supplementary-material

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EDITED BY
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

REVIEWED BY
Yanlin Song,
Sichuan University, China
Wenjin Yang,
Changhai Hospital, China
Qiwen Deng,
Nanjing Medical University, China

*CORRESPONDENCE
Yongpo Jiang
jyongpo8@163.com
Wei Hu
zzdxhuw@163.com
Renfei Shan
danrf@enzemed.com

†These authors have contributed equally to this work and share first authorship

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Development and validation of a predictive model for stroke associated pneumonia in patients after thrombectomy for acute ischemic stroke

Jingying Wang[1†], Chao Yang[1†], Ruihai Zhang[2†], Wei Hu[2*], Peng Yang[1], Yiqing Jiang[3], Weijun Hong[3], Renfei Shan[1,4*] and Yongpo Jiang[1,4*]

[1]Department of Emergency Medicine, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Taizhou, China, [2]Department of Neurosurgery, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Taizhou, China, [3]Department of Neurology, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Taizhou, China, [4]Department of Critical Care Medicine, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Taizhou, China

Objective: This study aims to identify the risk factors associated with stroke-associated pneumonia (SAP) in patients who have undergone thrombectomy for acute ischemic stroke and to develop a nomogram chart model for predicting the occurrence of pneumonia.

Methods: Consecutive patients who underwent thrombectomy for acute ischemic stroke were enrolled from three hospitals at Taizhou Enze Medical Center. They were randomly divided into a training group and a validation group in a 7:3 ratio. The training group data was used to screen for effective predictive factors using LASSO regression. Multiple logistic regression was then conducted to determine the predictive factors and construct a nomogram chart. The model was evaluated using the validation group, analyzing its discrimination, calibration, and clinical decision curve. Finally, the newly constructed model was compared with the AIS-APS, A2DS2, ISAN, and PANTHERIS scores for acute ischemic stroke-associated pneumonia.

Results: Out of 913 patients who underwent thrombectomy, 762 were included for analysis, consisting of 473 males and 289 females. The incidence rate of SAP was 45.8%. The new predictive model was constructed based on three main influencing factors: NIHSS ≥16, postoperative LMR, and difficulty swallowing. The model demonstrated good discrimination and calibration. When applying the nomogram chart to threshold probabilities between 7 and 90%, net returns were increased. Furthermore, the AUC was higher compared to other scoring systems.

Conclusion: The constructed nomogram chart in this study outperformed the AIS-APS, A2DS2 score, ISAN score, and PANTHERIS score in predicting the risk of stroke-associated pneumonia in patients with acute ischemic stroke after thrombectomy. It can be utilized for clinical risk prediction of stroke-associated pneumonia in patients after thrombectomy for acute ischemic stroke.

KEYWORDS

acute ischemic stroke, thrombectomy, stroke-associated pneumonia, risk factors, prediction model

Introduction

Stroke-associated pneumonia (SAP) is one of the most common complications in stroke patients. It is a pulmonary infection that occurs during the acute and sequelae phases of stroke (1). Stroke has a high incidence and mortality rate, with survey data showing approximately 13.7 million new stroke cases worldwide (2). Among these cases, stroke-associated pneumonia accounts for 7–22%, and the number of deaths attributable to stroke reaches up to 2 million every year (2). Studies have shown that pneumonia triples the risk of 30-day mortality in stroke patients (3). At the present, various studies have found that the pathophysiology of stroke-associated pneumonia is mainly related to the inflammatory mechanism and aspiration (4, 5). The systemic immunosuppression that occurs during the acute phase of stroke, known as post-stroke immunosuppressive syndrome, increases susceptibility to bacterial infections and is one of the major causes of infection in patients (6–8). The occurrence of stroke-associated pneumonia can cause a systemic inflammatory response, exacerbate neurological damage in stroke patients, prolong their hospitalization time, increase hospitalization costs and economic pressure, and lead to a poor prognosis, thereby increasing the probability of patient death (3, 9, 10). Early identification and treatment of stroke-associated pneumonia is of great significance for the prognosis of stroke patients.

Previous studies comparing the prognosis of thrombectomy and thrombolysis in patients with acute ischemic stroke have shown that thrombectomy alone is not inferior in improving functional outcomes compared to patients who underwent remove thrombus after thrombolysis within 4.5 h after symptom onset (11). Thrombectomy is the main treatment method for patients with large vessel stroke nowadays (12). With the increasing number of stroke patients undergoing thrombectomy, early warning of stroke-associated pneumonia in thrombectomy patients is becoming more important.

Presently, various scoring scales and inflammatory indicators are used in clinical practice to predict the occurrence of stroke-associated pneumonia. The AIS-AP score was proposed in 2013 through a study of the Chinese Stroke Login Database. It includes age, history, mRS (modified Rankin Scale) score, NIHSS (National Institutes of Health Stroke Scale) score, GCS (Glasgow Coma Scale) score, speech impairment, stroke classification, and blood sugar, but scoring using this scale is relatively cumbersome (13). The A2DS2 score was proposed based on a study of 15,335 registered stroke cases in Berlin, Germany. The score includes five items: gender, age, atrial fibrillation, dysphagia, and admission NIHSS score (14). The ISAN score was collected from 23,199 stroke patients in the UK and was scored based on four factors: age, gender, admission NIHSS, and ability to take care of oneself before the stroke (15). The PANTHERIS score was studied on 335 stroke patients in the intensive care unit of the hospital. The score includes four items: patient age, GCS upon admission, 24-h systolic and systolic blood pressure upon admission, and WBC (white blood cell) count (16). However, due to the differences in the samples studied by these scores and the fact that the sample study subjects are stroke patients, not targeted at stroke thrombus removal patients, there is no consensus on which score is more accurate in predicting SAP. Therefore, the main purpose of this study is to develop and validate a prediction model for SAP in patients after stroke thrombectomy. This model will serve as a basis for early clinical treatment, enabling healthcare providers to identify patients at risk and initiate timely interventions (17).

Methods

Participants

This study is a retrospective study involving 913 consecutive patients with acute ischemic stroke who underwent thrombectomy at Taizhou Enze Medical Center from January 2021 to August 2023. Out of these, 762 patients were selected and randomly divided into a training set of 528 and a validation set of 234 at a 7:3 ratio (see Figure 1). The research proposal has been reviewed by the Ethics Committee of Taizhou Enze Medical Center (Group) (Ethics Number: K20201104). **Inclusion criteria**: (1) Patients who meet the diagnostic criteria for acute ischemic stroke in the 2018 Early Management Guidelines for Acute Ischemic Stroke (18), with an onset time of less than 24 h; and (2) patients who undergo mechanical thrombectomy. **Exclusion criteria**: (1) Cerebral hemorrhage and intracranial mass; (2) Transient ischemic attack; (3) Existence of immune system and blood system diseases; (4) Serious infection present before admission; (5) Patients automatically discharged or dying within 24 h of admission; (6) Liver and kidney failure; (7) Trauma; (8) Pregnant women or under the age of 18; (9) Tracheal intubation under general anesthesia for surgery or Ventilator-associated pneumonia; and (10) Patients with incomplete data collection. We applied for an informed consent waiver while keeping patient information confidential.

Clinical data collection and grouping: Patient information will be collected through the hospital's medical record system, including age, gender, past medical history (diabetes, hypertension, cerebral infarction, atrial fibrillation, heart failure, COPD (Chronic obstructive pulmonary disease), smoking history), GCS score, NIHSS score on admission, systolic pressure on admission, thrombolysis, Blockage of blood vessels, tracheal intubation, dysphagia and blood test indicators [WBC, Lym (lymphocyte count), Neu (neutrophil count), Mono (mononuclear leucocyte), PLT (platelet), Hb (hemoglobin), FBG (fasting blood glucose), PT (prothronmbin time), INR (international normalized ratio), APTT (activated partial thromboplastin time), FIB (fibrinogen), TT (thrombin time), D-dimer, CL (chloride), NA (sodium), ALB (albumin)]. Additionally, the patient's LMR (lymphocyte/monocyte ratio), NLR (neutrophil/lymphocyte ratio), SII (platelet * neutrophil/lymphocyte count), PLR (platelet/lymphocyte ratio), SIS (inflammation score, a comprehensive score of ALB and LMR) (19), as well as AIS-APS score, A2DS2 score, ISAN score, and PANTHERIS score will be calculated based on the collected information. The laboratory indicators obtained by the patient within

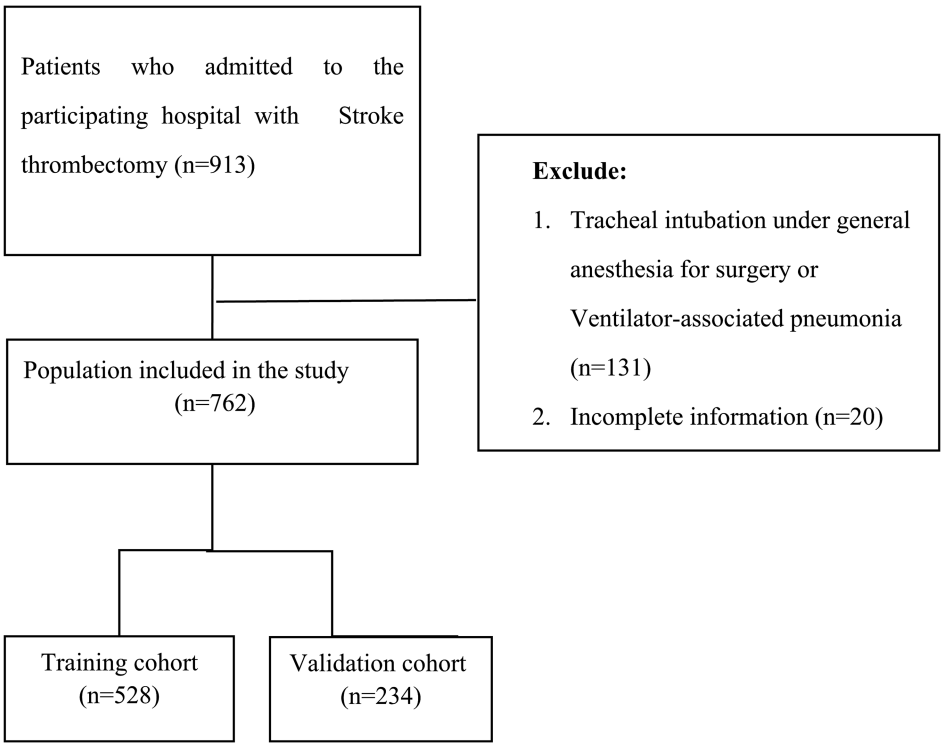

FIGURE 1
Study flow diagram.

24 h before surgery are used as preoperative indicators. The laboratory results 24 h after surgery are used as postoperative indicators.

Definition and indicators of pneumonia

The improved diagnostic criteria for stroke-associated pneumonia are based on the Centers for Disease Control and Prevention (CDC) guidelines (20). (1) Stroke-associated pneumonia refers to lower respiratory tract infections that occur within the first week (7 days) after a stroke in terms of onset time. (2) Diagnosis must comply with the modified SAP standards proposed by the US CDC, which include two levels: suspected SAP (compliance with CDC standards without typical chest X-ray changes even in repeated or continuous examinations) and confirmed SAP (compliance with CDC standards, including typical chest X-ray changes). (3) The diagnostic value of white blood cell count or C-reactive protein (CRP) for stroke-associated pneumonia is limited. (4) There is not enough evidence to suggest that other biomarkers, such as procalcitonin (PCT), are meaningful for the diagnosis of stroke-associated pneumonia. According to the standards of the CDC in the United States, patients are classified into SAP group and non-SAP group based on the occurrence of stroke-associated pneumonia.

Study procedure

The data collected and organized were analyzed using LASSO regression to determine the factors that influence stroke-associated pneumonia in patients undergoing acute ischemic stroke thrombectomy. Subsequently, stepwise regression analysis was performed to obtain a multivariate logistic regression model. The statistically significant factors ($p < 0.05$) identified in the model included NIHSS score ≥ 16, postoperative LMR, and dysphagia. These factors were included in the column chart to establish the model. After constructing the model, internal validation was conducted using the training set, and external validation was carried out using the validation set. The discriminability and calibration of the column chart were evaluated using ROC curves and calibration curves of the training and validation sets. Furthermore, the sensitivity and specificity of the new model in assessing the risk of stroke-associated pneumonia in patients undergoing acute ischemic stroke thrombectomy were compared with those of the AIS-APS, A2DS2 score, ISAN score, and PANTHERIS score using ROC curves. The aim of this analysis was to create a reliable model that effectively predicts the occurrence of stroke-associated pneumonia in patients undergoing acute ischemic stroke thrombectomy.

Statistical method

This study utilized the "glmnet" software package for performing LASSO regression analysis to identify the factors influencing stroke-associated pneumonia in acute ischemic stroke patients undergoing thrombectomy. Subsequently, the "glm" software package was employed to determine the significant risk factors through multiple logistic regression. A nomogram was constructed using the "rms" package in R software to represent the risk of stroke-associated

pneumonia in these patients. The model was validated using 500 resampled bootstrap methods and the ROC curve and calibration curve were drawn using the "fbroc" and "rms" packages in R software. The obtained results were then compared with AIS-APS, A2DS2 score, ISAN score, and PANTHERIS score. Moreover, the practical clinical value of the model was evaluated by drawing a clinical decision curve using the "rmda" package.

Results

This study included a total of 762 patients, with an incidence rate of 44% in the training set and 50% in the validation set. There was no statistically significant difference between the two groups ($p > 0.05$). The table indicated statistically significant differences ($p < 0.05$) in Thrombolysis, D-dimer, FBG, postoperative NLR, and postoperative SII, between the two patient groups, while no other data showed significant statistical differences ($p > 0.05$). Please refer to Table 1 for further details.

The influencing factors were identified through LASSO regression analysis, as depicted in Figure 2. The results of the multiple logistic regression analysis demonstrated that an NIHSS score of ≥16 points (OR 2.11; 95% CI 1.40–3.19; $p < 0.001$), postoperative LMR (OR 0.74; 95% CI 0.65–0.83; $p < 0.001$), and dysphagia (OR 5.14; 95% CI 3.452–7.73; $p < 0.001$) were found to be statistically significant risk factors for SAP, as demonstrated in Figure 3.

A predictive model for the risk of associated pneumonia in patients undergoing thrombectomy for acute ischemic stroke was established by creating a column chart based on NIHSS scores ≥16, postoperative LMR, and dysphagia. The chart shows the scores and risks of different risk factors in various line segments, where each risk factor corresponds to different risk scores. The total score reflects the overall risk of pneumonia occurrence, with higher scores indicating a higher likelihood of pneumonia, as illustrated in Figure 3.

The predictive model developed in this study aimed to assess the risk of associated pneumonia in patients who underwent thrombectomy for acute ischemic stroke. The area under the receiver operating characteristic (ROC) curve was 0.792 (95% CI 0.754–0.83) for the training set and 0.769 (95% CI 0.708–0.829) for the validation set, indicating good discriminative ability. In the calibration chart, the red line represents the correlation between actual and predicted values. The diagonal dashed line represents a perfect prediction, and the cross feature indicates the deviation between the predicted and actual values. The Hosmer-Lemeshow goodness-of-fit test revealed X-squared values of 6.0678 ($p = 0.6396$) for the training set and 12.008 ($p = 0.1509$) for the validation set, suggesting a good fit as the $p > 0.05$ indicated. The calibration curves for both the training and validation sets closely resemble the standard curve, indicating strong discrimination, calibration, and predictive value, as shown in Figure 4.

The clinical decision curve and column chart demonstrate high net benefits in both the training and validation sets. Within the threshold probabilities ranging from 7 to 90%, the column chart outperforms the "treat-all" or "no-treatment" strategies, resulting in increased net benefits, as illustrated in Figure 5.

Further predictions of the incidence of associated pneumonia in patients with acute ischemic stroke thrombectomy were performed using other scoring methods. The AIS-APS scoring method had AUCs of 0.698 (95% CI 0.654–0.742) and 0.636 (95% CI 0.563–0.709) for the

TABLE 1 Baseline characteristics of AIS patients in training and validation cohort.

Variables	Validation cohort $n = 229$	Training cohort $n = 533$	p-value
Stroke associated pneumonia			0.826
N	126 (55.0%)	287 (53.8%)	
Y	103 (45.0%)	246 (46.2%)	
Demographic characteristics			
Gender			0.916
Female	88 (38.4%)	201 (37.7%)	
Male	141 (61.6%)	332 (62.3%)	
Age			0.218
<70	105 (45.9%)	272 (51.0%)	
≥70	124 (54.1%)	261 (49.0%)	
Comorbidities and disease history			
History of hypertension			0.778
N	93 (40.6%)	209 (39.2%)	
Y	136 (59.4%)	324 (60.8%)	
History of diabetes			0.832
N	184 (80.3%)	423 (79.4%)	
Y	45 (19.7%)	110 (20.6%)	
History of smoking			0.422
N	163 (71.2%)	396 (74.3%)	
Y	66 (28.8%)	137 (25.7%)	
History of COPD			0.636
N	225 (98.3%)	519 (97.4%)	
Y	4 (1.75%)	14 (2.63%)	
History of cerebral infarction			0.005
N	201 (87.8%)	420 (78.8%)	
Y	28 (12.2%)	113(21.2%)	
History of Atrial Fibrillation			0.797
N	160 (69.9%)	379 (71.1%)	
Y	69 (30.1%)	154 (28.9%)	
History of heart failure			1.000
N	217 (94.8%)	506 (94.9%)	
Y	12 (5.24%)	27 (5.07%)	
History of Myocardial infarction			0.123
N	228 (99.6%)	521 (97.7%)	
Y	1 (0.44%)	12 (2.25%)	
Clinical parameters			
Thrombolysis			0.035
N	157 (68.6%)	406 (76.2%)	
Y	72 (31.4%)	127 (23.8%)	
Blockage of blood vessels			0.742
Post-loop	39 (17.0%)	84 (15.8%)	

(Continued)

TABLE 1 (Continued)

Variables	Validation cohort $n = 229$	Training cohort $n = 533$	p-value
Pre-cycle	190 (83.0%)	449 (84.2%)	
Difficulty swallowing			0.625
N	101 (44.1%)	247 (46.3%)	
Y	128 (55.9%)	286 (53.7%)	
Hyperperfusion (bleeding or oozing)			0.161
N	177 (77.3%)	437 (82.0%)	
Y	52 (22.7%)	96 (18.0%)	
GCS			0.978
<8	40 (17.5%)	91 (17.1%)	
≥8	189 (82.5%)	442 (82.9%)	
NIHSS			0.837
<16	144 (62.9%)	341 (64.0%)	
≥16	85 (37.1%)	192 (36.0%)	
Time of onset (hour, median [IQR])	4.00 [3.00;7.00]	4.50 [3.00;8.00]	0.236
Body temperature (°, median [IQR])	36.6 [36.5;36.8]	36.6 [36.5;36.8]	0.299
Preoperative Laboratory parameters			
Neu (10^9/L, median [IQR])	5.90 [4.30;8.10]	6.00 [4.40;8.20]	0.799
HB (g/L, median [IQR])	137 [125;147]	136 [123;149]	0.923
PLT (10^9/L, median [IQR])	206 [172;247]	202 [162;245]	0.409
Mono (10^9/L, median [IQR])	0.40 [0.30;0.60]	0.40 [0.30;0.60]	0.376
Lym (10^9/L, median [IQR])	3.50 [2.50;5.00]	1.40 [1.00;2.00]	0.234
LMR (median [IQR])	3.50 [2.42;4.96]	3.40 [2.33;4.83]	0.611
NLR (median [IQR])	3.93 [2.41;7.44]	4.19 [2.47;7.12]	0.507
SII (median [IQR])	782 [466;1,460]	816 [476;1,462]	0.618
PLR (median [IQR])	134 [91.8;201]	140 [100;197]	0.556
SIS (median [IQR])			0.480
0	23 (10.0%)	66 (12.4%)	
1	89 (38.9%)	217 (40.7%)	
2	117 (51.1%)	250 (46.9%)	
FBG (mmol/L,median [IQR])	7.64 [6.49;9.86]	7.12 [6.17;9.04]	0.008
ALB (g/L, median [IQR])	37.4 [34.1;40.2]	37.6 [34.6;41.3]	0.138
CL (mmol/L,median [IQR])	105 [102;107]	105 [102;107]	0.699
Na (mmol/L,median [IQR])	138 [137;140]	139 [137;140]	0.391
PT (s, median [IQR])	13.2 [12.6;13.8]	13.1 [12.6;13.7]	0.350
INR (median [IQR])	1.03 [0.96;1.08]	1.01 [0.96;1.07]	0.315
APTT (s, median [IQR])	33.9 [31.5;36.6]	33.8 [30.9;37.2]	0.813
Fib (g/L, median [IQR])	3.24 [2.68;3.78]	3.19 [2.72;3.78]	0.987
TT (s,median [IQR])	17.8 [17.0;18.8]	17.7 [16.9;18.9]	0.449
D-dimer (mg/L, median [IQR])	1.42 [0.73;2.38]	1.19 [0.57;2.32]	0.016
Postoperatively laboratory parameters			
WBC (10^9/L, median [IQR])	9.10 [7.30;11.1]	8.70 [7.10;10.5]	0.082
Neu (10^9/L, median [IQR])	7.50 [5.60;9.50]	7.00 [5.40;8.80]	0.082
HB (g/L, median [IQR])	125 [113;136]	124 [113;135]	0.670
PLT (10^9/L, median [IQR])	195 [163;229]	191 [155;230]	0.291
Mono (10^9/L, median [IQR])	0.40 [0.30;0.60]	0.43 [0.30;0.60]	0.508
Lym (10^9/L, median [IQR])	1.10 [0.80;1.50]	1.20 [0.80;1.60]	0.138
LMR (median [IQR])	2.50 [1.67;3.86]	2.50 [1.71;3.80]	0.922
NLR (median [IQR])	6.50 [4.03;11.0]	5.92 [3.90;9.22]	0.049
SII (median [IQR])	1,222 [787;2,112]	1,119 [729;1743]	0.039
PLR (median [IQR])	177 [124;241]	164 [122;229]	0.110
SIS (median [IQR])			0.375
0	11 (4.80%)	38 (7.13%)	
1	81 (35.4%)	198 (37.1%)	
2	137 (59.8%)	297 (55.7%)	

AIS, Stroke associated pneumonia; COPD, chronic obstructive pulmonary disease; GCS, Glasgow Coma Scale; NIHSS, National Institutes of Health Stroke Scale; Neu, neutrophil count; HB, hemoglobin; PLT, platelet; Mono, mononuclear leucocyte; Lym, lymphocyte count; LMR, lymphocyte/monocyte ratio; NLR, neutrophil/lymphocyte ratio; SII, platelet*neutrophil/lymphocyte count; PLR, platelet/lymphocyte ratio; SIS, inflammation score, a comprehensive score of ALB and LMR; FBG, fasting blood glucose; ALB, albumin; CL, chloride; Na, sodium; PT, prothronmbin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; Fib, fibrinogen; TT, thrombin time; WBC, white blood cell.

training and validation sets, respectively. For the A2DS2 scoring method, the AUCs were 0.757 (95% CI 717–0.798) for the training set and 0.743 (95% CI 0.68–0.807) for the validation set. The ISAN scoring method had AUCs of 0.684 (95% CI 0.62–0.749) and 0.672 (95% CI 0.602–0.741) for the training and validation sets, respectively. The AUCs for the PANTHERIS scoring method were 0.624 (95% CI 0.577–0.671) for the training set and 0.652 (95% CI 0.58–0.724) for the validation set, as shown in Figure 5. The discrimination of this model is higher compared to the AIS-APS, A2DS2 score, ISAN score, and PANTHERIS score.

Discussion

Stroke-associated pneumonia is a common complication in clinical practice following a stroke (19). Previous research reports have primarily focused on patients with cerebral hemorrhage or cerebral ischemia, without specifically screening out patients who underwent thrombus removal (16, 21). In a study by Yan et al., it was discovered that the probability of stroke-associated pneumonia occurring in patients with cerebral hemorrhage is 25.52% (10). Furthermore, the probability of pneumonia occurring in high-risk

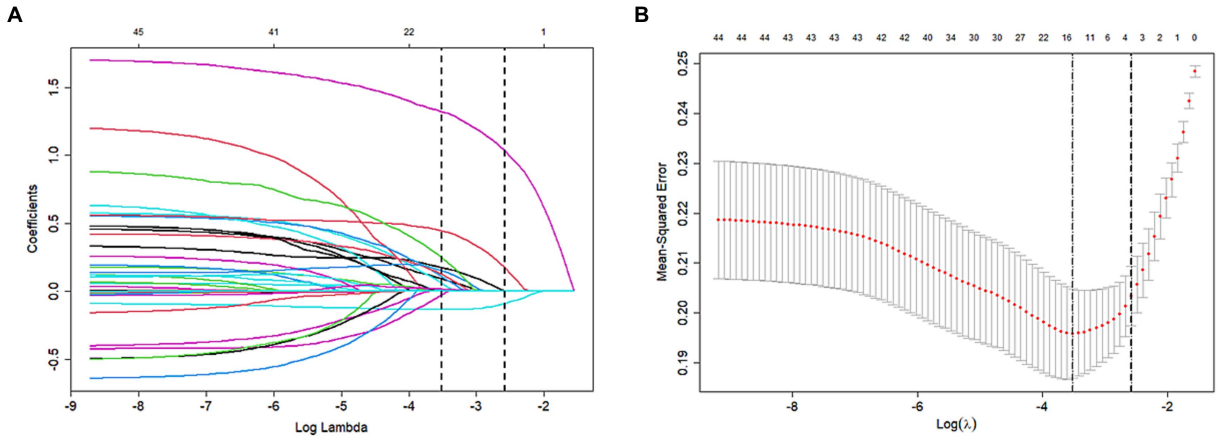

FIGURE 2
Variable selection by the LASSO binary logistic regression model. **(A)** LASSO coefficient profile of the clinical features. **(B)** The optimal penalization coefficient lambda was generated in the LASSO via tenfold cross-validation. We plotted the partial likelihood deviance (binomial deviance) curve versus log (lambda) and drew dotted vertical lines based on 1 standard error criteria. LASSO Least absolute shrinkage and selection operator.

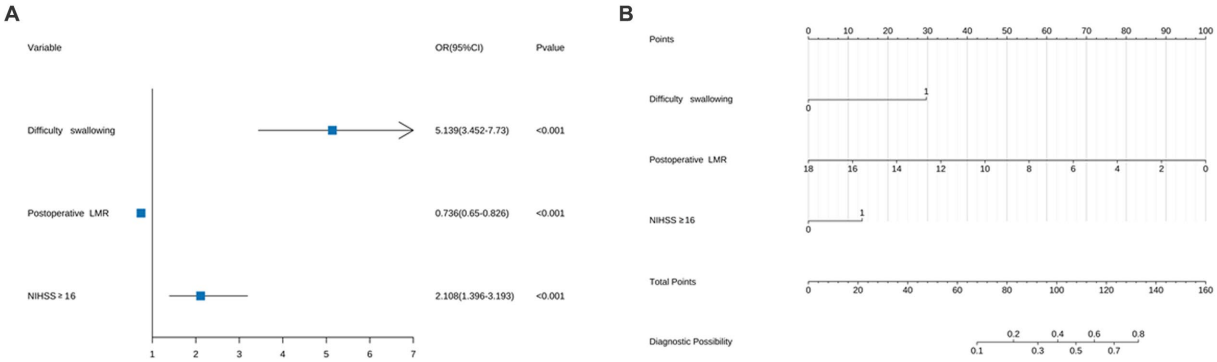

FIGURE 3
(A) Forest map of pneumonia risk in patients with acute ischemic stroke thrombectomy and **(B)** column chart of pneumonia risk in patients with acute ischemic stroke thrombectomy. For all patients, add up the scores of the three indicators, and the total score is located on the "Total Points" axis, which corresponds to the risk of SAP in patients with acute ischemic stroke thrombectomy.

populations can be as high as 40% (21). In a cohort study conducted in England and Wales, patients with ischemic or hemorrhagic stroke admitted to the stroke unit were included. It was found that the median prevalence of stroke-associated pneumonia (SAP) was 8.5%, with the highest recorded prevalence being 21.4% (22). The data from this study suggests that the incidence of stroke-related pneumonia in patients after thrombectomy is 45.8%, which is higher than the incidence reported in other research studies. This discrepancy may be attributed to the fact that our sample consisted of patients who underwent thrombectomy for acute ischemic stroke. Following thrombectomy, there is a risk of brain tissue ischemia–reperfusion injury, as well as stress-induced immune responses triggered by surgery and contrast agents. In a separate study, a rare complication of contrast agent encephalopathy was observed after carotid artery stent surgery (23). The underlying pathogenesis of this complication is currently unclear, but it may be associated with chemical toxicity and brain edema caused by the passage of contrast agents through the blood–brain barrier (23). All of these factors have the potential to alter the patient's immune and inflammatory response, thus promoting the occurrence of SAP.

