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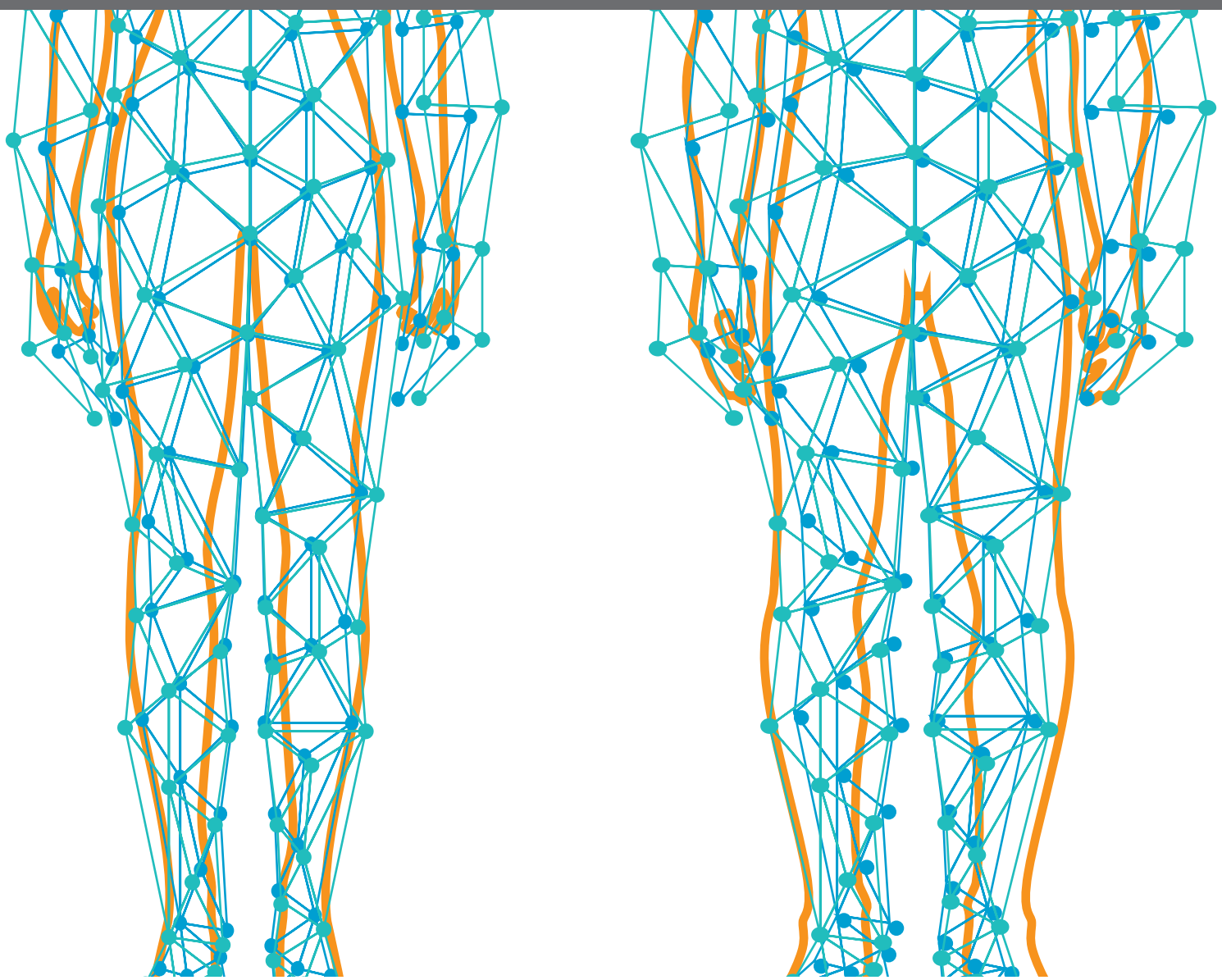

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INSIGHTS IN INTENSIVE CARE MEDICINE AND ANESTHESIOLOGY: 2021

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Sequential Organ Failure Assessment Score for Prediction of Mortality of Patients With Rhabdomyolysis Following Exertional Heatstroke: A Longitudinal Cohort Study in Southern China

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Background: Heatstroke is a medical emergency that causes multi-organ injury and death without intervention, but limited data are available on the illness scores in predicting the outcomes of exertional heat stroke (EHS) with rhabdomyolysis (RM). The aim of our study was to investigate the Sequential Organ Failure Assessment (SOFA) score in predicting mortality of patients with RM after EHS.

Methods: A retrospective cohort study was performed, which included all patients with EHS admitted into the intensive care unit (ICU) of General Hospital of Southern Theater Command of Peoples Liberation Army from January 2008 to June 2019. RM was defined as creatine kinase (CK) > 1,000 U/L. Data, including the baseline data at admission, vital organ function indicators, and 90-day mortality, were reviewed.

Results: A total of 176 patients were enrolled; among them, 85 (48.3%) had RM. Patients with RM had a significantly higher SOFA score (4.0 vs. 3.0, $p = 0.021$), higher occurrence rates of disseminated intravascular coagulation (DIC) (53.1 vs. 18.3%, $p < 0.001$) and acute liver injury (ALI) (21.4 vs. 5.5%, $p = 0.002$) than patients with non-RM. RM was positively correlated with ALI and DIC, and the correlation coefficients were 0.236 and 0.365, respectively (both p-values <0.01). Multivariate logistics analysis showed that the SOFA score [odds ratio (OR) 1.7, 95% CI 1.1–2.6, $p = 0.024$] was the risk factor for 90-day mortality in patients with RM after EHS, with the area under the curve (AUC) 0.958 (95% CI 0.908–1.000, $p < 0.001$) and the optimal cutoff 7.5 points.

Conclusions: Patients with RM after EHS have severe clinical conditions, which are often accompanied by DIC or ALI. The SOFA score could predict the prognosis of patients with RM with EHS. Early treatment strategies based on decreasing the SOFA score at admission may be pivotal to reduce the 90-day mortality of patients with EHS.

Keywords: exertional heatstroke, rhabdomyolysis, mortality, risk factors, SOFA score

INTRODUCTION

Heatstroke is an acute medical emergency characterized by the central nervous system (CNS) dysfunction, multi-organ failure, and extreme hyperthermia (usually >40.5°C) with a mortality rate of 40 to 70% and a disability rate of 30%. It is typically classified as exertional heat stroke (EHS) and classical heatstroke (CHS) (1). Rhabdomyolysis (RM) is one of the complications in patients with EHS, which ranges from an asymptomatic illness with an elevated creatine kinase (CK) level to a life-threatening condition associated with extreme elevations in CK, electrolyte imbalances, acute kidney injury (AKI), or disseminated intravascular coagulation (DIC). However, the relationships between RM and organ function and prognosis are poorly documented.

Some studies have also shown that RM caused by heatstroke may be an important factor in initiating sepsis (2–6) and will further cause the disturbance of blood coagulation, which can easily lead to the occurrence of DIC (7) and AKI (8, 9). Research showed that the Sequential Organ Failure Assessment (SOFA) score was an independent risk factor affecting the survival of patients (10); therefore, treatments based on reducing the SOFA score may be pivotal for reducing the mortality of EHS complicated with AKI (11). However, so far there are few clinical studies on the clinical characteristics and prognosis of EHS complicated with RM, and there is no scoring system that can predict prognosis in patients with RM with EHS. To provide a reference for timely and effective treatment, a retrospective cohort study was designed in a tertiary-care teaching hospital in southern China over 10 years, in which the clinical characteristics, the relationships between RM and organ function, and risk factors and 90-day mortality were analyzed.

METHODS

Study Design and Participants

This retrospective cohort study was performed in the intensive care unit (ICU) of General Hospital of Southern Theater Command of Peoples Liberation Army from January 2008 to June 2019. The inclusion criteria of EHS are as follows (1): patients exposed to high temperature, high humidity, and history of strenuous exercise, with an excessively high body temperature (central temperature higher than 40°C) or/and nervous system dysfunction (including delirium, cognitive impairment, coma, etc.). The exclusion criteria were as follows: (1) death or discharged within 24 h after admission, (2) incomplete data regarding key indicators, (3) incomplete outcome evaluation data obtained *via* telephone follow-up, and (4) a previous history of organ dysfunction, such as skeletal muscle disease and chronic kidney disease.

Comprehensive treatments were provided to all patients, such as body cooling, the volume of infusion, and anti-inflammation drugs. Meanwhile, organ function supports were provided for patients with RM under clinical guidelines if necessary, including appropriate hydration, alkalization of urine, blood purifications with polymer interception, and so on.

Research Procedures

The basic characteristics of patients were reviewed, including the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, SOFA score, Glasgow Coma Score (GCS), and inflammatory and organ function indicators at admission. The indicators included blood count (lymphocyte and platelets), kidney function markers [blood urea nitrogen (BUN) and serum creatinine (Scr)], liver function markers [total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST)], C-reactive protein (CRP), procalcitonin (PCT), cardiac markers [CK, MB isoenzyme of creatine kinase (CK-MB), MB, and cardiac troponin I (cTNI)], clotting factors [prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), and D-dimer], and blood transfusion during treatment. All patients were assigned to the RM group and the non-RM group according to the presence of RM. Survival time was defined as the duration from onset to death; when the survival time was longer than 90 days, it was recorded as 90 days. The main results, including the 90-day mortality, ICU time, and the total cost during hospitalization, were analyzed. The survival curve analysis was performed.

Definitions

(1) RM (12): General fatigue, muscle soreness, and soy sauce-like urine; elevated laboratory CK; and elevated non-cardiogenic MB. This study adopted the current consensus opinion that CK > 1,000 U/L or increased more than five times the normal level was considered as elevated CK, whereas an increase in CK due to cardiogenic shock (CK-MB/CK < 5%) was excluded.
(2) DIC (13): International Society for Thrombosis and Haemostasis (ISTH) standard: An ISTH score ≥5 points.
(3) AKI (14): The Kidney Disease: Improving Global Outcomes (KDIGO) standard: Scr increased to ≥26.5 μmol/L (≥0.3 mg/dl) within 48 h, Scr increased to ≥1.5 times the baseline within 7 days, or urine output <0.5 ml/(kg h) for 6 h.
(4) Acute liver injury (ALI) (15): Plasma TBIL ≥ 34.2 μmol/L and INR ≥ 1.5, or with any grade of hepatic encephalopathy.
(5) Lymphocytopenia (16): Absolute lymphocytes less than 0.8×10^9/L.

Statistical Analysis

The continuous variables conformed to a normal distribution are expressed as $\bar{x} \pm s$. For continuous variables that did not conform to a normal distribution are presented as medians and interquartile ranges (IQRs), and the categorical data were summarized as numbers and percentages. Continuous variables were compared using the independent two-sample *t* test or Mann–Whitney *U* tests. Categorical variables were compared using the Chi-Square test or Fisher's exact test. Significant indicators were analyzed using single factor analysis. Indicators with a *P* value < 0.1 were included in the multivariate logistic regression (LR) model: OR (odds ratio) and 95% confidence interval levels (95% CI), and forward stepwise regression was

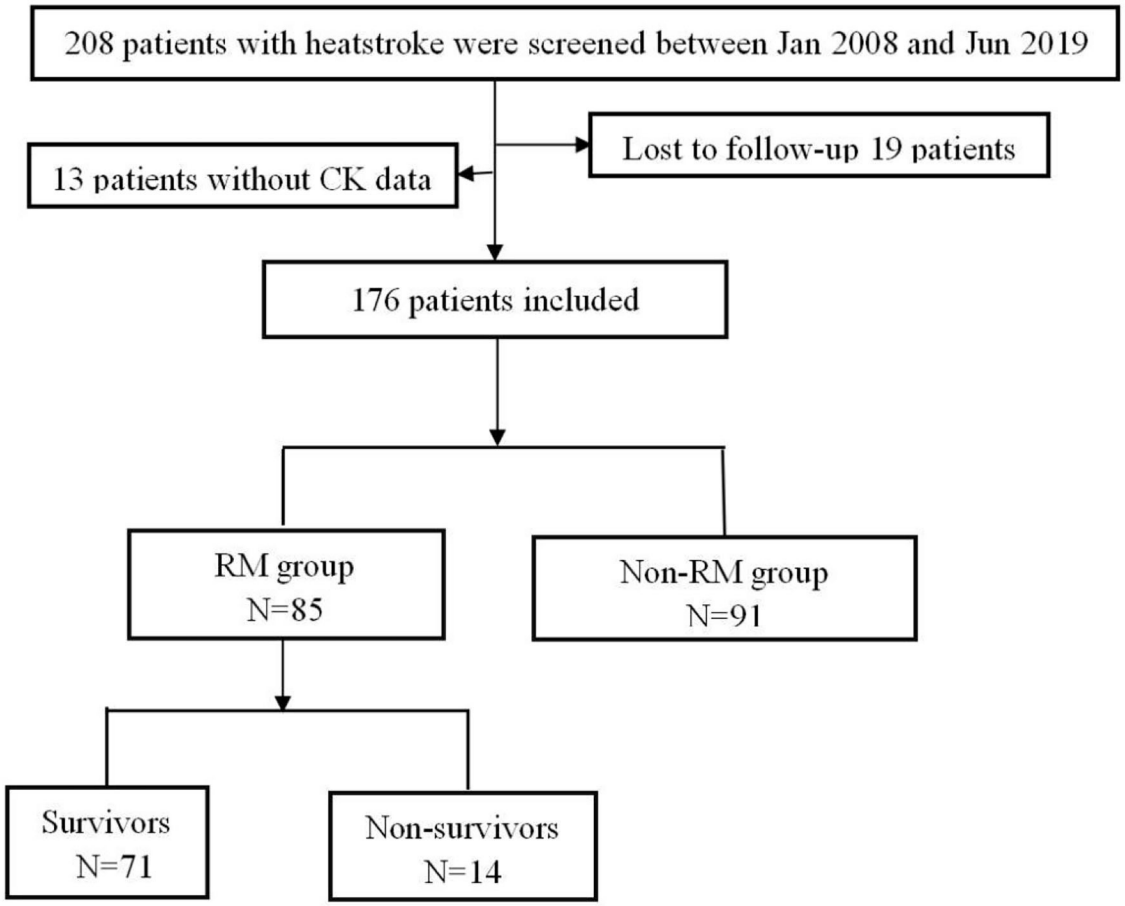

FIGURE 1 | Flowchart of all excluded and included patients.

used to gradually eliminate each variable. The predictive ability of SOFA score for 90-day mortality was assessed using the area under the receiver operating characteristic (AU-ROC) curve, and the optimal cutoff value was determined by Youden's index. We analyzed the 90-day mortality in the RM and non-RM groups using the Kaplan–Meier's method and assessed the differences by the log-rank test. The relationship between RM and organ injury was performed using the Pearson correlation analysis. Statistical analyses were performed using the IBM SPSS Windows version 23.0 (IBM Corp., Armonk, NY, USA), Empower (R) (http://www.empowerstats.com, X&Y solutions, Inc., Boston, MA, USA), and R (http://www.R-project.org) software. The *p*-values (two-tailed) less than 0.05 were considered statistically significant.

RESULTS

Clinical Characteristics of the Patients With EHS

A total of 208 patients fulfilled the inclusion criteria; among them, 32 patients were excluded because of loss to follow-up or missing CK data. Finally, 176 patients were included, who were all men; among them, there were 91 patients (51.7%) without RM, and 85 patients (48.3%) with RM (**Figure 1**). There was no statistical difference in age between the two groups [20.0 vs. 22.0 (years), $p = 0.472$]. Compared with the patients with non-RM, the patients with RM had a significantly higher SOFA score (4.0 vs. 3.0, $p = 0.021$), higher incidence of DIC (53.1 vs. 18.3%, $p < 0.001$) and ALI (21.4% vs. 5.5%, $p = 0.002$). These patients also had lower lymphocyte and platelet, significantly increased CK, MB, PCT, PT, APTT, D-dimer, ALT, and AST, and higher blood transfusion proportion than patients with non-RM (all $p < 0.05$). However, there were no statistically significant differences in APACHE II (12.0 vs. 10.0, $p = 0.285$) and GCS scores (10.0 vs. 12.0, $p = 0.429$) and also in the incidence of lymphocytopenia (44.6 vs. 33.0%, $p = 0.116$) and AKI (48.8 vs. 39.6%, $p = 0.218$). Interestingly, the 90-day mortality of patients with RM was not significantly increased (16.5% vs. 8.8%, $p = 0.124$), whereas the total cost of hospitalization was particularly higher [51,986.3 vs. 31,810.5 (RMB), $p = 0.036$] than that of patients with non-RM (**Table 1**).

Comparisons of Survivors and Non-survivors With EHS Complicated With RM

Among the patients with RM induced by EHS, 71 survived (83.5%) and 14 died (16.5%). Non-survivors had higher APACHE

TABLE 1 | Comparisons of clinical characteristics between patients with rhabdomyolysis (RM) and non-RM with exertional heat stroke (EHS).

Variables	Non-RM (n = 91)	RM (n = 85)	p-value
APACHE II score, median (IQR)	10.0 (8.0–15.0)	12.0 (8.0–17.8)	0.285
SOFA score, median (IQR)	3.0 (2.0–5.0)	4.0 (2.0–9.0)	0.021
GCS score, median (IQR)	12.0 (7.0–14.0)	10.0 (7.0–13.0)	0.429
Age (years), median (IQR)	20.0 (19.0–26.5)	22.0 (19.0–27.0)	0.472
Lymphocyte (1×10^9/L), median (IQR)	1.3 (0.7–2.3)	0.9 (0.5–1.7)	0.044
Platelets (1×10^9/L), median (IQR)	186.0 (139.0–230.5)	106.0 (59.0–177.5)	<0.001
TBIL (μmol/L), median (IQR)	12.2 (8.6–21.5)	19.9 (13.3–37.6)	<0.001
ALT (U/L), median (IQR)	22.0 (15.0–41.5)	91.0 (33.0–868.0)	<0.001
AST (U/L), median (IQR)	36.0 (26.0–62.0)	173.0 (88.0–837.0)	<0.001
BUN (mmol/L), median (IQR)	5.5 (4.4–6.6)	6.2 (4.9–8.1)	0.022
Scr (μmol/L), median (IQR)	127.0 (94.0–150.5)	131.0 (93.0–186.0)	0.164
Cystatin C (mg/L), median (IQR)	1.0 (0.8–1.2)	1.0 (0.8–1.2)	0.838
CK (U/L), median (IQR)	372.0 (212.0–636.0)	2,918.0 (1,536.0–5,568.0)	<0.001
CK-MB (ng/ml), median (IQR)	26.0 (19.5–34.5)	72.0 (45.0–128.5)	<0.001
MB (ng/ml), median (IQR)	239.1 (73.3–646.5)	954.0 (317.8–1000.0)	<0.001
cTNI (pg/ml), median (IQR)	90.0 (26.4–281.1)	200.0 (30.0–699.9)	0.481
PT (s), median (IQR)	14.7 (13.7–16.4)	18.1 (15.8–26.2)	<0.001
INR, median (IQR)	1.2 (1.0–1.3)	1.5 (1.2–2.1)	0.003
APTT (s), median (IQR)	35.0 (31.6–40.9)	43.3 (37.4–76.5)	0.001
TT (s), median (IQR)	17.3 (16.3–18.5)	18.1 (16.8–29.8)	0.003
FIB (g/L), median (IQR)	2.5 (2.1–3.0)	2.4 (1.9–2.8)	0.111
D-Dimer (mg/L), median (IQR)	0.9 (0.4–3.7)	3.7 (0.9–10.1)	<0.001
CRP (mg/dl), median (IQR)	2.0 (0.4–3.7)	3.4 (3.2–7.2)	<0.001
PCT (ng/ml), median (IQR)	1.2 (0.6–2.4)	3.1 (1.4–5.3)	0.008
Transfusion, *N* (%)	14/90 (15.6%)	34/80 (42.5%)	<0.001
Lymphocytopenia, *N* (%)	30/91 (33.0%)	37/83 (44.6%)	0.116
DIC, *N* (%)	13/71 (18.3%)	34/64 (53.1%)	<0.001
AKI, *N* (%)	36/91 (39.6%)	41/84 (48.8%)	0.218
ALI, *N* (%)	5/91 (5.5%)	18/84 (21.4%)	0.002
90-day mortality, *N* (%)	8/91 (8.8%)	14/85 (16.5%)	0.124
ICU time (days), median (IQR)	4.0 (3.0–8.5)	6.0 (4.0–10.0)	0.024
Survival time (days), median (IQR)	90.0 (90.0–90.0)	90.0 (90.0–90.0)	0.118
Hospitalization costs (RMB), median (IQR)	31,810.5 (20,639.3–56,200.3)	51,986.3 (33,642.8–132,146.6)	0.036

AKI, acute kidney injury; ALI, acute liver injury; APTT, activated partial thromboplastin time; APACHE II, Acute Physiology and Chronic Health Evaluation II; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, MB isoenzyme of creatine kinase; CRP, C-reactive protein; cTNI, cardiac troponin I; DIC, disseminated intravascular coagulation; FIB, fibrinogen; ICU, intensive care unit; INR, international normalized ratio; IQR, interquartile range; PCT, procalcitonin; PT, prothrombin time; Scr, serum creatinine; SOFA, Sequential Organ Failure Assessment; TT, thrombin time.