Acute ischemic stroke-associated pneumonia was initially proposed by Professor Hilker et al. (24). When patients experience acute ischemic stroke, it can result in immune suppression, and the incidence of aspiration may also contribute to the development of SAP. When brain tissue ischemia occurs, it can trigger a series of immune inflammatory reactions, leading to the production of toxic substances, such as inflammatory cytokines, chemokines, and reactive oxygen species. These substances can disrupt the blood–brain barrier. They can rapidly activate immune inflammatory cells, affecting their entry into the ischemic area and triggering immune responses that further damage neurons (25). In clinical practice, the primary diagnostic indicators for SAP include WBC, Neu, PCT, CRP, and other markers. Various studies have demonstrated that peripheral blood inflammatory markers, such as LMR, NLR, and SII, are closely associated with stroke-associated pneumonia, providing a scientific and theoretical foundation for the diagnosis and prediction of this

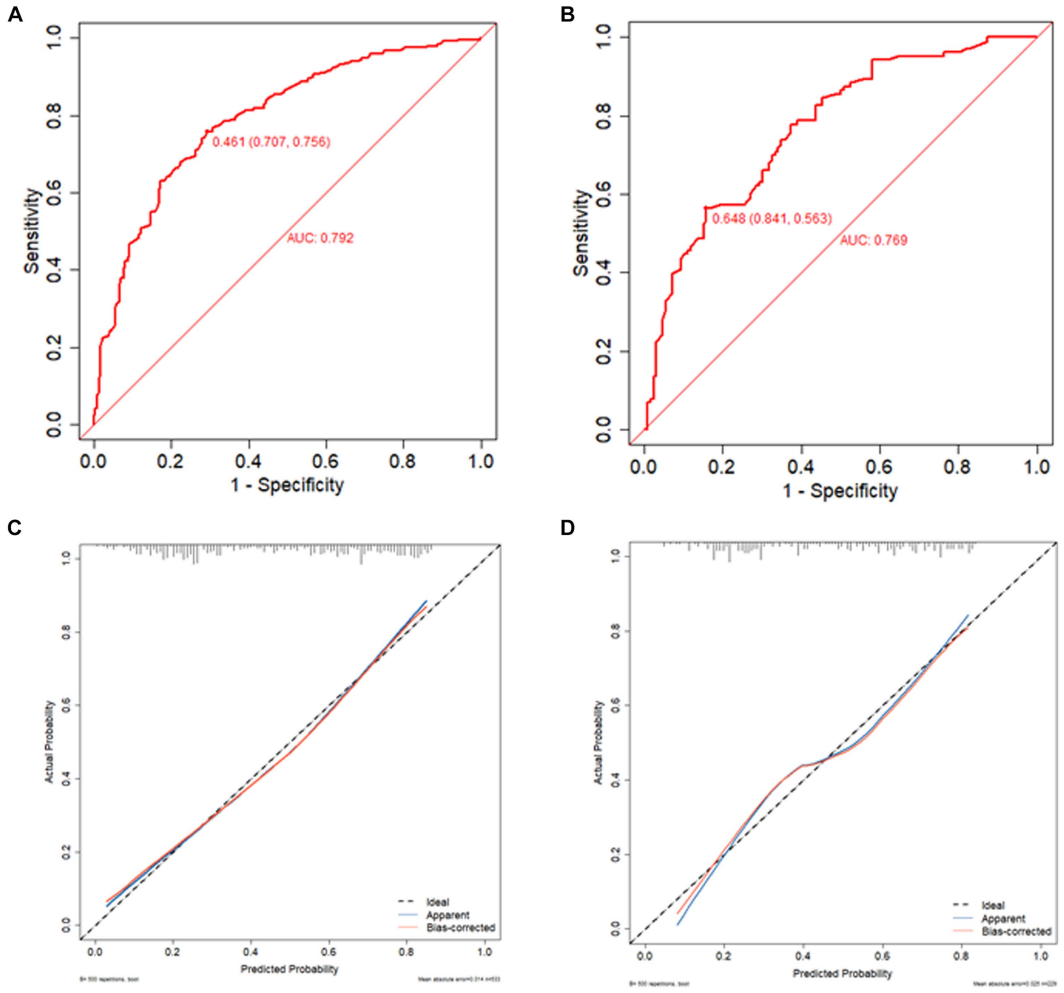

FIGURE 4
ROC curve of training set **(A)**, ROC curve of validation set **(B)**, calibration curve of training set **(C)**, and calibration curve of validation set **(D)**. The red line indicates the correlation between actual and predicted values. The diagonal dashed line represents the most perfect prediction, and the cross feature represents the correction between the predicted value and the actual value. $p > 0.05$, good fit.

condition (26). For example, Nam et al. discovered that NLR is correlated with the severity of SAP (27). LMR reflects the immune response of the body, and previous studies have mostly demonstrated it to be one of the prognostic factors for many cancers (28). In this study, it was found that LMR is one of the influencing factors in the development of SAP in patients. Therefore, in clinical practice, the dynamic monitoring of LMR can be used for early prevention, ultimately reducing the incidence of stroke-associated pneumonia.

Meanwhile, the degree of impairment in consciousness of stroke patients is closely related to the occurrence of stroke-associated pneumonia (29). In the acute phase of ischemic stroke, patients are susceptible to neurological and other complications. The NIHSS scoring system is a commonly utilized scale for assessing the severity of neurological damage in patients (30). The score covers various aspects, including the patient's level of consciousness, gaze, visual acuity, facial paralysis, upper and lower limb movement, limb ataxia, sensation, language, articulation disorders, and neglect. A higher NIHSS score indicates more severe nerve damage. When consciousness disorders worsen, it can inhibit the cough reflex, leading to weakened respiratory movement or even ventilator paralysis. This can result in poor drainage of respiratory secretions and lead to pneumonia. Additionally, studies have shown that while 45% of normal individuals can experience trace inhalation of oral secretions while falling asleep, the probability of this occurring increases to 90% in patients with consciousness disorders. In their research, Chumbler et al. found that the NIHSS score has high predictive significance for the occurrence of stroke-associated pneumonia (31). In this study, swallowing difficulties were identified as a risk factor. Stroke-induced damage to the swallowing center of patients disrupts the control ability of the pharyngeal muscles and nerves, resulting in swallowing dysfunction. This dysfunction increases the likelihood of foreign objects entering the airways and bronchi, thereby increasing the probability of pneumonia occurrence (32). Evaluating the patient's swallowing function, providing effective oral care, and conducting early rehabilitation exercises are measures that can reduce the risk of associated pneumonia, as shown by prior research (33).

Nonetheless, there are still limitations to this study as the collected data is limited to our hospital and has not yet been clinically validated by external hospitals. Additionally, our prediction model is built based on the clinical indicators we can collect, and it is not ruled out that

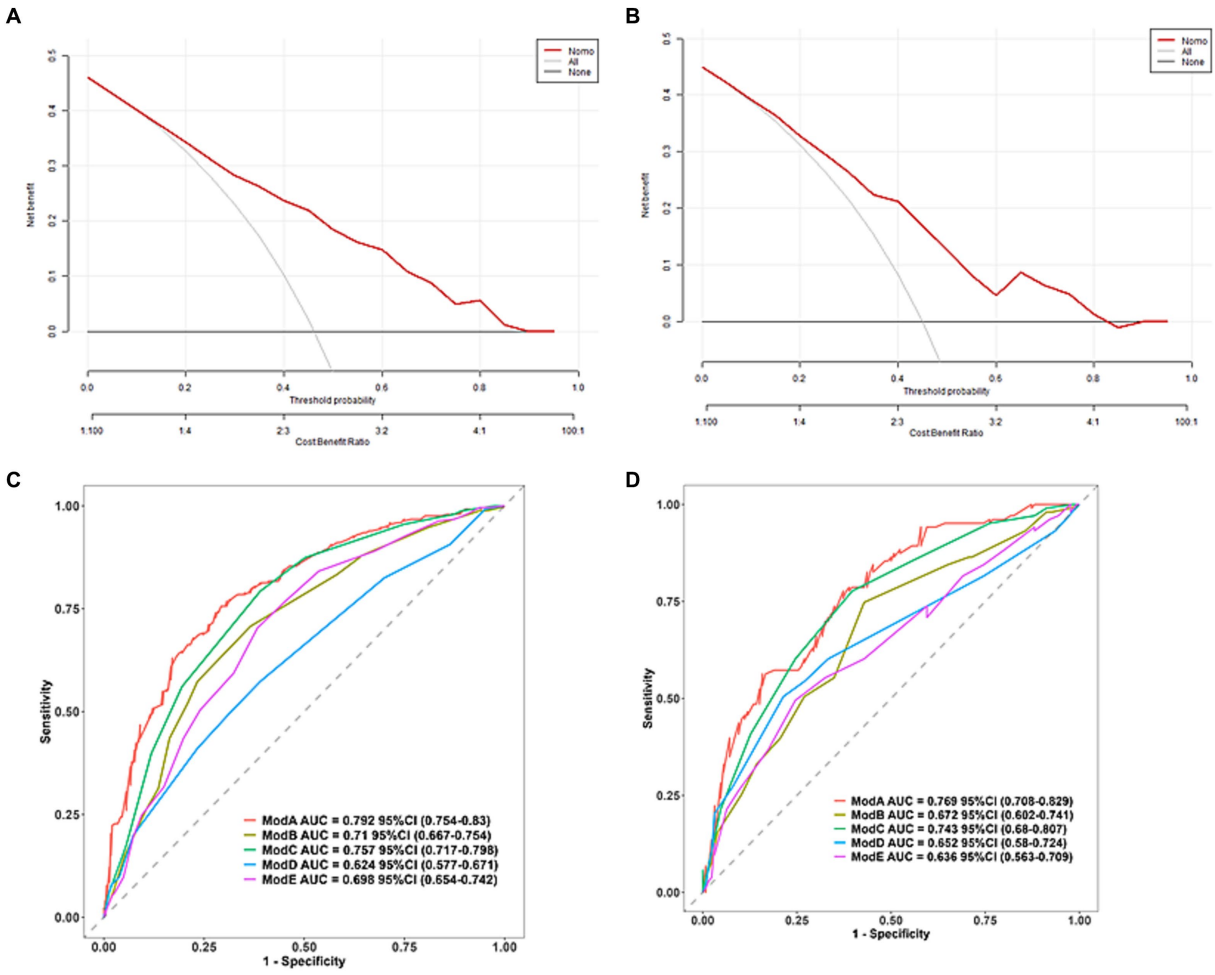

FIGURE 5
Clinical decision diagrams for training set **(A)** and validation set **(B)**. The net benefit is calculated by adding true positives and subtracting false positives. For cases where the threshold probability is greater than 7%, applying a column chart will increase net benefits compared to treating all strategies or not treating strategies. **(C)** Predicts the training set ROC curve of SAP using different scoring systems and **(D)** predicts the validation set ROC curve of SAP using different scoring systems (Model A: column chart, Model B: ISAN score, Model C: A2DS2, Model D: PANTHERIS, Model E: AIS-APS).

there are better clinical indicators to help us predict SAP. The heterogeneity of the study population should be acknowledged so that future work is needed to explore how subgroups of patients can have different results/conclusions (34). What's more, preventing SAP and advancing the treatment window for stroke-associated pneumonia to the ultra-early stages of stroke occurrence are important issues that require further exploration and study.

In conclusion, the nomogram chart that we constructed based on NIHSS≥16, postoperative LMR, and dysphagia as three influencing factors, has shown good discrimination and calibration in predicting the risk of associated pneumonia in patients with acute ischemic stroke thrombectomy. It is noteworthy that the predictive accuracy of this chart surpasses that of the AIS-APS, A2DS2 score, ISAN score, and PANTHERIS score.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Taizhou Enze Medical Center (Group) (Ethics Number: K20201104). Written informed consent was not required as per local legislation and institutional requirements.

Author contributions

JYW: Writing – original draft, Data curation. CY: Data curation, Writing – original draft. RHZ: Data curation, Writing – original draft. WH: Writing – review & editing, Validation. PY: Formal analysis, Supervision, Writing – original draft. YQJ: Investigation, Visualization, Writing – original draft. WJH: Methodology, Writing – review & editing. RFS: Writing – original draft, Project administration, Supervision. YPJ: Funding acquisition, Writing – review & editing, Conceptualization, Writing – original draft.

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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.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1370986/full#supplementary-material

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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Omer Iqbal,
Loyola University Chicago, United States
Ekaterina Baryshnikova,
IRCCS San Donato Polyclinic, Italy

*CORRESPONDENCE
Jingchun Song
songjingchun@126.com

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Time to maximum amplitude of thromboelastography can predict mortality in patients with severe COVID-19: a retrospective observational study

Lincui Zhong[1], Qingwei Lin[1], Longping He[1], Dongmei Liu[2,3], Lin Zhu[2,4], Qingbo Zeng[1] and Jingchun Song[1,2]*

[1]Intensive Care Unit, The 908th Hospital of Chinese PLA Logistic Support Force, Nanchang, Jiangxi, China, [2]Intensive Care Unit, Huoshenshan Hospital, Wuhan, Hubei, China, [3]Intensive Care Unit, The 940th Hospital of Chinese PLA Logistic Support Force, Lanzhou, Gansu, China, [4]Department of Critical Care Medicine, The 944th Hospital of Chinese PLA Logistic Support Force, Jiuquan, Gansu, China

Objective: To predict mortality in severe patients with COVID-19 at admission to the intensive care unit (ICU) using thromboelastography (TEG).

Methods: This retrospective, two-center, observational study involved 87 patients with PCR-and chest CT-confirmed severe COVID-19 who were admitted to at Wuhan Huoshenshan Hospital and the 908th Hospital of Chinese PLA Logistic Support Force between February 2020 and February 2023. Clinic demographics, laboratory results, and outcomes were compared between those who survived and those who died during hospitalization.

Results: Thromboelastography showed that of the 87 patients, 14 were in a hypercoagulable state, 25 were in a hypocoagulable state, and 48 were normal, based on the time to maximum amplitude (TMA). Patients who died showed significantly lower α angle, but significantly longer R-time, K-time and TMA than patients who survived. Random forest selection showed that K-time, TMA, prothrombin time (PT), international normalized ratio (INR), D-dimer, C-reactive protein (CRP), aspartate aminotransferase (AST), and total bilirubin (Tbil) were significant predictors. Multivariate logistic regression identified that TMA and CRP were independently associated with mortality. TMA had a greater predictive power than CRP levels based on time-dependent AUCs. Patients with TMA ≥ 26.4 min were at significantly higher risk of mortality (hazard ratio 3.99, 95% Confidence Interval, 1.92–8.27, $p < 0.01$).

Conclusion: TMA ≥26.4 min at admission to ICU may be an independent predictor of in-hospital mortality for patients with severe COVID-19.

KEYWORDS

thromboelastography, coronavirus, critical illness, time to maximum amplitude, mortality

Introduction

The world continues to experience outbreaks of novel coronavirus disease-2019 (COVID-19) (1), for which the overall case-fatality rate may be around 2.3%, increasing to 49–62% among critical cases (2, 3). One of the major causes of death among COVID-19 patients admitted to the intensive care unit is severe coagulation dysfunction (4). Patients who die in hospital tend to show higher levels of D-dimer and fibrin degradation product (FDP) on admission than those who survive hospitalization (5). In fact, a high incidence of arterial and venous thrombotic complications in COVID-19 patients including pulmonary embolism (PE), deep-vein thrombosis (DVT), ischemic stroke, myocardial infarction, or systemic arterial embolism gave rise to increased mortality (6–9). Most seriously, more than 70% of severe COVID-19 patients who developed disseminated intravascular coagulation (DIC) died in hospital (10).

While routine laboratory assays of plasma can provide valuable information about the hemostasis of patients with COVID-19, they have significant limitations in quantifying hypercoagulable states and evaluating cellular contribution to hemostasis by platelets and red blood cells (RBC) (11, 12). TEG may provide more comprehensive analysis of hemostatic capacity, because it can assess the coagulation function of cells and individual proteins (13, 14). Two coagulation states of sepsis-induced DIC were identified by TEG tracings: a hypercoagulable state of early DIC and a hypocoagulable state of late DIC, which provided evidence for further anticoagulation or alternative therapy (15, 16). Unfortunately, prognostic implications of TEG parameters in COVID-19 patients have not been reported. We compared the findings between patients who survived or died during hospitalization, and we assessed whether TEG parameters may help identify patients at higher risk of mortality in the ICUs.

Methods

Participants

This study retrospectively analyzed 87 patients with severe COVID-19 who were admitted to the ICU of Wuhan Huoshenshan Hospital (Wuhan, China) and the 908th Hospital of Chinese PLA Logistic Support (Nanchang, China) between February 2020 and February 2023. All 87 patients were diagnosed with COVID-19 based on World Health Organization interim guidance (17) and detection of SARS-CoV-2 RNA in the hospital's clinical laboratory. In addition, all patients were defined as having severe disease because they were admitted to the ICU and required mechanical ventilation or they experienced shock or organ failure (18). We excluded patients with congenital coagulation disorders, or pre-existing liver failure, as well as those who were taking systemic anticoagulation or antiplatelet medications.

Data collection

Data were collected on age, sex, in-hospital survival, and the following laboratory results on admission: complete blood count, analyzed using an XE-2100 Hematology Analyzer (Sysmex, Japan); blood chemistry, analyzed on an AU5800 system (Beckman Coulter, United States); coagulation status, analyzed using the CS5100 system (Sysmex, Japan); and thromboelastography after kaolin-activation, performed using an LEPU-8800 system (LEPU, China).

Patients were analyzed by TEG, and the following indices were determined (19–21): reaction time (R time), defined as the interval from when the blood was placed in the analyzer until the start of fibrin formation; kinetic time (K time), defined as the time to reach a certain level of clot strength; α angle, which reflects the level of fibrinogen; maximum amplitude (MA), which reflects platelet function; clot lysis 30 min following maximal amplitude (LY30), which reflects clot lysis; TMA, defined as the time needed to form a stable clot. Patients were classified as being in a hypercoagulable, normal, or hypocoagulable state based on whether TMA was, respectively, < 23, 23–33, or > 33 min (22, 23).

Statistical analysis

Data were reported as mean ± standard deviation, median (interquartile range), or *n* (%), as appropriate. Data were analyzed using R 4.2.1 (R Core Team, Vienna, Austria), and differences associated with $p < 0.05$ were considered significant. Continuous data were compared between survivors and non-survivors using Student's *t* test if data were normally distributed, or using the Mann–Whitney U test if the data were skewed. Categorical data were compared using the chi-squared test. Random Forest and correlation matrix were applied to select features. The ability of selected features to predict mortality was assessed using logistic regression. The ability of TMA and CRP to predict mortality in our sample was further assessed by the area assessed by the time-dependent receiver operator characteristic curve (AUC). Survival of patients below or above the optimal TMA cut-off value was compared using Kaplan–Meier curves and the log-rank test. Risk of mortality was expressed in terms of a hazard ratio (HR) and corresponding 95% confidence interval (95% CI).

Results

Of the 104 patients initially assessed for enrollment, one hemophilia B patient, three patients with cirrhosis, five patients receiving rivaroxaban and eight patients receiving aspirin were excluded. Finally, the final analysis included 87 patients with severe COVID-19, who were 73 (63, 89) years old and were predominantly male ($n = 55$). By the end of February, 2023, 56 (64.4%) patients had been discharged and 31 (35.6%) patients had died.

While patients who died showed similar age and gender as those who survived, those who died showed significantly greater inflammation on admission, based on counts of white blood cells count and neutrophils as well as the level of C-reactive protein (Table 1). Based on aspartate aminotransferase and serum creatinine levels, liver and kidney function was significantly worse on admission to non-survivors compared with survivors (Table 1). They also showed significantly lower platelet count, longer activated partial thromboplastin time and prothrombin time, and higher D-dimer levels, indicating worse coagulation function compared to survivors on admission.

Based on thromboelastography, 14 patients were in a hypercoagulable state and 25 were in a hypocoagulable state on admission. Patients who died during the study period showed lower

α angle, as well as longer R-time, K-time, MA, and TMA than those who survived (Table 2). The random forest is an important feature selection method, which is frequently applied to select key variables or features. In this study, there were 24 clinical variables which were selected for further analysis. Random forest selection showed eight variables were significant predictors: K, TMA, PT, INR, DD, CRP, AST, and Tbil (Figure 1). The indices were used for correlation matrix analysis, which showed high correlation coefficients for PT, INR, and DD (Figure 2). PT, INR, and DD were not included in the logistic regression analysis because of multicollinearity problems. Tbil was considered from a clinical specialty perspective and was also not included in the logistic regression analysis. The remaining four significant variables were directly analyzed by multivariate logistic regression, and the results identified that TMA and CRP were independently associated with mortality (Table 3). Figure 3 showed AUCs for TMA and CRP using time-dependent ROC analysis. TMA had greater predictive power than the CRP for in-hospital mortality based on time-dependent AUCs. Survival analysis was applied to investigate the impact of TMA on mortality. Stratifying patients using this cut-off showed that those with TMA ≥ 26.4 min were the significant predictor of subsequent deaths (Log-rank = 13.84, $p < 0.001$, HR = 3.99, 95%CI 1.92–8.27). Notably, TMA was significantly related to in-hospital mortality and the median survival time reached 27 days (Figure 4).

Discussion

To our knowledge, this is the first assessment of whether thromboelastographic parameters can be used to identify patients

TABLE 1 Characteristics of severe COVID-19 patients at admission.

Variable	Normal range	All (*n* = 87)	Survivors (*n* = 56)	Non-survivors (*n* = 31)	*p*
Age (years)		73 (63, 89)	73.5 (65, 89)	72 (60, 87.5)	0.598
Male/female		55/32	36/20	19/12	0.964
White blood cells (×10⁹/L)	3.5–9.5	8.6 (6.4, 13.6)	7.7 (6.2, 12.5)	10.3 (7.9, 16.1)	0.03
Neutrophils (×10⁹/L)	2.5–7.5	7.8 (5.1, 11.4)	6.3 (4.9, 10.8)	9.7 (6.8, 14.9)	0.045
Lymphocytes (×10⁹/L)	0.8–4	0.8 (0.5, 1.1)	0.8 (0.5, 1.1)	0.6 (0.4, 1.1)	0.373
Hemoglobin (g/L)	115–150	93.6 ± 21.2	93 ± 22.8	94.6 ± 18.2	0.72
CRP (mg/L)	0–4	65.4 (26.6, 103.3)	55.2 (17.6, 81.4)	103.3 (44.1, 144.4)	< 0.001
TBil (μmoI/L)	0–21	12.0 (7.4–23.4)	11.2 (7.0–17.5)	13.5 (10.7–45.7)	0.072
AST (U/L)	7–45	32.4 (23.2, 84.3)	29 (22.6, 47.4)	63.9 (28.9, 170.1)	0.006
ALT (U/L)	7–40	27.8 (17.7, 50.9)	23.6 (17.2, 40.4)	37.9 (20.4, 93)	0.039
SCr (μmoI/L)	41–81	75.4 (53.8, 156.1)	67.1 (48.7, 104.8)	100.8 (70.2, 179.3)	0.02
PLT (×10⁹/L)	125–350	172 (72.5, 235.5)	195.5 (105, 247.8)	120 (64, 194)	0.048
APTT (s)	21–37	32.7 (29.6, 38.3)	31.7 (28.7, 35.7)	37.2 (31.5, 42.9)	0.002
PT (s)	9.2–15	14.2 (12.8, 15.9)	13.9 (12.7, 15)	15.3 (13.6, 20)	0.002
INR	0.8–1.25	1.2 (1.1, 1.3)	1.1 (1.1, 1.2)	1.3 (1.1, 1.7)	0.002
FIB (g/L)	2–4	4.9 (3.8, 13.5)	4.8 (3.8, 8.5)	5.3 (4.1, 13.9)	0.28
TT (s)	10–20	15.1 (4.6, 16.3)	14.9 (10.8, 16)	15.2 (4.1, 17.5)	0.556
D-Dimer (μg/mL)	0–0.55	2.3 (0.8, 5)	1 (0.4, 3)	5.3 (2.7, 16)	<0.001

Values are *n*, mean ± SD, or median (interquartile range), unless otherwise noted. ALT, Alanine aminotransferase; APTT, Activated partial thromboplastin time; AST, Aspartate aminotransferase; CRP, C-reactive protein; FIB, Fibrinogen; HGB, Hemoglobin concentration; INR, International normalized ratio; PLT, Platelet count; PT, Prothrombin time; SCr, Serum creatinine; TBil, Total bilirubin; TT, Thrombin time.

TABLE 2 Thromboelastography parameters of severe COVID-19 patients at admission.

Parameter	Normal range	All (*n* = 87)	Survivors (*n* = 56)	Non-survivors (*n* = 31)	*p*
R-time (min)	5–10	7.2 (5.8, 10.5)	6.8 (5.5, 8.7)	9.4 (6.4, 12.3)	0.014
K-time (min)	1–3	1.8 (1.3, 4)	1.6 (1.2, 2.3)	3.1 (1.8, 5.2)	< 0.001
α angle (°)	53–72	60.5 (42.3, 69.8)	63.9 (53.4, 71.2)	50.3 (38.7, 66.2)	0.011
MA (mm)	50–70	65.2 (53.7, 71.8)	68.5 (58.4, 72.9)	62.9 (42, 68.8)	0.017
LY30 (%)	0–8	0 (0, 0.3)	0.1 (0, 0.5)	0 (0, 0.1)	0.056
TMA (min)	23–33	27.8 (24.8, 35)	25.9 (24, 30)	34.3 (27.3, 38.8)	< 0.001

Values are *n*, mean ± SD, or median (interquartile range), unless otherwise noted. K-time, Kinetic-time; LY30, Clot lysis 30 min following thromboelastography maximal amplitude; MA, Maximum amplitude; R-time, Reaction time; TMA, Time to maximum clot strength.

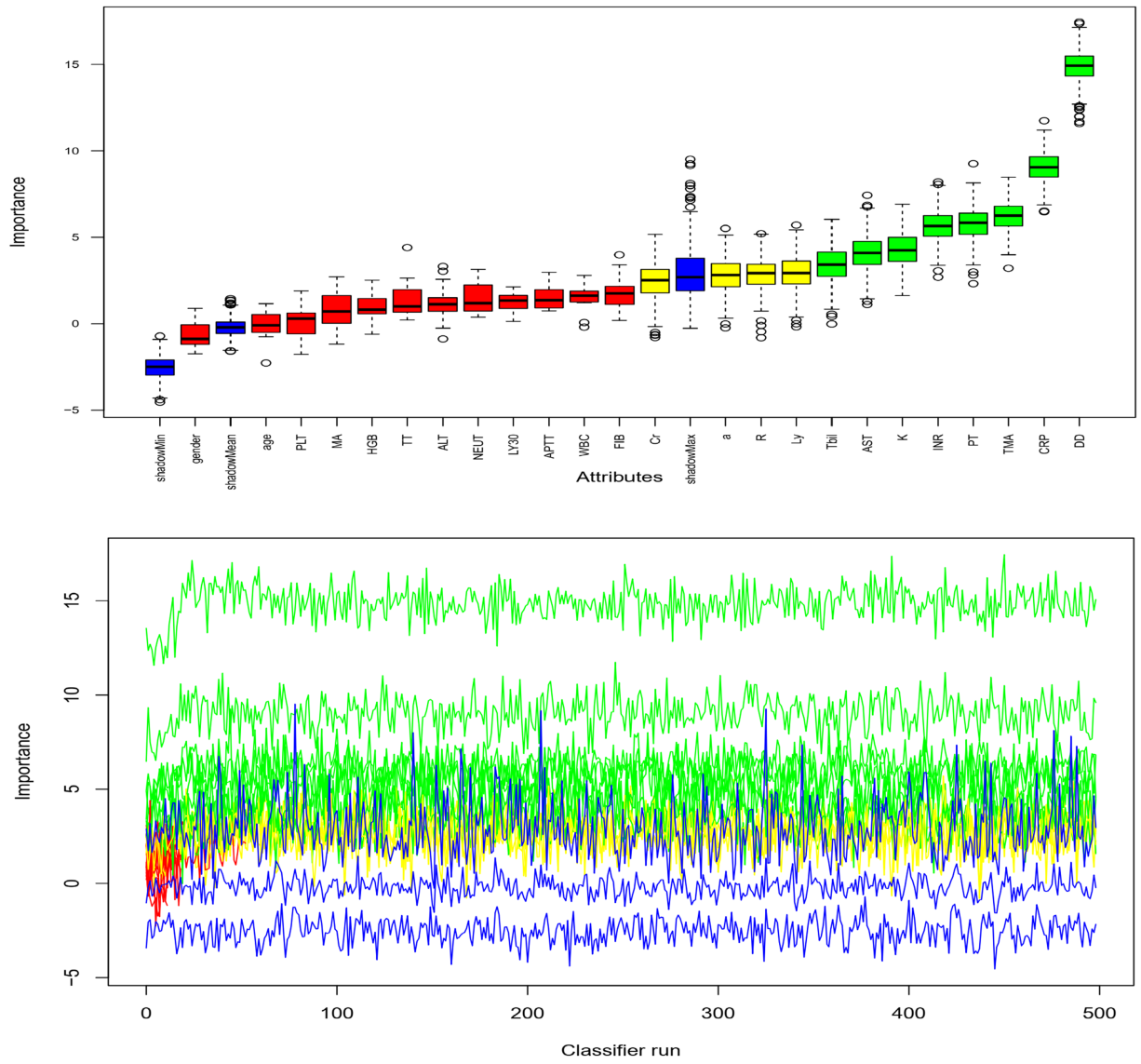

FIGURE 1
Machine learning analysis and selection of biomarker for prognosis.

with severe COVID-19 who are more likely to die within 30 days after ICU admission. Our results suggest that TMA may be useful as a prognostic indicator, which may reflect its ability to assess interaction between coagulation factors and platelets (24). Indeed, we found that TMA correlated with prothrombin time, international normalized ratio, and D-Dimer. Whatever the explanation, our data suggest that using TMA to screen COVID-19 patients for mortality risk may help optimize limited healthcare resources and provide timelier patient management and treatment, ultimately leading to better outcomes.

Studies have linked COVID-19 to hypercoagulability and decreased fibrinolysis, yet we found that only 14 of our 87 patients with severe disease were in a hypercoagulable state, which is similar to the proportion reported for a sample of patients from Asian (25), but much lower than the 50% reported for patients from Caucasian (26). Conversely, a high proportion of our non-survivors were in a hypocoagulable state which manifested as reduced platelet count, longer activated partial thromboplastin time and prothrombin time, and elevated D-dimer levels. Thromboelastography confirmed this hypocoagulable state by indicating reduced α angle as well as longer R-time, K-time, and TMA. Similarly, our incidence of hypocoagulable state is significantly higher than that in the COVID-19 patients from Caucasian (26). Ethnicity may help explain these differences, since Caucasians and African-Americans are generally at higher risk of thromboses than Chinese (13). Whatever the explanation, our results suggest that hypocoagulability is associated with mortality among patients with severe COVID-19, consistent with a previous report linking hypocoagulability to COVID-19 severity (14).

Many patients with severe pneumonia suffer disseminated intravascular coagulation and multiple organ failure when vascular endothelium, platelets, and leukocytes become activated and dysregulate the production of thrombin systemically and in the lungs (27). A similar process may occur in COVID-19, which has also been linked to disseminated intravascular coagulation and multiple organ failure (28, 29). Thus, treatments to restore or regulate hemostasis may

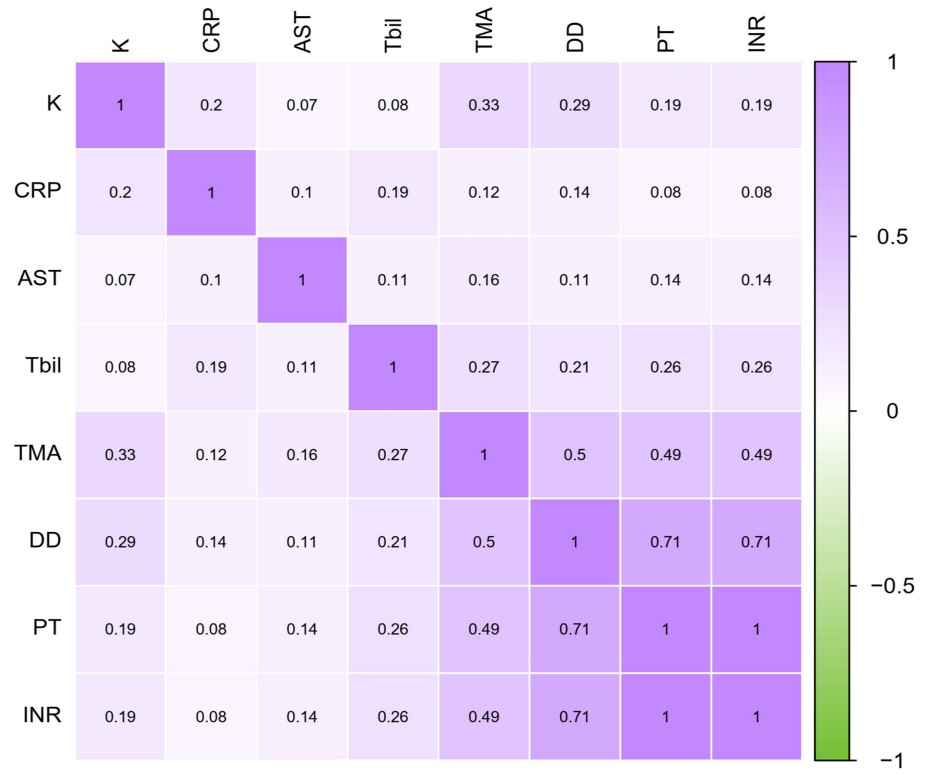

FIGURE 2
Correlation matrix.