II score [23.0 (21.0–23.5) vs. 11.0 (8.0–14.5), $p < 0.001$] and SOFA score at admission [12.0 (10.5–14.5) vs. 3.0 (2.0–6.0), $p < 0.001$], and lower GCS scores [6.0 (4.5–7.5) vs. 12.0 (8.0–13.5), $p = 0.005$]. In the non-survivor group, the organ function was worse than that in the survivor group, illustrated by the significantly increased total bilirubin, ALT, AST, Scr, Cystatin C, cTNI, and MB values (all $p < 0.05$), the worse blood coagulation (PT, INR, APTT, and D-dimer, all $p < 0.001$), and higher blood transfusion proportion (90 vs. 35.7%, $p < 0.001$). Furthermore, the non-survivors were easily complicated with DIC (100 vs. 42.3%, $p < 0.001$), AKI (100 vs. 38.6%, $p < 0.001$) and ALI (42.9 vs. 17.1%, $p = 0.032$) but there were no significant differences in the incidence of lymphocytopenia (61.5 vs. 41.4%, $p = 0.180$), inflammation index (PCT/CRP) ($p > 0.05$), and the length of ICU time [5.5 vs. 6.0 (days), $p = 0.381$] between survivors and non-survivors group. However, the total hospitalization costs in the non-survivor group were increased [156,820.3 vs. 45,182.6 (RMB), $p < 0.001$] (**Table 2**).

The Relationship Between RM and Another Organ Injury in EHS

It was found by the Pearson correlation that RM was positively correlated with ALI and DIC, with the correlation coefficients of 0.236 and 0.365, respectively (both $p < 0.01$). However, it was not associated with AKI ($p = 0.220$) and lymphocytopenia ($p = 0.117$) when CK > 1,000 U/L was used as the serological diagnostic standard of RM (**Table 3**).

TABLE 2 | Comparisons of clinical characteristics between survivors and non-survivors with RM induced by EHS.

Variables	Survivor(n = 71)	Non-survivor(n = 14)	p-value
APACHE II score, median (IQR)	11.0 (8.0–14.5)	23.0 (21.0–23.5)	<0.001
SOFA score, median (IQR)	3.0 (2.0–6.0)	12.0 (10.5–14.5)	<0.001
GCS score, median (IQR)	12.0 (8.0–13.5)	6.0 (4.5–7.5)	0.005
Age (years), median (IQR)	22.0 (19.0–27.5)	21.5 (20.2–23.0)	0.311
Lymphocyte (1×10^9/L), median (IQR)	1.0 (0.5–1.7)	0.5 (0.3–2.1)	0.368
Platelets (1×10^9/L), median (IQR)	127.0 (72.8–186.2)	65.0 (29.0–84.0)	0.004
TBIL (μmol/L), median (IQR)	17.6 (12.9–33.5)	37.4 (21.2–103.6)	<0.001
ALT (U/L), median (IQR)	64.0 (31.0–648.2)	546.5 (95.0–1,648.2)	0.028
AST (U/L), median (IQR)	133.0 (77.5–708.0)	408.5 (306.5–1,849.2)	0.020
BUN (mmol/L), median (IQR)	5.8 (4.5–7.7)	7.9 (6.2–9.0)	0.597
Scr (μmol/L), median (IQR)	114.0 (88.5–149.0)	245.5 (210.0–283.0)	<0.001
Cystatin C (mg/L), median (IQR)	1.0 (0.8–1.2)	1.5 (1.1–2.8)	<0.001
CK (U/L), median (IQR)	2,486.0 (1,462.5–4,927.0)	6,196.0 (2,231.8–8,251.5)	0.583
CK–MB (ng/ml), median (IQR)	71.0 (44.0–105.0)	298.0 (98.0–374.0)	0.332
MB (ng/ml), median (IQR)	658.0 (228.0–1,000.0)	1,000.0 (1,000.0–1,000.0)	0.019
cTNI (pg/ml), median (IQR)	110.0 (20.0–343.1)	1,530.0 (1,019.0–3,860.0)	<0.001
PT (s), median (IQR)	17.1 (15.4–21.9)	38.6 (24.8–45.3)	<0.001
INR, median (IQR)	1.4 (1.2–1.9)	4.2 (2.8–5.0)	<0.001
APTT (s), median (IQR)	41.2 (36.4–49.8)	93.8 (68.5–123.8)	<0.001
TT (s), median (IQR)	17.7 (16.6–22.2)	40.7 (28.9–58.7)	<0.001
FIB (g/L), median (IQR)	2.5 (2.1–2.8)	1.3 (0.9–1.8)	0.002
D–Dimer (mg/L), median (IQR)	2.9 (0.7–6.6)	10.1 (10.0–20.0)	<0.001
CRP (mg/dl), median (IQR)	3.6 (3.2–7.7)	3.3 (3.3–3.3)	0.454
PCT (ng/ml), median (IQR)	3.0 (1.3–4.8)	4.6 (1.7–8.1)	0.433
Transfusion, *N* (%)	25/70 (35.7%)	9/10 (90%)	<0.001
Lymphocytopenia, *N* (%)	29/70 (41.4%)	8/13 (61.5%)	0.180
DIC, *N* (%)	22/52 (42.3%)	12/12 (100.0%)	<0.001
AKI, *N* (%)	27/70 (38.6%)	14/14(100.0%)	<0.001
ALI, *N* (%)	12/70 (17.1%)	6/14 (42.9%)	0.032
ICU time (days), median (IQR)	6.0 (4.0–10.8)	5.5 (5.0–8.8)	0.381
Survival time (days), median (IQR)	90.0 (90.0–90.0)	5.5 (5.0–8.8)	<0.001
Hospitalization costs (RMB), median (IQR)	45,182.6 (29,738.0–93,106.3)	156,820.3 (133,525.5–214,730.5)	<0.001

AKI, acute kidney injury; ALI, acute liver injury; APTT, activated partial thromboplastin time; APACHE II, Acute Physiology and Chronic Health Evaluation II; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, MB isoenzyme of creatine kinase; CRP, C-reactive protein; cTNI, cardiac troponin I; DIC, disseminated intravascular coagulation; FIB, fibrinogen; ICU, intensive care unit; INR, international normalized ratio; IQR, interquartile range; PCT, procalcitonin; PT, prothrombin time; Scr, serum creatinine; SOFA, Sequential Organ Failure Assessment; TT, thrombin time.

Risk Factors of 90-Day Mortality for EHS Complicated With RM

The univariate analysis showed that APACHE II, SOFA, GCS, Cystatin C, MB $\geq$ 1,000 ng/ml, INR, FIB, and D-dimer were closely related to the 90-day mortality of patients with RM (all $p<$ 0.001). The multivariate logistic regression showed that the SOFA score [OR 1.7 (1.1, 2.6), p = 0.024] was an independent risk factor affecting 90-day mortality in patients with EHS complicated with RM (**Table 4**). The area under the ROC curve for prediction of mortality based on the SOFA score was 0.958 (95% CI 0.908–1.000, p < 0.001), the optimal cutoff was 7.5 points, with SEN 100% and SPE 83.7% (**Figure 2**). However, there was no significant difference in the 90-day mortality between patients with RM and non-RM (p = 0.11) (**Figure 3**).

DISCUSSION

In this study, we observed clinical characteristics and risk factors in critically ill patients with RM induced by EHS in southern China. When CK $\geq$ 1,000 U/L was used as the serological diagnostic standard of RM, the results showed that the SOFA score of patients with RM was higher than that of patients with non-RM, which were mainly involving ALI and DIC. While non-survivors with EHS complicated with RM had a higher incidence of DIC and AKI at admission. The SOFA score at admission was an independent risk factor for 90-day mortality in patients with RM following EHS.

There are many causes leading to RM. Patients with RM caused by EHS were often doing strenuous exercise under high temperature and humidity, which are different from crush

TABLE 3 | The relationship between RM and another organ injury in EHS.

Variables	RM		
	Correlation	95%CI	*p*-value
AKI	0.093	0.056, 0.238	0.220
ALI	0.236	0.090, 0.371	0.002
Lymphocytopenia	0.119	−0.030, 0.263	0.117
DIC	0.365	0.209, 0.503	<0.001

AKI, acute kidney injury; ALI, acute liver injury; DIC, disseminated intravascular coagulation.

TABLE 4 | Risk factors for 90-day mortality with RM induced by EHS.

Variables	Univariate OR (95%CI) *p*-value	Multivariate OR (95%CI) *p*-value
APACHE II score	1.5 (1.3, 1.9) < 0.001	1.3 (0.8, 1.9) 0.272
SOFA score	2.2 (1.5, 3.1) < 0.001	1.7 (1.1, 2.6) 0.024
GCS score	0.6 (0.4, 0.8) < 0.001	1.2 (0.7, 2.2) 0.497
DIC	18.4 (5.0, 67.2) < 0.001	9.1 (0.2, 489.7) 0.277
AKI	19.7 (4.4, 87.5) < 0.001	9.5 (0.1, 748.2) 0.312
Cystatin C	2.7 (1.6, 4.5) < 0.001	NA
MB ≥ 1,000 ng/ml	7.4 (2.7, 20.4) < 0.001	NA
INR	2.1 (1.4, 3.0) < 0.001	NA
FIB	0.2 (0.1, 0.5) < 0.001	NA
D-dimer	1.1 (1.0, 1.2) < 0.001	NA
ALI	1,486,691.7 (0.0, Inf) 0.992	NA

Adjust model adjust for: Age. AKI, acute kidney injury; ALI, acute liver injury; APACHE II, Acute Physiology and Chronic Health Evaluation II; DIC, disseminated intravascular coagulation; FIB, fibrinogen; GCS, Glasgow Coma Score; OR, odds ratio; SOFA, Sequential Organ Failure Assessment.

syndrome. Vascular endothelial cells are more severely damaged due to the high temperature, which leads to a higher incidence of DIC. On the other hand, heatstroke could induce ALI *via* IL-1β and HMGB1-induced pyroptosis (17). It may be related to the pathogenesis as followed. RM releases myoglobin that can be decomposed into myosin, which plays an important role in the coagulation cascades, including both coagulation factors and fibrinolysis (18). In addition, some nuclear proteins are released by muscle cell injuries, such as histone 3 (19, 20) and HMGB1 (21), which can activate platelets and then lead to the occurrence and development of DIC and ALI. Previous studies have found that the renal tubular injury in RM caused by crush syndrome is mainly caused by apoptosis (22), which shows that RM is prone to AKI. The mechanism of its occurrence may include the following two aspects or the result of a combination: the deficiency of effective circulating blood volume caused by fluid loss and dehydration (23), and the mechanical obstruction of renal tubules caused by MB released RM (24). It has even been reported that myoglobin oxidative stress directly leads to renal tubular epithelial injury, but the specific mechanism of this injury is not completely clear yet (25). Furthermore, our study also showed that non-survived patients with RM were more likely complicated with AKI and DIC ($p < 0.001$). The reasons may be caused by the direct damage to vascular endothelial cells due to heat shock (26) and significantly reduced renal perfusion due to DIC, which are different from crush syndrome. Therefore, it is necessary to further explore the mechanism of RM on renal tubules under heatstroke.

Due to the imbalance between production and dissipation of heat, the occurrence of EHS is high when doing strenuous exercise under high temperature conditions. It often damages from CNS, striated muscle, kidney, and the coagulation system. The mechanism may be related to vascular endothelial cell damage, and the activation of inflammatory cells and platelets (21). A single-center retrospective study of 140 critical patients with severe heatstroke found that RM at admission was an independent risk factor for mortality (27). However, there was no further study on the effects of RM and other key organ functions and mortality. Therefore, we analyzed the effect of RM on the indicators of key organ functions and 90-day mortality by using RM as an exposure factor. The results showed that patients with EHS complicated with RM had more severe organ injuries. RM was positively correlated with ALI and DIC (all $p < 0.01$) whereas not associated with AKI ($p = 0.220$) and lymphocytopenia ($p = 0.117$), but there was no difference in 90-day mortality ($p = 0.11$) between patients with RM and non-RM. We speculated that the underlying reason is the inaccuracy of CK ≥ 1,000 U/L as the serological diagnostic criteria for RM in evaluating RM and organ function. In addition, there were other factors leading to AKI, such as glomerular perfusion pressure decreased because of the lower cardiac output, renal tubular apoptosis, renal interstitial edema, inflammatory exudation, and so on (25, 28). Multicenter prospective studies are needed to confirm CK thresholds at different organ injuries.

Multivariate logistic regression showed that only the SOFA score was an independent risk factor for 90-day mortality in patients with EHS complicated with RM, but not the APACHE II score. The APACHE II score is an important scoring system for evaluating the prognosis of critically ill patients, which involves age and chronic health. However, our patients were previously healthy and had a median age of 21 years. In addition, because the APACHE II score excluded some vital acute organ functions including coagulation function and liver function, it is not as comprehensive as that of the SOFA score. Therefore, the APACHE II score is not appropriate to evaluate prognosis in young patients with EHS. The optimal cutoff for the prediction of 90-day mortality based on the SOFA score was 7.5 points, with SEN 100% and SPE 83.7%. Moreover, SOFA scores in survivors and non-survivors with RM induced by EHS were 3.0 and 12.0, respectively. This indicates that the SOFA score can accurately predict the 90-day mortality of patients with RM induced by EHS. Survival curves showed there was no significant difference in the 90-day mortality between patients with RM (CK ≥ 1,000 U/L) and non-RM. Because the serologic diagnostic standard of RM with CK ≥ 1,000 U/L is too lenient, it does not reflect the true organ function status and predict the prognosis of patients. Multicenter prospective studies are needed to confirm CK thresholds for 90-day mortality in different disease states.

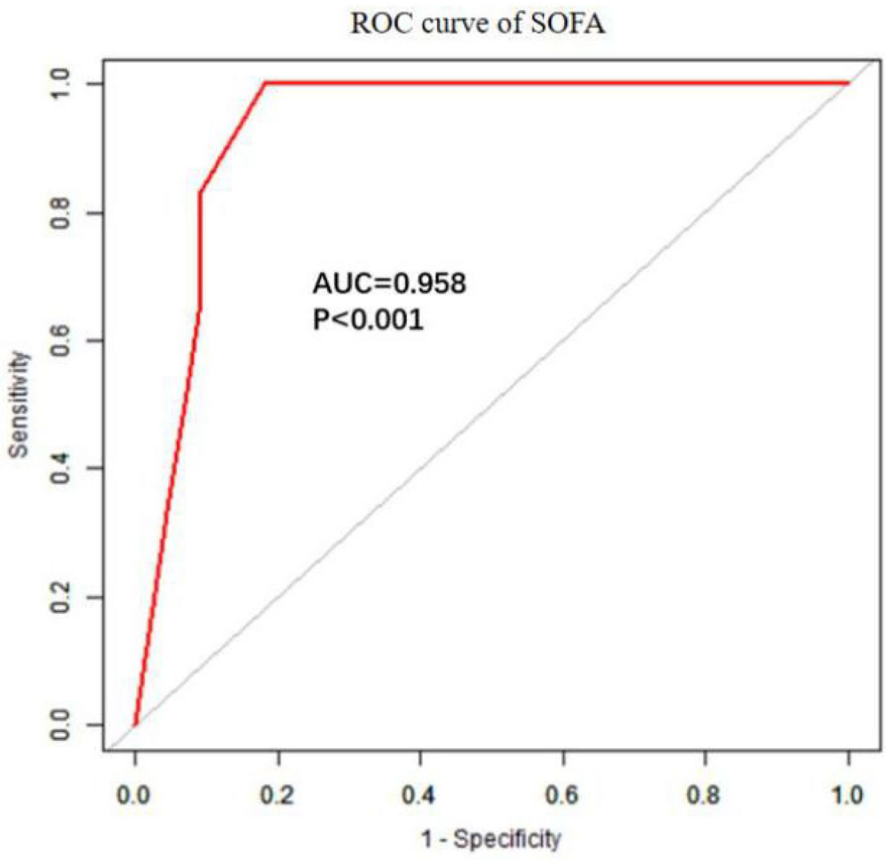

FIGURE 2 | The ROC curve of SOFA in predicting 90-day mortality with patients with RM induced by EHS. EHS, exertional heat stroke; RM, rhabdomyolysis; ROC, receiver operating characteristic; SOFA, Sequential Organ Failure Assessment.

There are many measures that can be used to treat RM in ICU, including removal of MB by blood purification (29), antioxidation (30), anti-inflammation (31), and so on. Only the SOFA score was an independent risk factor for mortality, suggesting that the follow-up treatment with the primary aim of protecting key organ function is an important way to reduce mortality.

This study has some limitations. It was a single-center retrospective cohort study with a comparatively small number of cases. In addition, this study excluded 32 patients, which may cause selective bias in the results. Because all the patients were male and the average age was relatively young, though the type of heatstroke was restricted to EHS, the results do not fully reflect the overall conditions of the heatstroke population. Expanding the sample size and employing a prospective cohort study should be designed to achieve higher-level clinical results in the subsequent studies.

CONCLUSIONS

Patients with RM after EHS have severe clinical conditions, which are often accompanied by DIC or ALI. The SOFA score was an important independent risk factor for 90-day mortality in patients with EHS complicated with RM. Early treatment strategies based on decreasing the SOFA score at admission may be pivotal to reduce the 90-day mortality rate of patients with EHS.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of the General Hospital of Southern Theatre Command of Peoples Liberation Army (HE-2020-09). Written informed consent for participation was not required for this study in

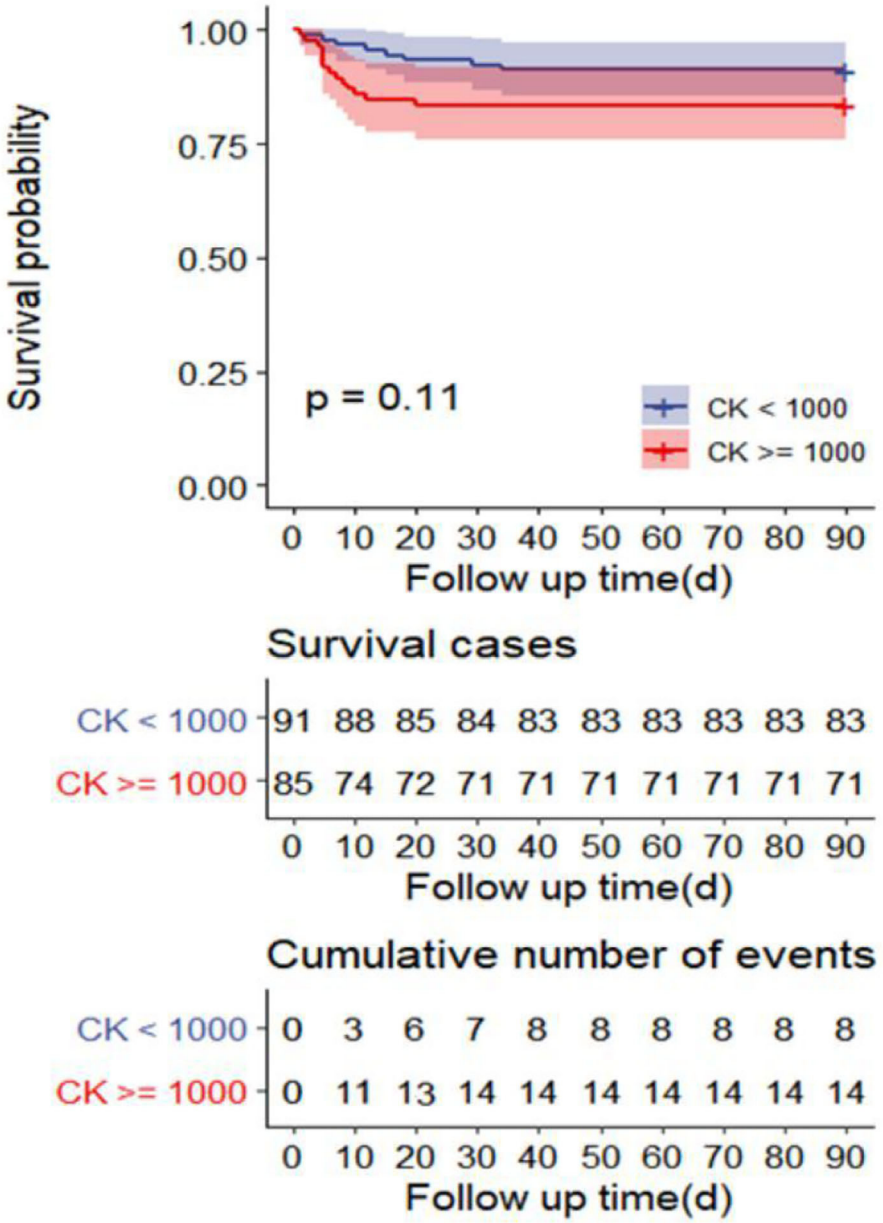

FIGURE 3 | Survival curves of 90-day mortality rate in the R) group (CK ≥ 1,000 U/L) and non-RM group (CK < 1,000 U/L). CK, creatine kinase; RM, rhabdomyolysis.

accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZFL and MW contributed to the study concept and design. MW, CW, and ZYL collected the data. MW and CW performed the statistical analysis. ZFL, MW, and CW drafted the manuscript. All the authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Association Between Platelet Levels on Admission and 90-day Mortality in Patients With Exertional Heatstroke, a 10 Years Cohort Study

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Background: Heatstroke is a common clinical symptom in summer with high mortality requiring identification of appropriate and rapid methods of assessment.