TABLE 3 Multivariate logistic regression to identify parameters associated with mortality in severe COVID-19 patients.

Parameter	Odds ratio (95%CI)	*p*
TMA (min)	1.068 (1.016–1.122)	0.01
AST (U/L)	1.002 (0.999–1.006)	0.22
CRP (mg/L)	1.011 (1.003–1.018)	0.006
K (min)	1.053 (0.956–1.159)	0.295

benefit individuals infected with coronavirus (30). For example, administering heparin to patients with severe COVID-19 with extremely high D-dimer levels can decrease mortality (31). While elevated D-dimer levels can signal the activation of coagulation and fibrinolysis (32), they can also occur due to bleeding. Therefore, thromboelastography may be better for determining the timing of anticoagulation therapy (33).

Our findings should be interpreted with caution in light of several limitations. First, our sample was retrospective and came from two centers. Second, we did not perform thromboelastography multiple times during the disease course. Third, we did not provide the number of days between the onset of symptoms and hospitalization for severe patients, due to the difficulty in obtaining their medical history upon admission. Our results should be verified and extended in larger studies with longitudinal monitoring.

Despite these limitations, our study demonstrates the usefulness of thromboelastography for assessing the coagulation state of patients with severe COVID-19. It also provides the first evidence that the thromboelastographic parameter TMA may accurately identify severe COVID-19 patients at higher risk of dying.

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 humans were approved by the Ethics Committee of Wuhan Huoshenshan Hospital and the 908th Hospital of Chinese PLA Logistic Support Force. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because all patients or their legal guardians gave written consent, at the time of admission, for patients' anonymized data to be analyzed and published for research purposes.

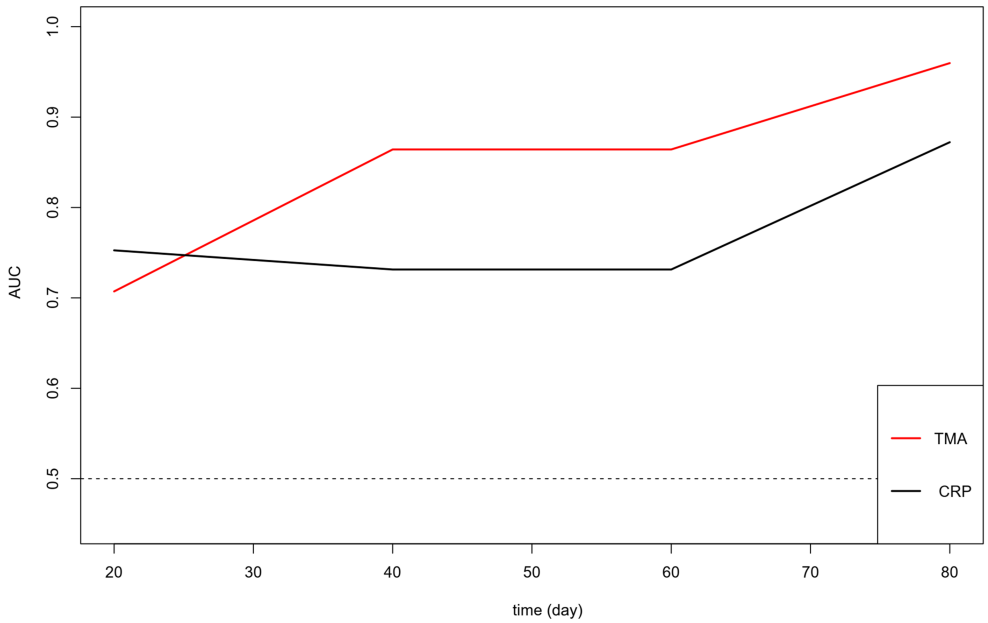

FIGURE 3
Time-dependent ROC for TMA and CRP for predicting mortality. AUC, Area under the curve; ROC, Receiver operating curves.

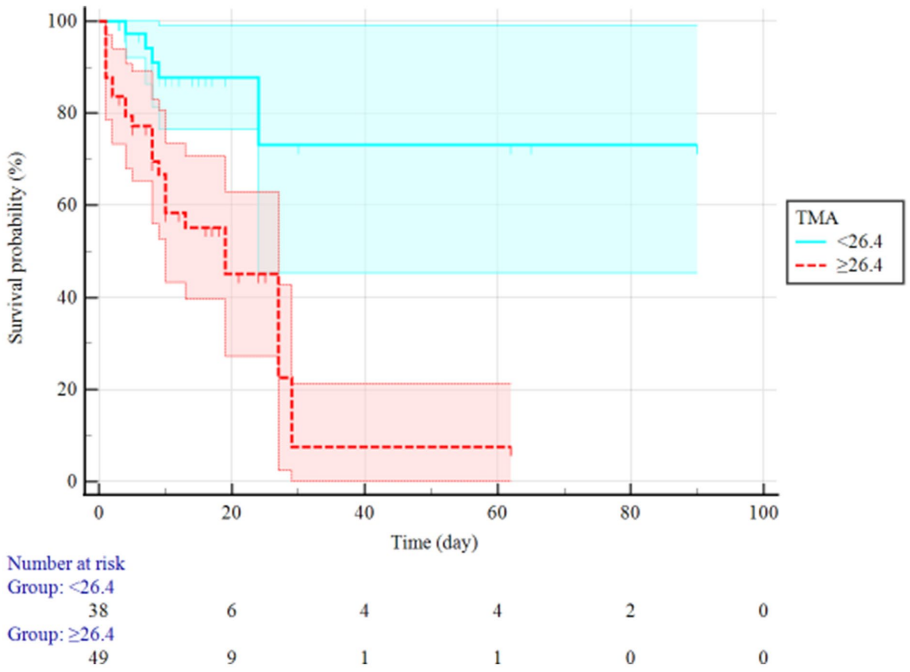

FIGURE 4
Kaplan–Meier survival curve of resected in severe COVID-19 patients with long TMA (≥26.4 min) and short TMA (<26.4 min).

Author contributions

LZho: Writing – original draft. QL: Data curation, Formal Analysis, Writing – original draft. LH: Software, Writing – original draft. DL: Data curation, Writing – review & editing. LZhu: Data curation, Writing – review & editing. QZ: Validation, Writing – review & editing. JS: Conceptualization, Funding acquisition, Writing – review & editing.

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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.2024.1356283/full#supplementary-material

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EDITED BY
Marcos Ferreira Minicucci,
São Paulo State University, Brazil

REVIEWED BY
Estela de Oliveira Lima,
São Paulo State University, Brazil
Kavin K. S.,
Anna University, India

*CORRESPONDENCE
Fang Xu
✉ fangxu@hospital.cqmu.edu.cn
Wen-jian Qin
✉ wj.qin@siat.ac.cn

†These authors have contributed equally to this work and share first authorship

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Identifying the risk factors of ICU-acquired fungal infections: clinical evidence from using machine learning

Yi-si Zhao[1,2†], Qing-pei Lai[3,4†], Hong Tang[1†], Ren-jie Luo[1], Zhi-wei He[1], Wei Huang[1], Liu-yang Wang[1], Zheng-tao Zhang[1], Shi-hui Lin[1], Wen-jian Qin[4*] and Fang Xu[1*]

[1]Department of Critical Care Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China, [2]Medical Data Science Academy, Chongqing Medical University, Chongqing, China, [3]Shenzhen College of Advanced Technology, University of Chinese Academy of Sciences, Shenzhen, China, [4]Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

Background: Fungal infections are associated with high morbidity and mortality in the intensive care unit (ICU), but their diagnosis is difficult. In this study, machine learning was applied to design and define the predictive model of ICU-acquired fungi (ICU-AF) in the early stage of fungal infections using Random Forest.

Objectives: This study aimed to provide evidence for the early warning and management of fungal infections.

Methods: We analyzed the data of patients with culture-positive fungi during their admission to seven ICUs of the First Affiliated Hospital of Chongqing Medical University from January 1, 2015, to December 31, 2019. Patients whose first culture was positive for fungi longer than 48 h after ICU admission were included in the ICU-AF cohort. A predictive model of ICU-AF was obtained using the Least Absolute Shrinkage and Selection Operator and machine learning, and the relationship between the features within the model and the disease severity and mortality of patients was analyzed. Finally, the relationships between the ICU-AF model, antifungal therapy and empirical antifungal therapy were analyzed.

Results: A total of 1,434 cases were included finally. We used lasso dimensionality reduction for all features and selected six features with importance ≥0.05 in the optimal model, namely, times of arterial catheter, enteral nutrition, corticosteroids, broadspectrum antibiotics, urinary catheter, and invasive mechanical ventilation. The area under the curve of the model for predicting ICU-AF was 0.981 in the test set, with a sensitivity of 0.960 and specificity of 0.990. The times of arterial catheter ($p = 0.011$, OR = 1.057, 95% CI = 1.053–1.104) and invasive mechanical ventilation ($p = 0.007$, OR = 1.056, 95%CI = 1.015–1.098) were independent risk factors for antifungal therapy in ICU-AF. The times of arterial catheter ($p = 0.004$, OR = 1.098, 95%CI = 0.855–0.970) were an independent risk factor for empirical antifungal therapy.

Conclusion: The most important risk factors for ICU-AF are the six time-related features of clinical parameters (arterial catheter, enteral nutrition, corticosteroids, broadspectrum antibiotics, urinary catheter, and invasive mechanical ventilation), which provide early warning for the occurrence of fungal infection. Furthermore, this model can help ICU physicians to assess

whether empiric antifungal therapy should be administered to ICU patients who are susceptible to fungal infections.

KEYWORDS

fungal infection, ICU-acquired fungi, machine learning, empiric antifungal therapy, risk factors

1 Introduction

Infections are been a key medical issue in in intensive care units (ICUs). In a recent survey of a worldwide sample of ICU patients, the prevalence of suspected or proven infection was 8,135 out of 15,202 (54%), and the in-hospital mortality rate was 2,404 out of 7,936 (30%) (1). Fungi are opportunistic pathogens that normally colonize the skin and mucous membranes of ICU patients (2). The entry of fungi into the body results in fungal infection when the body's defense barrier or immune system is disrupted (3, 4). Although this decade the prevalence of fungal infection has decreased from 963 out of 4,947 (19%) to 864 out of 5,259 (16%) in the ICU, it is still the third most common pathogen in ICU (1, 5). A study reported that invasive fungal infections have a mortality rate of more than 30% in critically ill patients (6). The mortality rate after *Candida* infection is more than 40% (7, 8). Furthermore, the mortality rate attributable to invasive aspergillosis >42% (9). Fungal infections occur at different sites with varying rates. The mortality rate of patients with candidemia was 28%, which was higher than that of patients with abdominal invasive candidiasis (16%) and non-abdominal sterile sites (10%) (10). Therefore, it is important to focus on the early characteristics of fungal infections to reduce the infection and mortality rates in the ICU.

A multicenter study involving global ICU infections found that 1706 out of 8,135 (16%) infections were ICU-acquired (1), which are summed up in hospital-acquired infections (HAPs) (11). The mortality rate of ICU-acquired infections (461 out of 1706, 27%) was higher than that of community-acquired infections (697 out of 3,474, 20%) and hospital-acquired infections (661 out of 2,724, 24.9%) (1). Among the 848 (30%) cases of fungal infections, 255 were "ICU-acquired fungal infections (ICU-AFIs)," which are attributed to the special pathophysiology of critically ill patients during ICU stay (1, 8). Mainstream diagnostic methods are classified as proven, probable, and possible (12). However, diagnosing fungal infection is difficult. The false-negative rate of ICU-acquired candidemia, which is a conventional fungal infection in the ICU, can reach 60% (13). It is puzzling that the basis for the initial diagnosis of ICU-AFI limits early identification because the fungal samples belong to the ICU (255 out of 848, 30%) or other medical units (300 out of 848, 35%) (1). It is very difficult for clinical doctors to accurately confirm and treat ICU-AFI. Therefore, distinguishing between ICU-acquired fungi (ICU-AF) and non-ICU-acquired fungi (non-ICU-AF) is beneficial for the early management of ICU-AFI.

In the real world, studies on the same target may yield different results owing to multiple confounding factors. A recent study by Poissy and Keighley on the risk factors of candidemia in the ICU produced conflicting conclusions regarding urinary catheters and liver disease (14, 15). This study is a retrospective clinical cohort study that used machine learning (ML) to identify the origin of ICU-AFIs and created a scoring chart to predict ICU-AF risk models.

2 Methods

2.1 Study design

This study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (reference number: 2021–366). The ethics committee waived the requirement for informed consent because of the retrospective nature of this study. Patient data were sourced from medical record systems and analyzed anonymously to protect patient privacy.

We included a cohort of patients who had culture-positive fungi during their admission to seven ICUs (GICU, general ICU; SICU, surgical ICU; RICU, respiratory ICU; NICU, neurology ICU; NSICU, neurosurgery ICU; CSICU, cardiothoracic surgery ICU; CCU, cardiovascular ICU) at the First Affiliated Hospital of Chongqing Medical University from January 1, 2015, to December 31, 2019. Culture-positive fungi refer to specimens obtained from ICU patients that were cultured positive for fungi by laboratory physicians in the microbiology room, and an official report was issued. Subsequently, all patient data, including basic information (age, gender and comorbidities), characteristics of fungi (microbiology and time to positivity of ICU), laboratory results (all results shall be obtained within 24 h after the fungal culture is positive), and clinical data (days in the ICU, department, Acute Physiology and Chronic Health Evaluation (APACHE) II Score, diagnosis on ICU admission, and clinical characteristics), were extracted from our internal electronic medical records (Table 1). The 28-day mortality rates after ICU admission were recorded. Data were collected by three investigators and were checked by two other investigators to avoid bias. Notably, these features were chosen on the basis of their availability in all patients rather than on any *a priori* assumptions about their ability to predict fungal acquisition, although the goal of our prediction model was to select the most influential factors in the collected data for the prediction of ICU-AF.

2.2 Definition

According to guidelines, infection after 48 h of hospitalization is defined as HAP (11). Therefore, we included patients whose first culture was positive for fungi longer than 48 h after ICU admission in the ICU-AF cohort and less than 48 h in the non-ICU-AF cohort. The cohort process and exclusion criteria are shown in Figure 1.

All antifungal treatment decisions were jointly made by two or more deputy chief physicians with >15 years of clinical experience in critical care medicine. Among these, empirical antifungal therapy prior to fungal culture is based on guidelines (6).

2.3 Machine learning

ML methods are computer algorithms that automatically recognize complex patterns on the basis of empirical data. The goal is to enable algorithms to learn from past or present data and to use this knowledge to make predictions or decisions regarding unknown future events (16). In the current study, we used the random forest (RF) ML algorithm. It is a "tree-based" algorithm in which multiple decision trees are constructed using random classifications of independent features that are used to predict outcome labels for random subsets of samples (17). On the one hand, the RF technique is a regression tree technique that uses bootstrap aggregation and randomization of predictor variables to achieve a high degree of predictive accuracy and is often used in medical field analysis to construct classification prediction models (18–20). On the other hand, RF may be more suitable for feature selection during classification tasks in bioinformatics and related sciences, where it has a relatively low tendency to overfit and produces more robust results (21).

2.4 Data set division

We randomly assigned 1,434 cases to the sample, with 50% of the cases used as the training set and the rest as the test set. We also ensured that there was no gender or age bias between the training set and testing set.

2.5 Feature extraction

For the training set, we first used the Least Absolute Shrinkage and Selection Operator (LASSO) to reduce the dimension of features according to whether the patient is ICU-AF. We performed feature reduction using LASSO on the training set. LASSO performs feature selection during model construction by penalizing the respective regression coefficients. As this penalty increases, more regression coefficients shrink to zero, thus resulting in a more regularized model (22). In this process, 49 significant features with nonzero coefficients were obtained. We then used them in the RF prediction model. By using a ten-fold cross-validation analysis, we selected the best model parameter on the basis of the accuracy of each fold of the model. At the same time, we ranked the features in this model by setting a threshold of 0.05 to select the features in reference to previous articles (23, 24). These features were retained, and the randomized forest model was trained to predict patient ICU-AF by using ten-fold cross-validation. Finally, the model was tested using the test set. Both downscaling and ten-fold cross-validation were used to prevent overfitting. Overfitting can occur when excessive features affect the predictive performance of a model. However, the use of nested k-fold cross-validation allows us to perform model training independently of hyperparameters optimization, which prevents overfitting or incorrect generalization estimates (25). The R language was used to

TABLE 1 The characteristics of training set and test set.

	Train *N* = 717	Test *N* = 717
Age	67.89 ± 15.645	68.32 ± 15.91
Gender (male)	479, 66.8%	452, 63.0%
Days in ICU	11 (5,18)	10 (5, 18)
APACHE II Score on ICU admission	20.90 ± 6.654	20.87 ± 6.05
Department		
GICU	230, 32.1%	216, 30.1%
SICU	148, 20.6%	150, 20.9%
RICU	202, 28.2%	209, 29.1%
NICU	92, 12.8%	99, 13.8%
NSICU	14, 2.0%	16, 2.2%
CSICU	13, 1.8%	8, 1.1%
CCU	18, 2.5%	19, 2.6%
Diagnosis on ICU admission		
Medical	450, 62.8%	449, 62.6%
Surgical	219, 30.5%	223, 31.1%
Trauma	48, 6.7%	45, 6.3%
Surgery	216, 30.1%	211, 29.4%
Comorbidities		
Diabetes	208, 29.0%	240, 33.5%
COPD	157, 21.9%	164, 22.9%
Heart failure	229, 31.9%	261, 36.4%
Chronic liver disease	37, 5.2%	57, 7.9%
Chronic kidney disease	68, 9.5%	70, 9.8%
Solid tumours	95, 13.2%	97, 13.5%
Haematological malignancy	5, 0.7%	9, 1.3%
Solid organ transplant	2, 0.3%	2, 0.3%
Acute pancreatitis	44, 6.1%	43, 6.0%
Sepsis	241, 33.6%	256, 35.7%
SOFA score	5.29 ± 3.06	5.38 ± 3.09
Microbiology		
Undefined Saccharomyces	185, 25.8%	191, 26.6%
Candida albicans	257, 33.1%	254, 35.4%
Candida tropicalis	75, 10.5%	86, 12.0%
Candida glabrata	88, 12.3%	83, 11.6%
Candida parapsilosis	24, 3.3%	24, 3.3%
Candida krusei	4, 0.6%	9, 1.3%
Other Candida	33, 4.6%	13, 1.8%
Undefined Aspergillus	20, 2.8%	10, 1.4%
Aspergillus fumigatus	30, 4.2%	30, 4.2%
Aspergillus niger	5, 0.7%	4, 0.6%
Aspergillus flavus	6, 0.8%	6, 0.8%
Other Aspergillus	1, 0.1%	1, 0.1%
Cryptococcus neoformans	2, 0.3%	3, 0.4%
Other fungi	6, 0.8%	3, 0.4%

(Continued)

TABLE 1 (Continued)

	Train *N* = 717	Test *N* = 717
Time to positivity of ICU admission over 48 h	321, 44.8%	303, 42.3%
Clinical characteristic		
Central venous catheter	482, 67.2%	464, 64.4%
Arterial catheter	399, 55.6%	389, 54.3%
Invasive mechanical ventilation	404, 56.3%	398, 55.5%
Non-invasive mechanical ventilation	318, 44.4%	323, 45.0%
Tracheotomy	127, 17.7%	124, 17.3%
Urinary catheter	617, 86.1%	604, 84.2%
Hemodialysis or continuous hemofiltration	88, 12.3%	94, 13.1%
Parenteral nutrition	433, 60.4%	414, 57.7%
Enteral nutrition	371, 51.7%	347, 48.4%
Corticosteroids	183, 25.5%	166, 23.2%
Broad-spectrum antibiotics	636, 88.7%	642, 89.5%
Candida score	1.80 ± 1.48	1.80 ± 1.47
Laboratory data		
T	37.05 ± 0.80	37.02 ± 0.77
P	100.74 ± 21.35	100.31 ± 20.49
R	22.79 ± 5.75	22.87 ± 5.37
WBC	12.59 ± 7.72	12.19 ± 6.55
N%	85.29 ± 8.77	84.71 ± 9.55
PLT	204.23 ± 126.61	197.28 ± 123.56
PCT	0.93 (0.23, 6.27)	1.06 (0.25, 5.46)
ALB	29.51 ± 6.31	29.81 ± 5.58
TBil	14.10 (9.50, 22.15)	14.3 (9.70, 23.55)
ALT	32.00 (22.00, 56.50)	32.00(22.00, 55.50)
AST	34.00 (22.00, 61.00)	34.00 (22.00, 59.50)
Ur	12.28 ± 9.22	12.57 ± 9.73
Cr	119.58 ± 117.72	123.31 ± 125.98
UA	272.70 ± 167.113	270.35 ± 164.375
PT	14.20 (12.80, 15.70)	14.40 (12.70, 15.65)
APTT	38.86 ± 24.76	39.38 ± 24.64
Death of 28 days	165, 23.0%	187, 26.1%

The distribution of features in the training set and test set is randomly divided equally. ICU, Intensive care unit; APACHE II, Acute Physiology and Chronic Health Evaluation II; GICU, general ICU; SICU, Surgical ICU; RICU, Respiratory ICU; NICU, Neurology ICU; NSICU, Neurosurgery ICU; CSICU, Cardiothoracic Surgery ICU; CCU, Cardiovascular ICU; COPD, Chronic obstructive pulmonary disease; SOFA, Sequential Organ Failure Assessment; T, Temperature; P, Pulse rate; R, Respiratory rate; WBC, White blood cell count; N%, Neutrophil count%; PLT, Platelet count; PCT, Procalcitonin; ALB, Albumin; TBil, Total bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Ur, Urea Nitrogen; Cr, Creatinine; UA, Uric acid; PT, Prothrombin time; APTT, Activated partial thromboplastin time.

draw the density map between each feature and APACHE II, and the *lm* function was used to fit the regression model. The "pheatmap" package implements heatmap to display sample survival and feature performance.

2.6 Model performance

In the ten-fold cross-validation of the model, we trained different model parameters and selected the model parameters with the best accuracy in one fold for application to the test set. The ability of the model to discriminate between acquired fungi was determined using the area under the curve (AUC), and the stability of the model was determined on the basis of sensitivity and specificity. From our learning models, we chose the model with the best discrimination ability.

2.7 Statistical analysis

All statistical analyses were performed using Stata 24.0 software. To divide the training and test sets, we used analysis of variance (ANOVA) to analyze whether there was a difference in the age distribution between the training and test sets, and the chi-square test was used to analyze whether there was a difference in the gender distribution between them. The main specification of ML is that the models constructed from selected features perform well for predicting patient outcomes, AUC, sensitivity, specificity, and accuracy and are only used to determine the performance of the models (26). Therefore, many previous studies have used ML and logic methods (27, 28). In our research, factors associated with antifungal therapy and empirical antifungal therapy for acquired fungi were analyzed using univariate and multivariate conditional logistic regression models for all features of the ML model. Its odds ratio (OR) and 95% confidence interval (CI), $p < 0.05$ was considered significant.

3 Results

3.1 Cohort characteristics

For the submission of the manuscript, we enrolled 2,147 cases. A total of 1,434 cases with complete data were obtained after exclusion and screening (Figure 1, step 1). The cases were randomly and equally divided into the training ($N = 717$) and test ($n = 717$) sets. The distribution of outcome labels for patients in the training and test sets showed no significant differences ($p = 0.37$, chi-square test, not shown). The features of the two data sets are shown in Table 1; age (ANOVA, $p = 0.60$, not shown) and gender (chi-square test, $p = 0.15$, not shown) had no statistical difference, and the other features are shown in Table 1. On the basis of whether the fungi were ICU acquired, LASSO was performed to reduce the dimension of features. Thereafter, by using ten-fold cross-validation, the average accuracy of the random forest model was 0.907 ± 0.042, among which the third-fold accuracy we applied was the highest, which was 0.972 (Figure 2). We took the third-fold model parameter as our optimal model parameter. We selected six features with importance ≥0.05 in the optimal mode, namely, times of arterial catheter, times of enteral nutrition, times of corticosteroids, times of broad-spectrum antibiotics, times of urinary catheter and times of invasive mechanical ventilation (Figure 3A).

3.2 The role of each feature

By using these features for ML analysis and testing on an independent test set, the results showed that the AUC for predicting ICU-AF was 0.981 in the test set, with a sensitivity of 0.960 and specificity of 0.990 (Figure 3B). Disease severity in ICU patients was represented by the APACHE II Score, which was analyzed separately with the continuous time of these six features. Only the times of invasive mechanical ventilation showed a significant linear correlation with the APACHE II Score ($p = 0.031$) (Figure 3C). The duration time of these features showed no significant differences in the 28-day mortality (Figure 3D).

3.3 Risk factors associated with antifungal therapy and empirical antifungal therapy

Considering the univariate and multivariate conditional logistic regression analyses of antifungal therapy in ICU-AF, the results showed that among these six features, times of arterial catheter ($p = 0.011$, OR = 1.057, 95%CI = 1.053–1.104) and times of invasive mechanical ventilation ($p = 0.007$, OR = 1.056, 95%CI = 1.015–1.098) were independent risk factors for antifungal therapy in ICU-AF (Table 2). In the sample on antifungal therapy, times of arterial catheter ($p = 0.004$, OR = 1.098, 95%CI = 0.855–0.970) was an independent risk factor for empirical antifungal therapy (Table 3).

4 Discussion

This retrospective clinical cohort study spanned 5 years, included 1,434 cases with complete data, and identified 6 risk factors for ICU-AF using ML. Fungal infection, which is accompanied by difficult treatment and poor prognosis, is an important component of ICU infections (1, 29). He et al. used ML to establish predictive models for secondary candidemia in patients with systemic inflammatory response syndrome (SIRS) patients in the ICU. These models have a potential guiding role in the antifungal treatment of critically ill patients with SIRS (30). Researchers often focus on the pathogenic state and non-pathogenic state of fungi, which are known as "infection" and "colonization," respectively (31, 32). Once a fungal infection emerges in critically ill patients in the ICU, colonization poses a high risk to individuals with immune disorders. Popular researches has considered fungal colonization, including multi-site colonization and the colonization of special strains, as a risk factors for fungal infection (33, 34). The risk of fungal infection increased significantly after fungal colonization in ICU patients. One study found that 93 out of 137 (68%) patients with candidemia had *Candida* colonization (30). The preconception was that fungal infection is opportunistic. However, the sensitivity of ICU blood cultures for invasive candidiasis (including intra-abdominal candidiasis) is approximately 40% (13). Up to 70% of patients with candidemia do not receive early empiric antifungal therapy early on (35). Generally, doctors in the ICU often value patients who already have the "fungi" label, but the preparation for a new onset one is insufficient. This could increase the risk of patients in the ICU. A study showed that a 12-h delay in starting antifungal therapy was associated with a 2.09-fold increase in mortality (36). Discovering the types of patients in the ICU who are at high risk for acquired fungal infections is an important part of critical illness warnings.

This study advances the warning line of fungal infection before colonization, which is called ICU-AF and is defined as fungi cultured after 48 h in the ICU. LASSO dimensionality reduction and ML methods were used to analyze patients admitted to the ICU over the past 5 years. Compared with non-acquired fungi, six features including times of arterial catheter, times of enteral nutrition, times of corticosteroids, times of broad-spectrum antibiotics, times of urinary catheter, and times of invasive mechanical ventilation, showed high significance in ICU-AF. These features are considered high-risk factors for fungal infection in the ICU (7, 37–42). The current study used ML to prove that ICU-AF has a higher risk of occurrence when ICU patients exhibit the above six features. However, the utility of risk factors in ICU-AF patients depends on differentiating between the dimensions of time, frequency, and intensity. ICU-AF is expected to provide an early warning for antifungal therapy or even empirical antifungal therapy.

Logistic regression analysis showed that the times of arterial catheter and invasive mechanical ventilation were independent risk factors for antifungal therapy in ICU-AF, and ductus arteriosus time was an independent risk factor for empirical antifungal therapy in ICU-AF. By using ML to study the early warning of ICU-AF, the times of arterial catheter insertion and invasive mechanical ventilation can be used to warn critical care physicians on whether antifungal therapy is needed. Patients with arterial catheters may require early empirical antifungal therapy.

4.1 Strengths and limitations

This study applied an unconventional method to study susceptibility to ICU-AF: First, we used efficient ML methods to analyze clinical data to reduce the bias of manual analysis. Second, we focused on the early warning of fungal infection, namely, ICU-AF, and this approach is more in line with the needs of treating ICU patients. Finally, we investigated the role of the ICU-AF early warning model in antifungal therapy and empirical antifungal therapy for guiding the management of ICU-AF.

This study has the following limitations. First, this was a retrospective, single-center study. It should be noted that this single-center study involved seven different ICU wards (GICU, RICU, SICU, NICU, NSICU, CSICU, and CCU), and some specific characteristics (such as major abdominal surgery and disturbance of consciousness) were diverse. However, even across seven different ICUs, each patient had these six features. As a routine treatment procedure for patients in the ICU, the duration of these six features was obtained via detailed nursing records and reflected the length of time that patients received treatment in the real world and the homogeneity of fungal infection risk factors across all ICUs. Second, there were more than 40 salient features in the optimal model (Figure 3A). However, we selected only six features with importance >0.05. When multiple features appear in the results, it is crucial to extract better and more convenient feature models for clinical applications. In addition to using 0.05 as a threshold to screen six features, we also explored the important role of these six features in ICU-AF on the basis of clinical practice. Other features (such as central venous catheter, abdominal surgery and SOFA

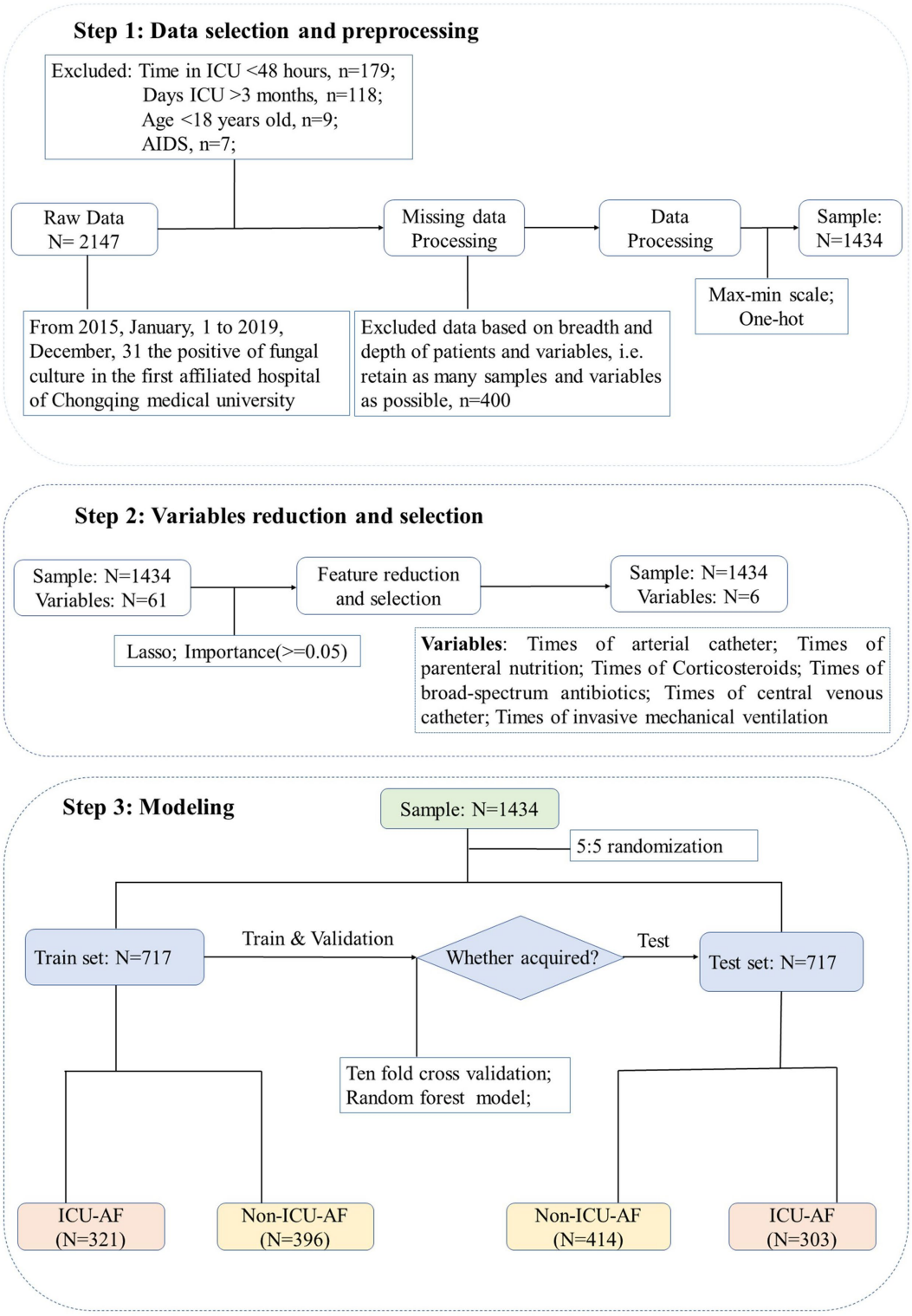

FIGURE 1
Flowchart for enrollment and screening. Step 1: Preliminarily screen the samples according to the inclusion and exclusion criteria. Step 2: Use lasso dimensionality reduction for all 61 features and select 6 features with importance ≥0.05. Step 3: All samples are randomly and equally divided into training set and test set. Max-min scale: normalization for continuous features, the formula is $x' = \frac{x - x_{\min}}{x_{\max} - x_{\min}}$. One-hot: setting unordered classification features to mutually exclusive dummy features.

scores) that were reported to be connected with ICU-AF (43, 44), could probably have hidden roles. They still have potential value for future discussion. The prevailing view supports that the six features, analyzed in the current study are good predictors of ICU-AF (7, 37–42). Controlling these six operations is an effective way to reduce ICU-AF. Blaize et al. found that controlling the use of

corticosteroids could reduce the risk of invasive pulmonary fungal infections in COVID-19 patients admitted to the ICU (45). Thirdly, regarding the question of whether ICU physicians can distinguish fungal colonization from fungal infection. The AUC of the optimal model for the fungal infection test obtained in this study was 0.670 (Supplementary Figure S1). In the clinical cohort, these were indistinguishable at the time of diagnosis; thus, we advanced the field of view to the acquired fungus. Finally, increasing the amount of training data can enable us to obtain more information and make diverse learning in most cases, as well as increase the chances of achieving better results. Some important studies use 70% or 80% of samples in the training set (46–48). We randomly assigned 1,434 cases to the sample, with 50% of the cases used as the training set and the rest as the test set, to improve the efficiency of model validation. Meanwhile, it was also ensured that there was no gender and age bias between the training set and the test set. Although this ratio is also a common ratio for dividing datasets in previous studies, such as in some studies on tumor diseases (49, 50), we will continue to collect and expand sample size data in future research to improve the sample ratio in the training set.

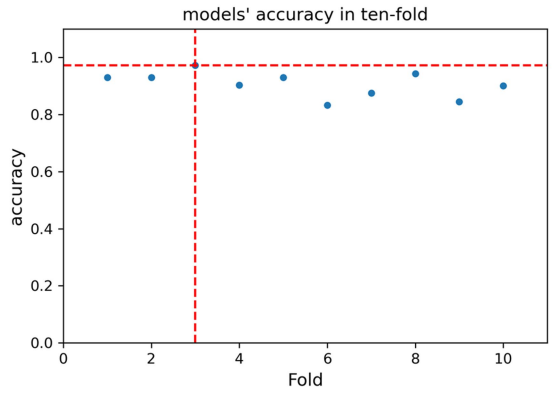

FIGURE 2
Accuracy of models in ten-fold cross-validation.

In summary, ML classifier models in clinical cohorts have the potential to predict the risk of ICU-AFI. The most important risk factors for ICU-AF are the six time-related clinical parameters (arterial catheter, enteral nutrition, corticosteroids, broad-spectrum antibiotics, urinary catheter, and invasive mechanical ventilation) that provide early warnings for the early prevention of fungal infection. Furthermore, this model, although needs to be more clinically validated, has the potential to help ICU physicians assess whether

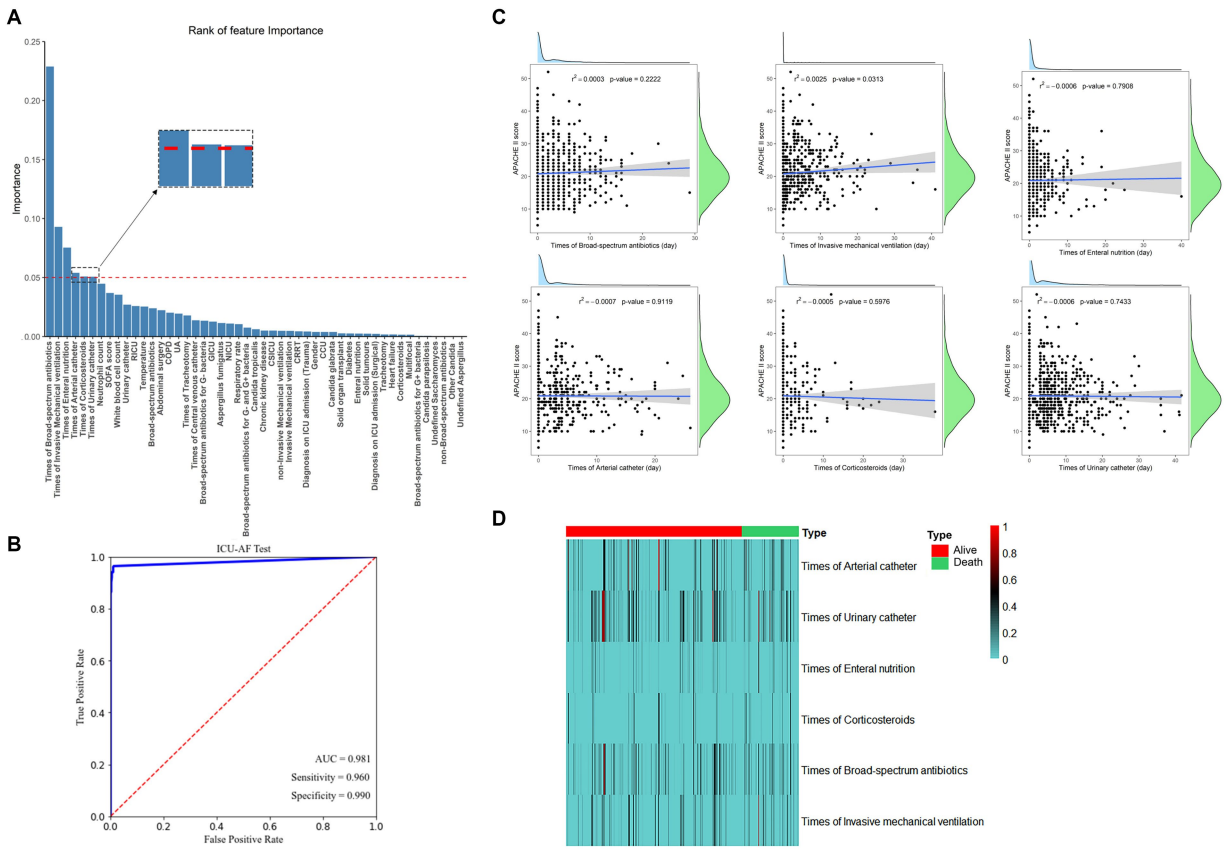

FIGURE 3
(A) The 49 features with the highest relative gain for model predicting ICU-AF and the 5 features with importance ≥0.05. **(B)** Receiver operating characteristic curve (ROC) of models. **(C)** Scatter plot with linear regression line of best fit with APACHE II score analyzed separately with six features. r^2: represents the degree of feature fitting; $p < 0.05$ were considered significant; **(D)** The heatmap of different features in dead vs. surviving patients, and colors in the heatmap indicate the time (days) for the corresponding feature. AUC, area under the subject curve. APACHE II, Acute Physiology and Chronic Health Evaluation II.

TABLE 2 Independent risk factors associated with antifungal therapy according to ICU-AF.

Variable (Times of)	Univariate			Multivariate		
	OR	95%CI	*p* value	OR	95%CI	*p* value
Arterial catheter	1.061	1.021–1.101	0.002	1.057	1.013–1.104	0.011
Invasive mechanical ventilation	1.056	1.023–1.089	0.001	1.056	1.015–1.098	0.007
Urinary catheter	1.028	1.003–1.053	0.027	0.984	0.950–1.020	0.378
Parenteral nutrition	1.036	0.989–1.085	0.136	1.01	0.957–1.066	0.718
Corticosteroids	1.029	0.980–1.080	0.253	0.999	0.947–1.054	0.978
Broad-spectrum antibiotics	1.037	0.996–1.079	0.077	1.005	0.959–1.052	0.846

OR stands for odds ratio, CI for confidence interval; The samples for logistic regression analysis were from all ICU-AF samples. Variables were selected with importance ≥ 0.05 in the training set.

TABLE 3 Independent risk factors associated with empirical antifungal therapy according to ICU-AF.

Variable (Times of)	Univariate			Multivariate		
	OR	95%CI	*p* value	OR	95%CI	*p* value
Arterial catheter	1.088	1.035–1.143	0.001	1.098	0.855–0.970	0.004
Invasive mechanical ventilation	1.028	0.987–1.070	0.188	1.053	0.894–1.009	0.094
Urinary catheter	1.018	0.980–1.057	0.362	0.947	0.992–1.125	0.089
Parenteral nutrition	0.99	0.924–1.060	0.769	0.98	0.931–1.118	0.666
Corticosteroids	1.039	0.971–1.111	0.265	1.015	0.910–1.066	0.714
Broad-spectrum antibiotics	1.068	1.007–1.134	0.028	1.052	0.884–1.023	0.176

OR stands for odds ratio, CI for confidence interval; The samples for logistic regression analysis were from all ICU-AF samples with antifungal therapy. Variables were selected with importance ≥ 0.05 in the training set.

empiric antifungal therapy should be administered to ICU patients who are susceptible to fungal infections.

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 humans were approved by Institutional Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

Y-sZ: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. Q-pL: Formal analysis, Methodology, Software, Supervision, Writing – original draft. HT: Conceptualization, Data curation, Investigation, Resources, Writing – original draft. R-jL: Data curation, Methodology, Writing – original draft. Z-wH: Data curation, Investigation, Software, Writing – original draft. WH: Data curation, Investigation, Writing – original draft. l-yW: Data curation, Writing – original draft. Z-tZ: Investigation, Writing – original draft. S-hL: Data curation, Investigation, Resources, Writing – original draft. W-jQ: Conceptualization, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing. FX: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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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.2024.1386161/full#supplementary-material

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EDITED BY
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

REVIEWED BY
Wasineenart Mongkolpun,
Mahidol University, Thailand
Anas Abu-Humaidan,
The University of Jordan, Jordan

*CORRESPONDENCE
Gabriel Eustache
✉ gabriel.eustache@chu-rennes.fr

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Management of spontaneous septic hypothermia in intensive care. A national survey of French intensive care units

Gabriel Eustache*, Pierre Le Balc'h and Yoann Launey

Critical Care Unit, Department of Anaesthesia, Critical Care and Perioperative Medicine, University Hospital of Rennes, Rennes, France

The benefit of temperature control in sepsis or septic shock is still under debate in the literature. We developed a national survey to assess the current state of knowledge and the practical management of spontaneous septic hypothermia in French intensive care units. Out of more 764 intensivists who were contacted, 436 responded to the survey. The majority of doctors (52.4%) considered spontaneous septic hypothermia to be a frequently encountered situation in intensive care, and 62.1% were interested in this problem. Definition of spontaneous septic hypothermia among French intensivists was not consensual. More than half of the doctors questioned (57.1%) stated that they did not actively rewarm patients suffering from spontaneous septic hypothermia.

KEYWORDS

spontaneous septic hypothermia, sepsis, septic shock, temperature control, hypothermia

Introduction

The benefit of temperature control in sepsis or septic shock is still under debate in the literature (1, 2). The concept of a protective and adaptive effect of fever is controversial (3, 4) and the current randomised trial, SEPSISCOOL 2 (NCT04494074), compares two thermal control strategies for febrile patients in septic shock undergoing mechanical ventilation: namely, maintaining fever and maintaining normothermia via external cooling. However, a recent pilot study of afebrile septic patients found all-cause mortality at 28 days to be lower when hyperthermia (increase in body temperature of +1.5°C) was induced by external rewarming (5). In contrast, spontaneous hypothermia is thought to be associated with increased mortality among patients with sepsis (6). The benefits of induced hypothermia have also been reported in animal studies (7, 8), but clinical benefits have not been demonstrated in mechanically ventilated human patients with septic shock (9).

Unlike accidental hypothermia (10), hypothermia associated with haemorrhagic shock (11), or perioperative-associated hypothermia (12), there is no consensus on the management of spontaneous septic hypothermia. Two surveys—one involving patients in the United Kingdom (13) and the other on a European scale (14)—looked at the practices of different intensivists with regards to hypothermia in septic patients. Both studies revealed great variability in the definition and clinical management of the condition.

The aim of this survey is to assess the current state of knowledge and the practical management of spontaneous septic hypothermia in French intensive care units.

Methods

We developed a national survey containing simple or multiple-choice questions and open questions. In the first phase, the survey was distributed to intensivists in the surgical intensive care unit at Rennes University Hospital. In the second phase, the survey was submitted to doctors working in intensive care units in other departments (medical and cardio-thoracic intensive care) for testing and validation. The questions were revised and adapted according to the comments received. It was then circulated to members of the SFAR (French Society of Anesthesia and Intensive Care Medicine) and SRLF (French Intensive Care Society) societies between 1 March and 4 July 2023. Respondents were asked to answer the survey anonymously, referring to the usual practices within their respective intensive care units. The survey was distributed by e-mail to SRLF members, and also distributed to SFAR members including through social networks.

Statistical analysis

All analyses and graphs were produced using Excel® software. Categorical variables were presented as counts and percentages.

Results

The survey was distributed by e-mail (764 e-mails) and through social networks. However, we do not know exactly how many intensivists actually received the survey and so cannot calculate an accurate response rate. The 2021 French demographic survey reported 2,350 intensivists practicing in France (15), of whom we hope to have contacted close to 50%.

Out of more 764 intensivists who were contacted, 436 responded to the survey. Of these, 405 worked in public hospitals, almost half in general intensive care units. The most represented specialisation was anaesthesiology. Over one-third of respondents declared 0–5 years' experience in intensive care. All French regions were represented, as were the majority of departments (96%). Table 1 presents the characteristics of survey respondents.

The majority of doctors (52.4%) considered spontaneous septic hypothermia to be a frequently encountered situation in intensive care, and 62.1% were interested in this problem. Table 2 illustrates the heterogeneity in the definition of spontaneous septic hypothermia among French intensivists.

More than half of the doctors questioned (57.1%) stated that they did not actively rewarm patients suffering from spontaneous septic hypothermia, but 42.3% of these reported using survival blankets to limit heat loss. The primary reason for not using active rewarming (reported by 76.1% of respondents) was the lack of evidence in the literature and 30% of intensivists who did not use it actually associated the practice with deleterious effects, particularly in terms of haemodynamics. Furthermore, 30% of doctors who did not use active rewarming considered hypothermia to be an adaptive response that should be tolerated, and 5.2% thought it may have beneficial effects.

Of the doctors prescribing active rewarming, 80% used it in cases of sepsis or septic shock and 17% used it for septic shock only. The vast majority (97.3%) administered rewarming using pulsed hot-air blankets, while rewarming through infusion fluids and targeted temperature

TABLE 1 Characteristics of respondents.

Characteristics	All respondents (*n* = 436)
Intensive care, *n* (%)	
Polyvalent	215 (49.3)
Medical	117 (26.8)
Surgical	100 (22.9)
Polyvalent	58 (59.8)
Neurosurgery-neurology	16 (16.5)
Cardiothoracic	23 (23.7)
Paediatrics	4 (0.9)
Hospital, *n* (%)	
Public	405 (92.9)
Academic	262 (60.1)
Nonacademic	143 (32.8)
Private	28 (6.4)
Armies	3 (0.7)
Years of experience, *n* (%)	
0–5 years	170 (39.1)
6–10 years	98 (22.5)
11–15 years	70 (16.1)
16–20 years	33 (7.6)
>20 years	64 (14.7)
Diploma in specialised studies, *n* (%)	
Anaesthesiology	238 (55.1)
Medical	114 (26.4)
Intensivist	52 (12)
Emergency medicine	28 (6.5)
Number of intensive care beds, *n* (%)	
<10	37 (8.5)
10–15	139 (32.1)
16–20	143 (33)
21–25	65 (15)
26–30	41 (9.5)
>30	8 (1.8)
Region, *n* (%)	
Auvergne-Rhône-Alpes	53 (12.3)
Bourgogne-Franche-Comté	12 (2.8)
Bretagne	67 (15.5)
Centre-Val de Loire	22 (5.1)
Corse	1 (0.2)
Grand Est	31 (7.2)
Hauts-de-France	15 (3.5)
Île-de-France	112 (25.9)
Normandie	32 (7.4)
Nouvelle-Aquitaine	16 (3.7)
Occitanie	17 (13.9)
Pays de la Loire	23 (5.3)
Provence-Alpes-Côte d'Azur	25 (5.8)
Outre-mer	6 (1.4)

TABLE 2 The temperatures below which respondents consider a patient with sepsis hypothermic.

Definition of hypothermia (°C)	Response rate (*N* = 418)
33°C	1 (0.2%)
34°C	6 (1.4%)
35°C	107 (25.6%)
35.5°C	36 (8.6%)
35.8°C	4 (1.0%)
36°C	241 (57.7%)
36.3°C	2 (0.5%)
36.5°C	19 (4.5%)
37°C	2 (0.5%)

TABLE 3 The trigger temperature at which respondents consider rewarming patients with hypothermic sepsis.

Trigger for rewarming (°C)	Response rate (*N* = 183)
32°C	1 (0.5%)
33°C	1 (0.5%)
34°C	13 (7.1%)
34.5°C	4 (2.2%)
35°C	66 (36.1%)
35.5°C	50 (27.3%)
36°C	46 (25.1%)
36.5°C	2 (1.1%)

control equipment were rarely used (by 6.5 and 14%, respectively). Most of the doctors who used active rewarming reported starting it at 35°C (Table 3) with a target temperature of 36°C (Table 4). The speed at which hypothermia was corrected was uncontrolled by 52.4% of doctors, with 36.8 and 9.2% reportedly aiming for 0.5 and 1°C/h, respectively.

Regarding the reasons for practising active rewarming, the majority of respondents (69.8%) wanted to combat coagulation disorders induced by hypothermia. Half of the intensivists surveyed used this practice because of the excess mortality associated with septic hypothermia, and 41.2% implemented rewarming for the immunomodulatory effects. Among the other responses, clinical tolerance of hypothermia and shivering were cited by 3% as justification for rewarming, while the prevention of hypothermia-induced cardiovascular events was reported by 4%.

Conclusion

There is currently no consensual definition of spontaneous septic hypothermia, and huge heterogeneity exists in the management of this condition with a poor prognosis. The results of the present survey provide an overview of the clinical practices in French intensive care units, which are mainly based on medical experience or extrapolation from the management of hypothermic non-septic patients (haemorrhagic shock, accidental hypothermia, peri-operative hypothermia). The data presented here highlight the gaps in the current literature on this subject.

TABLE 4 The target temperature to which respondents rewarm patients.

Rewarming target temperature (°C)	Response rate (*N* = 189)
35°C	2 (1.1%)
36°C	88 (49.2%)
36.3°C	1 (0.6%)
36.5°C	37 (20.7%)
37°C	48 (26.8%)
37.5°C	3 (1.7%)

The publications are mainly descriptive or pathophysiological and do not make it possible to identify a clear and consensual definition or a strategy for the therapeutic management of spontaneous septic hypothermia. No therapeutic trial has assessed the impact of active rewarming in terms of morbidity and mortality. This could explain the results of our survey, in which less than half of the doctors questioned practised active rewarming, with the methods and objectives varying widely from centre to centre. Our findings highlight the necessity of further therapeutic trials involving intensive care teams for improving the management of patients with spontaneous septic hypothermia.

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.

Author contributions

GE: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. PB: Writing – review & editing. YL: Writing – review & editing.

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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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EDITED BY
Guo-wei Tu,
Fudan University, China

REVIEWED BY
Fen Liu,
The First Affiliated Hospital of Nanchang University, China
Alberto Rodriguez-Archilla,
University of Granada, Spain

*CORRESPONDENCE
Yuetian Yu
✉ fishyyt@sina.com
Bing Li
✉ libing044162@163.com

†These authors have contributed equally to this work

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Causal relationship between type 1 diabetes mellitus and mycoses: a Mendelian randomization study

Xiaolan Chen[1†], Chen Chen[2†], Mingyan Wu[1], Shanmei Wang[1], Hongbin Jiang[1], Zhe Li[2], Yuetian Yu[2*] and Bing Li[3*]

[1]Department of Emergency, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai, China, [2]Department of Critical Care Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [3]Department of Respiratory and Critical Care Medicine, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai, China

Background: Type 1 diabetes mellitus (T1DM) is frequently associated with various infections, including mycoses; however, the direct link between T1DM and fungal infections remains under-researched. This study utilizes a Mendelian randomization (MR) approach to investigate the potential causal relationship between T1DM and mycoses.

Methods: Genetic variants associated with T1DM were sourced from the European Bioinformatics Institute database, while those related to fungal infections such as candidiasis, pneumocystosis, and aspergillosis were obtained from the Finngen database, focusing on European populations. The primary analysis was conducted using the inverse variance weighted (IVW) method, with additional insight from Mendelian randomization Egger regression (MR-Egger). Extensive sensitivity analyses assessed the robustness, diversity, and potential horizontal pleiotropy of our findings. Multivariable Mendelian randomization (MVMR) was employed to adjust for confounders, using both MVMR-IVW and MVMR-Egger to evaluate heterogeneity and pleiotropy.

Results: Genetically, the odds of developing candidiasis increased by 5% in individuals with T1DM, as determined by the IVW method (OR = 1.05; 95% CI 1.02–1.07, $p = 0.0001$), with a Bonferroni-adjusted p-value of 0.008. Sensitivity analyses indicated no significant issues with heterogeneity or pleiotropy. Adjustments for confounders such as body mass index, glycated hemoglobin levels, and white blood cell counts further supported these findings (OR = 1.08; 95% CI:1.03–1.13, $p = 0.0006$). Additional adjustments for immune cell counts, including CD4 and CD8 T cells and natural killer cells, also demonstrated significant results (OR = 1.04; 95% CI: 1.02–1.06, $p = 0.0002$). No causal associations were found between T1DM and other fungal infections like aspergillosis or pneumocystosis.

Conclusion: This MR study suggests a genetic predisposition for increased susceptibility to candidiasis in individuals with T1DM. However, no causal links were established between T1DM and other mycoses, including aspergillosis and pneumocystosis.

KEYWORDS

diabetes mellitus type 1, mycoses, candidiasis, Mendelian randomization analysis, infections

1 Introduction

Mycoses play a critical role in escalating morbidity and worsening outcomes for certain vulnerable populations, including patients with hematologic and solid organ malignancies, as well as those in critical care settings. The 2008 guidelines clarify the host factors linked with invasive pulmonary mycoses, highlighting conditions that severely weaken the immune system. These include recent occurrences of neutropenia, hematopoietic stem cell transplants, and Acquired Immune Deficiency Syndrome (AIDS) (1). The 2019 update to these guidelines incorporated additional host factors such as solid organ transplantation and the use of B-cell immunosuppressants (2). Despite these detailed guidelines, there are notable limitations in their scope concerning host factors. Approximately 30 to 70% of patients with invasive pulmonary mycoses do not fit the classical host factor criteria specified in the guidelines. In individuals with Type 1 diabetes mellitus (T1DM), compromised immune function related to abnormalities in purine metabolism, chemotactic inflammation, and macrophage activity may increase their vulnerability to fungal infections. For instance, a study involving 1,192 patients newly diagnosed with acute myeloid leukemia identified diabetes mellitus as a risk factor for developing invasive aspergillosis, although it did not show a similar risk for invasive candidiasis (3). Similarly, a retrospective study of deep fungal infections in elderly individuals found that diabetic patients were prone to developing invasive candidiasis, while invasive aspergillosis was less prevalent (4). Despite the established connection between diabetes and bacterial infections, the association between T1DM and mycoses remains a less explored area, presenting an intriguing path for future study (5).

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder characterized by the destruction of pancreatic islet beta cells. This leads to insulin deficiency and elevated blood glucose levels (6). While extensive scientific studies have elucidated the metabolic challenges associated with T1DM, recent inquiries have shed light on a growing concern: the heightened susceptibility of T1DM individuals to mycoses (7). Although diabetes is known to exacerbate fungal infections and lead to poor prognosis, there is limited evidence based on evidence-based medicine. Much of the literature is currently focused on the relationship between type 2 DM and fungal infections (8). As there are fewer retrospective and prospective clinical studies to demonstrate the relationship between type 1 diabetes and fungal infections, we used a Mendelian randomization study to fill an important knowledge gap and possibly the casual relationship between mycoses and T1DM.

Mendelian Randomization (MR) stands as a valuable analytical tool frequently employed in epidemiology to discern causality, leveraging the principles of Mendelian independent assortment. Establishing a plausible causal pathway for MR is paramount. Previous observational research has highlighted numerous associations between T1DM and mycoses, suggesting a potential correlation between these conditions. This study employs a comprehensive MR analysis to uncover the potential causal link between T1DM and mycoses. Furthermore, it utilizes the multivariable MR (MVMR) method to explore confounding factors such as glycated hemoglobin and blood immune cells in the interplay between T1DM and mycoses.

2 Methods

Univariable MR analysis was performed to detect the causal link between T1DM and mycoses in our study (9). MR utilizes genetic variation as a proxy for risk factors; therefore, the instrumental variables (IVs) used in causal inference must adhere to three critical assumptions: 1. Hypothesis of association: a significant correlation exists between single-nucleotide polymorphisms (SNPs) and exposure factors. 2. Hypothesis of independence: SNPs are not associated with confounding factors. 3. Assumption of exclusivity: The impact of SNPs on outcomes is mediated exclusively through exposure factors. The study design is shown on Figure 1. To investigate the direct influence of T1DM on mycoses, we conducted a multivariable Mendelian randomization (MR) analysis, which extends beyond univariable MR by enabling the identification of causal effects involving multiple risk factors concurrently. This paper adheres to the STROBE-MR principle (10).

2.1 Data source

Genetic variations linked to T1DM were sourced from the European Bioinformatics Institute (EBI) database (11). Data on candidiasis, aspergillosis, pneumocystis, and other mycoses were acquired from the FinnGen Consortium (12), involving participants. Adjustment variables such as BMI, HbA1c, lymphocyte count, monocyte count, neutrophil count, CD4 regulatory T cell, CD4+ T cell, CD8+ T cell, Nature Kill (NK) T cell count, and B cell absolute count were also sourced from the EBI database.

2.2 IVs selection

The threshold of instrumental variables (IVs) was established at 5×10^{-6} (13, 14). To filter out SNPs with a linkage disequilibrium (LD) threshold of $r^2 < 0.001$ within a 10,000 kb range, the clumping procedure was implemented using R software version 4.2.0. The F statistic was utilized as a key indicator of the statistical robustness of the IVs. Calculation of the F statistic was based on the formula $F = R^2(N-K-1)/K(1-R^2)$ (15).

2.3 Statistical analysis

The inverse variance weighted (IVW) method was used as the main approach, complemented by additional methods such as MR-Egger, weighted median, simple mode, and weighted mode (16). We calculated odds ratios (OR) along with their 95% confidence intervals (CI), considering statistical significance to be present at $p < 0.05$. We use the Bonferroni method to correct the p-value. SNP heterogeneity was assessed using Cochran's Q statistic and corresponding p-values to test for horizontal pleiotropy among the selected IVs. To assess heterogeneity, we employed both the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) method and the MR-Egger approach (9, 17). Funnel plots showed the robustness of the correlation and absence of heterogeneity. MVMR analyses were conducted to adjust for confounding factors (17) including BMI (18), HbA1c, neutrophil count, lymphocyte count,

monocyte count, and lymphocyte classification. MVMR-IVW and MVMR-Egger were performed to detect heterogeneity and potential pleiotropy (19). HbA1c was selected as a confounding variable based on research suggesting that elevated blood sugar levels, rather than diabetes alone, significantly increase morbidity and mortality from infectious diseases. Patients with diabetes mellitus exhibit reduced neutrophil chemotaxis, phagocytosis, intracellular bactericidal activity, and limited lymphocyte activation, potentially increasing susceptibility to fungal infections (20). Therefore, we corrected for confounding factors such as neutrophil count, lymphocyte count, monocyte count, and lymphocyte classification (including CD4 regulatory T cell, CD8+ T cell, CD4+ CD8dim T cell, Natural Killer T cell, and B cell Absolute Count). All analyses were conducted as two-sided tests using the Two Sample MR package (version 0.5.7) within R software (version 4.2.0).