Method: This is a retrospective study that included the recent 10 years clinical data of heatstroke patients. A total of $n = 186$ patients were included in this study and grouped based on platelet (PLT) abnormality observed on admission.

Results: In the study group, $n = 120$ patients (64.5%) patients had normal PLT and $n = 66$ patients (35.5%) had abnormal PLT. Compared with PLT-normal group, PLT-abnormal group had higher Acute Physiology and Chronic Health Evaluation II (APACHE II) scores [median 15.0 (IQR 11.5–21.5) vs. 9.0 (IQR 7.0–12.5)] and SOFA scores [median 6.0 (IQR 4.0–10.0) vs. 2.0 (IQR 2.0–4.0)], lower Sequential Organ Failure Assessment (GCS)[median 8.0 (IQR 5.0–12.0) vs. 13.0 (IQR 9.0–14.0)]. The PLT-abnormal group had severe organ damage, including damage to the coagulation system, liver, and kidney (all $p < 0.05$). Significant differences were noted in 90-day survival between the two groups even after correction for Age, GCS, White blood cell count (WBC), Neutrophil, International normalized ratio (INR), Activated partial thromboplastin time (APTT), Procalcitonin (PCT), Alanine aminotransferase (ALT), Creatine (CR), D-Dime (D-D) (Before correction $P < 0.001$; After correction $P = 0.009$).The area under the ROC curve for the prediction of mortality based on PLT was 80.7% (95% CI 0.726–0.888, $P < 0.001$), the optimal cutoff value was 94, the sensitivity was 77.3%, and the specificity was 82.6%.

Conclusion: Patients with heatstroke with platelet abnormalities during admission have more severe organ impairment and a lower 90-day survival rate even when adjusted for other factors.

Keywords: heatstroke, platelets, APACHE II scores, SOFA scores, mortality

INTRODUCTION

Heatstroke is a common clinical symptom in any season especially in summer, manifesting as multi-system inflammation caused by central hyperthermia and eventually multi-organ dysfunction, mortality up to 40% (1, 2). Heatstroke can be clarified in exertional heatstroke or classic heatstroke. Classic heatstroke occurs mostly in older patients, especially those with chronic diseases, they have weak ability to regulate thermal stimulation and are easily affected by high temperature stimulation. Exertional heatstroke usually occurs in athletes, soldiers or worker engaged in outdoor physical labor and it usually occurs under the condition of continuous high temperature and high intensity exercise. The best way to diagnose heatstroke is with rapid body temperature assessment and evaluation of central nervous system status. Since much of the research on exertional heatstroke indicates cellular damage occurs when body temperature exceeds 40.5 C for 30 min. It is very important to find an effective indicator to quickly evaluate the severity and prognosis of the disease. Platelets stop bleeding and participate in thrombosis (3). Platelet abnormality is a risk factor for evaluating the prognosis of a variety of diseases (4–6). Hence this study, retrospectively analyzed the clinical data of patients admitted to hospital for severe heatstroke in the past 10 years, and evaluated early platelet changes on the prognosis of patients with heatstroke for timely and effective clinical treatment.

METHODS

Study Design and Participants

This single-center retrospective case-control study collected all patients with heatstroke admitted to the ICU of the General Hospital of Southern Theater Command in China from October 2008 to May 2019.

The inclusion criteria were as follows: (1) age $> = 18$ years old; (2) met the diagnostic criteria of severe heatstroke described below; (3) The diagnostic criteria for heatstroke were as follows: classic or exertional heatstroke with a history of exposure to hot and humid weather or strenuous activity, concurrent hyperthermia (central temperature above 40°C), and neurological dysfunction, including delirium, cognitive disorders, and disturbed consciousness. The exclusion criteria were as follows: (1) existing irreversible underlying diseases affecting mortality and (2) pregnancy or breastfeeding.

Research Procedures

The base data of patients were collected, including inflammatory and organ function parameters on admission. Patients were divided into the PLT-normal and PLT-abnormal group according to whether their PLT is normal or not. The main result was 90-day mortality. Survival curve analysis was also performed and ROC curve analysis of PLT to assess the 90-day mortality of heatstroke patients. The PLT-abnormal group was further divided into the Survivors subgroup and Non-survivors subgroup, and the characteristics of the subgroups were compared.

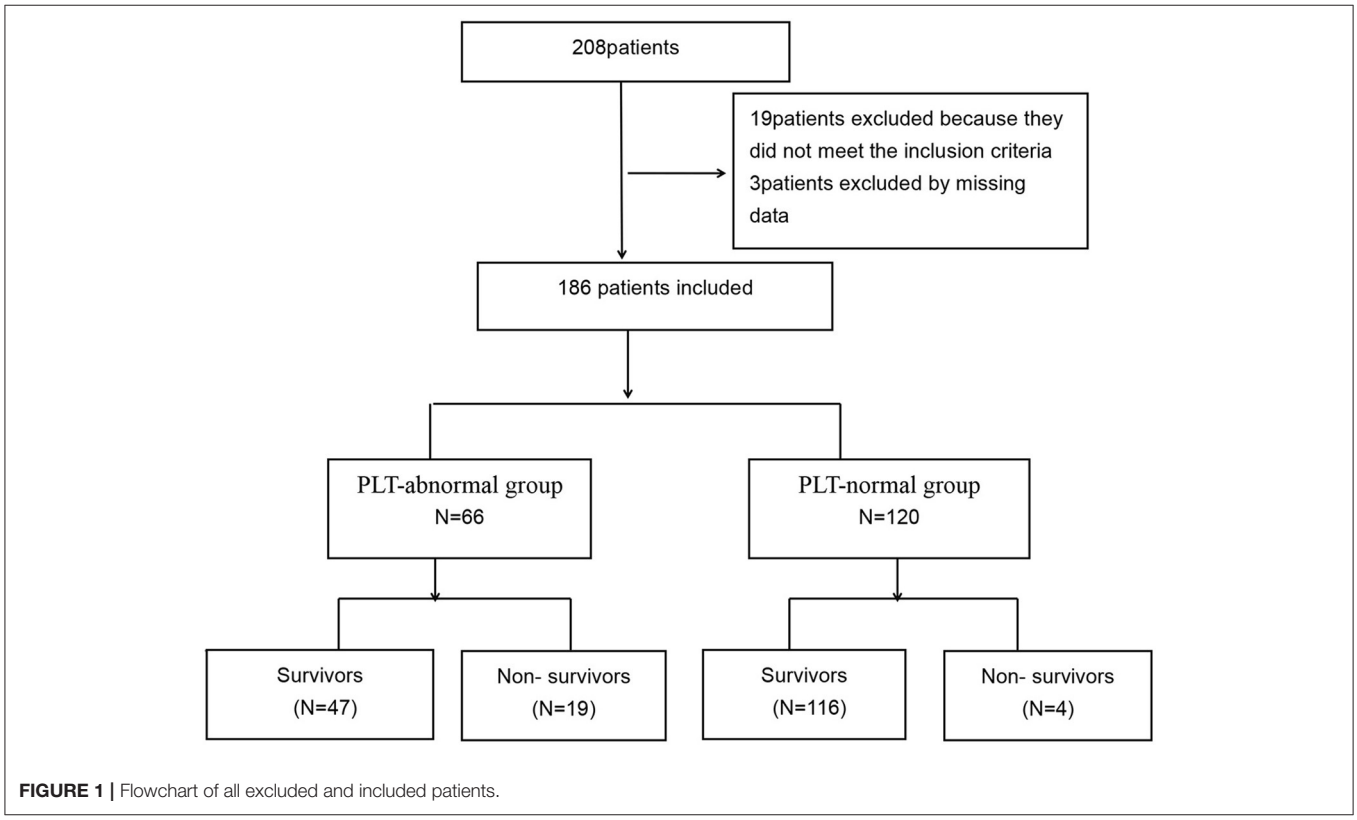

FIGURE 1 | Flowchart of all excluded and included patients.

Definitions

Diagnostic criteria for platelet abnormalities: platelet count (PLT) $<100(1 \times 10^9/L)$ or platelet count $>300(1 \times 10^9/L)$.

Statistical Analysis

The categorical data are expressed as numbers and percentages, and intergroup comparisons were performed with the Mann-Whitney U test, χ^2 test, or Fisher's exact test. Continuous variables are expressed as the medians and interquartile ranges. Continuous data with a Gaussian distribution were compared with Student's *t*-test or one-way ANOVA, and those with a non-Gaussian distribution were compared with the Wilcoxon rank-sum test. The patient endpoint event was death within 90 days after onset. The survival curve was drawn using the Kaplan-Meier method. Significant indicators were identified using single-factor analysis, ROC curves were used to predict the prognosis of patients according to PLT. Statistical analyses were performed using the SPSS Windows version 11.0 statistical package (SPSS Inc, Chicago, IL), and *P* values (two-tailed) <0.05 were considered statistically significant.

RESULTS

Demographics and Baseline Characteristics

A total of 208 patients were included. After screening, 28 patients were excluded because they did not meet the inclusion criteria,

TABLE 1 | Baseline characteristics of clinical and laboratory findings in PLT-normal group and PLT-abnormal group.

	Total (*N* = 186)	PLT-normal (*N* = 120)	PLT-abnormal (*N* = 66)	*P* value
APACHII score, median (IQR)	11.0 (8.0–16.0)	9.0 (7.0–12.5)	15.0 (11.5–21.5)	< 0.001
SOFA score, median (IQR)	3.0 (2.0–6.0)	2.0 (2.0–4.0)	6.0 (4.0–10.0)	< 0.001
GCS score, median (IQR)	12.0 (7.0–14.0)	13.0 (9.0–14.0)	8.0 (5.0–12.0)	< 0.001
Age (years), median (IQR)	21.0 (19.0–27.0)	20.0 (19.0–25.0)	23.0 (19.8–37.3)	0.003
WBC (1×10^9/L), median (IQR)	11.4 (8.6–14.7)	11.7 (9.0–15.6)	10.1 (7.9–13.3)	0.020
Neutrophil (1×10^9/L), median (IQR)	8.9 (6.5–12.5)	9.3 (6.6–13.2)	8.6 (6.5–10.9)	0.237
Lymphocyte (1×10^9/L), median (IQR)	1.1 (0.6–1.9)	1.4 (0.8–2.2)	0.6(0.4–1.4)	< 0.001
Monocytes (1×10^9/L), median (IQR)	0.7 (0.4–1.0)	0.8 (0.4–1.1)	0.6 (0.3–0.7)	0.001
Platelets (1×10^9/L), median (IQR)	165.0 (81.5–219.0)	187.0 (158.0–226.8)	62.0 (34.5–86.8)	< 0.001
Mean platelet volume (%)	10.7 (10.1–11.5)	10.6 (10.1–11.1)	10.9 (10.2–11.7)	< 0.001
Platelet distribution width (%)	12.5 (11.5–13.8)	12.0 (11.2–13.3)	13.5 (11.8–15.2)	< 0.001
TBIL (μmol/L), median (IQR)	15.9 (10.1–29.5)	11.8 (8.7–18.8)	31.2 (16.1–65.3)	< 0.001
ALT (U/L), median (IQR)	34.5 (20.0–228.8)	26.0 (17.0–47.0)	221.0 (53.8–1,606.3)	< 0.001
AST (U/L), median (IQR)	66.5 (34.3–228.0)	44.0 (28.0–84.5)	290.0 (110.0–1,587.0)	< 0.001
BUN (mmol/L), median (IQR)	5.8 (4.5–7.6)	5.6 (4.2–6.8)	6.4 (5.0–8.7)	0.009
CR (μmol/L), median (IQR)	127.5 (92.0–163.0)	125.0 (94.0–146.0)	147.5(87.8–223.5)	0.009
Cystatin C (mg/L)	1.0 (0.9–1.2)	1.0 (0.8–1.2)	1.1 (0.9–1.5)	0.003
CK (U/L), median (IQR)	912.0 (341.0–2,554.0)	585.5 (252.0–1,601.8)	1,688.5 (814.5–5,465.8)	< 0.001
CKMB (ng/ml), median (IQR)	8.9(4.5–20.4)	5.7(3.8–14.8)	18.0(8.4–37.7)	< 0.001
Mb (ng/ml), median (IQR)	468.9 (122.0–1,000.0)	265.4 (69.2–840.9)	789.0 (219.0–1,000.0)	0.001
CTNI (ng/ml), median (IQR)	0.11 (0.02–0.43)	0.06 (0.01–0.2)	0.28 (0.11–1.11)	< 0.001
PT (s), median (IQR)	15.9 (14.1–20.6)	15.0 (13.9–16.3)	23.5 (16.6–37.4)	< 0.001
INR median (IQR)	1.3 (1.1–1.8)	1.2 (1.1–1.3)	2.0 (1.3–3.7)	< 0.001
APTT (s), median (IQR)	39.0 (33.5–49.5)	35.6 (32.2–40.9)	58.1 (41.4–99.9)	< 0.001
TT(s), median (IQR)	17.6 (16.6–21.3)	17.2 (16.3–18.0)	22.6 (17.6–54.0)	< 0.001
FIB (g/L), median (IQR)	2.5 (2.0–2.9)	2.5 (2.2–3.1)	2.2 (1.5–2.8)	0.003
D-D (mg/L), median (IQR)	1.7 (0.5–7.0)	0.8 (0.4–2.7)	6.6 (2.0–13.3)	< 0.001
CRP (mg/dl), median (IQR)	3.3 (1.4–6.6)	3.2 (0.7–5.6)	3.4 (3.2–9.8)	0.016
PCT (ng/ml), median (IQR)	1.9 (0.8–4.3)	1.3 (0.6–3.3)	3.2 (1.4–5.9)	< 0.001
RM	83/174(47.7%)	40 (48.2%)	43 (51.8%)	< 0.001
AKI	80/185 (43.2%)	41 (51.2%)	39 (48.8%)	0.001
RM&AKI	40/181 (22.1%)	13 (32.5%)	27 (67.5%)	< 0.001

IQR, Inter-Quartile Range; APACHE II, Acute Physiology and Chronic Health Evaluation II score; SOFA, Sequential Organ Failure Assessment; GCS, Glasgow Coma Score; WBC, White blood cell count; TBIL, Total bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; CR, Creatine; CK, Creatine Kinase; CK-MB, Creatine kinase isoenzymes; CTNI, Cardiac troponin I; PT, Prothrombin time; INR, International normalized ratio; APTT, Activated partial thromboplastin time; TT, Thrombin time; FIB, Fibrinogen; D-D:D-Dimer; CPR, C-reactive protein; PCT, Procalcitonin.

3 patients were excluded by missing data, 19 patients excluded because they did not meet the inclusion criteria, and 186 patients were finally included (**Figure 1**). All 186 patients were male, with a mean age of 21.0 years (IQR 19.0–27.0), Acute Physiology and Chronic Health Evaluation II (APACHE II) scores of 11.0 (IQR 8.0–16.0), Sequential Organ Failure Assessment (SOFA) scores of 3.0 (IQR 2.0–6.0), and GCS score of 12.0 (IQR 7.0–14.0). There were 120 patients (64.5%) patients with normal PLT and 66 patients (35.5%) with abnormal PLT.

Comparison of Groups

Compared with PLT-normal group, PLT-abnormal group had higher APACHE II scores [median 15.0 (IQR 11.5–21.5) vs. 9.0 (IQR 7.0–12.5)] and SOFA scores [median 6.0 (IQR 4.0–10.0) vs. 2.0 (IQR 2.0–4.0)], lower GCS [median 8.0 (IQR 5.0–12.0) vs. 13.0 (IQR 9.0–14.0)]. PLT-abnormal group had severe organ damage, including damage to the coagulation system, liver and kidney (all $p < 0.05$) (**Table 1**).

Main Outcomes

The 90-day mortality rate of the PLT-abnormal group was 29% (19/66), while that of the PLT-normal group was 3.3% (4/120). There were statistically significant differences in 90-day survival between the two groups even after correction for Age, GCS, White blood cell count (WBC), Neutrophil, International normalized ratio (INR), Activated partial thromboplastin time (APTT), Procalcitonin (PCT), Alanine aminotransferase (ALT), Creatine (CR), D-Dime (D-D) (Before correction $P < 0.001$; After correction $P = 0.009$) (**Table 2**). Survival analysis showed that the 90-day survival time of patients with PLT-abnormal group was shorter than that of the PLT-normal group ($P < 0.001$) (**Figure 2**).

Subgroup Analysis

In the subgroup analysis of the PLT-abnormal group, there were 47 survivors (71.2%) and 19 Non-survivors (28.8%).Compared with survivors, non-survivors had higher APACHE II scores [median 22.0 (IQR 17.8–23.5) vs. 14.0 (IQR 10.0–18.0)], higher SOFA scores [median 13.5 (IQR 8.8–15.0) vs. 6.0 (IQR 3.0–7.0)], lower GCS [median 4.0 (IQR 3.0–7.3) vs. 10.0 (IQR 6.0–14.0)]. In addition, kidney and blood coagulation system was more serious in the non-survivors ($P < 0.05$), (**Table 3**).

Risk Factor Analysis

The area under the ROC curve for the prediction of mortality based on PLT was 80.7% (95% CI 0.726–0.888, $P < 0.001$), the optimal cutoff value was 94 (1×10^9/L), the sensitivity was 77.3%, and the specificity was 82.6% (**Figure 3**).

DISCUSSION

In this retrospective cohort study, patients with heat stroke and abnormal platelets at the time of admission had more serious organ function damage, mainly blood coagulation, liver, and kidney damage. Compared with patients without platelet abnormalities, patients with platelet abnormalities had a reduced 90-day survival rate. The cut-off value of PLT predicted by the ROC curve was 94(1×10^9/L), the sensitivity was 77.3%, and the specificity was 82.6%.

TABLE 2 | Outcomes of PLT-normal group and PLT-abnormal group.

All cohort	PLT-normal ($n = 120$)	PLT-abnormal ($n = 66$)	*P*-value	*P*-value*
90-day fatality			< 0.001	0.009
Survivor	116(96.7%)	47(71%)		
Non-survivor	4(3.3%)	19(29%)		

**Adjusted: Age, GCS, WBC, Neutrophil, INR, APTT, PCT, ALT, SCR, D-D.*

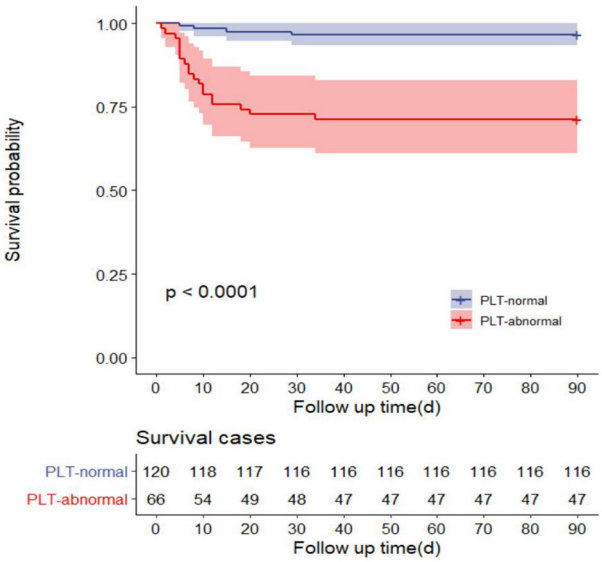

FIGURE 2 | Survival curves of 90-day mortality rate in PLT-abnormal group and PLT-normal group.