3 Results

3.1 Exploration of the causal effect of T1DM onset on candidiasis

A total of 153 SNPs were identified as IVs for this study, each demonstrating robustness with F-statistics exceeding 10 (ranging from 18.09 to 1772.13), which underscores their suitability for evaluating the relationship between T1DM and candidiasis. All SNP details are provided in Supplementary Table S1. The GWAS data for T1DM were visually represented on a Manhattan plot (Figure 2). Figure 3 illustrates the individual impact of each SNP on the risk of developing candidiasis. Various analytical methods consistently demonstrated an increased risk of candidiasis among T1DM patients: the IVW method showed an OR of 1.05 (95% CI

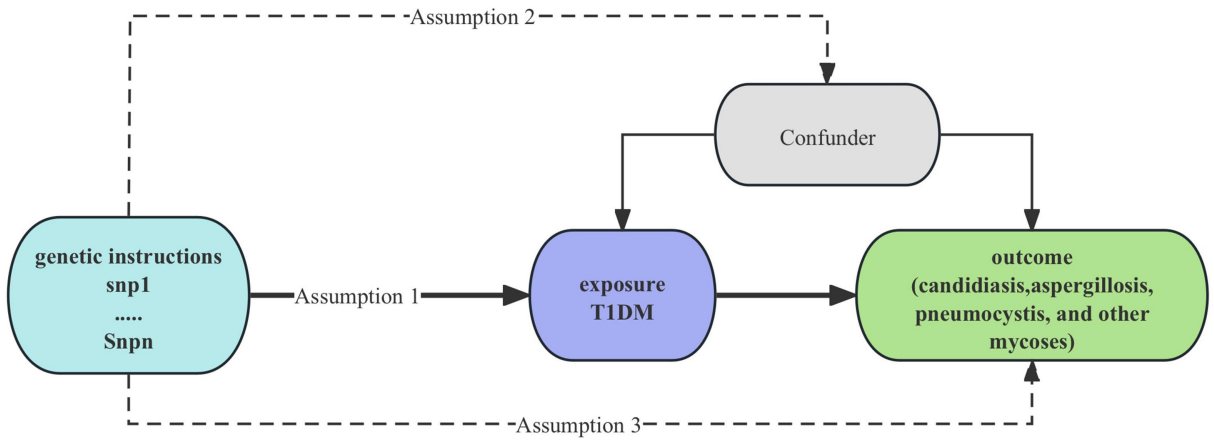

FIGURE 1
Study design of causality Between T1DM and mycoses. T1DM, type 1 diabetes mellitus; snp, single-nucleotide polymorphisms.

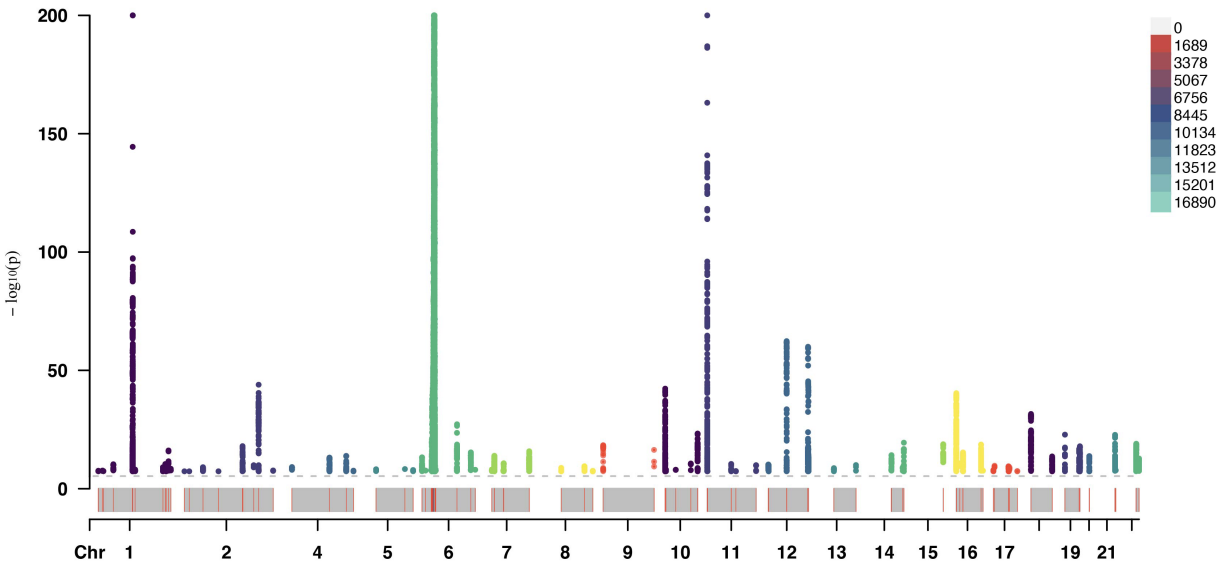

FIGURE 2
GWAS information of T1DM. GWAS, genome-wide association study.

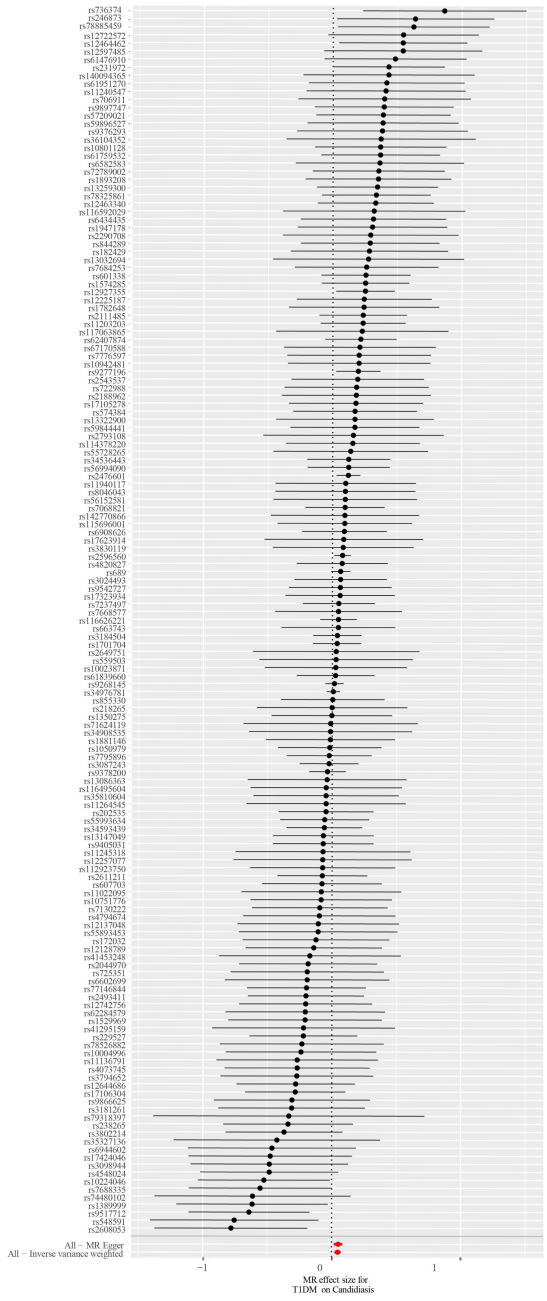

FIGURE 3
The forest plot depicts the impact of each SNP on candidiasis. Black lines represent the effect of an individual SNP, while a red line signifies the causal estimation using all ivs. If the solid line is positioned entirely to the left of 0, it suggests that T1DM may reduce the likelihood of candidiasis based on this SNP. Conversely, it implies that T1DM might potentially increase the risk of candidiasis. An intersection of the solid line with 0 indicates an insignificant result. SNP, single-nucleotide polymorphisms; ivs, instrumental variables; T1DM, type 1 diabetes mellitus.

1.02–1.07, $p < 0.001$), MR-Egger yielded an OR of 1.05 (95% CI 1.02–1.09, $p = 0.003$), the weighted median method reported an OR of 1.05 (95% CI 1.01–1.09, $p = 0.025$), and the weighted mode method indicated an OR of 1.04 (95% CI 1.01–1.07, $p = 0.01$), with all tests showing consistent beta directions (Table 1; Figure 4). Figure 5 presents a scatter plot which validates the increased risk of candidiasis in T1DM patients. The univariate MR analysis indicated no significant heterogeneity, as shown by the MR-Egger ($p = 0.17$) and IVW ($p = 0.18$) tests. Furthermore, no significant pleiotropy was detected, with the MR-Egger intercept revealing no substantial influence (intercept = −0.00099; $p = 0.76$). The MR-PRESSO test confirmed the absence of outliers (Table 2), and the symmetry of selected SNPs was clear in the funnel plot (Figure 6). Additionally, the scatter plot visually reinforced the causal link between T1DM and candidiasis. The forest plot displayed effect sizes for individual SNPs on the risk of candidiasis, confirming the causal relationship. Notably, the "Leave-one-out" plot analysis demonstrated that no single SNP significantly influenced the estimated causal association (Figure 7).

3.2 MVMR analyses the causal effect of T1DM onset candidiasis

The MVMR-IVW estimates revealed a significant causal relationship between T1DM and candidiasis, even after adjusting for BMI, HbA1c, and counts of neutrophils, lymphocytes, and monocytes (OR=1.08; 95% CI, 1.03–1.13; p=0.0006). The analysis showed no heterogeneity (MR-IVW, p=0.30; MR-Egger, p=0.29) and no potential pleiotropy (mv-pleiotropy, p=0.30). The robustness of these instruments is underscored by an F-statistic of 14.98. Further adjustments for immune cell classifications, including CD4 regulatory T cells, CD8+ T cells, CD4+ T cells, NK T cells, and B cell absolute counts, reinforced the causal link between T1DM and candidiasis. The adjusted MVMR-IVW analyses yielded an OR of 1.04 (95% CI, 1.02–1.06; p=0.0002), with no detected heterogeneity (MR-IVW, p=0.77; MR-Egger, p=0.75) or pleiotropy (mv-pleiotropy, p=0.95). The strength of this model is highlighted by an F-statistic of 86.73, suggesting that the instrumental variables are highly relevant and the findings are statistically sound.

3.3 Exploration of the causal effect of T1DM onset pneumocystosis, aspergillosis, and other mycoses

In the MR analyses exploring the effects of T1DM on pneumocystosis, aspergillosis, and other mycoses, we utilized 153, 147, and 153 SNPs as IVs, respectively. All IVs demonstrated robustness with F-statistics exceeding 10, confirming their suitability for the analysis. Detailed SNP information for each condition can be found in Supplementary Tables S2–S4. Our findings indicated no causal relationship between T1DM and pneumocystosis, aspergillosis, or other mycoses when analyzed using the IVW method. Similar results were consistent across additional MR methods as detailed in Table 1. Furthermore, the analyses showed no signs of pleiotropy or heterogeneity in the relationship between T1DM and these mycoses, as shown in Table 2. Visual aids such as forest plots, scatter plots, funnel plots, and leave-one-out plots for each mycosis have been detailed in Supplementary Figures S1–S12, respectively. These visualizations further corroborate the robustness and transparency of our findings, illustrating the analytical process and outcomes comprehensively.

TABLE 1 Results of MR analyses.

Outcome	MR methods	SNP number	OR(95%CI)	*p* value
Candidiasis	MR Egger	153	1.05 (1.02–1.09)	0.003
	Weighted median	153	1.05 (1.01–1.09)	0.025
	Inverse variance weighted	153	1.05 (1.02–1.07)	<0.001
	Simple mode	153	1.02 (0.94–1.11)	0.586
	Weighted mode	153	1.04 (1.01–1.07)	0.01
Pneumocystosis	MR Egger	153	1.07 (0.96–1.18)	0.24
	Weighted median	153	1.09 (0.97–1.22)	0.17
	Inverse variance weighted	153	1.03 (0.95–1.11)	0.50
	Simple mode	153	1.13 (0.84–1.52)	0.42
	Weighted mode	153	1.09 (0.99–1.20)	0.093
Aspergillosis	MR Egger	147	0.89 (0.71–1.10)	0.28
	Weighted median	147	0.99 (0.76–1.30)	0.95
	Inverse variance weighted	147	0.94 (0.80–1.10)	0.43
	Simple mode	147	0.91 (0.51–1.63)	0.75
	Weighted mode	147	0.91 (0.74–1.11)	0.35
Other mycoses	MR Egger	153	1.04 (0.81–1.32)	0.76
	Weighted median	153	1.04 (0.78–1.39)	0.80
	Inverse variance weighted	153	1.09 (0.91–1.30)	0.35
	Simple mode	153	1.08 (0.55–2.12)	0.83
	Weighted mode	153	1.14 (0.9–1.44)	0.28

MR, Mendelian randomization; SNP, single-nucleotide polymorphisms; OR, odd ratio; CI, confidence intervals; MR Egger, Mendelian randomization-egger regression.

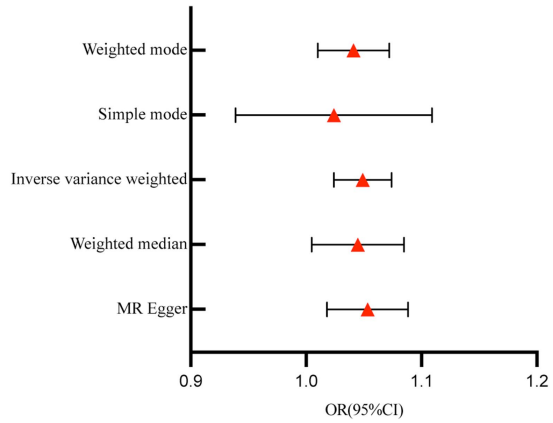

FIGURE 4
Forest plot for the effect of T1DM on candidiasis. T1DM, type 1 diabetes mellitus; OR, odds ratios; CI, confidence intervals.

4 Discussion

Our comprehensive MR investigation revealed a significant causal association between T1DM and candidiasis. Cochran's Q test indicated no heterogeneity, a finding further supported by the symmetrical distribution observed in the funnel plot. Moreover, both the MR PRESSO and MR Egger tests detected no evidence of horizontal pleiotropy. Figure 5 illustrates that all MR test results pointed in the same positive direction, confirming a consistent correlation between T1DM and the occurrence of candidiasis. Figure 6 confirms the absence of heterogeneity in the results, while Figure 7 demonstrates that no single SNP significantly impacts the estimated causal association. These sensitivity analyses underscore the robustness of our results. Further, in the MVMR analyses, the significant association between T1DM and candidiasis persisted even after adjustments for BMI, HbA1c, and counts of neutrophils, lymphocytes, and monocytes. Additionally, our study indicates that T1DM may not be implicated in the development of certain other mycoses, including aspergillosis and pneumocystis, suggesting a more specific immunological interaction with candidiasis. This nuanced understanding could guide more targeted approaches in the management and prevention of mycoses in patients with T1DM.

The main findings of our research align with prior investigations in the field. In a study involving 32 young females with T1DM, Candida species were detected in 52.5% of cases (21), significantly higher than the control group's rate of 18.2%. Prevalent species included *C. albicans* (72.7%), *Candida glabrata* (22.7%), *Candida tropicalis* (2.3%), and *Candida parapsilosis* (2.3%) (22). In a study, a 75.5% prevalence of gastrointestinal candidiasis was reported among patients diagnosed with T1DM, while other investigations indicated rates ranging from 2.5 to 9.7% (23). Moreover, elevated HbA1c levels were associated with oral candidiasis and gingivitis in pediatric T1DM patients. Candidiasis affecting skin folds and mucous membranes, have been documented in more than 5% of patients (24). Candida species are the primary fungal colonizers of the urinary tract in diabetic individuals (25), with infection occurring in the presence of symptoms or pyuria. Moreover, investigations into candidemia among intensive care unit patients underscore diabetes mellitus as a notable

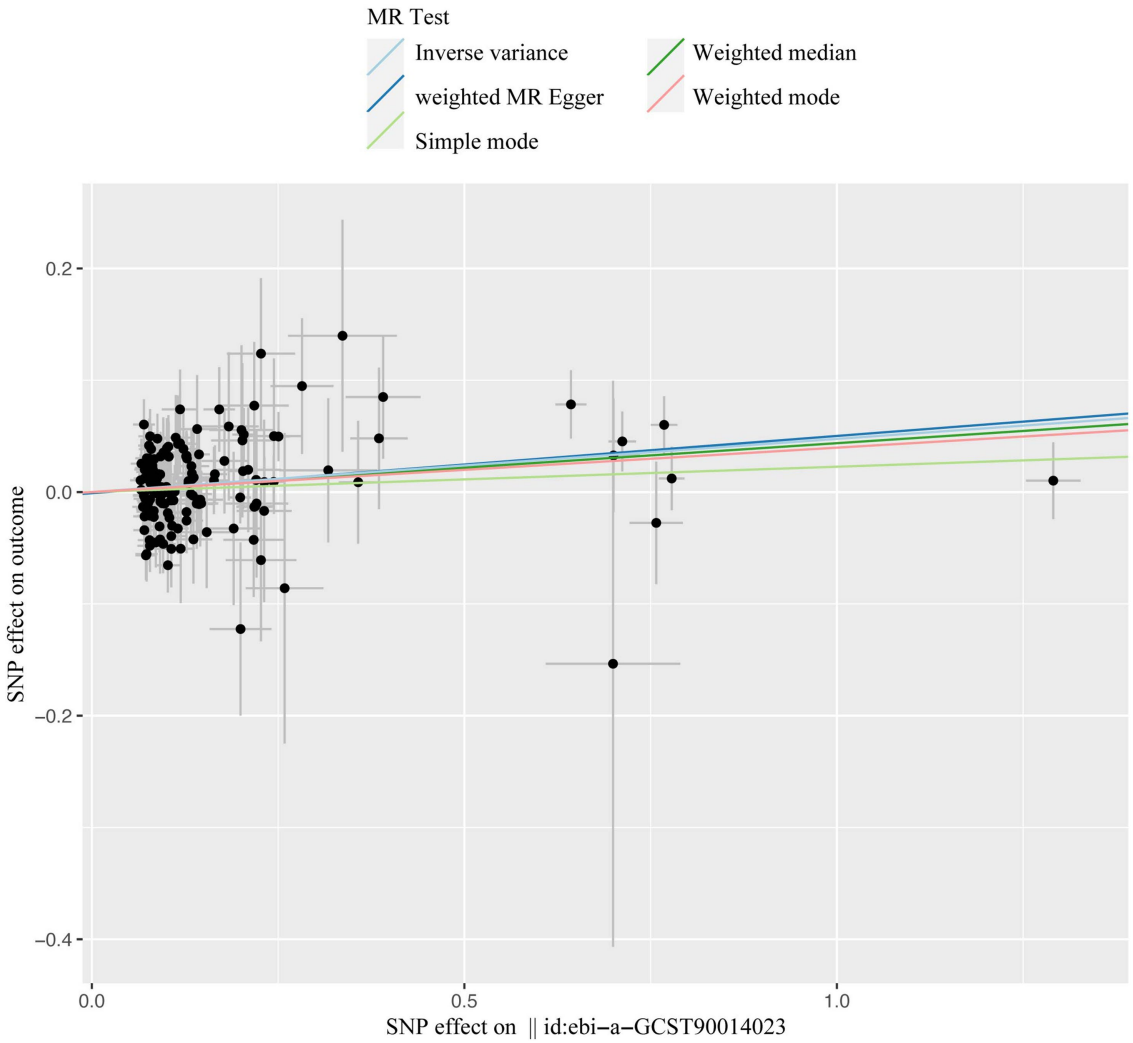

FIGURE 5
The scatter plot of casual effect of T1DM on candidiasis. Black data points represent ivs, with the horizontal axis indicating the influence of SNPs on T1DM, and the vertical axis indicating the influence of SNPs on candidiasis occurrence. Colored lines depict the results of MR analysis utilizing five different methods. T1DM, type1 diabetes mellitus; ivs, instrumental variables; SNP, single-nucleotide polymorphism; MR, Mendelian randomization.

TABLE 2 Sensitive analysis of T1DM on outcomes.

Outcome	Heterogeneity		Pleiotropy		Outliers (MR-PRESSO)
	IVW	MR Egger	Intercept	*p* value	
Candidiasis	0.18	0.17	−0.00099	0.76	No
Pneumocystosis	0.95	0.95	−0.0102	0.31	No
Aspergillosis	0.59	0.58	0.016	0.45	No
Other mycoses	0.62	0.61	0.013	0.58	No

IVW, inverse variance weighted; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; MR Egger, Mendelian randomization-egger regression.

risk factor for candidemia onset. Dissemination of candidemia to the lungs can lead to secondary pulmonary candidiasis (26).

A study investigated the association between diabetes and both the isolation and infection with *aspergillus species* (27). In a study by Sun et al. (28) in 2017, it was highlighted that diabetes mellitus had a prevalence of 18.4% among 407 patients diagnosed with invasive pulmonary aspergillosis (IPA), ranking as the second most common underlying condition after hematological malignancies. Additionally, Xu et al. (29) observed that 30% of patients with chronic obstructive pulmonary disease (COPD) who developed IPA also had diabetes, contrasting with only 6.7% of COPD patients without aspergillosis having diabetes ($p<0.05$). Collectively, these studies suggest that

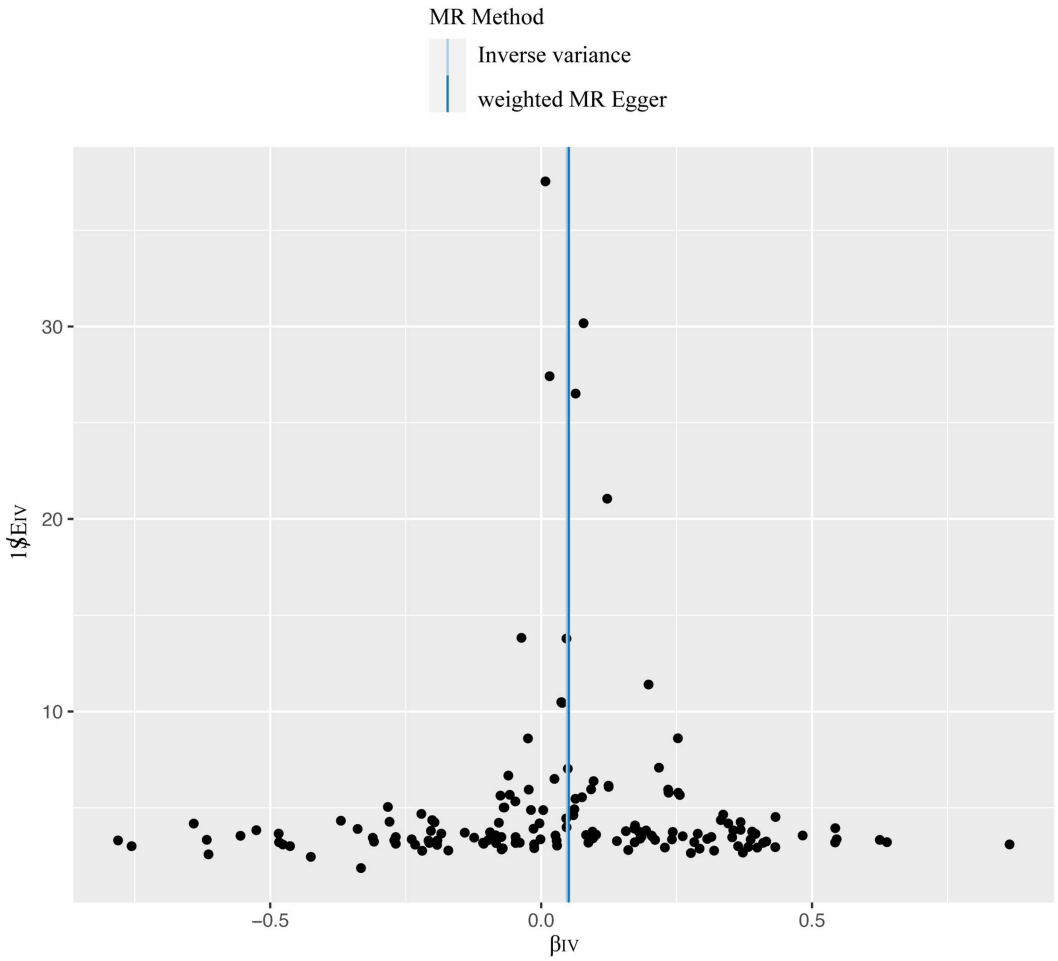

FIGURE 6
The overall heterogeneity test was conducted to assess the impact of T1DM on candidiasis. SNPs are depicted by black points, and their distribution is evenly spread around the IVW and MR-Egger line. T1DM, type 1 diabetes mellitus; SNP, single-nucleotide polymorphism; IVW, inverse variance weighting.

diabetes mellitus may heighten the likelihood of developing invasive pulmonary aspergillosis, particularly when combined with other risk factors. Nonetheless, larger epidemiological studies are needed to validate the association between diabetes and aspergillosis (30), and the current research does not genetically support a higher predisposition to aspergillosis in patients with T1DM.

A comprehensive review of current literature highlights several factors contributing to the occurrence of pneumocystis pneumonia (PCP). Advanced age, lymphopenia, the administration of high-dose steroids, triple immunosuppression, and specific comorbidities such as chronic obstructive pulmonary disease (COPD) have been identified as influential factors. Furthermore, epidemiological research conducted in Japan has shed light on prevalent comorbidities associated with PCP among adults. This study revealed that hematologic malignancies (31%) and diabetes (30%) were the most commonly observed comorbidities in individuals diagnosed with PCP. These findings underscore the importance of considering these risk factors in clinical assessments and management strategies for PCP (31). Studies have indicated that older age, gender, type of transplant, cytomegalovirus infection, allograft rejection, and immunosuppression are significant variables that increase the risk of developing pneumocystis pneumonia post-transplant (32). However, these studies have primarily stemmed from studies conducted in individual medical centers. Reports suggest that around 1 to 2% of patients with rheumatologic disorders (33), particularly those undergoing immunosuppressive treatment, have developed PCP. Overall, the limited direct research on the correlation between diabetes mellitus and pneumocystis pneumonia mainly consists of retrospective epidemiological investigations from single-center studies with numerous confounding factors. Our study proposes that T1DM may not have a genetic association with pneumocystis pneumonia, a hypothesis that warrants further confirmation through prospective studies.

In Europe and the United States, *Trichinella* infections primarily affect patients undergoing chemotherapy for hematologic malignancies, solid organ transplants, or bone marrow transplants. Conversely, in Asian countries, diabetic patients experience a higher incidence of *Trichinella* infections. *Cryptococcal* infections, on the other hand, are most prevalent in immunocompromised patients. In a retrospective study examining *cryptococcosis* in patients with diabetes mellitus, it was found that 62% of the patients had poor glycemic control. This observation suggests a potential relationship between blood glucose levels and the occurrence of *cryptococcal* infections (34). Similarly, we found no exceptions to their association

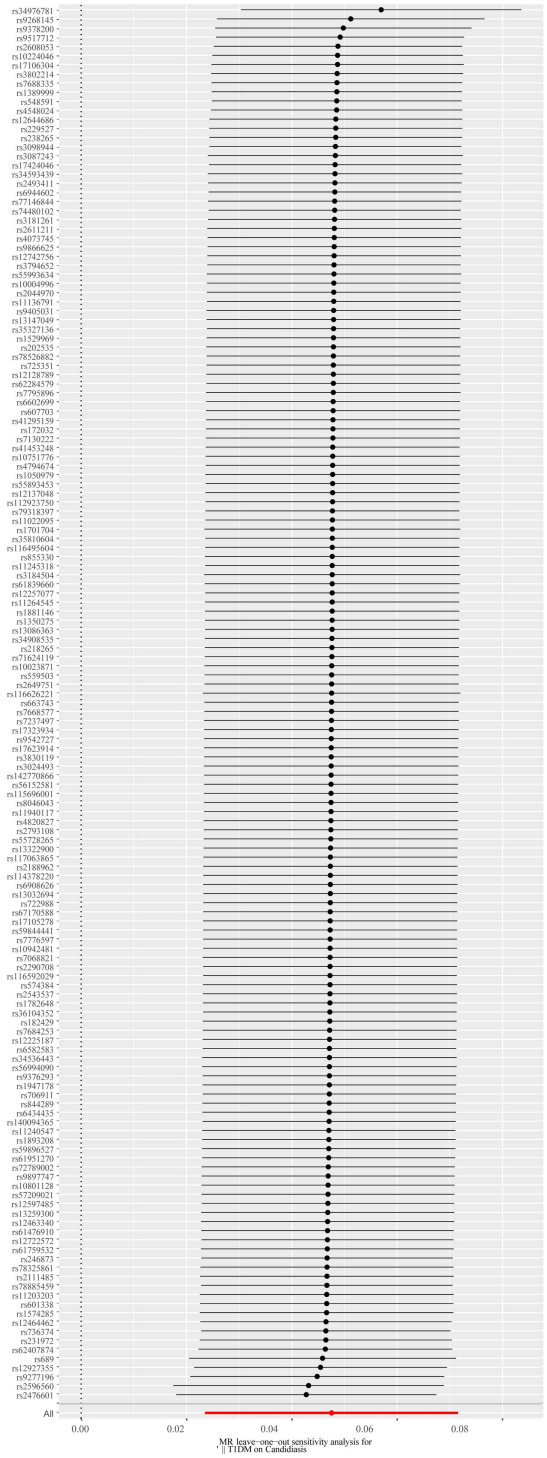

FIGURE 7
The forest plot of the leave-one-out analysis. The red data point's position above 0 suggests a positive outcome. The black dots are situated to the right of the invalid line, suggesting that the removal of any of the SNPs will have minimal impact on the overall results. SNP, single-nucleotide polymorphism.

with T1DM genetics. High glucose and acidic environments facilitate the growth and proliferation of molds. Iron ions are also essential for mold growth. In diabetic ketoacidosis, when serum pH decreases due to acidosis, the transport capacity of transferrin for iron is inhibited, leading to an increase in serum free iron, which promotes mold growth. The results of a prospective multicenter study showed that among 50 patients with pulmonary trichinosis (clinically diagnosed or above), 57% had poorly controlled diabetes, 18% had ketoacidosis, and observations highlighted diabetes as an autonomous risk factor for the initiation of pulmonary trichinosis (35, 36). However, there is a lack of separate GWAS databases for *Trichoderma* and *Cryptococcus*, so separate Mendelian randomization studies are not possible.

Elevated blood sugar levels have adverse effects on the immune system, altering tissues, skin, and blood circulation, thereby increasing susceptibility to infections. Specifically, high blood sugar levels and inadequate insulin levels suppress vital components of the body's innate immune response to pathogens and impede the production of pro-inflammatory cytokines. Studies have shown that individuals with Type 1 diabetes mellitus exhibit reduced secretion of interleukin 1 and interleukin 6 by mononuclear cells and monocytes (7, 37). Additionally, chronic hyperglycemia impairs the mobilization, chemotaxis, and phagocytosis of polymorphonuclear leukocytes, further compromising the body's ability to combat infections (38, 39). Elevated blood sugar levels, also known as hyperglycemia, disrupt antimicrobial activity through various mechanisms. These include increased apoptosis, diminished mobility of polymorphonuclear cells across endothelial layers, and inhibition of glucose 6-phosphate dehydrogenase (40). Moreover, individuals diagnosed with T1DM demonstrate compromised complement activity, marked by diminished C4 levels, impaired cytokine production, and dysfunction of polymorphonuclear cells (37).

The design of the MR study, aimed at mitigating confounding factors and addressing reverse causality inherent in epidemiological studies, stands out as a crucial strength of this research. Furthermore, a comprehensive exploration of the causal links between T1DM and fungal infections was meticulously conducted. All selected IVs exhibited robustness, as indicated by F-statistics exceeding 10. Moreover, the absence of pleiotropy provided further support for the accuracy of our findings. Upon establishing a causal relationship between T1DM and candidiasis, three separate MVMR analyses were performed to validate the results by adjusting for BMI and HbA1c. Notably, MVMR Egger yielded reliable estimates despite significant pleiotropy, with no observed heterogeneity. The consistency across outcomes obtained through the three methodologies underscores the credibility of our findings. It is important to acknowledge that the datasets used for exposure and outcome variables primarily comprised European populations.

The current study presents several advancements over previous research. Most notably, it is the first MR study to elucidate the genetic causal links between T1DM and mycoses. A major strength of this approach is the MR design itself, which significantly reduces the risk of reverse causality and confounding factors affecting the results. By focusing on populations of the same ethnicity, this study also effectively minimizes racial and ethnic disparities that could skew findings. However, the study is not without limitations. Firstly, it's important to recognize that the results may still be influenced by other potential confounders not accounted for in the analysis. Secondly, the reliance on Finnish and EBI databases could introduce bias and limit the generalizability of the findings. Additionally, the absence of GWAS data for *mucormycosis* and *cryptococcosis* in the EBI database compelled us to rely solely on data from the FinnGen database for these and other mycoses. Lastly, setting a *p*-value threshold of 5×10^{-6} may increase the risk of false positives in our findings.

In conclusion, our MR analysis supports a genetic predisposition for increased susceptibility to candidiasis in individuals with T1DM. However, the study did not establish a causal relationship between T1DM and other fungal infections, such as aspergillosis, pneumocystis, and other types of mycoses. This specificity in the interaction between T1DM and candidiasis may inform future research and clinical approaches.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

XC: Data curation, Formal analysis, Resources, Writing – original draft, Writing – review & editing. CC: Data curation, Formal analysis, Writing – original draft. MW: Investigation, Writing – review & editing. SW: Supervision, Writing – review & editing. HJ: Resources, Writing – review & editing. ZL: Methodology, Project administration, Writing – review & editing. YY: Data curation, Funding acquisition, Resources, Software, Validation, Writing – original draft, Writing – review & editing. BL: Data curation, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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.2024.1408297/full#supplementary-material

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EDITED BY
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

REVIEWED BY
Martin Helán,
St. Anne's University Hospital, Czechia

*CORRESPONDENCE
Xiangcheng Zhang
✉ Zhxc0318@163.com

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Progress in the study of pentraxin-3(PTX-3) as a biomarker for sepsis

Yi Zhang, Xuelin Li, Xiaobei Zhang, Tiantian Wang and Xiangcheng Zhang*

Department of Critical Care Medicine, The Affiliated Huai'an No 1 People's Hospital of Nanjing Medical University, Huai'an, Jiangsu, China

Sepsis is a intricate pathological process characterized by life-threatening organ dysfunction resulting from a dysregulated host response to infection. It stands as a prominent cause of mortality among critically ill patients globally. The pivotal focus in sepsis management lies in the early identification and prompt administration of antimicrobial agents. Owing to the constraints of current diagnostic methodologies, marked by insufficient sensitivity and delayed outcomes, extensive research has been undertaken to ascertain novel biomarkers for sepsis. In this review, we provide an overview discussing the latest advancements in the study of PTX-3 as a biomarker for sepsis. We acknowledge pivotal discoveries from preceding research and engage in discourse regarding the challenges and limitations confronted by PTX-3 as a sepsis biomarker.

KEYWORDS

sepsis, pentraxin-3 (PTX-3), biomarker, diagnosis, prognosis

1 Introduction

Sepsis is characterized by life-threatening organ dysfunction resulting from a dysregulated host response to infection (1). Recent research estimates globally recorded sepsis cases at 489 million, with reported sepsis-related deaths reaching 11 million, accounting for 19.7% of global mortality (2). In China, a study indicated a higher burden of sepsis in hospitalized patients compared to estimates (3). Therefore, early diagnosis of sepsis is crucial, as it allows for the prompt initiation of supportive and antibiotic therapy to reduce sepsis mortality. However, due to the complexity and heterogeneity of sepsis, accurate early diagnosis and prognosis assessment are challenging. Traditional diagnostic methods are both time-consuming and expensive, and require operation by professionally medically trained personnel (4). Blood culture is the gold standard for diagnosing sepsis; however, it has a high false-negative rate and often results in delayed outcomes (5, 6). Over the past few decades, extensive research has explored various biomarkers, utilizing them to diagnose and predict outcomes in sepsis patients, playing crucial roles in the pathophysiology of sepsis.

Pentraxin-3 (PTX-3), also known as human serum penetratin-3, is a representative member of the long-chain pentraxin subfamily in the pentraxin family (7). PTX-3 is a new type of acute-phase inflammatory factor and plays an important role in infectious diseases. In recent years, it has been found that the PTX-3 level increases sharply in the blood of sepsis patients, and may be superior to traditional biomarkers in judging the severity of their condition and prognosis assessment. The purpose of this review is to summarize the current literature on one of the sepsis biomarkers, PTX-3, to provide a comprehensive overview of the research progress on PTX-3 as a sepsis biomarker. This aims to offer a brief outlook for further research on the sepsis biomarker PTX-3.

2 Pentraxin-3(PTX-3)

Pentraxins is an evolutionarily conserved protein superfamily characterized by a structural motif, that is, the pentraxin domain (8). Pentraxin-3 is a 45-kilodalton protein that forms high-molecular-weight oligomers through interchain disulfide bonds (9). The C-terminal domain (203 amino acids) of PTX-3 has homology with classic short pentameric proteins such as C-reactive protein (CRP) and serum amyloid protein P (SAP), while its N-terminal domain (178 amino acids) has no significant homology with other known proteins.

Unlike classic short pentameric proteins such as CRP and SAP that are systemically produced by hepatocytes, PTX-3 is produced by various cell types and shows differences in genomic organization, cellular source, and ligand-binding characteristics (8). Due to significant differences in sequence and regulation, CRP (which is not an acute-phase protein in mice) cannot be used in genetic methods to study its *in vivo* functions, while PTX-3 remains highly conserved throughout the evolutionary process. Notably, PTX-3 plays a complex and irreplaceable role *in vivo*, being able to recognize various pathogens, regulate complement activity by binding to C1q, and promote the recognition of pathogens by macrophages and dendritic cells. PTX-3, as a liquid-phase effector molecule of the innate immune system, its production is stimulated by cytokines and bacterial endotoxins. PTX-3 can bind to specific pathogens, activate complement, promote cell recognition and clearance, and can also act as a matrix component (8).

PTX-3 is an acute-phase reactant with relatively low blood levels under normal circumstances (about 25 ng/mL in mice and < 2 ng/mL in humans). It has been reported that human plasma PTX-3 levels are related to gender, significantly lower in men than in women, and increase significantly with age. In infectious shock, sepsis, and other inflammatory and infectious states, plasma PTX-3 levels increase sharply, reaching a peak level within 6–8 h (10), and its concentration can increase up to 100 ng/mL during sepsis (11). When injury occurs, the PTX-3 level in the blood is extremely high, which is related to the release of pre-formed PTX-3 in neutrophils. Maugeri et al. (12) clearly described this, reporting that neutrophils led to an increase in plasma PTX-3 concentration within 6 h after the appearance of clinical symptoms of acute myocardial infarction and returned to normal within 48 h. Studies using *in vivo* neutrophil depletion and multiple vascular proteomics have concluded that PTX-3 may be stored and released in a polymeric form. Proteomic analysis of the aorta of mice injected with lipopolysaccharide (LPS) showed that PTX-3 was the most upregulated protein, and polymeric PTX-3 was deposited along with other neutrophil-derived proteins as early as 2 h after LPS injection. In healthy volunteers, a rapid degranulation reaction was observed 6 h after LPS injection, followed by an acute-phase response (13). In addition, studies have shown that plasma PTX-3 levels increase as the glomerular filtration rate (GFR) decreases (14), and the increase in liver PTX-3 levels in liver necrosis may be a marker of acute histological liver injury (15).

PTX-3 has a tendency to rise faster in the pathogenesis of sepsis than the previously used biomarkers and is superior to traditional biomarkers, which may be due to the fact that it is locally produced at the site of infection or tissue damage rather than relying on other molecules to trigger the synthesis of body organs. In contrast, CRP is systemically generated by liver cells in response to IL-6 stimulation, and the process takes longer and only begins to change significantly after 24–30 h (16). The comparison of PTX-3 with other common biomarkers is shown in Table 1.

The overexpression of PTX-3 will exacerbate the inflammatory response and reduce the survival rate of mice subjected to intestinal ischemia–reperfusion injury (17). The data collected from various pathological processes indicate that there is a correlation between plasma PTX-3 level and disease severity, suggesting the potential role of PTX-3 as a pathological biomarker. Whether the significant correlation between the result and the severity reflects its role in the pathogenesis of the injury mechanism, such as amplifying the complement and coagulation cascade reactions, remains to be elucidated (18). Elevated PTX-3 levels have been observed in various infectious diseases including sepsis, septic shock, aspergillus infection, tuberculosis and dengue fever (19, 20). PTX-3 is also expressed in aseptic inflammation. It has been reported that the PTX-3 level of patients with acute coronary syndrome increases by about 6–7 ng/mL (21, 22), the PTX-3 level of patients with congestive heart failure increases (about 3–4 ng/mL) (23), the PTX-3 level in renal failure increases by about 5–6 ng/mL (14, 24), and the PTX-3 level in acute respiratory distress syndrome increases by about 70 ng/mL (25). In addition, in patients with AMI with ST-segment elevation, PTX-3 can predict the 3-month mortality rate after adjusting important risk factors and other acute phase prognostic indicators (26). Early detection of PTX-3 level is an independent indicator for predicting multiple organ dysfunction syndrome (MODS) and premature death in patients with cardiac arrest (27, 28). In autoimmune diseases, PTX-3 mediates the complement regulatory mechanism, leading to inflammation and tissue damage in RA (29–31). PTX-3 may be a new non-invasive biomarker indicating the clinical arthritis activity of RA patients (32). In addition, PTX-3 is significantly correlated with the activity degree of SLE (33, 34). PTX-3 may participate in the pathogenesis of psoriasis and can indicate the disease activity of psoriasis (35, 36). The increase of PTX-3 level during the acute attack of acute rheumatic fever may help predict the clinical course (37). PTX-3 can also be used for the early severity assessment and prediction of acute pancreatitis (AP) (38). However, PTX-3 is not as good as CRP and APACHE II score in predicting the mortality of AP, and the combination of PTX-3 and CRP cannot improve the predictive value of CRP (39).

In conclusion, PTX-3 is a multifunctional protein at the intersection of immunity, inflammation, extracellular matrix

TABLE 1 Comparison of PTX-3 with other commonly used biomarkers in sepsis.

	PTX-3	CRP	PCT
Normal values	< 2.0 ng/mL	0.8 mg/dL	< 0.5 ng/mL
Source	Neutrophils and diverse cells	Liver	Macrophages and diverse cells
Time to increase after insult	2 h	4-6 h	3-4 h
Time to peak concentration	6-8 h	36-50 h	6-24 h
Half-life	NR	19 h	22-35 h
Chronic liver failure	Elevation	Slight decrease	No effect
Renal failure	Elevation	No effect	Elevation
Renal replacement therapy	NR	No effect	Decrease

PTX-3, Pentraxin-3; CRP, C-reactive protein; PCT, Procalcitonin; NR, Not Report.

construction, and female reproduction (8, 40). In all these cases, the PTX-3 level is correlated with the clinical activity of the disease, making it a candidate biomarker for disease monitoring (41).

3 Diagnostic value

As an acute-phase reactant protein, PTX-3 has been widely studied as a biomarker to distinguish common bacterial infections from sepsis or septic shock. The results of one study showed that the area under the curve (AUC) for the discrimination between the healthy control group and the SIRS group was 0.922 (cut-off value 16.0 ng/mL, sensitivity 89.1%, specificity 85%), and the difference was statistically significant (42). At the same time, among suspected infection patients visiting the emergency department, the AUC of PTX-3 in predicting severe sepsis from day 0 to day 28 was 0.73 (cut-off value 14.1 ng/mL, sensitivity 63%, specificity 80%) (43). Another study showed that in adult febrile patients in the emergency department, the AUC for predicting bloodstream infection was 0.71, the critical value was 16.1 ng/mL, and the sensitivity was 76% and the specificity was 61% (44). Hamed et al. (45) studied the PTX-3 level on the 1st, 3rd, and 8th days of treatment in ICU sepsis patients and found that at the cut-off value of 5 μg/L, the lowest sensitivity and specificity were 92% and 64%, respectively. Moreover, this study defined a unified diagnostic boundary, diagnosing sepsis at least (≥5.0 ng/mL) and septic shock (≥9.0 ng/mL) (45).

Similar to adults, PTX-3 can be used as a reliable biomarker for neonatal sepsis with high sensitivity and specificity. One study showed that when using the PTX-3 cut-off value of 5.6 μg/L to diagnose sepsis, the sensitivity was 98.3%, the specificity was 96.7%, the positive predictive value (PPV) was 98%, and the negative predictive value (NPV) was 96%. While the critical value of CRP was 8 mg/dL, the sensitivity was 96.7%, the specificity was 96.7%, the positive predictive value (PPV) = 98.3%, and the negative predictive value (NPV) = 93.5%, and the accuracy (area under the curve) = 0.989 (46). Although this study may have some limitations, for the diagnosis of neonatal sepsis, the sensitivity of biomarkers is more important than the specificity, so PTX-3 seems to be superior to CRP as a diagnostic biomarker for neonatal sepsis.

4 Prognostic value

Abnormally elevated plasma PTX-3 levels are closely related to the mortality rate of sepsis and have important significance in predicting the risk of death for sepsis patients. Multiple studies have shown that the systemic PTX-3 level of critically ill patients with a fatal outcome is significantly higher than that of surviving critically ill patients (25, 42, 47–50). Among sepsis patients, the maximum PTX-3 value of non-surviving patients in the first to fourth days is significantly higher than that of surviving patients (44.8 vs. 6.4 ng/mL, $P<0.001$), and the AUC for predicting the case fatality rate is 0.82 (cut-off value 15 ng/mL, sensitivity 72%, specificity 81%) (51). Similarly, when predicting the mortality rate on the 28th day, the AUC of suspected infected emergency patients is 0.69 (95% confidence interval 0.58 ~ 0.79, $p < 0.001$), and the critical value is 7.7 ng/mL, (sensitivity 70%, specificity 63%) (43). Wang et al.'s (52) meta-analysis included 17 studies with 3,658 sepsis patients, and the results showed that the PTX-3 level of sepsis patients who died was significantly higher than that of surviving patients, indicating that a high level of PTX-3 is significantly related to the risk of death in sepsis and can predict the patient mortality rate. Lee et al.'s (53) meta-analysis found that an increase in PTX-3 doubles the risk of death in sepsis patients. Another trial conducted a prospective study on 160 sepsis patients and showed that when predicting the 28-day mortality rate of sepsis, at the cut-off value of 26.90 ng/mL, its sensitivity is 88.9% and specificity is 49.5%, and the AUC is 0.734 (95% CI 0.656 ~ 0.811) (54). A prospective cohort study by Kim et al. found that compared with PCT, neutrophil count and CRP, PTX-3 has a larger AUC value (0.819, 95% CI 0.677 ~ 0.961) in predicting the 28-day all-cause mortality rate of severe sepsis patients. At the same time, by establishing a Cox proportional hazards model, it was found that the plasma PTX-3 level measured at admission is an independent predictor of the 28-day all-cause mortality rate of severe sepsis patients (HR = 7.16, 95% CI 2.46 ~ 15.85) (55).

The above research findings suggest that PTX-3 is an early biomarker for predicting the mortality rate of sepsis. Timely detection of PTX-3 can provide guidance for subsequent treatment and management, which has significant clinical importance. Additionally, a single-center prospective study reveals that in comparison to PCT, IL-6, CRP, lactic acid, and platelet count, PTX-3 demonstrates a higher diagnostic value in differentiating between patients with infectious shock and those without ($p < 0.001$) (56). However, when compared to the traditional Simplified Acute Physiological Score II (SAPS II), the ability of PTX-3 to predict mortality is poorer (49).

In different studies, the optimal cut-off value of PTX-3 for predicting bacterial infection, sepsis, septic shock and mortality varies, and the reported sensitivity and specificity are also discrepant. The comparison with other indicators is presented in Table 2.

The correlation between the PTX-3 level and the disease severity and organ dysfunction exceeds that of other measured mediators such as tumor necrosis factor-α, interleukin-6, and C-reactive protein (62). Although PTX-3 alone may not perform as well as the Simplified Acute Physiology Score II (SAPS II), this does not necessarily rule out its potential application in prognosis. In recent decades, scoring systems such as the Acute Physiology and Chronic Health Evaluation System (APACHE II), the Simplified Acute Physiology Score (SAPS), and the Sequential Organ Failure Assessment (SOFA) have become increasingly popular in assessing the risk of death in critically ill patients. However, these scoring systems have significant limitations in clinical practice. Data collection requires multiple laboratory measurements and the calculation involving numerous variables, making it both laborious and costly (63, 64).

5 Discussion

Although PTX-3 has good prospects as a biomarker for sepsis, challenges and limitations still need to be addressed. Due to the heterogeneity of research and selection criteria, an accurate cut-off value has not been determined yet. Therefore, laboratory test results should be interpreted in combination with the clinical situation and used in combination with other laboratory findings to ensure accurate diagnosis and guide appropriate management. Previous studies have included studies involving both adults and children, as well as individuals with different severities in infectious diseases. Some scholars strongly recommend subgroup analysis according to age and different disease manifestations (65). Future research should focus on refining the role of PTX-3 in different etiologies of sepsis and evaluating its potential as a therapeutic target, such as whether the

TABLE 2 The cut-off values of PTX-3 and other commonly used indicators in differencing infection, sepsis and predicting mortality.

Parameter	Clinical relevance	Cut-off	AUC	Sensitivity (%)	Specificity (%)	References
PTX-3	Diagnosis of sepsis	5 ng/mL	0.92	98	79	Hamed et al. (45)
	Diagnosis of septic shock	9 ng/mL	0.81	93	45	Hamed et al. (45)
	Diagnosis of Sepsis	NR	0.73	NR	NR	Muller et al. (49)
	Diagnostic value of sepsis	5.84 ng/mL	0.68	0.667	0.697	Chen et al. (56)
	Diagnostic value of septic shock	11.12 ng/mL	0.73	0.555	0.828	Chen et al. (56)
	Prognostic value of ICU mortality in patients with sepsis	NR	0.63	NR	NR	Muller et al. (49)
	Predicting 28-day mortality in patients with sepsis	7.7 ng/mL	0.69	70	63	Rottman et al. (43)
	Predicting 28-day mortality in patients with sepsis	26.9 ng/mL	0.734	88.9	49.5	Song et al. (54)
	Prediction of bloodstream infection	7.96 ng/mL	NR	90	29	de Kruif et al. (44)
	Prediction of bloodstream infection	16.1 ng/mL	NR	76	61	de Kruif et al. (44)
CRP	Septic patients compared to control group	8.02 mg/L	0.98	88.4	100	Hou et al. (57)
	Differentiating infectious and non-infectious SIRS	50 mg/L	NR	98.5	75.0	Póvoa et al. (58)
	Prognostic value of ICU mortality in patients with sepsis	NR	0.55	NR	NR	Muller et al. (49)
PCT	Cut-off for sepsis	1.57 ng/mL	NR	67.33	65.79	Aksaray et al. (59)
	Differentiating infectious and non-infectious SIRS	1.1 ng/mL	NR	97	78	Harbarth et al. (60)
	Differentiating infectious and non-infectious SIRS	1.8 ng/mL	NR	95	88	Rau et al. (61)
	Diagnostic value of sepsis	1.62 ng/mL	0.79	0.815	0.697	Chen et al. (56)
	Diagnostic value of septic shock	7.27 ng/mL	0.73	0.328	0.672	Chen et al. (56)
	Predicting 28-day mortality in patients with sepsis	0.47 ng/mL	0.689	92.1	41.2	Song et al. (54)
Lactate	Diagnostic value of sepsis	2.3 mmol/L	0.70	0.723	0.621	Chen et al. (56)
	Diagnostic value of septic shock	3.9 mmol/L	0.73	0.453	0.914	Chen et al. (56)
SOFA	Predicting 28-day mortality in patients with sepsis.	8 points	0.712	81.0	51.5	Song et al. (54)
SAPS II	Prognostic value of ICU mortality	NR	0.76	NR	NR	Muller et al. (49)

PTX-3, Pentraxin-3; CRP, C-reactive protein; PCT, Procalcitonin; SOFA, Sequential Organ Failure Assessment; SAPS II, Simplified Acute Physiological Score II; AUC, Area under curve; NR, Not Report.

PTX-3 level can guide the use of antibiotics, etc., and its molecular biological mechanism still needs to be further clarified.

Summary

PTX-3 appears to be a promising prognostic biomarker for critically ill patients. Currently, research is limited to observational designs estimating the predictive potential for mortality risk. Further investigation is needed to determine whether monitoring PTX-3 levels during treatment can be used to guide therapeutic decisions. In conclusion, PTX-3 emerges as a valuable candidate as a biomarker for sepsis, providing insights into its structural characteristics, physiological functions, and potential diagnostic applications. The progress in understanding the role of PTX-3 in sepsis paves the way for improving diagnostic accuracy, prognostic assessment, and targeted therapeutic interventions.

Author contributions

YZ: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Funding acquisition. XL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. XiaoZ: Conceptualization, Investigation, Methodology, Software, Writing – review & editing. TW: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. XianZ: Methodology, Supervision, Validation, Writing – review & editing.

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EDITED BY
Yuetian Yu,
Shanghai Jiao Tong University, China

REVIEWED BY
Paraskevi C. Fragkou,
Evaggelismos General Hospital, Greece
Marios Karvouniaris,
University General Hospital of Thessaloniki AHEPA, Greece

*CORRESPONDENCE
Hou-Tai Chang
houtai38@gmail.com

†These authors have contributed equally to this work and share first authorship

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Optimizing patient outcomes in severe pneumonia: the role of multiplex PCR in the treatment of critically ill patients

Jia-Hao Zhang[1,2,3†], San-Fang Chou[4†], Ping-Huai Wang[3], Chia-Jui Yang[5], Yi-Horng Lai[6], Mei-Yun Chang[3] and Hou-Tai Chang[1,7*]

[1]Department of Critical Care Medicine, Far Eastern Memorial Hospital, Taipei, Taiwan, [2]Department of Nursing, Cardinal Tien College of Healthcare and Management, New Taipei, Taiwan, [3]Department of Chest Medicine, Far Eastern Memorial Hospital, Taipei, Taiwan, [4]Department of Medical Research, Far Eastern Memorial Hospital, Taipei, Taiwan, [5]Department of Infection Medicine, Far Eastern Memorial Hospital, Taipei, Taiwan, [6]Department of Healthcare Administration, Asia Eastern University of Science and Technology, Taipei, Taiwan, [7]Department of Industrial Engineering and Management, Yuan Ze University, Taoyuan, Taiwan

Herein, we evaluated the optimal timing for implementing the BioFire® FilmArray® Pneumonia Panel (FA-PP) in the medical intensive care unit (MICU). Respiratory samples from 135 MICU-admitted patients with acute respiratory failure and severe pneumonia were examined using FA-PP. The cohort had an average age of 67.1 years, and 69.6% were male. Notably, 38.5% were smokers, and the mean acute physiology and chronic health evaluation-II (APACHE-II) score at initial MICU admission was 30.62, and the mean sequential organ failure assessment score (SOFA) was 11.23, indicating sever illness. Furthermore, 28.9, 52.6, and 43% of patients had a history of malignancy, hypertension, and diabetes mellitus, respectively. Community-acquired pneumonia accounted for 42.2% of cases, whereas hospital-acquired pneumonia accounted for 37%. The average time interval between pneumonia diagnosis and FA-PP implementation was 1.9 days, and the mean MICU length of stay was 19.42 days. The mortality rate was 50.4%. Multivariate logistic regression analysis identified two variables as significant independent predictors of mortality: APACHE-II score (p = 0.033, OR = 1.06, 95% CI 1.00–1.11), history of malignancy (OR = 3.89, 95% CI 1.64–9.26). The Kaplan–Meier survival analysis indicated that early FA-PP testing did not provide a survival benefit. The study suggested that the FA-PP test did not significantly impact the mortality rate of patients with severe pneumonia with acute respiratory failure. However, a history of cancer and a higher APACHE-II score remain important independent risk factors for mortality.

KEYWORDS

BioFire® FilmArray, intensive care unit, pneumonia, respiratory tract infections, APACHE-II score, SOFA score

1 Introduction

According to statistics from the World Health Organization (WHO), lower respiratory tract infections and pneumonia are the fourth leading cause of death worldwide. Moreover, pneumonia is a prevalent condition encountered in intensive care units. Pneumonia-related mortality is higher in elderly patients and individuals with a history of malignancy (1, 2). In

clinical practice guidelines provided by various organizations, the recommended antibiotic choices for patients with severe pneumonia include penicillin, fluoroquinolones, or agents effective against methicillin-resistant *Staphylococcus aureus* (MRSA). The extensive use of antibiotics, however, has contributed to the emergence of drug-resistant bacteria, causing a shift in the spectrum of pathogenic bacteria involved in community- or hospital-acquired pneumonia. Further, the prevalence of pneumonia caused by multidrug-resistant (MDR) bacteria ranges from 14.1 to 62%, leading to increased morbidity and mortality among patients, and imposing a substantial economic burden in terms of social costs (3–5). According to the American Thoracic Society/Infectious Disease Society of America (ATS/IDSA), sputum Gram staining, aerobic culture, and blood culture collection should be performed prior to administering broad-spectrum antibiotics. This approach aims to ensure an accurate diagnosis and appropriate treatment. In addition, compliance with these guidelines can help to prevent the overuse of broad-spectrum antibiotics, a critical factor in the development of antibiotic resistance (6). However, it is important to note that only 31.9% of the patients diagnosed with community-acquired pneumonia (532 of 1,669) managed to produce high-quality sputum samples. Additionally, among these cases, only 14.4% (240 of 1,669) of the collected sputum samples could be cultured to identify the predominant morphotype. The limited availability of high-quality sputum samples poses challenges in accurately diagnosing and treating pneumonia (7). Moreover, traditional sputum cultures generally require a waiting period of 2–5 days before the final report is available. In contrast, the utilization of a rapid diagnostic test, such as the BioFire® FilmArray® Pneumonia Panel (FA-PP; BioFire Diagnostics, Salt Lake City, UT, United States), which employs multiplex polymerase chain reaction (PCR) technology, offers a significantly higher detection rate (ranging from 74.6 to 92%) than sputum cultures obtained from endotracheal aspirate or bronchoalveolar lavage samples. This expeditious and more accurate diagnostic approach could facilitate prompt and targeted treatment decisions for patients with pneumonia (8–10).

The FA-PP can detect eight viruses, 18 bacteria, and seven antibiotic resistance genes within a remarkably short timeframe of only 60 min, offering a rapid and comprehensive diagnostic solution for patients with pneumonia. Hence, the implementation of a multiplex PCR system to analyze respiratory samples from patients with hospital-acquired and ventilator-associated pneumonia has the potential to improve empirical antimicrobial therapy and reduce the use of broad-spectrum antibiotics. This change could increase the de-escalation rate from 39 to 48.2% (8, 11, 12).

However, the impact of multiplex PCR on the mortality rate of patients with severe pneumonia in the intensive care unit remains uncertain. In addition, the optimal timing of application in cases of severe pneumonia remains unknown. Hence, this retrospective cohort study sought to ascertain the most effective timing for implementing multiplex polymerase chain reaction (PCR) in the management of patients with severe pneumonia, and to assess its potential to improve the survival rate of these patients.

2 Materials and methods

This retrospective single-center cohort study was conducted at a medical center in Taiwan between July 1, 2021 and July26, 2022. This study used anonymous data and was approved by the Medical Ethics Committee of the Far Eastern Memorial Hospital (approval number: 111211-E).

Patients admitted to the medical intensive care unit (MICU) with acute respiratory failure and severe pneumonia were included in accordance with the diagnostic criteria outlined by the Infectious Diseases Society of America (IDSA). These criteria necessitated the presence of either new or progressive chest X-ray consolidations combined with clinical symptoms, such as dyspnea, cough, sputum production, fever, and abnormal breathing sounds indicative of pulmonary consolidation. All the above conditions were important criteria for enrolling study patients (6, 13). Specific exclusion criteria were implemented to maintain a focused analysis. Patients who did not receive invasive mechanical ventilation were excluded.

Respiratory samples were collected via tracheal aspiration (TA) or bronchoalveolar lavage (BAL). Two distinct methods were employed for the sample analysis. The first involved traditional microbiological techniques, including sputum Gram staining and aerobic culturing. The second employed a multiplex PCR system, FA-PP, operated according to the manufacturer's recommended protocol.

Clinical data were collected retrospectively and anonymized. The collected data encompassed various patient aspects, including age, gender, pneumonia type (e.g., community-acquired pneumonia (CAP), healthcare-associated pneumonia (HCAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP)), acute physiology and chronic health evaluation-II (APACHE-II) score, sequential organ failure assessment (SOFA) scores, serum lactate level, smoking history, major underlying medical conditions (e.g., malignancy, diabetes mellitus, hypertension, etc.), time interval between pneumonia diagnosis and FA-PP procedure, intubation duration, length of stay, and in-hospital mortality. The term HCAP indicates that patients must meet the diagnosis of pneumonia and also have one of the following conditions: hospitalization in an acute care hospital for more than 2 days within the past 90 days, residing in a nursing home or long-term care facility, receiving intravenous antibiotics, chemotherapy, or dialysis within the past 30 days. HAP refers to patients meeting the diagnosis of pneumonia occurring more than 48 h after hospital admission, or within 14 days after discharge from a previous hospitalization. Factors that influenced mortality and survival outcomes in patients with severe pneumonia who underwent FA-PP treatment, were subsequently identified.

2.1 Statistical analysis

Categorical data were expressed as frequency and percentage, while numerical data were expressed as mean ± standard deviation (SD) or median (1st quartile, 3rd quartile) (Q1, Q3). The difference between groups was examined using independent-samples *T* test or Mann–Whitney U test for continuous data. The Pearson Chi-Square test was used to compare differences between groups for categorical data, unless otherwise noted. We further performed multivariate logistic regression analysis to identify potential risk factors associated with mortality in patients with severe pneumonia. Kaplan–Meier analysis was performed to assess the 28-day survival and in-hospital survival. The Kaplan–Meier survival curves were compared using the log-rank test. Statistical analysis was conducted using IBM SPSS

Statistics 27 software. The results were considered statistically significant at the $p < 0.05$ level.