In our study, it was found that 35.5% of heatstroke patients had abnormal platelets, of which thrombocytopenia was the majority. Those with platelet abnormalities will suffer more severe organ function damage, mainly blood clotting, liver, and kidney damage, which is consistent with previous studies (7). Platelet count and function play an important role in the coagulation lesions of heatstroke, and platelet activation is triggered by heat and/or endothelial injury (7). Heatstroke can lead to inflammation, coagulation, and direct cytotoxic effects, damage to endothelial cells, leading to microthrombosis, and eventually platelet depletion, which can lead to coagulation disorders and even DIC (8). Thrombocytopenia is an important finding in the development of HS-induced Acute kidney injury (AKI) (9, 10). AKI patients are combined with renal blood flow reduction, renal tissue ischemia, and hypoxia, fibrinolytic system disorders, causing abnormal coagulation, that are mainly manifested as platelet function loss (11). Heatstroke can lead to dysfunction of multiple organs, including liver damage. Serotonin from platelets are released and involved

TABLE 3 | Baseline characteristics of survivors and non-survivors in patients with PLT-abnormal.

	Total (*N* = 66)	Survivors (*N* = 47)	Non-survivors (*N* = 19)	*P* value
APACHII score, median (IQR)	15.0 (11.5–21.5)	14.0 (10.0–18.0)	22.0 (17.8–23.5)	0.001
SOFA score, median (IQR)	6.0 (4.0–10.0)	6.0 (3.0–7.0)	13.5 (8.8–15.0)	< 0.001
GCS score, median (IQR)	8.0 (5.0–12.0)	10.0 (6.0–14.0)	4.0 (3.0–7.3)	0.002
Age (years), median (IQR)	23.0 (19.8–37.3)	26.0 (20.0–39.0)	22.0 (18.0–24.0)	0.077
WBC (1×10^9/L), median (IQR)	10.1 (7.9–13.3)	10.1 (8.3–12.5)	9.8 (7.6–14.2)	0.927
Neutrophil (1×10^9/L), median (IQR)	8.6 (6.5–10.9)	8.6 (6.8–10.9)	7.4 (5.9–11.8)	0.620
Lymphocyte (1×10^9/L), median (IQR)	0.6 (0.4–1.4)	0.6 (0.4–1.1)	0.5 (0.3–3.1)	0.788
Monocytes (1×10^9/L), median (IQR)	0.6 (0.3–0.7)	0.5 (0.3–0.7)	0.7 (0.2–0.8)	0.854
Platelets (1×10^9/L), median (IQR)	62.0 (34.5–86.8)	59.0 (37.0–91.0)	71.0 (29.0–84.0)	0.600
Mean platelet volume (%)	11.2 (10.5–11.8)	11.2 (10.4–11.9)	11.1 (10.5–11.9)	0.837
Platelet distribution width (%)	13.3 (11.8–15.2)	13.3 (11.5–11.4)	13.2 (12.0–16.6)	0.430
TBIL (μmol/L), median (IQR)	31.2 (16.1–65.3)	27.6 (15.1–61.6)	37.4 (21.2–127.8)	0.183
ALT (U/L), median (IQR)	221.0 (53.8–1,606.3)	162.0 (44.0–1,314.0)	246.0 (87.0–1,897.0)	0.350
AST (U/L), median (IQR)	290.0 (110.0–1,587.0)	190.0 (81.0–160.0)	406.5 (112.5–2,270.0)	0.315
BUN (mmol/L), median (IQR)	6.4 (5.0–8.7)	5.8 (4.9–7.7)	8.1 (6.2–10.0)	0.058
CR (μmol/L), median (IQR)	147.5 (87.8–223.5)	109.0 (81.0–160.0)	228.0 (187.0–286.0)	< 0.001
Cystatin C (mg/L)	1.1 (0.9–1.5)	1.0 (0.8–1.3)	1.5 (1.1–3.4)	0.001
CK (U/L), median (IQR)	1,688.5 (814.5–5,465.8)	1,796.5.0 (799.3–4,572.3)	1,672.0 (863.3–8,240.5)	0.500
CKMB (ng/ml), median (IQR)	18.0 (8.4–37.7)	16.4 (7.8–31.9)	19.9 (9.7–50.4)	0.299
Mb (ng/ml), median (IQR)	789.0 (219.0–1,000.0)	456.2 (124.3–1,000.0)	1,000.0 (935.1–1,000.0)	0.003
CTNI (ng/ml), median (IQR)	0.28 (0.11–1.11)	0.16 (0.02–0.46)	1.9 (0.90–3.86)	< 0.001
PT (s), median (IQR)	23.5 (16.6–37.4)	20.3 (17.0–35.4)	37.5 (25.6–45.6)	0.003
INR median (IQR)	2.0 (1.3–3.7)	1.7 (1.3–2.5)	3.8 (2.4–5.0)	0.001
APTT (s), median (IQR)	58.1 (41.4–99.9)	46.3 (39.2–77.9)	91.2 (76.1–124.8)	0.003
TT (s), median (IQR)	22.6 (17.6–54.0)	20.3 (17.0–35.4)	38.7 (27.2–82.5)	0.004
FIB (g/L), median (IQR)	2.2 (1.5–2.8)	2.5 (1.9–2.8)	1.3 (0.9–2.1)	0.003
D-D (mg/L), median (IQR)	6.6 (2.0–13.3)	3.9 (0.9–11.4)	10.1 (9.8–20.0)	0.001
CRP (mg/dl), median (IQR)	3.4 (3.2–9.8)	3.4 (2.5–11.0)	3.4 (3.3–6.7)	0.886
PCT (ng/ml), median (IQR)	3.2 (1.4–5.9)	2.9 (1.2–5.4)	3.8 (1.7–8.8)	0.182

IQR, Inter-Quartile Range; APACHE II, Acute Physiology and Chronic Health Evaluation II score; SOFA, Sequential Organ Failure Assessment; GCS, Glasgow Coma Score; WBC, White blood cell count; TBIL, Total bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; CR, Creatine; CK, Creatine Kinase; CK-MB, Creatine kinase isoenzymes; CTNI, Cardiac troponin I; PT, Prothrombin time; INR, International normalized ratio; APTT, Activated partial thromboplastin time; TT, Thrombin time; FIB, Fibrinogen; D-D, D-Dimer; CPR, C-reactive protein; PCT, Procalcitonin.

in pathophysiological processes such as liver injury response, regulation of liver function, and regeneration (12).

PLT has been evaluated in sepsis, diabetes, cardiovascular disease, and other diseases, and is correlated with disease severity and prognosis (13–16). PLT count is also part of the scoring for heatstroke patients (17). What makes our study different from other studies is that we investigated the effect of early platelet changes on 90-day prognosis in a population with exertional heat stroke, not limited to patients with AKI. In addition, we had a relatively long follow-up period, and prognostic parameters were adjusted for statistical analysis. The clinical characteristics of patients with platelet abnormality were analyzed in detail, which was not mentioned in other literatures. ROC curve analysis of platelets, and prediction of cut-off values, which further clarified the prognostic value of platelets for heatstroke patients. In our study, it was also found that compared with patients without platelet abnormality, the 90-day survival rate of patients with platelet abnormality was lower, and the sensitivity and specificity were 77.3% and 82.6% when the PLT of 94 (1×10^9/L) was taken as the critical point for predicting the 90-day death of patients with severe heatstroke. In the pathophysiological process of heatstroke, the relationship between heatstroke and platelets is very complex. Heatstroke damages platelets through heat stress and inflammation, and platelets aggravate the condition by activating coagulation response and further amplifying inflammation response (18). Therefore, platelets can assess the severity of heatstroke patients to a certain extent, contribute to the evaluation of the prognosis of heatstroke patients, and provide a reference for the selection of clinical treatment options.

This study has some limitations. First, it is a single-center cohort study with a relatively small number of cases. A multi-center study will be conducted in the later stage to increase the sample size and increase the statistical reliability. Second, exertional heatstroke is a special disease that occurs mainly in

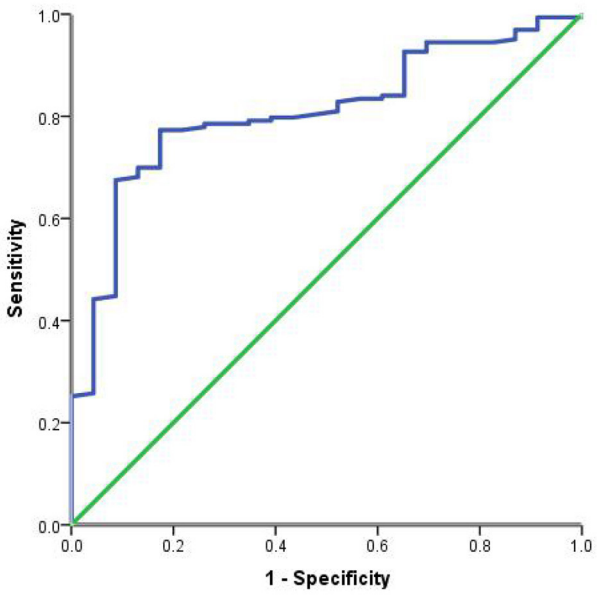

FIGURE 3 | ROC curve analysis of PLT to assess the 90-day mortality of heat stroke patients.

people who are engaged in heavy physical labor and exercise, often in a predominantly male group. In addition, due to estrogen protection and other factors, women under equal conditions rarely develop heatstroke. Therefore, our study subjects are also mainly male and may not fully reflect the changes of platelets in the overall population. Third, it is true that early recognition and treatment is crucial for heatstroke survivability, and the patients in our study usually received basic treatment before admission, including simple cooling and symptomatic support therapy. However, these information were pre hospital data. This study is a retrospective study, so we can not accurately obtain all these information, which is also one of the limitations of this study.

CONCLUSION

Patients with heatstroke who are admitted to hospital with platelet abnormalities have more severe organ impairment and a lower 90-day survival rate even when adjusted for other factors.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

LZ and MW: data collection and analysis and manuscript preparation. JJ and CW: data collection and analysis. ZL: study designed, data collection and analysis, manuscript preparation, and review. All authors read and approved the final manuscript.

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PERSPECTIVE
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Tools Are Needed to Promote Sedation Practices for Mechanically Ventilated Patients

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Suboptimal sedation practices continue to be frequent, although the updated guidelines for management of pain, agitation, and delirium in mechanically ventilated (MV) patients have been published for several years. Causes of low adherence to the recommended minimal sedation protocol are multifactorial. However, the barriers to translation of these protocols into standard care for MV patients have yet to be analyzed. In our view, it is necessary to develop fresh insights into the interaction between the patients' responses to nociceptive stimuli and individualized regulation of patients' tolerance when using analgesics and sedatives. By better understanding this interaction, development of novel tools to assess patient pain tolerance and to define and predict oversedation or delirium may promote better sedation practices in the future.

Keywords: mechanical ventilation, suboptimal sedation, patient tolerability, assessment tools, sedation depth

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INTRODUCTION

Mechanically ventilated (MV) patients can have a wide variety of discomforts resulting from multiple sources, including pathophysiological abnormalities (such as fever, hypoxia, and shock), emotional alterations (anxiety or fear), and intensive care procedures as well (such as non-physical ventilation, immobilization, frequent puncturing, and turning over, etc.) (1–4). Analgesics and sedatives are often used to maintain MV patients' comfort (5). In the last two decades, it has been observed that MV patients were deeply sedated very frequently in intensive care units (ICU) (6–8). Significantly, this behavior has been associated with poor outcomes, including prolonged duration of mechanical ventilation, increased incidence of ventilator associated pneumonia decline in cognitive ability, and even increased long-term mortality (6–10). Therefore, it has been strongly recommended to optimize sedation practices, such as implementing a light sedation protocol and the eCASH concept (early Comfort using Analgesia, minimal Sedatives and maximal Humane care) in MV patients (11, 12). Recently, more days without occurrence of coma or delirium were demonstrated in the patients receiving no sedation protocol than in those who were maintained at even light levels of sedation during the stay in the ICUs (13). These findings indicated that the lighter the level of sedation, the better outcomes would be for MV patients.

However, the frequency of deep sedation remains high in clinical practice based on recently published data from various studies (13–15), although a strong recommendation of minimizing sedation for MV patients has been published in the updated guidelines for several years. For instance, the mean depth of sedation was below RASS−2 (mean RASS = −2.3) on day 1 in the sedation group (i.e., the usual care arm) of Olsen's randomized control trial in ICUs where no sedation strategy was initiated 10 years ago (13, 16). It was previously recognized that the low adherence to a minimal sedation protocol was multifactorial, including inadequate assessments

because of shortage of nurses, lack of multidisciplinary cooperation, and even misperception as well (17–20). However, the barriers to translating a minimal sedation protocol into standard care for MV patients are not well-defined. It is necessary to reveal fresh insight into the fact that the outcome favored minimal sedation protocol was poorly implemented in MV patients.

LIGHTLY SEDATED PATIENTS' TOLERANCE TO MECHANICAL VENTILATION

Lack of knowledge on patient intolerance to MV has been an important barrier to implementing a minimal sedation protocol in MV patients. Among the common signs of patient intolerance, agitation affected nearly half of ICU MV patients in previous reports (21, 22). Moreover, accumulating evidence has demonstrated that the risks of agitation or agitation-associated events were significantly increased while maintaining MV patients at light levels of sedation (usually defined as at levels of RASS from −2 to 1) (16, 23, 24). Notably, severe agitation has been associated with unplanned self-extubation, removal of important intralumenal tubes and vascular catheters, poor patient–ventilator synchrony, and increased morbidity, including PTSD (25–28). Accordingly, agitation or agitated adverse events have been of serious concern in most ICU nurses and physicians, which in turn has affected their willingness to implement light sedation practices in their routine clinical care (18, 29). In a nationwide cross-sectional survey, we also found that ICU physicians' perception of patients' tolerance to the support levels of ventilation with light sedation was highly varied across institutions. Importantly, their perceptions were largely translated into clinical practices (14). In addition, bolus administration of sedatives was usually given as a rescue intervention for agitation, which often led to unjustified deep sedation (18).

Actually, measurement of MV patients' tolerance (who are unable to communicate) remains problematic. Tools to evaluate patient tolerance or sedation depth in mechanical ventilation have evolved since the Ramsay sedation scale first used in 1974 as shown on **Table 1**. RASS offers broader discrimination in the mild-to-moderate sedation range. It is the most commonly used tool in clinical practice (41), and has demonstrated greater inter-rater reliability between clinical staff (37, 38, 42, 44). Therefore, frequent assessment of RASS has been strongly recommended to optimize the depth of sedation for MV patients and has been associated with improvement in outcomes (56). However, RASS, like other tools, is actually a transient result of patient tolerance to nociceptive stimuli as regulated by the infused analgesics and sedatives in MV. It is not a scale to directly assess the intensity of stimuli that patients experience instantaneously. Being complementary to RASS, the pain assessment tools such as Behavioral Pain Score (BPS) or Clinical Pain Observation Tool (CPOT) were suggested to improve the overall assessment of comfort of critically ill patients. However, the intensity of nociceptive stimuli might change over time because of occurrence of fever, thirst, drainage tube pain, or intestinal colic, etc., that would raise the risk of patient intolerance to MV (or vice versa). No matter how frequent the RASS assessment is, titration of analgesics and sedatives always lags behind patient intolerance (or oversedation), which partially at least accounts for frequent and unpredictable agitation. In fact, there is a lack of reliable criteria to scale responses to the stimuli that patients experience during MV. Accordingly, it is difficult for ICU physicians to properly estimate the intensity of patients' responses as well as their tolerance when patients are lightly sedated, which might be an important source of suboptimal sedation practices.

Burk et al. (26) previously reported several predictors of agitation within 24 h in adult critically ill patients, including Sequential Organ Failure Assessment score, $PaO_2/FiO_2 < 200$ mmHg, receiving MV, using restraints, etc. Based on the variables relating to fever, ventilator settings, alterations in respiratory physiology, and dosage of sedatives and analgesics, our study group recently developed an ensemble model for the prediction of agitation in invasive MV patients under light sedation (57). The model showed good calibration and discrimination in an independent dataset. However, the effectiveness of interventions based on the prediction model need to be investigated in further experimental trials. These findings indicate that agitation (i.e., severe patient intolerance in MV) is predictable by evaluating variables related to nocioceptive stimuli. Thus, development of a tool for evaluating the balance between the intensity of stimuli and patient tolerance when analgesics and sedatives are used is needed to implement a minimal sedation protocol in the future.

RECOGNITION, ESTIMATION, AND PREVENTION OF OVERSEDATION IN MV PATIENTS

Suboptimal sedation practices include both oversedation and undersedation. In the literature, numerous studies have shown that deep sedation continues to be common in the ICU (8, 9, 13–15). Generally, it has been recognized that deep sedation (below RASS−2) remains relevant only for the management of some situations in MV patients, such as severe acute respiratory distress syndrome with ventilator–patient asynchrony or with use of neuromuscular blocking agents, severe brain injury with intracranial hypertension, status epilepticus, etc. (58–61). For the vast majority of ICU MV patients, deep sedation is unnecessary and should be avoided (62). Oversedation is therefore suspected when MV patients are sedated at the depths below RASS−2. However, this concept is mainly based on expert opinions rather than empirical evidence, which is misleading for appropriate sedation practices. For instance, sedatives could be overused while maintaining the level of sedation at RASS−2 for MV patients ready for weaning. On the other hand, the sedation depth at RASS−3 (or even the deeper levels) might be necessary for acute critically ill patients with multiple organ dysfunction caused by aggressive inflammatory responses (63, 64). In fact, no consensus on the definitions of deep sedation and oversedation is available because of gaps in the evidence. There is a dearth of information regarding the interaction among sedative choice,

TABLE 1 | Tools for assessment of sedation depth or pain in mechanically ventilated patients.

Tools	Describe	Advantage	Disadvantage	Clinical studies	
				Comparator	Findings
RSS Dawson et al. (30)	The RSS is a single-item tool to measure consciousness across three levels in critically ill patients who are awake and three levels in patients who are judged to be asleep (31).	The earliest and the most widely used scale (32).	Use of a single item to assess two or more different aspects of sedation can lead to loss of clinically important information and systematic or random measurement error (33).	SAS (33)	No difference was found in validity between two scales
NICS Mirski et al. (34)	The NICS is a simpler, more intuitive sedation scale that is both easy to use and recall and favored by nurses as a sedation communication tool (34).	NICS ranked highest in nursing preference and ease of communication and may thus permit more effective and interactive management of sedation (34).	Subjective.	• RASS • RSS • SAS	NICS is a valid and reliable sedation scale for use in a mixed population of intensive care unit patients (34).
SAS Mirski et al. (34)	The SAS is a single-item seven point scale developed by Riker and colleagues and commonly used within ICU (35).	Both reliable and valid (36)	Not suitable for patients with hearing impairment, nerve damage, and hemiplegia (37)	RSS (33, 38)	• The SAS provides additional information by stratifying agitation into three categories without sacrificing validity or reliability (33). • The SAS showed the best correlation and the best agreement results in all professional categories (38).
ATIC De Jonghe et al. (39)	The ATICE consists of five items: Awakeness and Comprehension combined in a Consciousness domain, and Calmness, Ventilator Synchrony, and Face Relaxation combined in a Tolerance domain (39).	Evaluates sedation and tolerance; longitudinal validity demonstrated; explicit instructions provided (39).	Studied in medical patients; only properties may differ in surgical population; more complex scoring method-requires (39).	• RASS • RSS • SAS	Offers assessment of tolerance to the ICU environment;longitudinal validity demonstrated (39).
RASS Sessler et al. (40)	The RASS is a single-item scale that has 10 levels of response, which range from minus five to plus four.	Longitudinal validity demonstrated in diverse patient. It offers broader discrimination in the mild-to-moderate sedation range (41, 42).	If there are visual or auditory obstacles, it will affect the accuracy of the evaluation results (43) physical stimulation can increase anxiety of patient.	• RSS (34, 38, 41, 44, 45) • SAS (34, 37, 41) • MAAS (34)	• The RASS correlated more highly with BIS compared to RSS (39), and demonstrated greater inter-rater reliability between clinical staff compared to RSS and SAS (37, 38, 42, 44). • The RASS showed high levels of reliance and ease of use in scoring and communicating sedation, agitation and intuitiveness, compared to the RSS, MAAS, and SAS (34).
BIS Watson and Kane-Gill (46)	The BIS measures the level of sedation by integrating information from the electroencephalography and a mathematical technique referred to as bispectral analysis (46).	Offers objective monitoring; offers continuous monitoring; Continuous monitoring (47)	Variability; conflicting ICU validity results; muscle activity alters values; Unable to distinguish between natural sleep and drug-induced sleep (48).	• RASS • RSS • SAS • ATICE • MAAS	The BIS monitor has potential benefits in the ICU environment, although optimal use requires further investigation (46).
MAAS Devlin et al. (49)	The MAAS is also a single-item tool with seven response-defined categories of behavior, which originated from the SAS and is therefore structurally similar to the SAS (49).	The MAAS was superior to the LSS based upon the observation that MAAS scores were less variable (50).	There is insufficient evidence to warrant use of the MAAS as a new method of evaluating critically ill patients requiring sedation in the emergency department (50).	LSS (50)	The MAAS is a valid and reliable sedation scale for use with mechanically ventilated patients in the SICU (49).