3 Results

Data were initially collected from 141 patients between July 2021 and July 2022. The exclusion criteria were applied as follows: five patients were excluded for not receiving tracheal intubation therapy; one outlier was excluded because the FA-PP test was performed 22 days after being diagnosed with HAP, and the patient died after the FA-PP test was conducted. Finally, 135 patients were enrolled in this study (Figure 1). An overview of the basic characteristics and clinical outcomes of the 135 patients who underwent FA-PP testing for pathogens and received mechanical ventilation support at the time of enrollment was shown in Table 1. The patient cohort had an average age of 67.10 ± 13.92 years, with 69.6% male. Of the 135 patients, 38.5% had a prior history of smoking, and the averages of severity indexes, including APACHE-II score, SOFA score, and serum lactate, were 30.62 ± 8.46, 11.23 ± 3.88, and 4.50 ± 4.19, respectively. Community-acquired pneumonia accounted for 42.2% of pneumonia cases, whereas HAP accounted for 37.0%. The time interval between pneumonia diagnosis and FA-PP implementation was 1.90 ± 1.62 days, and the average length of MICU stay was 19.42 ± 12.93 days. The overall in-hospital mortality rate was 50.4%.

The differences in basic characteristics and clinical outcomes between the survived and deceased groups were examined. The results suggested that APACHE-II score, SOFA score, history of malignancy, and the time interval between pneumonia diagnosis and FA-PP might have been the risk factors associated with the mortality (Table 2). To reveal the impact of those factors on the mortality, multivariate logistic analysis was performed (Table 3). APACHE-II score and history of malignancy were associated with death, with ORs of 1.06 ($p = 0.033$) and 3.89 ($p = 0.002$), respectively. However, the time interval between pneumonia diagnosis and the FA-PP test did not reach statistical significance (OR = 1.24, $p = 0.067$).

According to Kaplan–Meier survival analysis, the early test group, who underwent the FA-PP test within 1 day, did not show statistically significant differences in survival at 28 days and during hospitalization (Log Rank test: $p = 0.221$ and 0.210, respectively) (Figure 2). The results indicated that early FA-PP testing did not provide a survival benefit for the patients with severe pneumonia combined with acute respiratory failure.

4 Discussion

This retrospective study showed that among pneumonia patients requiring ventilator support, FA-PP testing, regardless of when it was administered during the treatment process, did not significantly improve patient survival rates.

Prior investigations have emphasized the importance of using the FA-PP test as a valuable tool for accurately diagnosing pathogenic bacteria and selecting appropriate antibiotics for patients with pneumonia (8, 9, 11). Monard et al. further evaluated a multiplex PCR test, which offered a promising approach to the early adaptation of antimicrobial therapy in adult patients with pneumonia (8). The capacity of this technology to simultaneously detect multiple pathogens enhances the precision of treatment decisions, aligning with the broader goal of reducing antibiotic misuse. Prior research by on the BioFire® FilmArray® Pneumonia Panel Gastli et al. demonstrated the capacity of this technique for rapid and accurate identification of pneumonia-causing bacteria (9). This tool can significantly improve bacteriological documentation, which is vital for pneumonia treatment. Buchan et al. compared the BioFire® FilmArray® Pneumonia Panel with conventional diagnostic methods (11), showing the potential impact of antimicrobial stewardship on adults with lower respiratory tract infections, highlighting the importance of this technology in optimizing antibiotic use. Collectively, these studies underscore the significance of enhancing pneumonia diagnostics to provide more efficient and precise diagnostic tools. The use of multiplex PCR and the BioFire®

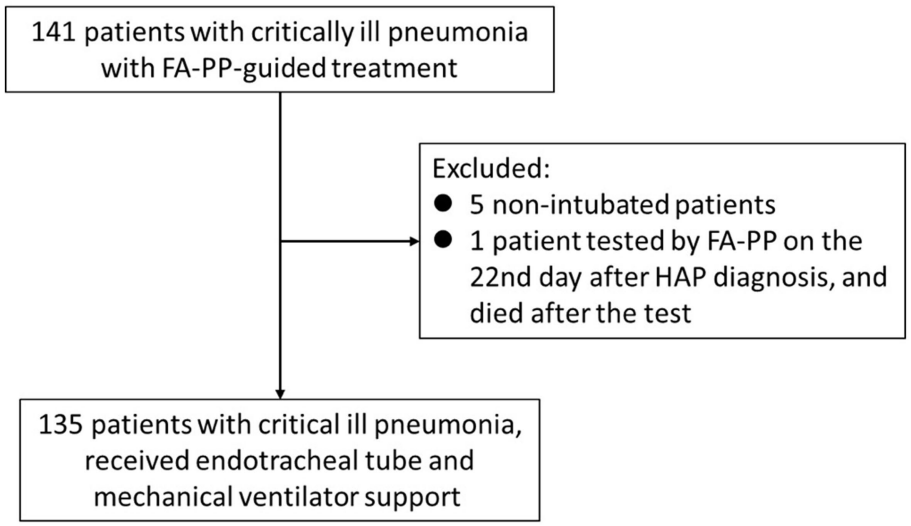

FIGURE 1
Flow chart of the screening process.

TABLE 1 Baseline demographic characteristics and clinical outcomes of critically ill patients.

Patient characteristics	Results	Min–Max
Age, years, mean±SD	67.10±13.92	22–97
Sex		
Male, *n* (%)	94 (69.6)	
Female, *n* (%)	41 (30.4)	
Smoker, *n* (%)	52 (38.5)	
APACHE-II score, mean±SD	30.62±8.46	14–57
SOFA score, mean±SD	11.23±3.88	3–21
Serum lactate, mmol/L, mean±SD	4.50±4.19	0–22.33
Comorbidities		
Malignancy, *n* (%)	39 (28.9)	
Chronic obstructive pulmonary disease, *n* (%)	15 (11.1)	
Asthma, *n* (%)	1 (0.7)	
Bronchiectasis, *n* (%)	2 (1.5)	
Hypertension, *n* (%)	71 (52.6)	
Diabetes mellitus, *n* (%)	58 (43.0)	
Coronary artery disease, *n* (%)	27 (20.0)	
Congestive heart failure, *n* (%)	22 (16.3)	
End stage renal disease, *n* (%)	15 (11.1)	
Liver cirrhosis, *n* (%)	12 (8.9)	
Type of pneumonia		
Community-acquired pneumonia, *n* (%)	57 (42.2)	
Healthcare-associated pneumonia, *n* (%)	15 (11.1)	
Hospital-acquired pneumonia, *n* (%)	50 (37.0)	
Ventilator-associated pneumonia, *n* (%)	13 (9.6)	
Interval time between pneumonia diagnosis and FA-PP, days, mean±SD/median (Q1, Q3)	1.90±1.62/1 (1, 2)	1–10
Outcome parameter		
Duration of intubation, mean±SD	21.56±18.98	1–101
ICU length of stay, mean±SD	19.42±12.93	2–65
Hospital length of stay, mean±SD	37.08±27.15	2–159
In-hospital mortality, *n* (%)	68 (50.4)	

Numerical data was expressed as mean ± SD or median (Q1, Q3) while categorical data was expressed as frequency and percentage. APACHE-II, acute physiology and chronic health evaluation-II; SOFA, sequential organ failure assessment; FA-PP, BioFire® FilmArray® pneumonia panel; ICU, intensive care unit.

FilmArray® Pneumonia Panel holds promise for guiding personalized, rapid, and effective treatment decisions. These advancements address the challenges associated with pneumonia management and contribute to improved patient care, while reducing unnecessary antibiotic use.

Rapid and accurate diagnosis and treatment are crucial for patients with sepsis. However, in our hospitals, sputum bacterial culture results typically take an average of 3 ± 2.6 days, and this delay can allow exacerbation of the underlying disease. In contrast, the FA-PP testing offers a faster alternative, with an average turnaround time of only 1 h. The rapidity of this test is of paramount importance in sepsis management. According to the Surviving Sepsis Campaign 2021 guidelines, for patients with suspected septic shock or a high likelihood of sepsis, antibiotics should be administered immediately, ideally within 1 h and 3 h of recognition, respectively (14). However, traditional bacterial culture methods often require several days to yield specific bacterial infection information, potentially leading to delayed antibiotic treatment. Under such circumstances, the FA-PP testing can provide rapid bacterial infection information, enabling healthcare professionals to make timely decisions regarding the appropriate antibiotic therapy. This not only enhances patient outcomes, but can also facilitate adherence to the recommendations of the Surviving Sepsis Campaign, and strengthen antibiotic stewardship for patients with sepsis. In this retrospective study, we initially hypothesized that for patients with severe pneumonia combined with respiratory failure, early intervention with FA-PP testing during the treatment process would lead to an increase in survival rates due to adjustments in antibiotic therapy.

The judicious use of antibiotics can drastically reduce patient mortality. A previous study found that patients in the ICU with an APACHE-II score of >30 had an average mortality rate of 73% (15). In 2011, Richards et al. reported that patients with community-acquired pneumonia combined with severe sepsis and an APACHE-II score > 25 had an in-hospital mortality rate of 48.2% (16). More recently, a study of 6,374 critically ill patients found that non-survivors had an average APACHE-II score of 19.8 ± 6.1 on the first day after admission (17). Our study revealed that, among patients with severe pneumonia with an average APACHE-II score of 30.62 ± 8.46, the use of FA-PP testing to guide antibiotic therapy resulted in an overall mortality rate of 50.4%. In the multivariate logistic regression analysis (Table 3), FA-PP testing was also found to be non-significantly associated with mortality ($p = 0.067$). Additionally, the typical treatment duration for pneumonia is approximately 14 days. We attempted to determine whether the timing of FA-PP testing during the treatment process affected survival rates at different time points. However, Kaplan–Meier survival analysis revealed that neither early nor late FA-PP testing resulted in significant differences in 28-day survival rates or in-hospital survival rates (Figure 2). This phenomenon may be attributed to the numerous factors affecting pneumonia prognosis, including age, sex, number of organ dysfunctions, and underlying diseases (18, 19). Through multivariate analysis, our retrospective study demonstrated that the APACHE-II score and history of malignancy were significant independent predictors of mortality among patients with severe pneumonia. Additionally, the financial situation of the patient's family can also influence the direction of medical care.

Although this retrospective study found a negative result for FA-PP testing, showing no benefit in patient survival rates, it does provide a clearer role for FA-PP testing. The FA-PP test can help reduce the use of broad-spectrum antibiotics and unnecessary antibiotic treatments without increasing the risk of treatment failure (8, 20). Additionally, the FA-PP test can lower overall healthcare expenditures and social costs (21). A recent meta-analysis found that, compared to traditional diagnostic methods, the use of the FA-PP test in cases of viral pneumonia resulted in a shorter diagnosis time (mean difference − 24.22 h, 95% CI −28.70 to −19.74 h), leading to improved medication control and a

TABLE 2 Association between risk factors and mortality among critically ill patients with pneumonia who underwent FA-PP-guided treatment.

Variable	Survival (*n* = 67)	Death (*n* = 68)	*p*-value
Age, years, mean±SD	65.67±16.21	68.50±11.15	0.241
Sex			0.896
Male, *n* (%)	47 (70.1)	47 (69.1)	
Female, *n* (%)	20 (29.9)	21 (30.9)	
Smoker, *n* (%)	30 (44.8)	22 (32.4)	0.138
APACHE-II score, mean±SD	28.79±7.94	32.43±8.63	0.012*
SOFA score, mean±SD	10.19±3.78	12.25±3.73	0.002*
Serum lactate, mmol/L, mean±SD	4.03±3.81	4.96±4.51	0.196
Comorbidities			
Malignancy, *n* (%)	11 (16.4)	28 (41.2)	0.002*
Chronic obstructive pulmonary disease, *n* (%)	8 (11.9)	7 (10.3)	0.761
Asthma, *n* (%)	0 (0.0)	1 (1.5)	1.000a
Bronchiectasis, *n* (%)	1 (1.5)	1 (1.5)	1.000a
Hypertension, *n* (%)	34 (50.7)	37 (54.4)	0.670
Diabetes mellitus, *n* (%)	34 (50.7)	24 (35.3)	0.070
Coronary artery disease, *n* (%)	13 (19.4)	14 (20.6)	0.836
Congestive heart failure, *n* (%)	13 (19.4)	9 (13.2)	0.332
End stage renal disease, *n* (%)	9 (13.4)	6 (8.8)	0.394
Liver cirrhosis, *n* (%)	5 (7.5)	7 (10.3)	0.764a
Type of pneumonia			0.038*b
Community-acquired pneumonia, *n* (%)	32 (47.8)	25 (36.8)	
Healthcare-associated pneumonia, *n* (%)	9 (13.4)	6 (8.8)	
Hospital-acquired pneumonia, *n* (%)	24 (35.8)	26 (38.2)	
Ventilator-associated pneumonia, *n* (%)	2 (3.0)	11 (16.2)	
Interval time between pneumonia diagnosis and FA-PP, days, mean±SD/ median (Q1, Q3)	1.67±1.40/1 (1, 2)	2.13±1.79/1.5 (1, 2)	0.098/0.035*c
Outcome parameter			
Duration of intubation, mean±SD	19.15±14.92	23.94±22.13	0.142
ICU length of stay, mean±SD	17.76±10.25	21.06±15.01	0.138
Hospital length of stay, mean±SD	37.73±20.61	36.44±32.49	0.783

Numerical data was expressed as mean ± SD or median (Q1, Q3) while categorical data was expressed as frequency and percentage. The differences between 2 groups were analyzed using either Independent-Samples *T* test, Mann–Whitney U test[c] or Chi-Square test, depending on the data was numerical or categorical. a, Fisher's Exact test used due to more than one cell count < 5 or < 20% in 2×2 crosstab. b, Chi-Square/Likelihood Ratio test used due to more than one cell count < 5 or < 20% in crosstab more than 2×2 cells. APACHE-II, acute physiology and chronic health evaluation-II; SOFA, sequential organ failure assessment; FA-PP, BioFire® FilmArray® pneumonia panel; ICU, intensive care unit. *, *p* value less than 0.05 indicates statistical significance.

TABLE 3 Multivariate logistic regression analysis of the association between risk factors and mortality among critically ill patients with pneumonia receiving FA-PP-guided treatment.

Variable	Multivariate logistic regression	
	OR (95% CI)	*p*-value
APACHE-II score	1.06 (1.00–1.11)	0.033*
SOFA score	1.11 (0.99–1.24)	0.067
Malignancy	3.89 (1.64–9.26)	0.002*
Interval time between pneumonia diagnosis and FA-PP, day	1.24 (0.99–1.56)	0.067

APACHE-II, acute physiology and chronic health evaluation-II; SOFA, sequential organ failure assessment; FA-PP, BioFire® FilmArray® pneumonia panel. *, *p* value less than 0.05 indicates statistical significance.

consequent reduction in hospitalization days (mean difference − 0.82 days, 95% CI −1.52 to −0.11 days) (22).

5 Limitations

This study had several limitations that warrant consideration. First, it should be noted that the research design is retrospective and confined to a single center, which may curtail the generalizability of the findings to broader populations. Second, the sample size, although adequate for the study's scope, was relatively small. Third, an important aspect not addressed in this study is the evaluation of the influence of various pathogens on patient outcomes, which may merit exploration in future endeavors. In addition, this study lacked a

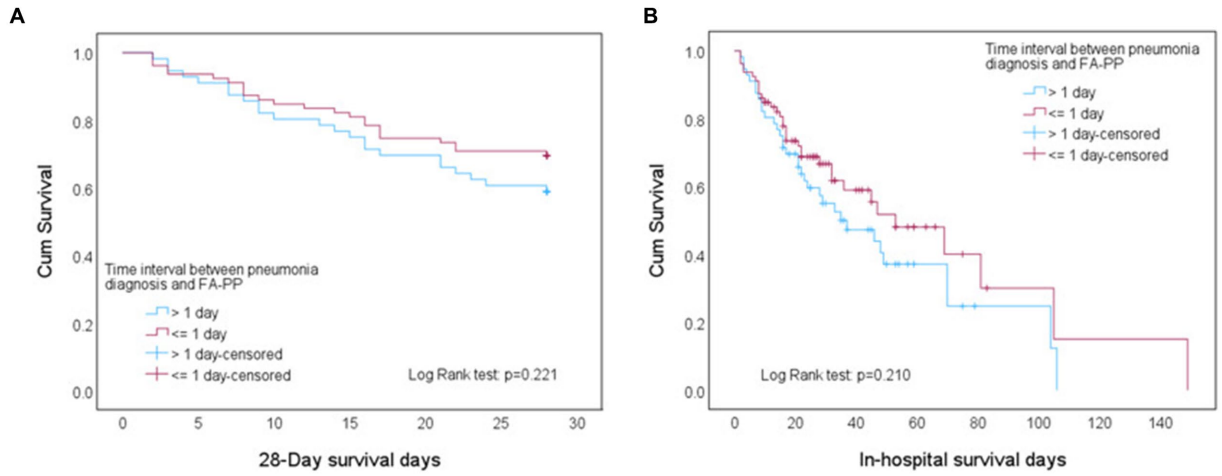

FIGURE 2
Kaplan–Meier curves showing the **(A)** 28-day survival rate and **(B)** in-hospital survival rate.

control group of patients who did not undergo the FA-PP testing, which may provide valuable comparative insights.

6 Conclusion

Overall, the results of this study suggest that the FA-PP test appears to have no impact on the mortality rate of patients with severe pneumonia and respiratory failure, regardless of whether it is performed early or later. However, a history of malignancy and a higher APACHE-II score remain important independent risk factors for mortality. Further research is required to validate these findings and explore the impact of different pathogens on patient outcomes.

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 humans were approved by Research Ethics Review Committee Far Eastern Memorial Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because this retrospective single-center cohort study was conducted at a medical center in Taiwan between July 1, 2021, and July26, 2022. This study used anonymous data and was approved by the Medical Ethics Committee of the Far Eastern Memorial Hospital (approval number: 111211-E).

Author contributions

J-HZ: Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. S-FC: Data curation, Formal analysis, Methodology, Software, Validation, Writing – review & editing. P-HW: Formal analysis, Investigation, Resources, Supervision, Writing – review & editing. C-JY: Methodology, Supervision, Validation, Data curation, Formal analysis, Writing – review & editing. Y-HL: Conceptualization, Methodology, Software, Validation, Writing – review & editing. M-YC: Project administration, Resources, Supervision, Writing – review & editing. H-TC: Funding acquisition, Supervision, Writing – review & editing.

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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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EDITED BY
Luo Zhe,
Fudan University, China

REVIEWED BY
Xiuling Shang,
Fujian Provincial Hospital, China
Lingai Pan,
Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, China

*CORRESPONDENCE
Yuetian Yu
✉ fishyyt@sina.com
Cheng Zhu
✉ zhucheng1203@163.com

†These authors have contributed equally to this work

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Correlation between the gut microbiota characteristics of hosts with severe acute pancreatitis and secondary intra-abdominal infection

Lihui Wang[1†], Weijun Zhang[1†], Simin Dai[1], Yuan Gao[1], Cheng Zhu[2*] and Yuetian Yu[1,3,4*]

[1]Department of Critical Care Medicine, Renji Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China, [2]Department of Disease Prevention and Control, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China, [3]Key Laboratory of Intelligent Pharmacy and Individualized Therapy, Zhejiang, China, [4]Key Laboratory of Multiple Organ Failure (Zhejiang University), Ministry of Education, Zhejiang, China

Objective: The objective of the study is to investigate the changes in the composition of intestinal microecology in severe acute pancreatitis (SAP) patients with or without intra-abdominal infection and also to analyze the expression of antibiotic resistance genes to provide evidence for early warning of infectious diseases and the rational use of antibiotics.

Methods: Twenty patients with SAP were enrolled in the study. According to whether the enrolled patients had a secondary intra-abdominal infection, they were divided into two groups, each consisting of 10 patients. Stool specimens were collected when the patients were admitted to the emergency intensive care unit (EICU), and nucleic acid extraction was performed. Next-generation gene sequencing was used to compare the differences in intestinal microflora diversity and drug resistance gene expression between the two groups.

Results: The gut microbiota of patients in the infection group exhibited distribution on multiple clustered branches with some intra-group heterogeneity, and their flora diversity was compromised. The infected group showed an enrichment of various opportunistic bacteria in the gut microbiota, along with a high number of metabolic functions, stress functions to external signals, and genes associated with pathogenesis. Drug resistance genes were expressed in the gut microbiota of both groups, but their abundance was significantly lower in the non-infected group.

Conclusion: The intestinal microbiota of patients in the infection group exhibited distribution on multiple clustered branches with some intra-group heterogeneity, and their flora diversity was compromised. Additionally, drug resistance genes were expressed in the gut microbiota of both groups, although their abundance was significantly lower in the non-infected group.

KEYWORDS

severe acute pancreatitis, intestinal microecology, intra-abdominal infection, metagenomic next-generation sequencing, intensive care unit

1 Introduction

The prevalence of secondary intra-abdominal infection in severe acute pancreatitis (SAP) is notably high, primarily due to pathogens originating from the host's intestinal tract (1, 2). Over 2000 species of intestinal microbes, constituting the gut microbiota, represent the most complex and significant micro-ecosystem in the human body. Metagenomic sequencing reveals that microbes within the human body encode genes exceeding the human genome's gene count by a factor of 150 (3, 4). The role of the host's intestinal microecology diversity, resistance, and metabolomic changes in SAP secondary intra-abdominal infection has been increasingly recognized. However, data from further controlled studies remain incomplete, lacking a foundation for developing diagnostic prediction systems or precision treatments.

Over the past decade, rapid advancements in metagenomic next-generation sequencing (mNGS) technology have enabled medical professionals to analyze the composition, structure, diversity, and drug resistance of gut microbiota (5–7). This progress facilitates further investigation into the potential pathogenesis of infectious diseases (8–11). Furthermore, gut microbes are crucial for the stability of gut microecology (12, 13). They establish a stable symbiotic relationship with both intestinal mucosal immune cells and intestinal epithelial cells. In critically ill patients, alterations in gut microecology can disrupt the stable co-existence of gut flora, resulting in complications such as microbial homeostasis loss, bacterial translocation, and enterogenic sepsis (14).

Secondary intra-abdominal infection in SAP has a high prevalence and is associated with a poor prognosis, predominantly caused by *Enterobacteriaceae*. Changes in the intestinal microbiota and bacterial translocation are hypothesized to significantly contribute to the disease's pathogenesis. Consequently, we conducted a prospective cohort study using metagenomic sequencing to examine the compositional changes in the intestinal microbiota of SAP patients, both infected and non-infected, to facilitate early detection of infectious diseases. Furthermore, we seek to identify the expression patterns of dominant antibiotic resistance genes to inform the judicious use of prophylactic antimicrobials in clinical settings.

2 Materials and methods

2.1 Study design and setting

This prospective cohort study was conducted at the emergency intensive care unit (EICU) of Ruijin Hospital from July 2023 to December 2023. The Ethics Committee of Shanghai Jiao Tong University School of Medicine approved the study (No. 2023-RES-184), and the study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all the enrolled patients. The design, implementation, and presentation of this study were strictly in accordance with the STROBE statement (15).

2.2 Participants

Adult patients admitted to the EICU and diagnosed with SAP were enrolled in our study. Patients were excluded if they met any of the following criteria: (1) upon admission to the EICU, in addition to SAP, concurrent one or more infectious diseases; (2) with a history of broad-spectrum antibiotic use 90 days before EICU admission; and (3) those who developed infectious diseases other than secondary abdominal infection during the course of hospital stay such as hospital-acquired pneumonia (HAP). They were categorized into two groups: the secondary intra-abdominal infection group and the non-infection group, each comprising 10 patients. Fresh stool samples were collected immediately following the definitive SAP diagnosis and admitted to the EICU. Both groups of patients did not receive antimicrobial therapy before sample collection.

2.3 Disease definition

According to the guidelines from the World Society of Emergency Surgery (WSES), acute pancreatitis can be diagnosed by meeting any two of the following three criteria: (1) sudden onset of acute upper abdominal pain radiating to the waist or back; (2) serum amylase and/or lipase levels in blood samples at least three times higher than the normal upper limit; (3) typical pancreatic lesions can be detected by contrast-enhanced computed tomography (CT) scan or magnetic resonance imaging (MRI) of the upper abdomen. If the patient has organ (one or more) dysfunction lasting more than 48 h after adequate fluid resuscitation, then they are diagnosed with SAP (16).

Secondary intra-abdominal infection may be suspected (16) when patients with acute pancreatitis meet one of the following criteria: (1) newly onset (body temperature $\geq$ 38.5°C) or persistent fever; (2) elevated levels of inflammatory markers [white blood cell count (WBC), neutrophil count, procalcitonin (PCT), or C-reactive protein (CRP)]; and (3) clinical symptoms continue to deteriorate, leading to secondary organ dysfunction; and at the same time, combined with any of the following: (1) the presence of gas bubbles in necrotic pancreatic tissue can be detected by an abdominal enhanced CT scan and (2) positive culture results can be obtained from percutaneous fine needle aspiration of the abdomen.

2.4 Nucleic acid extraction, library construction, and sequencing

The QIAGEN QIAamp PowerFecal DNA Kit facilitates the extraction of whole-genomic DNA from fecal samples. The purity and integrity of the extracted DNA are assessed via agarose gel electrophoresis, while its concentration is accurately measured using the Qubit 2.0 system. Samples exhibiting no diffuse degradation of nucleic acids and containing more than 10 ng of nucleic acid proceed to library construction. Following quality control, libraries are pooled based on their effective concentration and the desired data output volume. Sequencing is then performed using Illumina PE150, generating a minimum of 60 million reads per sample.

2.5 Gene prediction

Gene prediction is conducted using MetaGeneMark, followed by deduplication of the predicted genes to construct a gene catalog. This catalog forms the foundation for a comprehensive analysis of the clean data from each sample, yielding abundance information for the gene catalog in each instance.

2.6 Species annotation, diversity analysis, and differential microbiota analysis

The gene catalog, derived from gene prediction, is aligned with the MicroNR database and integrated with gene abundance data to generate species abundance profiles across different taxonomic levels. Species and functional abundance profiles are analyzed using methods such as abundance clustering, PCA, NMDS dimensionality reduction, and ANOSIM. Composition differences among samples, in terms of species and functions, are examined through LEfSe multivariate statistical analysis and comparative metabolic pathway analysis.

2.7 Annotation of antibiotic resistance genes

Utilizing the gene catalog and antibiotic resistance gene database for annotation provides sequential insights into the abundance, species attribution, and resistance mechanisms of antibiotic resistance genes.

2.8 Statistical analysis

Categorical variables are presented as counts (n) and percentages (%). They were compared using the chi-square (χ^2) test or Fisher's exact test when the sample size was less than five. Non-normally distributed data were compared using the Wilcoxon rank-sum test and are reported as medians [with an interquartile range (IQR)]. A two-sided p-value of <0.05 was considered statistically significant.

3 Results

3.1 Patient characteristics

The study included 20 patients with SAP, with 10 patients in each group, all of whom had biliary pancreatitis. Every enrolled patient had an intestinal feeding tube using a gastroscope for enteral nutrition within 48 h of admission to the EICU, and none of them received probiotic therapy during the course of the disease. Both groups of patients did not receive antimicrobial therapy during hospitalization before sample collection. After fecal sample collection, all patients with biliary pancreatitis were treated with a combination of third-generation cephalosporins (ceftazidime) and metronidazole. The median time for a secondary peritoneal infection to occur is 11 days. None of the 10 patients in the infected group developed intestinal fistula or necrosis. The main pathogens causing infection are *Escherichia coli* and *Klebsiella pneumoniae*, with three cases each. The baseline characteristics, inflammatory markers, intra-abdominal pressure, and prognosis indicators of the two groups of patients are listed in Table 1.

3.2 Analysis of microbial community structure

Clustering analysis was performed using the Bray–Curtis distance metric, constructing a sample clustering tree to examine similarities. Clustering results across taxonomic levels were combined with species' relative abundances in each sample for visualization. The analysis revealed that bacteria, with their higher abundance, are the

TABLE 1 Baseline characteristics of the study population.

Characteristic	Infection group (n = 10)	Non-infection group (n = 10)	p-value
Male gender, n (%)	5 (50.0)	5 (50.0)	1.00
Age, median [IQR]	41 [33, 56]	43 [31, 53]	0.32
Body mass index, median [IQR]	22.9 [18.3, 24.6]	22.1 [19.5, 25.3]	0.19
SOFA score	8 [5, 12]	7 [5, 11]	0.12
APACHE II score	15 [10, 21]	10 [6, 18]	0.03
Scr, median [IQR], umol/L	74 [57, 117]	68 [52,108]	0.17
Hb, median [IQR], g/L	85 [73, 99]	80 [71,103]	0.31
WBC, median [IQR], 10*^{9}count/L	12.9 [7.3, 14.7]	10.7 [6.3,13.5]	0.02
PLT, median [IQR], 10*^{9}count/L	118 [78, 225]	121 [98,262]	0.11
ALT, median [IQR], U/L	45 [17, 67]	41 [21,59]	0.21
AST, median [IQR], U/L	42 [18, 57]	47 [20, 59]	0.12
ALB, median [IQR], g/L	33.1 [28.7, 38.1]	34.3 [29.8, 36.1]	0.32
GLU, median [IQR], mmol/L	8.5 [6.4, 12.9]	9.6 [4.8, 12.7]	0.23
PCT, median [IQR], ng/mL	7.8 [5.2, 10.2]	5.8 [3.1, 7.6]	0.02
CRP, median [IQR], mg/L	156.3 [76.3, 205.9]	79.5 [55.2, 98.4]	0.04
IAP, median [IQR], mmHg	15 [7, 24]	14 [8, 23]	0.32
30-day mortality, n (%)	3 (30)	1 (10)	0.58

ALB, Albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; APACHE II, acute physiology and chronic health evaluation II; CRP, C-reactive protein; GLU, Glucose; Hb, hemoglobin; IAP, intra-abdominal pressure; PCT, procalcitonin; PLT, platelet; Scr, serum creatinine; SOFA, sequential organ failure assessment; WBC, White blood cells.

predominant microbial communities in the feces of both infected and uninfected patients. The primary differences in microbial taxa between groups predominantly arise from bacteria (Figure 1). In the non-infected group, patients 1–7 exhibited highly similar microbial community structures, whereas patients 8–10 were more akin to those in the infected group. Furthermore, a 2-week follow-up revealed that these three patients developed peritoneal infections. Samples from the infected group spanned multiple clustering branches, suggesting a degree of intra-group heterogeneity.