(Continued)

TABLE 1 | Continued

Tools	Describe	Advantage	Disadvantage	Clinical studies	
				Comparator	Findings
BPS Payen et al. (51)	The BPS was based on a sum score of three items: facial expression, movements of upper limbs, and compliance with mechanical ventilation (51).	• Higher reliability shown for the muscular domain (52). Simplicity, easiness; Descriptors clear or precise (53).	• Discriminant validation seems less satisfactory in sedated or agitated patients (52). • Less specific (53).	CPOT	Both CPOT and the BPS showed good reliability and validity and were good options for assessing pain during painful procedures with intensive care unit patients unable to self-report on pain (54).
CPOT Gélinas et al. (55)	The CPOT scale includes four behavioral indicators:facial expression;body movements;muscle tension; and compliance with the ventilator (for intubated patients) or verbalization (for extubated patients) (55).	• Have particularly good reliability and validity in assessing pain during procedures (54). • Descriptors more detailed; Descriptors better described (53).	• Discriminant validation seems less satisfactory in sedated or agitated patients (52). • Descriptors less well-detailed or confusing (53).	BPS	Because of the discriminant validation, the CPOT is to be preferred (54).

SAS, Ramsay Sedation Scale; SAS, Riker Sedation-Agitation Scale; RASS, Richmond Agitation Sedation Scale; AITCE, Adaptation to the Intensive Care Environment; NICS, Nursing instrument for the communication of sedation; BIS, Bispectral index; LSS, Luer Sedation Scale; BPS, Behavioral Pain Scale.

sedation depth, and patient-specific factors that affect outcomes (65). Therefore, determining optimal sedation and oversedation in MV patients remains challenging.

Ambiguity in definition is an important barrier to the development of protocols to prevent oversedation in practice. Previously, the ABCDEF bundle (Assess, prevent, and manage pain; Both spontaneous awakening and breathing trials; Choice of analgesia and sedation; Delirium assess, prevent, and manage; Early mobility and exercise; Family engagement/empowerment) was developed to promote appropriate sedation practices by creating a safe and comfortable environment for MV patients (66). Although reduction in the rate of deep sedation and improvement in outcomes were demonstrated in patients who did receive more of the bundle elements each day, the major limitation was low adherence in clinical practice because of too many unresolved issues involved in this protocol (67). A novel sedation-monitoring technology (the Responsiveness Index, RI) based on facial electromyography was developed to provide an alert for possible deep sedation. Results showed that use of the monitor increased optimal sedation-analgesia quality but just by 7% (68). Results from the AWARE study (69) revealed that by decreasing use of intravenous hypnotics, the oversedation prevention protocol was feasible in clinical practice and resulted in a significantly earlier time to spontaneous breathing trial and reduced duration of mechanical ventilation (69). However, mortality was not significantly different between the study group and the control group. It should be interpreted with caution that the rate of oversedation or deep sedation was prevented in this study. Therefore, a precision definition is fundamental for development of a reliable scale for estimation as well as an effective protocol for prevention of oversedation in MV patients.

DELIRIUM PREDICTION

Delirium is a well-established syndrome in the ICU that is considered to be an acute onset of brain dysfunction (70). There are two motor subtypes of delirium that are categorized according to its clinical presentation, namely, the hyperactive and hypoactive subtypes (71, 72). The primary presentation of hyperactive delirium is agitation, which is reported to occur in many ICU patients (26). Although agitated delirium is found to be less harmful than the hypoactive type with respect to 12-month mortality (72, 73), potential serious consequences of agitation as opposed to its hypoactive counterpart, mentioned above included medical device removal (such as urinary catheter, venous or arterial line, or surgical drain), falling out of bed, immobilization device removal, or self-aggression or aggression toward medical staff (25–28, 74). Thus, the prediction and appropriate prevention of agitated delirium is of paramount importance in the management of MV patients.

The mechanism of delirium remains unclear (75). Risk factors for delirium include illness-related acute pathophysiological abnormalities (e.g., hypotension, acidosis, hypoxia, and sepsis), environmental factors (e.g., lighting, alarm sounds, and noise); and iatrogenic harm (e.g., frequent suctions, puncture, immobilization, and even use of analgesic and

sedative drugs) (76–79). Among these, there are potentially modifiable risk factors, for example, minimizing sedation and benzodiazepine use (80). Significantly, numerous studies have reported that patients receiving deep sedation were more susceptible to post-traumatic stress disorder syndrome, ICU memory disorder, and delirium (81, 82). On the other hand, two recently published meta-analyses revealed that delirium occurred more frequently in the light than in the deep sedation group of MV patients (24, 83). Because of multiple etiologies, therefore, prediction and prevention of delirium remains problematic.

Some prediction models have been developed for delirium, but limitations remain. For example, the prediction model for delirium (PRE-DELIRIC) and early prediction model for delirium (E-PRE-DELIRIC) were initially developed in a single hospital and validated in four hospitals (84). However, the discriminatory ability of these models in an external dataset was less than satisfactory (area under curve: 0.68–0.79, respectively) (85–87). These studies are limited in several aspects. First, previous studies typically used variables collected on the day of ICU admission, and the delirium event may happen several days later. Some physiological variables change significantly in this interval. Second, there is no model to specifically predict hypoactive delirium. Third, previous models were usually developed in a single center, which partly explains the models' suboptimal performance in an external dataset. Foruth, the previous models were developed as generalized linear models that failed to capture higher- order and interaction terms between predictors. Therefore, a novel delirium prediction model is needed for MV patients.

CONCLUSION

Suboptimal sedation practices are common, which are largely attributable to the evidence gaps concerning the intensity of nociceptive stimuli that patients experience and patients' tolerance and its treatment by using analgesics and sedatives. Development of novel tools to assess patient tolerance and to define and predict oversedation or delirium are needed to implement better sedation practices in the future.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

TW and PM were the major contributors in writing the manuscript. DZ and ZZ helped to revise the manuscript. PM critically reviewed the manuscript and agreed with the final version. All authors read and approved the submitted manuscript.

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Pathophysiology of Coagulopathy Induced by Traumatic Brain Injury Is Identical to That of Disseminated Intravascular Coagulation With Hyperfibrinolysis

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Background: Traumatic brain injury (TBI)-associated coagulopathy is a widely recognized risk factor for secondary brain damage and contributes to poor clinical outcomes. Various theories, including disseminated intravascular coagulation (DIC), have been proposed regarding its pathomechanisms; no consensus has been reached thus far. This study aimed to elucidate the pathophysiology of TBI-induced coagulopathy by comparing coagulofibrinolytic changes in isolated TBI (iTBI) to those in non-TBI, to determine the associated factors, and identify the clinical significance of DIC diagnosis in patients with iTBI.

Methods: This secondary multicenter, prospective study assessed patients with severe trauma. iTBI was defined as Abbreviated Injury Scale (AIS) scores ≥4 in the head and neck, and ≤2 in other body parts. Non-TBI was defined as AIS scores ≥4 in single

body parts other than the head and neck, and the absence of AIS scores ≥3 in any other trauma-affected parts. Specific biomarkers for thrombin and plasmin generation, anticoagulation, and fibrinolysis inhibition were measured at the presentation to the emergency department (0 h) and 3 h after arrival.

Results: We analyzed 34 iTBI and 40 non-TBI patients. Baseline characteristics, transfusion requirements and in-hospital mortality did not significantly differ between groups. The changes in coagulation/fibrinolysis-related biomarkers were similar. Lactate levels in the iTBI group positively correlated with DIC scores (rho = −0.441, $p = 0.017$), but not with blood pressure (rho = −0.098, $p = 0.614$). Multiple logistic regression analyses revealed that the injury severity score was an independent predictor of DIC development in patients with iTBI (odds ratio = 1.237, $p = 0.018$). Patients with iTBI were further subdivided into two groups: DIC ($n = 15$) and non-DIC ($n = 19$) groups. Marked thrombin and plasmin generation were observed in all patients with iTBI, especially those with DIC. Patients with iTBI and DIC had higher requirements for massive transfusion and emergency surgery, and higher in-hospital mortality than those without DIC. Furthermore, DIC development significantly correlated with poor hospital survival; DIC scores at 0 h were predictive of in-hospital mortality.

Conclusions: Coagulofibrinolytic changes in iTBI and non-TBI patients were identical, and consistent with the pathophysiology of DIC. DIC diagnosis in the early phase of TBI is key in predicting the outcomes of severe TBI.

Keywords: disseminated intravascular coagulation, fibrinolysis, shock, thrombin, trauma-induced coagulopathy, traumatic brain injury

INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of death and disability, with more than 10 million people hospitalized for TBI every year (1). Although primary damage to the brain is inevitable, secondary injuries are potentially avoidable and can affect the therapeutic interventions and outcomes of patients with TBI. TBI-associated coagulopathy is widely recognized as a risk factor for secondary brain damage and contributes to poor clinical outcomes. Two meta-analyses reported that the overall prevalence of TBI-associated coagulopathy was 32.7–35.2%, and a strong association was confirmed between the incidence of coagulopathy and poor outcomes (2, 3). TBI-associated coagulopathy contributes to poor outcomes in both hypocoagulability, leading to the expansion of intracranial hemorrhage, and hypercoagulability, leading to secondary cerebral ischemia due to intravascular thrombosis in the injured brain (4–8). Therefore, elucidation of the pathophysiology of TBI-induced coagulopathy may contribute to improving the outcomes of TBI patients, but no consensus has been reached thus far.

Disseminated intravascular coagulation (DIC) is acknowledged as the primary pathophysiological mechanism of trauma-induced coagulopathy (TIC), which is caused by multiple factors, such as anemia, hemodilution, hypothermia, acidosis, hemorrhagic shock, and serious trauma itself (9). Coagulopathy that occurs in patients with TBI is consistent with the definition of DIC proposed by the International Society on Thrombosis and Haemostasis: the activation of coagulation with the loss of localization and damage to the microvasculature (10, 11). Previous studies have clearly demonstrated consumption coagulopathy and disseminated microvascular thrombosis formation in the brain and other organs, which is the pathophysiology of DIC itself (12, 13).

A recent consensus statement from the International Society on Thrombosis and Haemostasis described that TIC is driven by two distinct and synergic insults: hypovolemic shock due to blood loss and extensive tissue disruption (14). However, it is unclear whether this concept can be applied to patients with TBI, since these patients do not suffer substantial blood loss, indicating that they are less likely to develop hemorrhagic shock.

Therefore, this study aimed to elucidate the pathophysiology of TBI-induced coagulopathy by comparing the coagulofibrinolytic changes in isolated TBI (iTBI) with those in non-TBI trauma, and to determine the associated factors, particularly shock-related factors such as blood pressure and serum lactate levels. To gain a deeper insight into the pathomechanisms of TBI-induced coagulopathy, coagulofibrinolytic changes were compared between iTBI patients with and without DIC, and the clinical significance of DIC diagnosis in patients with iTBI was evaluated.

TABLE 1 | Demographics and parameters at the scene and admission to the emergency department in patients with isolated traumatic brain injury and non-traumatic brain injury.

	Non-TBI $n = 40$	iTBI $n = 34$	*p*-value
Demographics			
Age (years)	67 (55–79)	59 (48–76)	0.633
Male sex, *n* (%)	27 (67.5)	23 (67.6)	0.989
Charlson comorbidity index	0 (0–1)	0 (0–0)	0.711
ISS	20 (17–26)	17(16–25)	0.196
AIS			
Head	0 (0–0)	4 (4–5)	< 0.001
Face	0 (0–0)	0 (0–0)	0.814
Neck	0 (0–0)	0 (0–0)	0.357
Thorax	4 (0–4)	0 (0–0)	< 0.001
Abdomen	0 (0–2)	0 (0–0)	0.001
Spine			
Cervical	0 (0–0)	0 (0–0)	0.019
Thoracic	0 (0–0)	0 (0–0)	0.019
Lumber	0 (0–0)	0 (0–0)	0.105
Upper extremity	0 (0–0)	0 (0–0)	0.022
Lower extremity	0 (0–2)	0 (0–0)	< 0.001
External	0 (0–1)	0 (0–0)	0.048
DIC 0 h, *n* (%)	8 (20.0)	9 (26.5)	0.510
DIC score 0 h	3 (2–3)	3 (1–4)	0.920
DIC 3 h, *n* (%)	10 (28.6)	10 (38.4)	0.416
DIC score 3 h	3(1–4)	3(1–4)	0.875
DIC 24 h, *n* (%)	10 (26.3)	3 (11.1)	0.131
DIC score 24 h	3 (1–4)	3 (0–3)	0.042
SIRS criteria	2 (1–2)	2 (1–3)	0.205
Shock (ABP) at scene, *n* (%)	6 (15.4)	3 (8.8)	0.489
Shock (ABP) at ED, *n* (%)	8 (20.0)	1 (2.9)	0.033
Shock (lac), *n* (%)	21 (56.8)	20 (69.0)	0.310
Tranexamic acid, *n* (%)	8 (20.0)	14 (41.2)	0.047
At the scene			
Systolic blood pressure (mmHg)	119 (103–136)	150 (121–169)	0.004
Diastolic blood pressure (mmHg)	72 (63–87)	80 (65–104)	0.187
Heart rate (bpm)	83 (69–99)	84 (74–97)	0.747
Respiratory rate (breaths/min)	20 (18–24)	18 (18–20)	0.001
At the ED			
Systolic blood pressure (mmHg)	140 (107–151)	144 (131–161)	0.007
Diastolic blood pressure (mmHg)	77 (6,693)	84 (71–95)	0.110
Heart rate (bpm)	82 (65–93)	84 (72–97)	0.520
Respiratory rate (breaths/min)	20 (18–26)	18 (16–20)	< 0.001
Lactate (mmol/L)	1.7 (1.1–3.1)	3.1 (1.7–4.3)	0.372
Body temperature (°C)	36.4 (36.1–36.8)	36.3 (35.9–37.0)	0.853

Reported proportions (counts) for categorical variables and medians (interquartile ranges) for continuous variables. Shock (ABP) represents a systolic blood pressure of <90 mmHg. Shock (lac) represents lactate levels of >2 mmol/L in the emergency department (ED). AIS, Abbreviated Injury Scale; DIC, disseminated intravascular coagulation; ISS, Injury Severity Score; iTBI, isolated traumatic brain injury; SIRS, systemic inflammatory response syndrome; TBI, traumatic brain injury.

TABLE 2 | Requirement for transfusion and emergency surgery, and in-hospital mortality.

	Non-TBI $n = 40$	iTBI $n = 34$	*p*-value
Operation within 24 h after admission, *n* (%)	17 (42.5)	14 (42.4)	0.995
Massive transfusion, *n* (%)	2 (5.0)	4 (11.8)	0.404
3-h Transfusion			
Packed red blood cells (mL)	0 (0–0)	0 (0–0)	0.909
Fresh frozen plasma (mL)	0 (0–0)	0 (0–0)	0.636
Platelet concentrate (U)	0 (0–0)	0 (0–0)	1.000
Cryoprecipitate (U)	0 (0–0)	0 (0–0)	1.000
24-h Transfusion			
Packed red blood cells (mL)	0 (0–280)	0 (0–0)	0.772
Fresh frozen plasma (mL)	0 (0–0)	0 (0–480)	0.693
Platelet concentrate (U)	0 (0–0)	0 (0–0)	0.705
Cryoprecipitate (U)	0 (0–0)	0 (0–0)	1.000
In-hospital mortality, *n* (%)	1 (2.5)	4 (12.1)	0.169

Reported median (interquartile range) for continuous variables. iTBI, isolated traumatic brain injury; TBI, traumatic brain injury.

MATERIALS AND METHODS

Study Design, Setting, and Ethical Approval

This descriptive study was performed as a secondary analysis of a multicenter prospective study conducted by the Japanese Association for Acute Medicine (JAAM) Focused Outcomes Research in Emergency Care in Acute Respiratory Distress Syndrome, Sepsis, and Trauma (FORECAST) study group (15). The JAAM FORECAST TRAUMA study recruited participants between April 1, 2016 and January 31, 2018, from 39 emergency departments (EDs) and intensive-care units (ICUs) in tertiary hospitals and was registered at the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR ID: UMIN000019588). This study was approved under the condition that written informed consent was obtained from the patient or next of kin by the JAAM and the Ethics Committee of each hospital (JAAM, 2014-01; Hokkaido University Graduate School of Medicine, Head Institute of the FORECAST group, 014-0307) and was performed in accordance with the Declaration of Helsinki.

Participants

The JAAM FORECAST TRAUMA study enrolled adult trauma patients with severe trauma injury (aged ≥16 years old) with an Injury Severity Score (ISS) of ≥16 who were directly transported from the scene by emergency medical services. Patients with a history of cardiac arrest and resuscitation, who were receiving anticoagulants, who had hemorrhagic diathesis or coagulopathy due to any cause, or who had been transferred from other hospitals were excluded before registration. The size of the study population was dependent on the study period. All patients were followed up until discharge. Twenty-seven healthy volunteers who were not age- or sex-matched were enrolled to obtain the control values of the measured markers.

Definition and Diagnosis

Injury severity was assessed using the ISS. A DIC diagnosis was made based on the JAAM DIC diagnostic criteria (16) (**Supplementary Table 1**). The DIC scores were calculated at 0,

3, and 24 h, and the DIC group was defined as patients who met the DIC criteria at least once during the study period. In the present study, the prothrombin time international normalized ratio (PT-INR) was used as a substitute for the prothrombin time ratio for the diagnosis of DIC. Transfusion of packed red blood cells of more than the estimated circulating blood volume (7.5% of body weight) within 24 h after presentation to the ED met the definition of massive transfusion. Shock was defined as a systemic inflammatory response syndrome criteria were used to assess systemic inflammation (17). A systolic blood pressure of <90 mmHg at the scene or at the ED and lactate levels >2 mmol/L at the ED. The Charlson comorbidity index was used to assess comorbidities (18).

iTBI was defined as an Abbreviated Injury Scale (AIS) score of ≥4 in the head and neck and ≤2 in other body parts. To match the injury severity of iTBI, non-TBI was defined as an AIS score of ≥4 in one body part other than the head and neck and the absence of AIS score of ≥3 in any part affected by trauma.

Data Collection and Measurements

Immediately after arrival at the ED (0 h) and 3 h after admission (3 h), 15 mL of blood was collected in citrate containing tubes at each sampling point. The samples were immediately centrifuged at 4°C in the laboratories of each hospital, and the obtained plasma was stored at −80°C. All plasma samples were measured at the center laboratory of the LSI Medience Corporation (Tokyo, Japan). We measured the following molecular markers: (1) soluble fibrin (marker of direct thrombin generation) (LA, IATRO SFII; LSI Medience), (2) antithrombin (marker of anti-thrombin) (chromogenic assay, HemosIL Antithrombin LQ; Instrumental Laboratory), (3) protein C (marker of anticoagulation) (LPIA, LPIA-ACE PCII; LSI Medience), (4) plasmin antiplasmin complex (marker of plasmin generation) (LPIA, LPIA-ACE PPI II; LSI Medience), (5) plasminogen activator inhibitor-1 (PAI-1) (marker of inhibition of fibrinolysis) (LA, LPIA-tPAI test; LSI Medience), (6) D-dimer (marker of fibrinolysis) (LPIA, LPIA GENESIS D-dimer; LSI Medience),

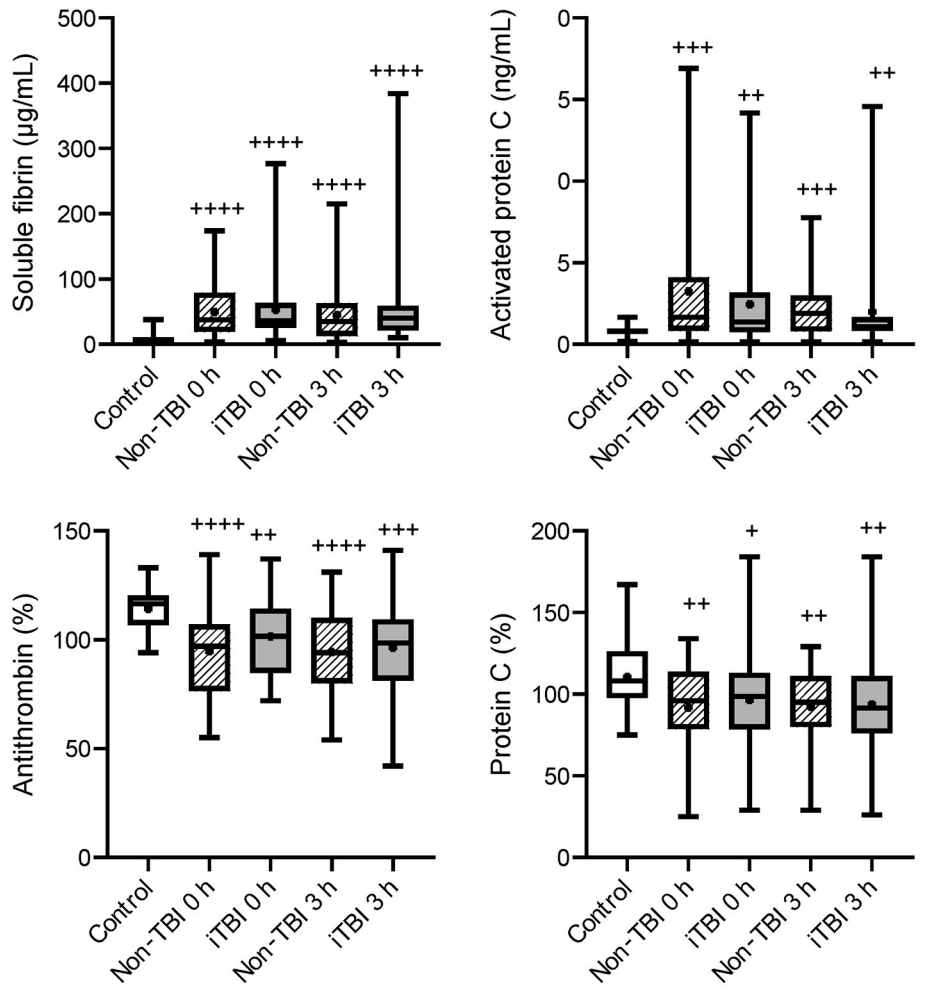

FIGURE 1 | Serial changes in the coagulation-related molecular markers. Healthy controls (white box), non-traumatic brain injury (TBI) (hatched boxes), and isolated TBI (iTBI) (gray boxes) at presentation to the emergency department (0 h) and 3 h after hospital arrival (3 h). The horizontal bars in the box indicate the median (middle) and interquartile ranges (upper 25% and lower 75%). Black boxes are mean values. $+p < 0.05$ vs. healthy controls; $++p < 0.01$ vs. healthy controls; $+++p < 0.001$ vs. healthy controls; $++++p < 0.0001$ vs. healthy controls. None of the markers significantly differed between the iTBI and non-TBI groups.

and (7) circulating activated protein C (APC) (marker of inhibition of thrombin) (EIA, EIA Kit For Activated Protein C Cloud-Clone Corp.). In addition to routine laboratory tests and blood gas analysis, measurements of platelet counts, PT-INR, activated partial thromboplastin time (APTT), fibrinogen, fibrin/fibrinogen degradation products (FDPs), and the FDP/D-dimer ratio, a surrogate marker of fibrin(ogen)olysis, were measured at 0, 3, and 24 h after arrival at the ED.

Statistical Analyses

Measurements are expressed as the median with the 25th−75th interquartile range or as numbers (percentages). Missing values were used without manipulation. Differences in demographics and measured parameters between the two groups were compared using the Mann–Whitney *U*-test for continuous variables and using either the chi-square test or Fisher's exact test for nominal variables when required. Platelet counts and global markers of coagulation and fibrinolysis were also evaluated using the Mann–Whitney *U*-test. Correlations were evaluated using Spearman's rank test when required. Survival probability curves with and without a DIC diagnosis in iTBI were constructed using the Kaplan–Meier method. The receiver operating characteristic (ROC) curve was constructed, and the area under the ROC curve (AUC) was used to assess the ability of DIC scores to predict in-hospital mortality. Differences were considered statistically significant at a two-tailed $p < 0.05$. SPSS software (version 26; IBM Japan, Tokyo, Japan) was used for all statistical analyses and calculations.

RESULTS

Baseline Characteristics, Transfusion, and In-Hospital Mortality

In total, 295 consecutive patients were registered during the study period in the FORECAST TRAUMA cohort. Ultimately,

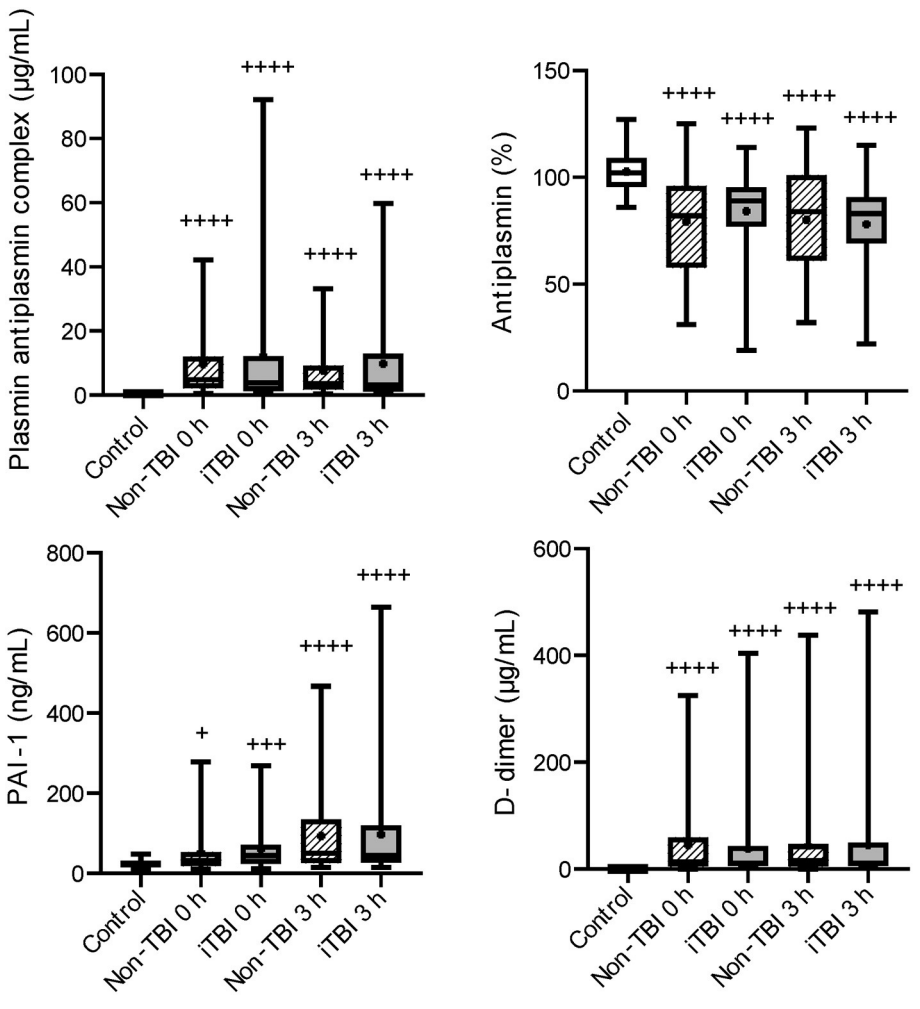

FIGURE 2 | Serial changes in the fibrinolysis-related molecular markers. Healthy controls (white box), non-traumatic brain injury (TBI) (hatched boxes), and isolated TBI (iTBI) (gray boxes) at presentation to the emergency department (0 h) and 3 h after hospital arrival (3 h). Horizontal bars in the box indicate the median (middle) and interquartile ranges (upper 25% and lower 75%). Black boxes are mean values. $+p < 0.05$ vs. healthy controls, $+++p < 0.001$ vs. healthy controls, $++++p < 0.0001$ vs. healthy controls. None of the markers significantly differed between the iTBI and non-TBI groups.

276 patients who met the eligibility criteria were divided into the iTBI ($n = 34$) and non-TBI ($n = 40$) groups (**Supplementary Figure 1**).

There were no significant differences in age, sex, comorbidities, or ISS between the groups. In addition, the DIC scores and prevalence at 0 and 3 h did not significantly differ between the groups (**Table 1**). The requirement for massive transfusion, transfusion volumes, and in-hospital mortality did not significantly differ between the two groups (**Table 2**).

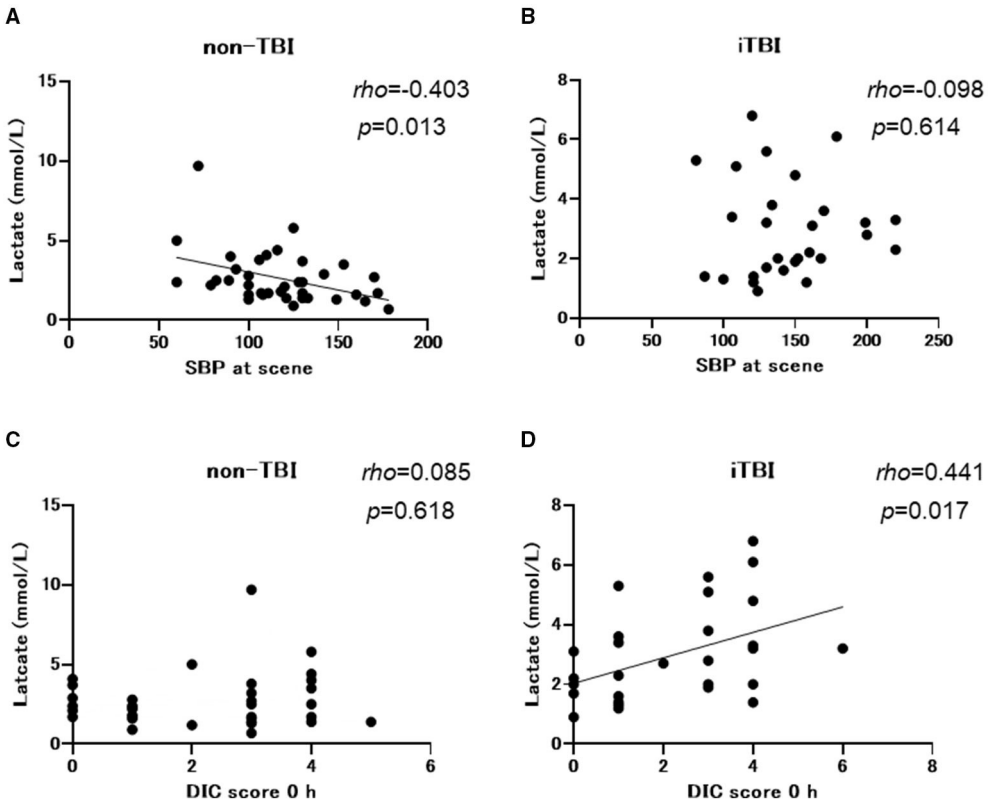

FIGURE 3 | Spearman's rank correlation coefficients between the lactate levels and systolic blood pressure (SBP) at scene and the disseminated intravascular coagulation (DIC) score at arrival to the emergency department. TBI, traumatic brain injury; iTBI, isolated traumatic brain injury.

TABLE 3 | Results of logistic regression analyses of variables predicting the development of disseminated intravascular coagulation in patients with isolated traumatic brain injury.

	Univariable			Multivariable		
	Odds ratio	***p*-value**	**95% CI**	**Odds ratio**	***p*-value**	**95% CI**
Age	1.027	0.202	0.986–1.070			
Sex	3.281	0.120	0.733–14.683			
ISS	1.220	0.018	1.034–1.439	1.237	0.018	1.034–1.439
SBP at scene	1.004	0.595	0.989–1.020			
Shock (ABP) at scene	2.769	0.425	0.226–33.879			
SBP at ED	1.025	0.078	0.997–1.053			
Shock (ABP) at ED, *n* (%)	0.000	1.000	0–0			
Lactate 0 h	1.403	0.177	0.858–2.295			
Shock (lac), *n* (%)	3.500	0.172	0.579–21.161			

Shock (ABP) represents a systolic blood pressure of <90 mmHg. Shock (lac) represents lactate levels of >2 mmol/L at the emergency department (ED).
CI, confidence interval; ED, emergency department; ISS, injury severity score; iTBI, isolated traumatic brain injury; SBP, systolic blood pressure; TBI, traumatic brain injury.

TABLE 4 | Demographics and parameters at the scene and admission to the emergency department in isolated traumatic brain injury patients with and without disseminated intravascular coagulation.

	Non-DIC $n = 19$	DIC $n = 15$	*p*-value
Demographics			
Age (years)	59 (51–73)	58 (49–74)	0.242
Male sex, *n* (%)	15 (78.9)	8 (53.3)	0.112
Charlson comorbidity index	0 (0–0)	0 (0–1)	0.190
Glasgow coma scale	14 (13–15)	7 (3–9)	0.004
ISS	17 (16–17)	25 (16–25)	0.120
AIS			
Head	4 (4–4)	5 (4–5)	0.004
Face	0 (0–0)	0 (0–0)	0.023
Neck	0 (0–0)	0 (0–0)	0.451
Thorax	0 (0–0)	0 (0–0)	1.000
Abdomen	0 (0–0)	0 (0–0)	0.811
Spine			
Cervical	0 (0–0)	0 (0–0)	1.000
Thoracic	0 (0–0)	0 (0–0)	1.000
Lumber	0 (0–0)	0 (0–0)	1.000
Upper extremity	0 (0–0)	0 (0–0)	0.811
Lower extremity	0 (0–0)	0 (0–0)	1.000
External	0 (0–0)	0 (0–0)	0.918
SIRS criteria	1 (1–2)	3 (1–3)	0.116
Shock (ABP) at scene, *n* (%)	1 (5.3)	2 (13.3)	0.409
Shock (ABP) at ED, *n* (%)	1 (5.3)	0 (0)	0.559
Shock (lac), *n* (%)	10 (58.8)	10 (83.3)	0.160
At the scene			
Systolic blood pressure (mmHg)	138 (121–155)	144 (138–179)	0.537
Diastolic blood pressure (mmHg)	78 (60–86)	83 (70–109)	0.708
Heart rate (bpm)	84 (77–87)	80 (66–98)	0.421
Respiratory rate (breaths/min)	18 (18–21)	18 (16–18)	0.167
At the ED			
Systolic blood pressure (mmHg)	138 (128–155)	144 (138–179)	0.083
Diastolic blood pressure (mmHg)	82 (65–90)	84 (74–98)	0.202
Heart rate (bpm)	74 (70–86)	95 (81–98)	0.401
Respiratory rate (breaths/min)	18 (16–19)	17 (15–20)	0.319
Lactate (mmol/L)	2.0 (1.3–2.9)	4.3 (3.2–6.1)	0.152
Body temperature (°C)	36.8 (36.0–37.0)	36.0 (35.7–36.8)	0.132

Reported proportions (counts) for categorical variables and medians (interquartile ranges) for continuous variables. Shock (ABP) represents a systolic blood pressure of <90 mmHg. Shock (lac) represents lactate levels of >2 mmol/L in the emergency department (ED). AIS, Abbreviated Injury Scale; DIC, disseminated intravascular coagulation; ISS, Injury Severity Score; iTBI, isolated traumatic brain injury; SIRS, systemic inflammatory response syndrome.

TABLE 5 | Requirement for transfusion and emergency surgery, and in-hospital mortality in isolated traumatic brain injury patients with and without disseminated intravascular coagulation.

	Non-DIC $n = 19$	DIC $n = 15$	*p*-value
Operation for TBI within 24 h after admission, *n* (%)	4 (21.1)	9 (60.0)	0.024
Massive transfusion, *n* (%)	0 (0)	4 (26.7)	0.029
3-h transfusion			
Packed red blood cells (mL)	0 (0–0)	0 (0–0)	0.451
Fresh frozen plasma (mL)	0 (0–0)	0 (0–0)	0.681
Platelet concentrate (U)	0 (0–0)	0 (0–0)	1.000
Cryoprecipitate (U)	0 (0–0)	0 (0–0)	1.000
24-h transfusion			
Packed red blood cells (mL)	0 (0–0)	560 (0–5,880)	0.096
Fresh frozen plasma (mL)	0 (0–0)	1,080 (480–8,160)	0.077
Platelet concentrate (U)	0 (0–0)	0 (0–32)	0.202
Cryoprecipitate (U)	0 (0–0)	0 (0–0)	1.000
In-hospital mortality, *n* (%)	0 (0)	4 (26.7)	0.033

Reported median (interquartile range) for continuous variables. TBI, traumatic brain injury; DIC, disseminated intravascular coagulation.

Comparison of Coagulofibrinolytic Changes Between the iTBI and Non-TBI Groups

Serial changes in the molecular markers of coagulation are shown in **Figure 1**. The levels of anticoagulation factors such as antithrombin and protein C were lower, while the levels of soluble fibrin and APC were significantly higher in the iTBI and non-TBI groups than in the healthy control group. These coagulation markers showed similar changes in patients with and without TBI. As shown in **Figure 2**, increased levels of plasmin-antiplasmin complex, D-dimer, and PAI-1 were observed in iTBI and non-TBI patients than in healthy controls. The levels of antiplasmin significantly decreased in the iTBI and non-TBI groups compared to those in the control group. These fibrinolysis-related molecular markers also underwent similar changes in the iTBI and non-TBI groups. In addition, serial changes in platelet counts and global markers of coagulation and fibrinolysis were similar between the groups (**Supplementary Table 2**).

Factors Associated With Lactate Levels

Non-TBI patients exhibited lower systolic blood pressure at the scene and at the ED with a higher incidence of shock defined as a systolic blood pressure of <90 mmHg at the ED than the iTBI group. However, the incidence of shock defined by lactate levels of >2 mmol/L was identical between the two groups (**Table 1**). There was a negative correlation between systolic blood pressure and lactate levels in the non-TBI group (Spearman's rho $= -0.403$, $p = 0.013$; **Figure 3A**), while no significant correlation was observed between these variables in the iTBI group (Spearman's rho $= 0.098$, $p = 0.614$; **Figure 3B**). By contrast, no correlation was observed between lactate levels and the DIC score at 0 h in the non-TBI group (Spearman's rho $= 0.085$, $p = 0.618$; **Figure 3C**), but a positive correlation was observed between these values in the iTBI group (Spearman's rho $= 0.441$, $p = 0.017$; **Figure 3D**).

Factors Associated With the Development of DIC in the iTBI Group

Logistic regression analysis was performed to evaluate the factors associated with the development of DIC. Multiple logistic regression analysis indicated that ISS was an independent

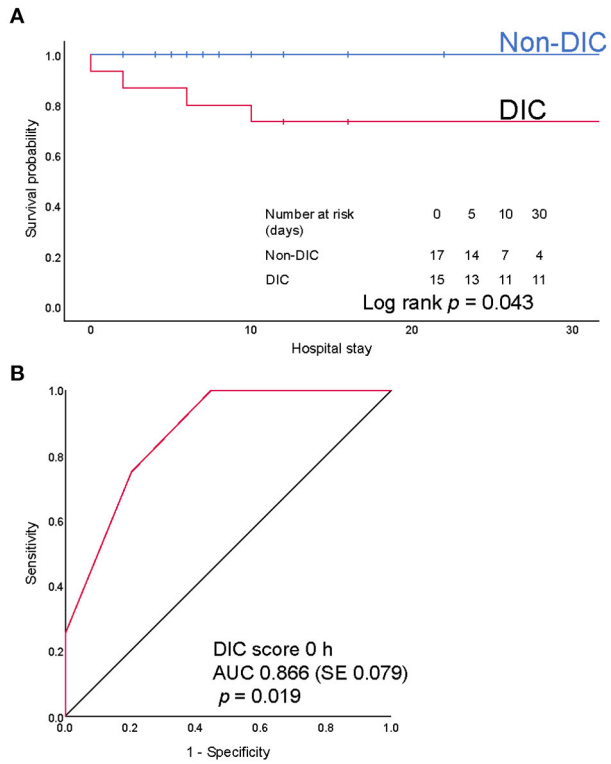

FIGURE 4 | (A) Kaplan-Meier survival provability curves for mortality during hospitalization. Numbers at risk represent the number of patients with isolated traumatic brain injury (iTBI) with or without disseminated intravascular coagulation (DIC) at risk of death on the indicated days. **(B)** Receiver operating characteristics (ROC) curves of the DIC score immediately after arrival at emergency department. AUC, area under the ROC curve; SE, standard error.

predictor of DIC development in patients with iTBI (odds ratio=1.237, p=0.018; **Table 3**).

Baseline Characteristics, Transfusion, and In-Hospital Mortality in iTBI Patients With and Without DIC

Next, we focused on iTBI patients and divided them into the DIC group (n = 15) and the non-DIC group (n = 19). The baseline characteristics of the patients are presented in **Table 4**. The median ISSs in the DIC and non-DIC groups were 25 and 17, respectively. DIC patients had a higher AIS score in the head and worse Glasgow Coma Scale scores than non-DIC patients. The physiological parameter values at the scene and ED did not differ between the groups. Only few patients had blood pressure-defined shock in both groups, whereas >50% of the patients had lactate-defined shock (DIC, 83.3%; non-DIC, 58.8%). The median lactate levels in the DIC and non-DIC groups were 4.3 and 2.0 mmol/L, respectively. The requirements for massive transfusion and emergency surgery for TBI were significantly higher, and in-hospital mortality was higher in the DIC group than in the non-DIC group (**Table 5**). The survival probability was significantly lower among iTBI patients diagnosed with DIC immediately after presentation to the ED than among patients without DIC (log rank p=0.043; **Figure 4A**). ROC curves indicated that the DIC scores immediately after arrival to the ED were a good predictor of in-hospital mortality in patients with iTBI (AUC 0.866, p=0.019; **Figure 4B**).