The analysis of the microbial structure within and across samples reveals distinct differences between the infected and non-infected groups. In the non-infected group, the predominant bacterial phyla in feces are *Firmicutes* and *Bacteroidetes*, with a lower abundance of *Proteobacteria*. This microbial composition

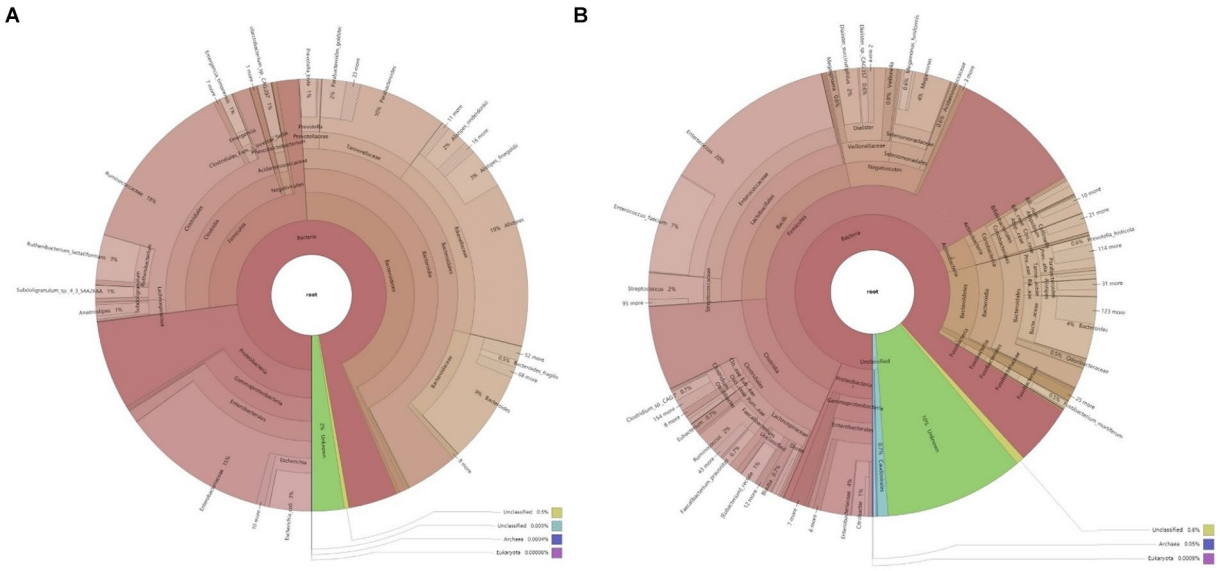

FIGURE 1
Krona chart of microbial composition in the samples. **(A)** Microbial composition of fecal samples from infected patients; **(B)** Microbial composition of fecal samples from non-infected patients.

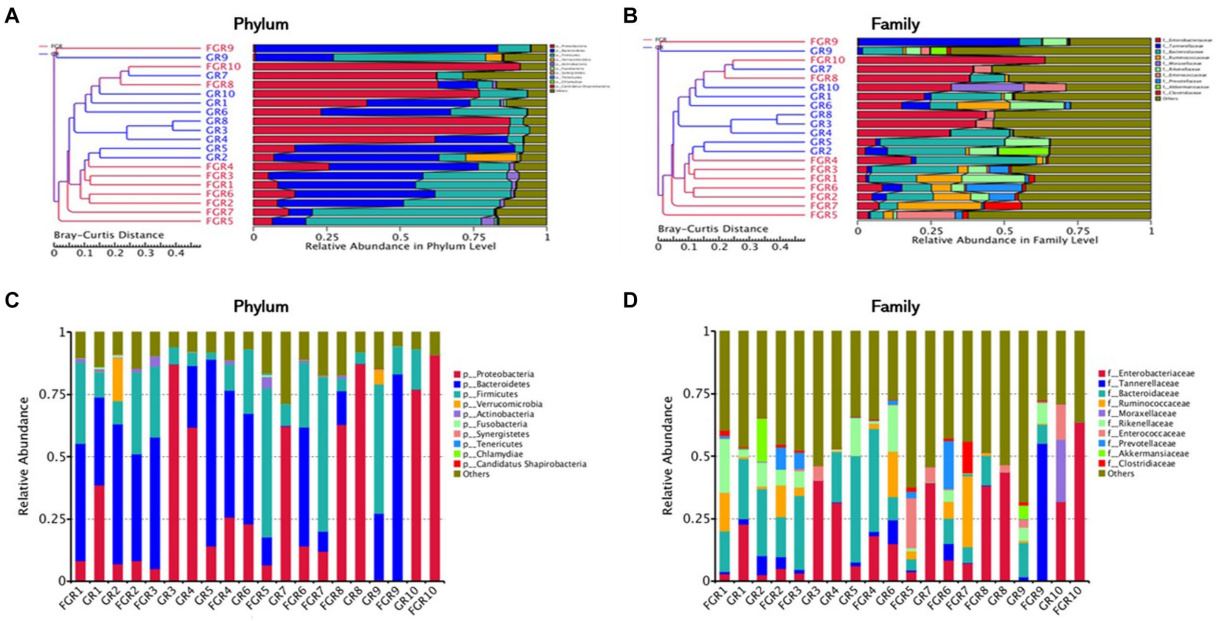

FIGURE 2
Cluster analysis of samples. **(A)** Cluster analysis of samples based on Bray–Curtis distance metric at the phylum level; **(B)** cluster analysis of samples based on Bray–Curtis distance metric at the family level; **(C)** stacked chart of the top 10 microbial compositions at the phylum level; **(D)** stacked chart of the top 10 microbial compositions at the family level.

mirrors that of healthy individuals. The microbial composition of the infected group varies among individuals. Comparing the phylum-level abundance between groups reveals a higher abundance of *Proteobacteria* in the feces of infected patients. At the family level, *Enterobacteriaceae* are more abundant in the infected group than in the non-infected group, comprising various opportunistic pathogens. Genus-level analysis indicates a higher abundance of *Klebsiella pneumoniae* in the feces of some infected patients (Figure 2). *Klebsiella pneumoniae*, an opportunistic pathogen, typically exists in low abundance within the intestinal microecology in a non-pathogenic symbiotic form. The increased abundance of *Klebsiella pneumoniae* in the infected group suggests alterations in the intestinal microbial composition, disruption of the normal steady state, and a potential shift from a non-pathogenic symbiotic state to a pathogenic state.

3.3 Diversity analysis

This study employed PCA and NMDS analyses to examine species abundance across various classification levels among samples and between infected and non-infected groups. Microbial species similarity is reflected by their proximity in PCA and NMDS plots. PCA and NMDS clustering analyses at the phylum level revealed variations in fecal microbiota composition between infected and non-infected groups, as illustrated in Figure 3.

3.4 Analysis of differential microbial communities

This study employed the Metastats method for statistical analysis of species abundance data, highlighting significant intergroup differences. It identified species with notable disparities and illustrated the abundance distribution using box plots. Figure 4 shows the 12 bacterial species exhibiting the most pronounced differences at the species level between the groups. This includes *Klebsiella pneumoniae* and *Actinobacter baumannii*, two conditionally pathogenic bacteria significantly more prevalent in the infected group. In the non-infected group, bacterial communities, including *Faecalibacterium*, *Ruminococcus*, and *Precotella*, which are involved in intestinal substance metabolism and short-chain fatty acid synthesis, showed higher abundance compared to the infected group. This finding suggests that in the infected group, the normal intestinal flora

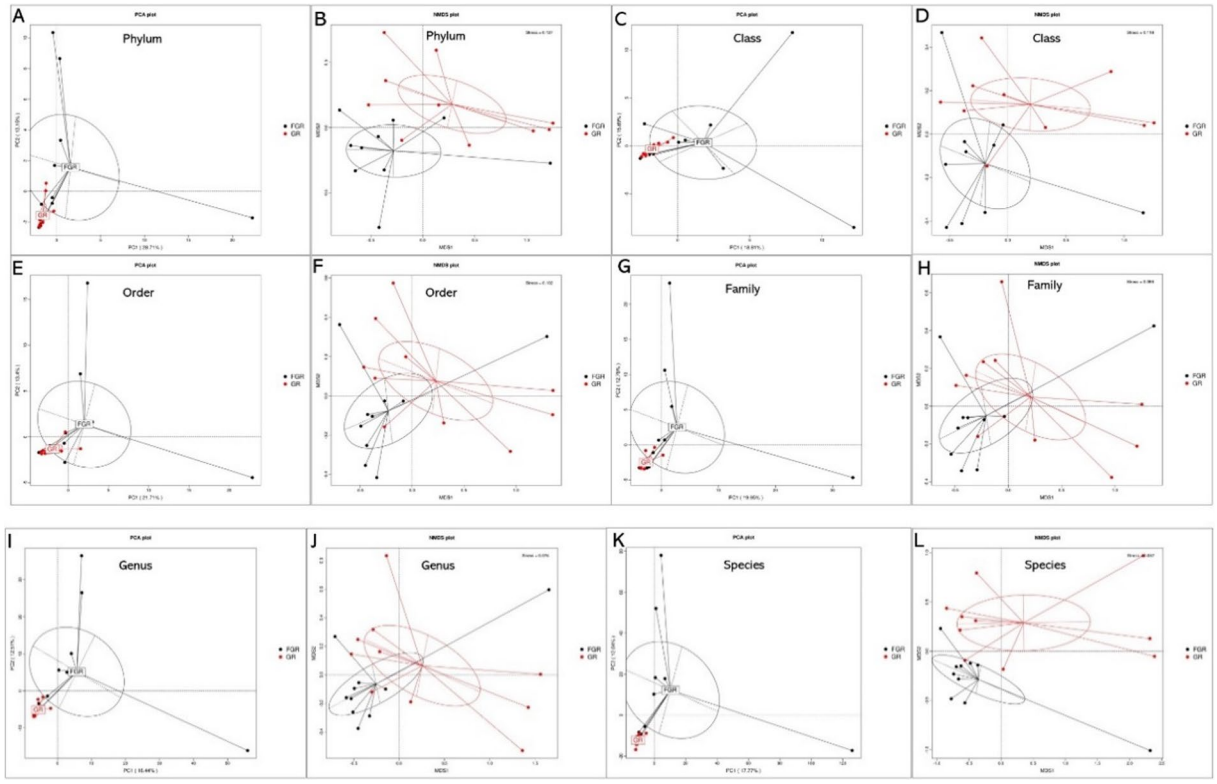

FIGURE 3
PCA and NMDS results of species at different levels between the two groups. **(A)** Phylum-level PCA; **(B)** phylum-level NMDS; **(C)** class-level PCA; **(D)** class-level NMDS; **(E)** order-level PCA; **(F)** order-level NMDS; **(G)** family-level PCA; **(H)** family-level NMDS; **(I)** genus-level PCA; **(J)** genus-level NMDS; **(K)** species-level PCA; **(L)** species-level NMDS. In PCA analyses, the *x*-axis and *y*-axis denote the first and second principal components, respectively, with the percentages reflecting the contribution of each principal component. Each sample is represented by a point in the figure, with samples from the same group depicted in identical colors. In NMDS analyses, points in the figure represent samples, with the distance between points within the same group reflecting sample repeatability. The closeness of samples within a group indicates the variation in hierarchical distance among group samples. The distance between points signifies the level of dissimilarity among samples, with samples from the same group shown in the same color. An NMDS stress value below 0.2 denotes a meaningful graphical analysis.

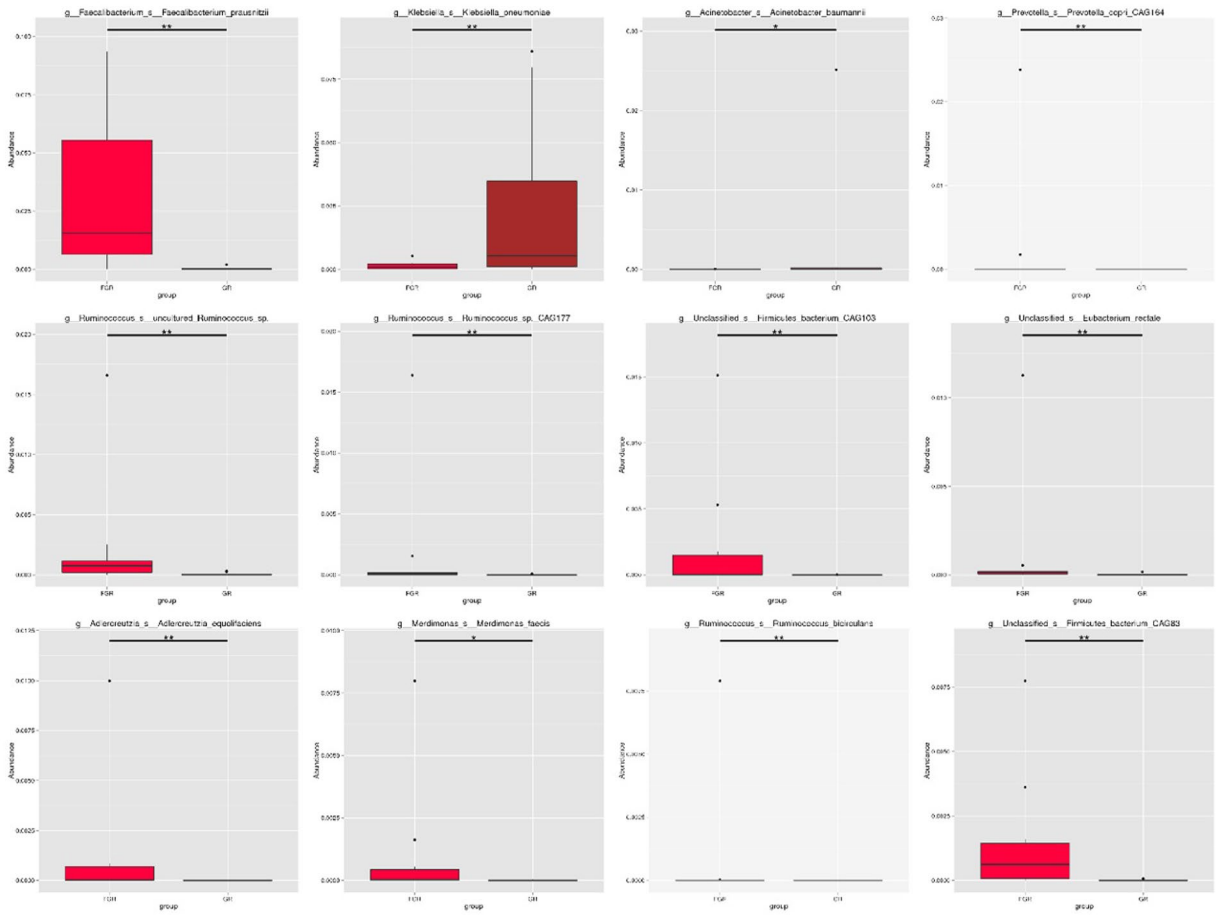

FIGURE 4
Box plot showing species with statistical differences at the species level. The horizontal axis denotes sample grouping, while the vertical axis indicates the relative abundance of the corresponding species. A horizontal line signifies the presence of statistical differences between two groups; its absence suggests no statistical difference exists for that species between the groups. $*p < 0.05$, $**p < 0.01$.

structure may be disrupted due to factors such as disease state and the use of antimicrobial drugs (Figure 5).

3.5 Functional gene structure

Upon analyzing the functional genes across all specimens, it was observed that the fecal microbiota from the 20 patients contained a significant number of functional genes associated with metabolic processes (Figure 6), particularly those involved in carbohydrate metabolism. These findings support the role of the gut microbiome in facilitating the digestion and breakdown of nutrients.

By analyzing the functional gene types of each sample and group, differences in the microbial structure between the infection group and the non-infection group can be observed. Both KEGG and eggNOG annotations show that microbial metabolism, stress response to external signals, and pathogen-related genes are more abundant in patients from the infection group. ANOSIM indicates that the differences between groups are greater than those within samples, confirming the existence of disparities between the infection group and the non-infection group. Level 1 analysis of eggNOG reveals significant differences in gene functions between the two groups.

3.6 Resistance gene analysis

Human intestinal microbiota frequently harbor antibiotic resistance genes. Antimicrobial drug usage has disrupted the stable co-existence of microbial species, impacting human health and ecological stability. Consequently, resistance gene research has garnered widespread attention. This study's specimens contained a significant number of resistance genes (Figure 7). Focusing solely on the types of resistance genes, 222 types were common to both infected and non-infected groups. The non-infected group had a slightly higher number of unique resistance genes compared to the infected group. Despite no difference in gene types (ARO), a significant disparity in resistance gene abundance was observed between the groups. ANOSIM, based on resistance gene abundance, revealed a significant difference between the infected and non-infected groups. The heatmap (Figure 8) indicated an accumulation of resistance genes in the feces of infected patients, likely due to the selective pressure from antimicrobial drug use.

4 Discussion

We collected fecal samples from SAP patients with and without secondary intra-abdominal infection. Metagenomic deep sequencing

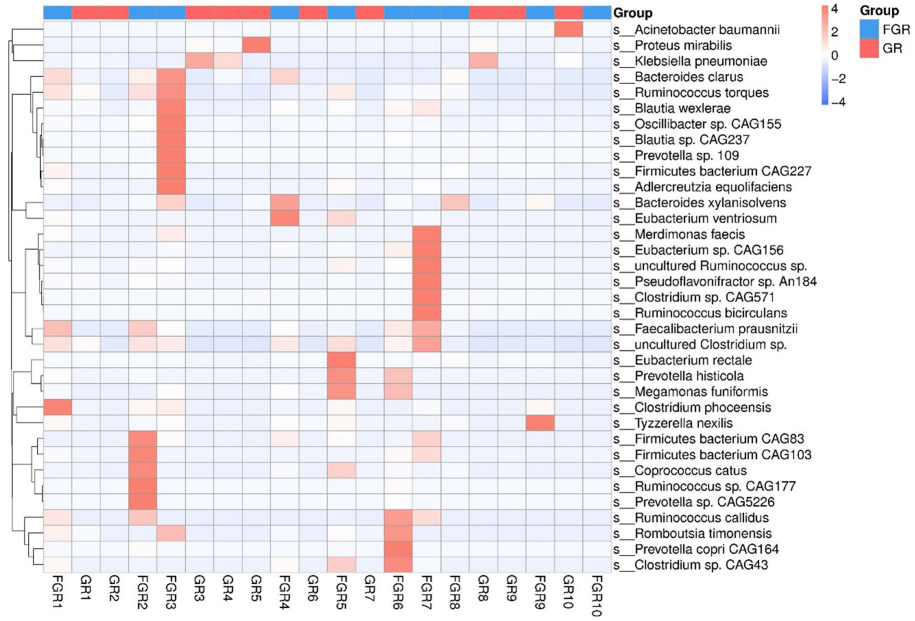

FIGURE 5
Heatmap of microbes detected in patients at the species level.

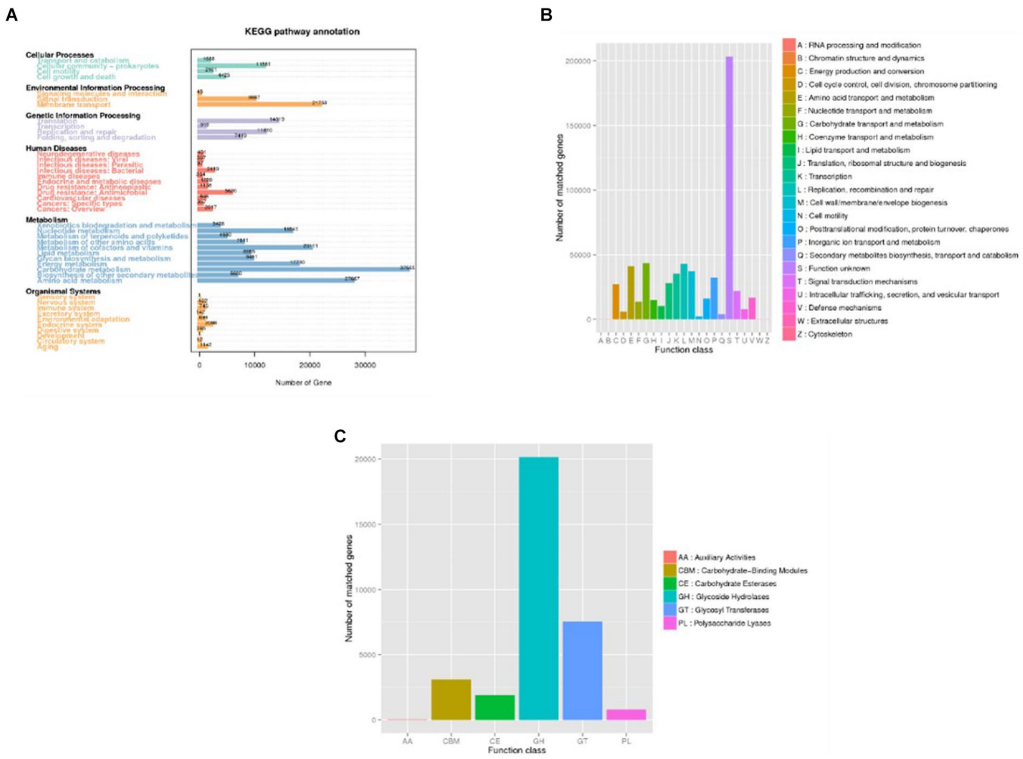

FIGURE 6
Distribution of annotated genes (Unigenes) across databases. **(A)** KEGG; **(B)** eggNOG. **(C)** CAZy. In panel **(A)**, the horizontal axis represents the number of annotated genes, while in panel **(B,C)** this role is assumed by the vertical axis. Conversely, the vertical axis in panel **(A)** and the horizontal axis in panel **(B,C)** depict the predicted levels of description, thereby illustrating the distribution of sample annotations across various functional categories.

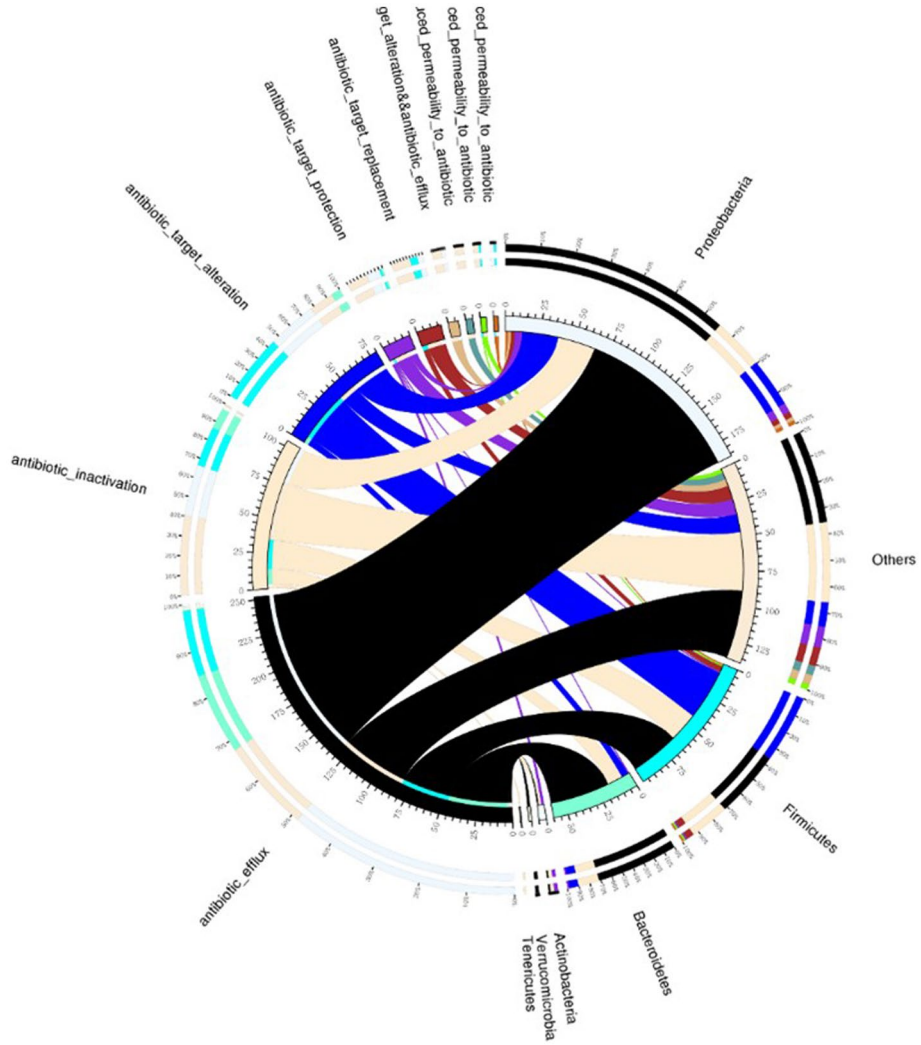

FIGURE 7
Venn diagram illustrating resistance mechanisms and species across all samples. The right side of the Venn diagram displays species information at the phylum level, and the left side outlines resistance mechanisms. The large circle on the left shows the percentage of resistance genes in each species, sorted by resistance mechanism. In contrast, the right side depicts the proportion of resistance genes in each mechanism, linked to specific species. The color of each small circle represents the corresponding species and resistance mechanisms, with the scale indicating the number of resistance genes. The left side shows the total number of genes associated with a specific resistance mechanism, whereas the right side presents the overall count of resistance genes.

analysis revealed that the gut microbiota of the infected group displayed distribution across multiple clustering branches, indicating intra-group heterogeneity and affected microbial diversity. The gut microbiome of the infected group was enriched with various opportunistic pathogens, exhibiting enhanced metabolic and stress response functions, as well as an increase in pathogenesis-related genes. Both groups showed antibiotic resistance gene expression in their gut microbiome. However, the non-infected group had significantly lower levels of these genes compared to the infected group.

The gut microbiota maintains host physiological balance and acts as a crucial barrier against pathogen invasion. It is essential for nutrition, energy metabolism, immune responses, and defending against infections (3). Research indicates that alterations in gut microecology significantly contribute to the development of inflammatory diseases, especially in the abdominal cavity. In SAP and similar conditions, a rapid release of inflammatory mediators can alter gut microbiota composition, degrade the colonic mucus layer, and increase intestinal mucosal permeability. Consequently, this leads to reduced colonization resistance, facilitating gut flora translocation, higher rates of enterogenic infections (e.g., *Enterobacteriaceae*, *Pseudomonadaceae*, and *Clostridium difficile*), and the emergence of metabolic diseases (4).

Colonization resistance refers to the gut microbiota's role in preventing colonization by foreign bacterial pathogens (17, 18). As described in the 1960s, this phenomenon is primarily attributed to direct microbial inhibition (19). The abundant gut microbiota compete for scarce nutrients and epithelial cell adhesion sites, thus preventing overgrowth and potential pathogen invasion. Besides directly competing for nutrients and ecological niches, the gut microbiota indirectly combats invading pathogens by boosting the host's gut immune defense (immune-mediated colonization resistance). Overall, these direct and indirect mechanisms synergistically prevent potential

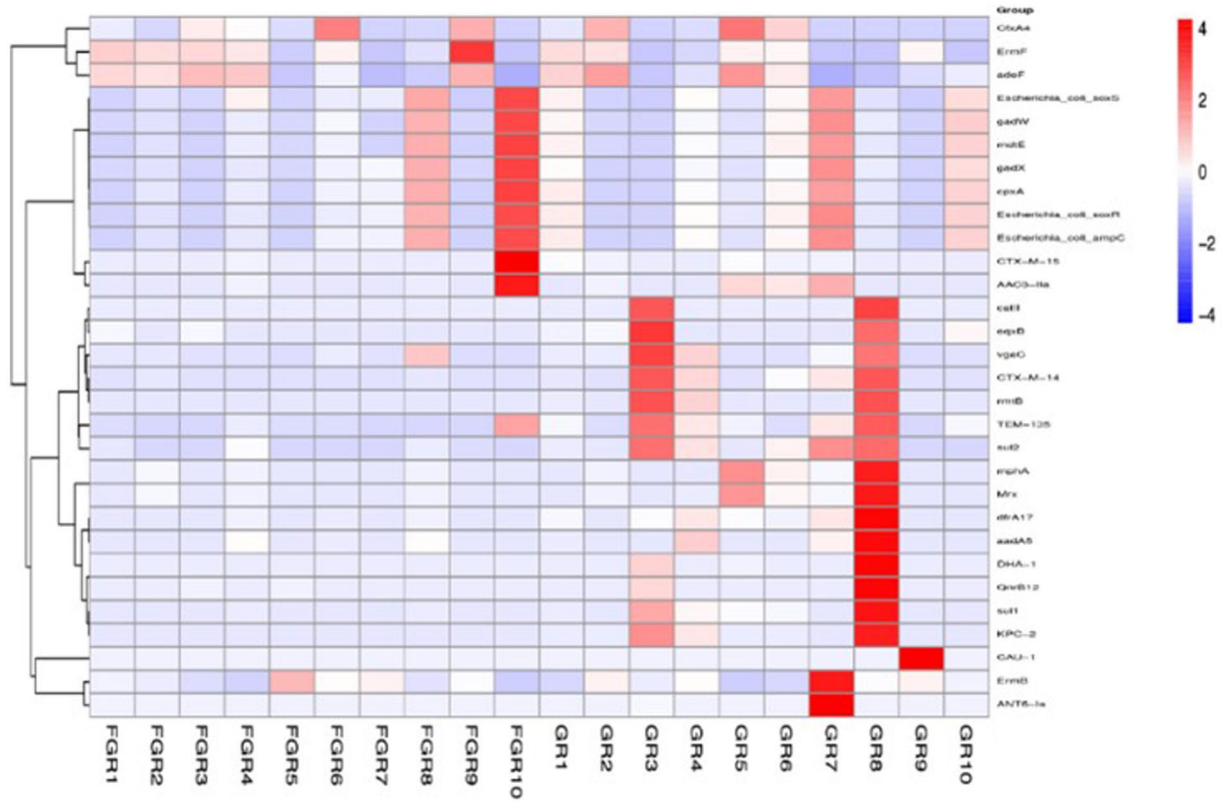

FIGURE 8
ARO distribution and abundance clustering heatmap. The horizontal axis represents the sample names, and the vertical axis on the right represents the names of the antibiotic resistance gene types (ARO).

pathogen colonization and invasion, thereby inhibiting bacterial translocation and enterogenic infection (20, 21). The controversial use of prophylactic antimicrobial drugs in SAP patients can reduce the diversity of intestinal symbiotic bacteria and their direct inhibitory effects. Consequently, antimicrobial-resistant bacteria like vancomycin-resistant *Enterococci*, carbapenem-resistant *Enterobacteriaceae*, and *Clostridium difficile* can proliferate, occupy the mucosal surface, and cause bacterial translocation, severe enterogenic infections, intra-abdominal infections, and bloodstream invasion (22). This aligns with findings from the group with secondary infections in severe acute pancreatitis in this study, characterized by various conditional pathogens and a high abundance of resistance genes. Although the exact mechanisms of colonization resistance remain unclear, this study indicates that alterations in gut microecology diversity could be a contributing factor. A future research direction involves diminishing colonization of conditional pathogens, lowering the expression of resistance genes, and mitigating secondary infections through the restoration of gut microbiota integrity using probiotics (23).

In summary, our study indicates that severe acute pancreatitis secondary infections impact the diversity of intestinal microecological flora in patients. The intestines of these patients exhibit a rich presence of various opportunistic pathogens, enhanced metabolic and stress response functions to external signals, and an increased number of pathogenesis-related genes, along with a high expression of antibiotic resistance genes.

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 humans were approved by the Ethics Committee of Ruijin Hospital School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LW: Data curation, Formal analysis, Writing – original draft. WZ: Conceptualization, Data curation, Formal analysis, Software, Writing – original draft. SD: Data curation, Formal analysis, Project administration, Writing – original draft. YG: Formal analysis, Funding acquisition, Resources, Validation, Writing – review & editing. CZ: Conceptualization, Formal analysis, Writing – original draft. YY: Writing – original draft, Writing – review & editing.

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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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