Comparison of Coagulofibrinolytic Changes Between iTBI Patients With and Without DIC

Higher levels of soluble fibrin and lower levels of antithrombin were confirmed in iTBI patients with DIC than in those without DIC (**Figure 5**). As shown in **Figure 6**, there were increased plasmin generation and fibrinolysis and lower levels of antiplasmin at 0 and 3 h in the iTBI with DIC group than in the iTBI without DIC group. The PAI-1 levels in iTBI patients with DIC at 3 h after hospital arrival tended to be higher than those in patients without DIC (p = 0.091). Higher levels of FDP at 0 h and lower platelet counts were observed in iTBI patients with DIC than in those without DIC (**Supplementary Table 3**).

DISCUSSION

This study aimed to elucidate the pathophysiology of TBI-induced coagulopathy by comparing the coagulofibrinolytic changes in iTBI patient with those in non-TBI trauma patients, to determine the associated factors, and to identify the clinical significance of DIC diagnosis in patients with iTBI. In the present study, similar requirements for massive transfusion and in-hospital mortality were observed between iTBI and non-TBI patients with a similar severity of trauma. The changes in the levels of coagulofibrinolytic biomarkers were also identical between the groups. The lactate levels in the iTBI group were positively correlated with DIC scores but not with blood pressure. Moreover, the ISS was an independent predictor of DIC development in patients with iTBI. iTBI patients with DIC showed a significantly higher requirement for emergency surgery for TBI, and a higher incidence of massive transfusion than did those without DIC. DIC development was significantly associated with poor hospital survival, and DIC scores at 0 h were predictive of in-hospital mortality. In patients with iTBI with DIC, marked thrombin and plasmin generation was confirmed.

Although numerous mechanisms that are potentially associated with coagulopathy after TBI have been suggested, we previously demonstrated that the main pathomechanism of TIC, with or without TBI, is DIC (19–21). DIC is characterized by increased thrombin generation and subsequent consumption coagulopathy due to damage-associated molecular patterns derived from injured cells and tissues (22). That is, DIC is caused by trauma itself (23). However, another theory advocates that TBI alone does not cause early coagulopathy (24) and that coagulopathy occurs only in patients with shock-induced profound acidosis and high lactate concentrations (25–27). The results of the present study indicated no correlation between lactate levels and systolic blood pressure (**Figure 3B**) in iTBI patients, but the positive correlation between lactate levels and DIC score (**Figure 3D**) in those patients implies that the increase

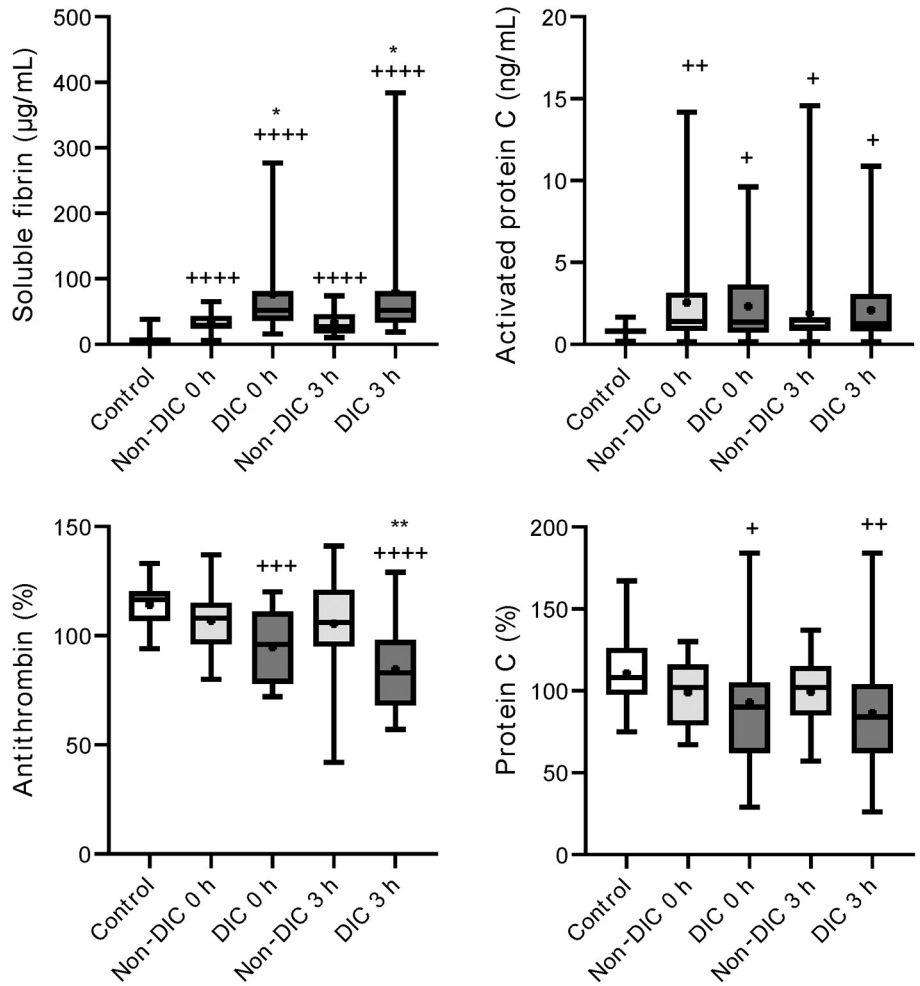

FIGURE 5 | Serial changes in the coagulation-related molecular markers in patients with isolated traumatic brain injury. Healthy controls (white box), non-disseminated intravascular coagulation (DIC) (light gray boxes), and DIC (dark gray boxes) at presentation to the emergency department (0 h) and 3 h after hospital arrival (3 h). Horizontal bars in the box indicate the median (middle) and interquartile ranges (upper 25% and lower 75%). Black boxes represent the mean values. $+p < 0.05$ vs. healthy controls, $++p < 0.01$ vs. healthy controls, $+++p < 0.001$ vs. healthy controls, $++++p < 0.0001$ vs. healthy controls, $^{*}p < 0.05$ vs. non-DIC, $^{**}p < 0.01$ vs. non-DIC.

in lactate levels in TBI patients is caused by DIC-mediated secondary tissue hypoperfusion rather than by hypotension due to hemorrhagic shock. The results demonstrated that ISS was an independent predictor of pathological coagulofibrinolytic changes, namely DIC, in iTBI patients (**Table 2**), which also supports the concept that TIC is caused by trauma itself, with or without shock (23). The current results revealed similar coagulofibrinolytic changes, characterized by DIC with hyperfibrinolysis, between iTBI and non-TBI (**Figures 1**, **2**), which was in line with the findings of a previous study (28).

iTBI patients with DIC had a higher requirement for massive transfusion and emergency surgery and had a higher in-hospital mortality than did those without DIC (**Table 5**). In addition, the DIC score in the iTBI group immediately after arrival to the hospital was predictive of in-hospital mortality (**Figure 4B**). Similar results have been reported not only in the setting of iTBI (2, 3), but also in other trauma settings (29). These results suggest the importance of DIC diagnosis in severely injured patients with or without TBI.

The present study revealed marked thrombin generation with lower levels of antithrombin in iTBI patients, particularly those with DIC (**Figure 5**). Previous studies confirmed such thrombin generation after iTBI, as evaluated by the high levels of the thrombin–antithrombin complex, prothrombin fragment 1 + 2, fibrinopeptide A, and fibrin monomers (28, 30, 31). High levels of soluble tissue factors have also been found (30, 32). Injury to the blood–brain barrier, caused by direct cerebral vascular disruption, potentially releases brain tissue factors into systemic circulation (5, 33). Tissue factors exposed to blood bind extensively to factor VIIa, followed by the initiation of the extrinsic coagulation pathway and subsequent consumption coagulopathy (23). Massive thrombin generation due to the activation of coagulation may lead to the consumption of anticoagulant factors, such as antithrombin

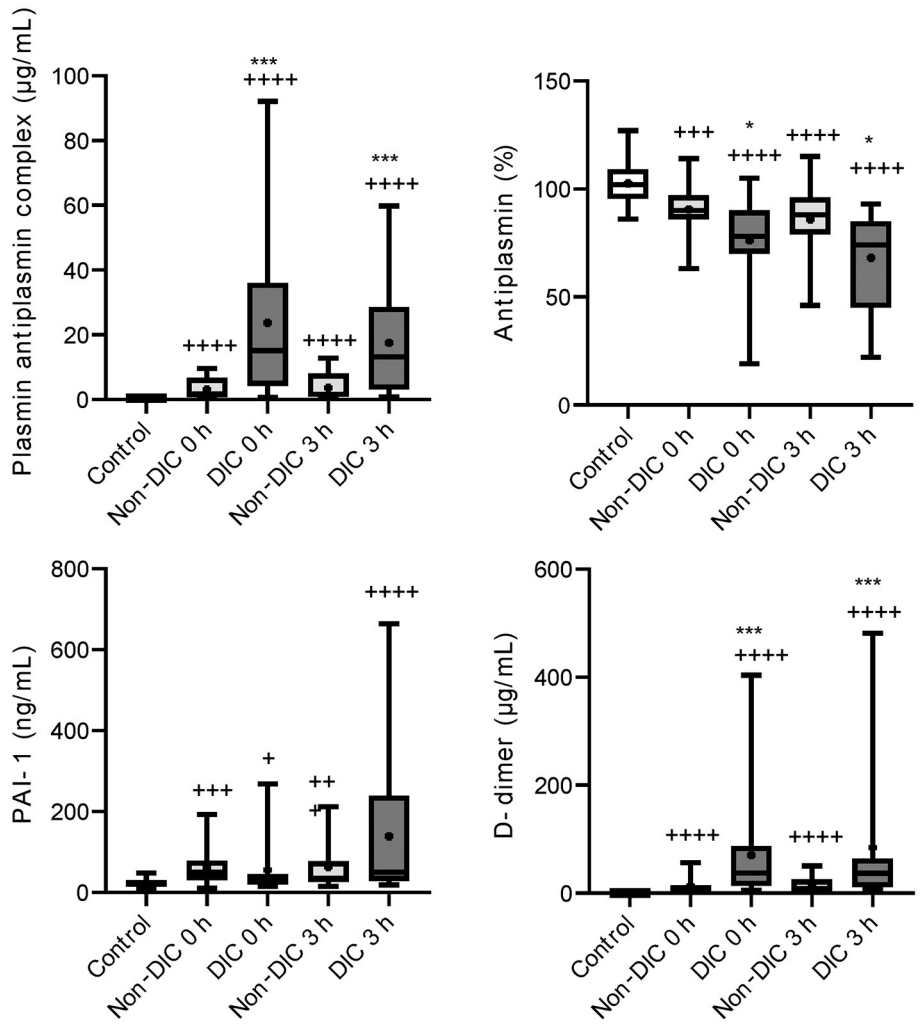

FIGURE 6 | Serial changes in the fibrinolysis-related molecular markers in patients with isolated traumatic brain injury. Healthy controls (white box), non-disseminated intravascular coagulation (DIC) (light gray boxes) and DIC (dark gray boxes) at presentation to the emergency department (0 h) and 3 h after hospital arrival (3 h). Horizontal bars in the box indicate the median (middle) and interquartile ranges (upper 25% and lower 75%). Black boxes represent the mean values. $+p < 0.005$ vs. healthy controls, $++p < 0.001$ vs. healthy controls, $+++p < 0.001$ vs. healthy controls, $++++p < 0.0001$ vs. healthy controls, $^{*}p < 0.05$ vs. non-DIC, $^{***}p < 0.001$ vs. non-DIC.

and protein C, followed by further activation of coagulation. These changes contribute to hypercoagulability inside the vessels, leading to intravascular microthrombosis in the brain and other organs, and hypocoagulability outside the vessels due to consumptive coagulopathy, leading to the progression of intracranial hemorrhage (34).

This study demonstrated marked plasmin generation with lower levels of antiplasmin in iTBI and these changes were prominent in iTBI patients with DIC (**Figure 6**). Hyperfibrinolysis after trauma is caused by the release of tissue-type plasminogen activator (t–PA) from the endothelial Weibel-Palade bodies due to traumatic shock-induced tissue hypoperfusion (22). However, patients with iTBI with high lactate levels had no low blood pressure (**Figure 3B**), and similar results were reported previously (35). These results indicate that increases in lactate levels are not caused by low blood pressure-related hypoperfusion, but by DIC-induced tissue-hypoperfusion. That is, high lactate levels in iTBI with coagulopathy are a result rather than a cause of TIC. Instead of traumatic shock-induced tissue hypoperfusion, the direct release of t-PA from injured brain tissue has been acknowledged as another explanation of hyperfibrinolysis in patients with iTBI (11). A previous study demonstrated that endogenous t-PA increases the lysis of plasma clots and contributes to intracerebral hemorrhage after TBI (36).

STUDY LIMITATIONS

Several limitations of our study need to be considered. First, although the present dataset was prospectively collected, causal relationships could not be defined because of the retrospective study design and missing values. Second, this study included a

small number of patients. Third, we did not distinguish types of TBI (e.g., acute subdural hematoma, traumatic subarachnoid hemorrhage, and contusion), which may affect the pathology and severity of TBI-induced coagulopathy. Fourth, although more patients received tranexamic acid in the iTBI group than in the non-TBI group, the effects of tranexamic acid on the levels of coagulation and fibrinolysis-related markers were not determined. Fifth, bias may exist in trauma types in the non-TBI group, as suggested by the finding that many patients in this group had severe thoracic injury. Finally, this was a single nationwide study conducted in a developed country, which may have limited the global generalization of the results.

CONCLUSIONS

The present study demonstrated similar coagulofibrinolytic changes between iTBI and non-TBI patients, which are consistent with DIC. Marked generation of thrombin and plasmin was confirmed in iTBI patients, and these changes were more prominent in iTBI patients with DIC. The development of DIC in patients with iTBI was associated with a poor survival outcome, and DIC scores immediately after hospital arrival could predict in-hospital death. Therefore, the diagnosis of DIC in the early phase of trauma is important for predicting the outcome of severely injured patients, including those with iTBI.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was approved under the condition that written informed consent was obtained from the patient or next of kin by the JAAM and the Ethics Committee of each hospital (JAAM, 2014-01; Hokkaido University Graduate School of Medicine, Head institute of the FORECAST group, 014-0307). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TW analyzed the study results, interpreted the data, and drafted the manuscript. AS checked the statistical methods and results. AS, SG, KY, SFujis, DS, SK, HO, TA, TM, JS, JK, NT, RT, KT, SS, YSh, TN, KO, YSa, AH, SFujim, YU, and YO planned the study, decided the methods, developed a web-based registration system, discussed the results, and critically revised the manuscript. All authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.767637/full#supplementary-material

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CASE REPORT
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The Application of a SaCoVLM™ Visual Intubation Laryngeal Mask for the Management of Difficult Airways in Morbidly Obese Patients: Case Report

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We report insertion of the SaCoVLM™ in three awake morbidly obese patients (BMI 46.7–52.1 kg/m^2). The patients were given intravenous atropine and midazolam injections after entering the operating room and then inhaled an anesthetic with 2% lidocaine atomization. After SaCoVLM™ insertion while patients were awake, when the vocal cords were visualized, controlled anesthetic induction commenced with spontaneous ventilation. The entire anesthesia induction and intubation process was completed under visualization, and no adverse events such as hypoxemia occurred. No patient had an unpleasant recall of the procedure. We conclude that the SaCoVLM™ is easy to use, well tolerated and suitable for awake orotracheal intubation in patients with known difficult airways.

Keywords: SaCoVLM™, visual intubation laryngeal mask, difficult airways, morbidly obese patients, laryngeal mask airway

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INTRODUCTION

Awake tracheal intubation is recommended in patients with known or predicted difficult airways (1). The most widely used technique for awake intubation is the fibreoptic bronchoscope (FOB). New optical intubation devices have been developed and are currently being used as an alternative method for this purpose, but only a few cases of awake intubation have been reported (2–4). The SaCoVLM™ visible intubation laryngeal mask was independently developed in China. It is a three-cavity laryngeal mask that provides ventilation with a visual interpolation cavity and gastric duct cavity (**Figure 1**). Through the visual appliance external display screen, the laryngeal housing and tracheal intubation can be visualized, and the position of the laryngeal mask can be continuously monitored during the perioperative period. The intubating laryngeal mask airway (ILMA) is a supraglottic airway that facilitates ventilation and blind tracheal intubation (5). When using the ILMA, however, anesthesiologists must blindly try to optimize the position of the throat mask to meet the appropriate intubation conditions. Using SaCoVLM™, we can directly observe the impact of standard reset operations (e.g., Chandy and "UP-Down" operations (6, 7)) and visually insert the tracheal intubation device. We report the first case series utilizing the SaCoVLM™ LMA

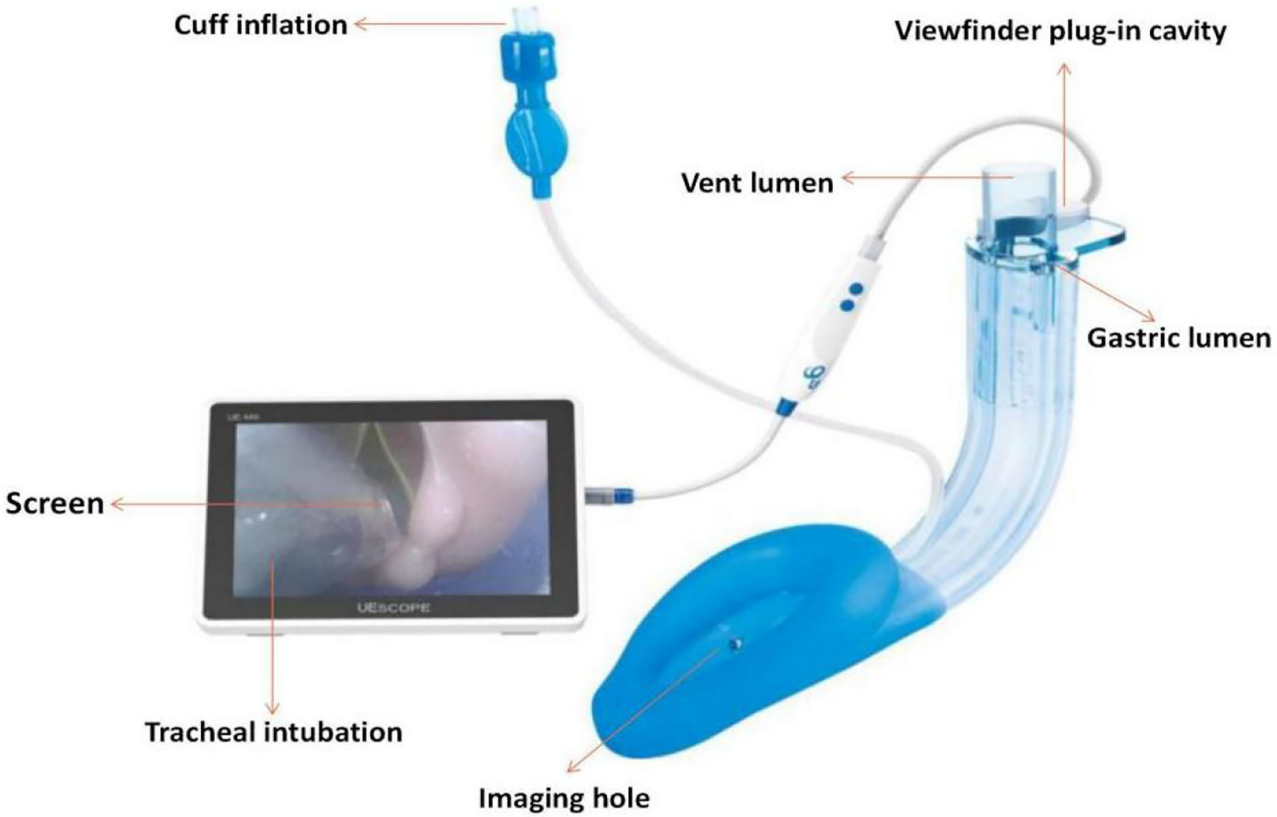

FIGURE 1 | SaCoVLM^{TM} visible intubation laryngeal mask,photo courtesy of Youyi Medical Instrument Co. Ltd. (HangZhou, China).

(Youyi Medical Instrument Co. Ltd., HangZhou, China) as an awake tracheal intubating device in patients with an anticipated difficult airway management.

CASE PRESENTATION

With Institutional Review Board approval and written informed consent from all patients, we collected SaCoVLM^{TM} insertion data on three morbidly obese patients who then received robot-assisted laparoscopic sleeve gastrectomies under general anesthesia. All three patients denied any history of gastric reflux, and all had been fasting for a minimum of 10 h.

Patient One

A 38-years-old, 124 kg, 163 cm, BMI 46.7 kg/ m^2, American Society of Anesthesiologists Classification (ASA) III level female. She had a history of 5 years hyperthyroidism (propylthiouracil 300 mg qd.) and Obstructive sleep apnea (OSA) for over 10 years, abdominal hernia for over 2 years, habitual mandibular dislocation, thyroidectomy and two cesarean deliveries without any significant personal or familial past history. Long-term high calorie diet and eating habits led her to gain weight gradually. Preoperative airway assessment included a Mallampati class III, a 7.5 cm thyromental distance, a 5 cm interincisor distance and a 46 cm neck circumference. Physical examination showed nutritional obesity, abdominal distension, two old transverse scars in the lower abdomen, and a transverse scar of about 5 cm in the neck. Admission diagnosis: Morbidly obese, Obstructive sleep apnea, Abdominal hernia, Hyperthyroidism, After thyroidectomy, After cesarean section.

Patient Two

A 48-years-old, 140 kg, 164 cm, BMI 52.1 kg/m^2, ASA III female. She was hospitalized for symptomatic angina. Owing to a gigantic appetite mainly on rice and pasta, her weight gradually increased in the past 20 years. Both her parents and sibling 3 were over obese. She had a history of hypertension (Bisoprolol 5 mg, Amlodipine 10 mg, and Olmesartan 40 mg), OSA, Diabetes, fatty liver and umbilical hernia, aortic stenosis and hypercholesterolemia. Preoperative airway assessment included a Mallampati class III, a 7.0 cm thyromental distance, a 5 cm interincisor distance and a 41 cm neck circumference. Physical examination showed nutritional obesity and mild cyanosis of the lips. Hematologic tests shows: WBC 10.93 $\times$ 10^9 /L, NEU% 0.798, CRP 11.7 mg/mL. CT showed fatty liver, umbilical hernia and dilatation of surrounding intestine. Admission diagnosis: Morbid obesity, Umbilical hernia, Hypertension, Fatty liver.

Patient Three

A 29-years-old, 145 kg, 172 cm, BMI 49.0 kg/m^2, ASA III male. His past medical history include liposuction surgery 3 years ago, hypertension, OSA and diabetes. progressive weight gain over 20 years, sleep snoring and gasping suppression for 5 years. By restricted diet, exercises he didn't get a good weight control and at home for Intermittently ventilator assisted breathing therapy at present. Preoperative airway assessment included a Mallampati class III, a 7.0 cm thyromental distance, a 5 cm interincisor distance and a 45 cm neck circumference. Physical examination showed nutritional obesity, abdominal distension, generalized fat accumulation. Hematologic tests shows: AST 52.10 U/L, UA 559.0 μmol/L, INS μIU/mL, UCP 5.25 ng/mL, $SpO_2$70.00 mmHg, GLU 6.40 mmol/L. Admission diagnosis: Morbid obesity, Obstructive sleep apnea.

All three patients were managed using the same protocol. The patients underwent routine monitoring and were given 2 mg of midazolam and 0.4 mg of atropine intravenously after being transferred to the operating room (OR). Aerosolized inhalation of 2% lidocaine was performed in the semisitting position on the transfer bed for 15–20 min without subglottic anesthesia. Each patient was placed in a slope position, the radial artery was punctured and catheterization under local anesthesia was performed to monitor invasive arterial blood pressure, and an intravenous micropump infusion of dexmedetomidine was given (load 1 $\mu g \cdot kg^{-1} \cdot 10\ min^{-1}$, maintenance amount 0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ until 40 min before the end of the operation).

A laryngeal tube was used to test the sensation in the back of the oropharynx. If there was a pharyngeal reflex, 2 mL of 2% lidocaine was sprayed. The laryngeal mask model was chosen according to the patient's lean body mass. The SaCoVLM^{TM} No. 4 was used for patients one and two and the SaCoVLM^{TM} No. 5 was used for patient three chooses. All patients tolerated the procedure well, were connected to the anesthesia circuit, and received 100% oxygen. The glottis was directly observed in 2 patients, and the epiglottis was folded down in 1 patient. Through the "UP-Down" operation (slowly withdrawing the cuff from the pharynx 5-6 cm to aid in unfurling the epiglottis, and then re-inserting), a glottis view was obtained (**Figure 2A**). Once we observed the glottis, we observed the waveform of end-breathing carbon dioxide.

For the induction of anesthesia, 2.5 $mg \cdot kg^{-1}$ of propofol, 0.6 $mg \cdot kg^{-1}$ of rocuronium and 0.3 $\mu g \cdot kg^{-1}$ of sufentanil were immediately injected. When the BIS value reached 40–60, visual descending tracheal intubation was performed, and the 3 patients successfully completed tracheal intubation (**Figure 2B**). The tracheal intubation and the entrance of the LMA was fixed with the infusion paster, the laryngeal mask was kept to evacuate the cuff gas, and tracheal intubation was used to maintain anesthesia during the operation (**Figure 2C**). Forty milliliters of 0.375% ropivacaine was chosen, and bilateral transversus abdominis nerve blocks were conducted under ultrasound guidance. An intraoperative pump injection of propofol 4–12 $mg \cdot kg^{-1} \cdot h^{-1}$ and remifentanil 0.2–0.5$\mu g \cdot kg^{-1} \cdot min^{-1}$ was used to maintain anesthesia; after the operation, tracheal intubation was removed under deep anesthesia. The laryngeal mask was kept and transferred to the post-anesthesia care unit (PACU). During the resuscitation period, sugammadex sodium was used to antagonize muscle relaxation at a dose of 2–4 $mg \cdot kg^{-1}$. After the patients were awake, the laryngeal mask was well tolerated. After 1 h of observation, the laryngeal mask was removed. All 3 patients had no adverse reactions. They were safely transferred to the ward after continued observation for 1 h. The dosage of the above patients was calculated based on their lean body mass, the anesthesia management timeline are depicted on **Figure 3**.

On the first postoperative day, an interview was conducted with each patient on their induction and intubation experience. All 3 patients could recall being pushed to the operating room and had memories of procedures such as indwelling intravenous needles and aerosol inhalation. One patient had no memory of SaCoVLM^{TM} implantation, and two patients described it as if they had swallowed a very large object. After midazolam was given, all patients clearly denied fear or discomfort. All patients were willing to experience the same anesthesia again, and their satisfaction with anesthesia was high. The postoperative VAS scores were all below 3, and no complications, such as hypoxemia, nausea or vomiting, occurred.

DISCUSSION

This is the first reported series of awake insertion of the SaCoVLM^{TM} in morbidly obese patients. SaCoVLM^{TM} has shown advantages in clinical applications since its creation in 2018. Visualization of laryngeal mask placement and endotracheal intubation and Airway patency as well as 100% oxygen delivery was achieved. we can continuous monitoring the LMA location during Perioperative period. SaCoVLM^{TM} is comparable in price to a common LMA that can easily clinical promoted, nevertheless, it's easy to use, well tolerated and safe (with a 30 cm H_2O expected seal pressure). However, SaCoVLM^{TM} has some limitations. Its imaging was not sufficiently sharp and the only way to improve imaging quality by adjusting the LMA position was not flexible enough. Supplementary intubation instruments such as fibrobronchoscopy and bougie are sometimes required. Susceptibility to airway secretions makes suppressive secreting drugs (e.g., atropine, pentylenetrin) a reliance which is limited to exceptional patients (Glaucoma patients). At present, clinical research to confirm the application of SaCoVLM^{TM} is still needed.

Studies have shown that ILMA can be successfully placed in conscious patients (5). Combes et al., for example, reported the effectiveness of ILMA in morbidly obese patients (8). LMA CTrach^{TM} is the world's first visual intubation laryngeal mask and was applied in clinical practice in 2004. In 2006, Liu et al. (9) first reported the use of the LMA CTrach^{TM} in 84 normal volunteers. The first intubation success rate was 100% when the glottis could be seen in the center of the monitor. A number of studies have shown that LMA CTrach^{TM} has greater advantages in the treatment of difficult airways, including in morbidly obese patients, and for airway resuscitation, abnormal head and neck

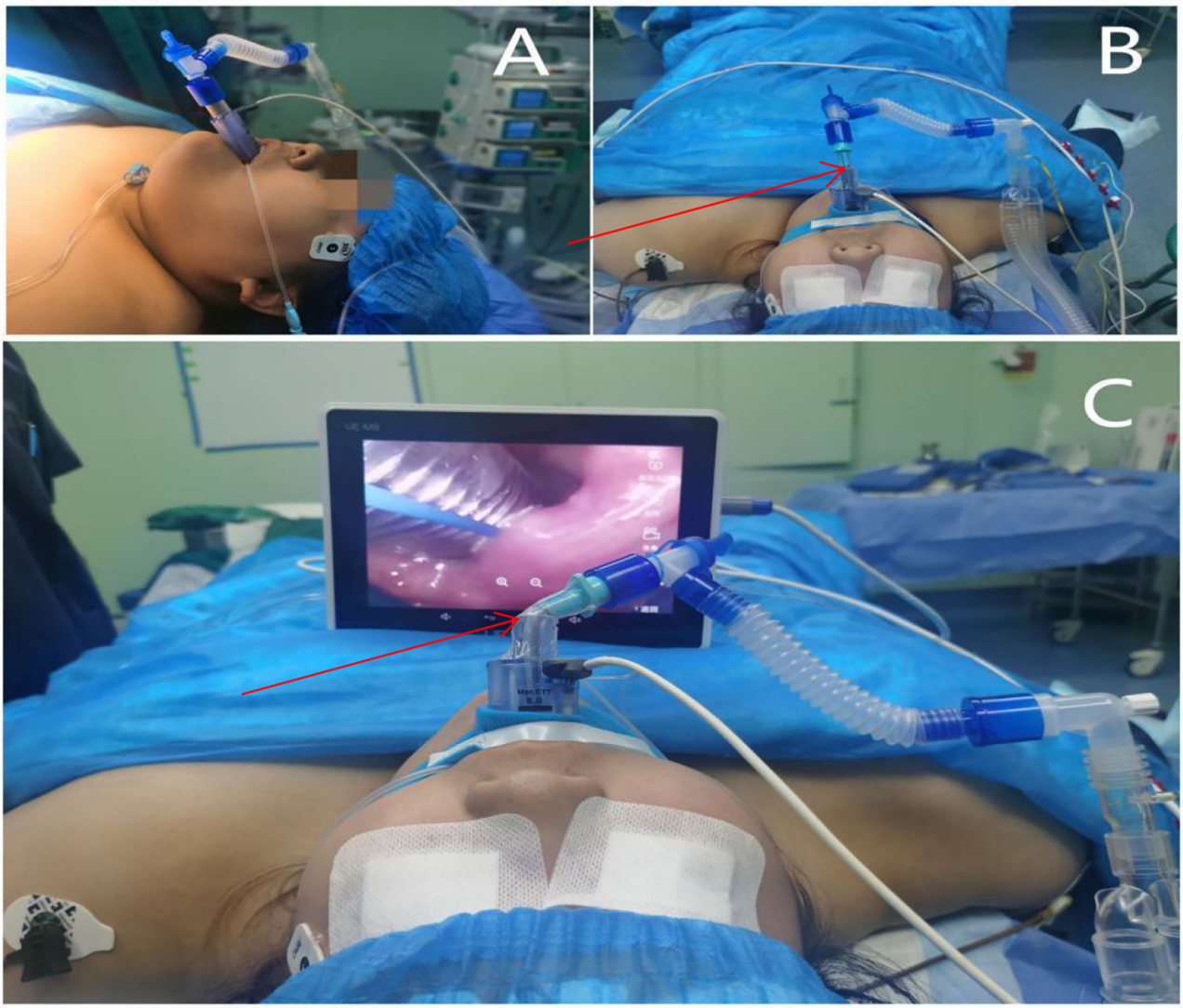

FIGURE 2 | Airway management, Fixed place of infusion paster (Ventilation lumen entrance and tracheal intubation).

movements and other difficult airway treatment problems (10). In addition, LMA CTrach™ was not well clinically promoted due to its exorbitant price. The usage of the SaCoVLM™ has overcome such difficulties. Although it does not provide a clear image like other fiber optic devices, the glottis can usually be seen and centered on the monitor, which is enough to successfully complete the first tracheal intubation.

Video-guided insertion can better locate the glottis, provide a more unobstructed airway, and improve the first success rate of tracheal intubation and gastric drainage tube insertion. Blind probe insertion into the supraglottic airway leads to poor positioning, downward folding of the epiglottis, and the use of more auxiliary devices, so the incidence of complications is higher (11). In fact, the Difficult Airway Society (DAS) guidelines state that "blind" airway management techniques are unreliable and are related to the high incidence of airway trauma (12, 13). Unlike traditional video laryngoscopes, the SaCoVLM™ reduces the need for assistive devices. It can control the airway within a few seconds and establish the best ventilation for tracheal intubation, reducing the time of apnea, which is suitable for patients with poor physiological reserve. The SaCoVLM™ is especially beneficial for certain patients such as morbidly obese patients or pregnant women.

The main cause of anesthesia-related injuries is respiratory safety, which cannot be ensured (12, 13). Actually, the most critical anesthetic skill for such patients is safe airway management, and a key component of this skill is the mastery of multiple techniques for securing the airway. When faced with a known or anticipated difficult-to-manage airway, although awake fiberoptic bronchoscopy may constitute "plan A" for intubation, a "plan B" is required, as fiberoptic bronchoscopy may fail to lead to successful tracheal intubation (e.g., due to airway secretions

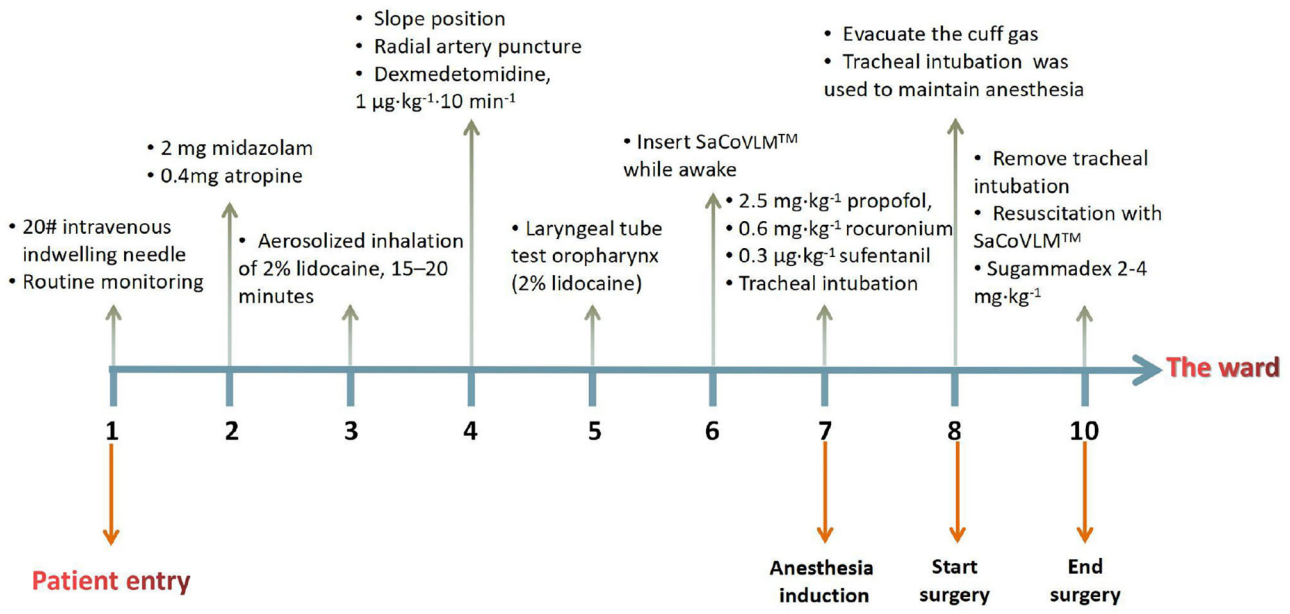

FIGURE 3 | Anesthesia management timeline.

or blood). All our patients had both a high Mallampati score and a thick neck circumference, a combination that is known to be a strong predictor of difficult airway (14). The use of the SaCoVLM™ helped achieve safe, successful intubation while maintaining spontaneous respiration. As the vocal cords were always kept in view, we did not need to be concerned about the displacement of laryngeal structures that may occur with the induction of anesthesia. Awake SaCoVLM™ insertion requires only that patients have adequate topical anesthetic applied to the oropharynx, whereas awake tracheal intubation using fiberoptic bronchoscopy requires that the infraglottic structures be anesthetized as well. The issue of infraglottic anesthesia is important in considering a patient's ability to protect his or her airway in the event of reflux or frank vomiting. Although none of the patients in our series reported having gastric reflux, a strong history of reflux may be an indication for rapid-sequence or awake intubation. When vocal cords and the immediate infraglottic areas are anesthetized, as is necessary for awake fiberoptic intubation, the cough reflex is suppressed, and it may become more difficult for patients to manage secretions. The use of rapid-sequence induction in patients who fasted with no risk factors for aspiration other than obesity is debatable.

Airway management in morbidly obese patients is extremely challenging and can result in serious adverse events and even death if mishandled. Poor satisfaction rates have been recorded despite fiberoptic bronchoscopy-guided awake intubation being the best choice for obese patients. The advantage of oxygenating morbidly obese patients with 100% oxygen cannot be overestimated, given the low functional residual capacity and rapid desaturation rates in this population. Thus, the ability to deliver high oxygen rates while using the CTrach™ to secure a view of the vocal cords is a significant feature of the SaCoVLM™. Furthermore, it has been observed that ILMA is actually placed better in obese patients than in patients with a normal body habitus (8, 15).

Anatomical and /or physiological compromise can result in morbidity and mortality, which were common problems for Obesity and obstructive sleep apnea patients in the extubation time. Based on the management guideline for adult perioperative extubation issued by DAS in 2012 and the practical guide for difficult airway management issued by ASA in 2013 (1, 16), LMA as a replacement of tracheal intubation is superior to either awake or deep extubation (17, 18). However, it is inappropriate in patients in whom re-intubation would be difficult or if there is a risk of regurgitation. If SaCoVLM™ can effectively ensure ventilation after implantation, we choose to remove tracheal intubation under deep anesthesia. Resuscitation with SaCoVLM™ can be monitored visually while avoiding the risk of re-intubation. All 3 patients recovered well. This is the first time to report on this method. Of course, we will continue to study it in depth to support this view.

CONCLUSION

In summary, this is the first reported series of awake insertions of the SaCoVLM™ LMA in morbidly obese patients with difficult airways. Intubation with the SaCoVLM™ LMA can provide oxygen for patients at any time. Its simple operation, well tolerated, and high success rate makes it a better airway management implementation tool in our practical work.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital of Shandong First Medical University. The patients/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

YS and YW conceived and wrote the manuscript. LH, LX, and YG collected patient's clinical information. YS and MZ analyzed and evaluated the treatment and curative effects. YW guided the entire process in terms of theory and practice and revised the manuscript. All other authors contributed to the analysis, reviewed results, and reviewed the manuscript.

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ORIGINAL RESEARCH
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Urine Output Is Associated With In-hospital Mortality in Intensive Care Patients With Septic Shock: A Propensity Score Matching Analysis

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Background: The relationship between urine output (UO) and in-hospital mortality in intensive care patients with septic shock is currently inconclusive.

Methods: The baseline data, UO, and in-hospital prognosis of intensive care patients with septic shock were retrieved from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database. By drawing receiver operating characteristic (ROC) curves and comparing the areas under the ROC curves (AUC) to determine the predictive value of UO for in-hospital mortality, and by drawing the Kaplan-Meier curves to compare the difference in in-hospital mortality between different groups of UO.

Results: Before and after the propensity score matching (PSM) analysis, UO was always a risk factor for in-hospital mortality in patients with septic shock. The AUC of UO was comparable to the Sequential Organ Failure Assessment (SOFA) scoring system, while the AUC of combining UO and SOFA was greater than that of SOFA. The median survival time of the high-UO group (UO $>$ 0.39 ml/kg/h, before PSM; UO $>$ 0.38 ml/kg/h, after PSM) was longer than that of the low-UO group. Compared with the high-UO group, the hazard ratios (HR) of the low-UO group were 2.6857 (before PSM) and 1.7879 (after PSM).

Conclusions: UO is an independent risk factor for septic shock. Low levels of UO significantly increase the in-hospital mortality of intensive care patients with septic shock. The predictive value of UO is comparable to the SOFA scoring system, and the combined predictive value of the two surpasses SOFA alone.

Keywords: septic shock, urine output, MIMIC-IV, in-hospital mortality, propensity score matching

INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is the most severe form of sepsis, which is characterized by persisting hypotension requiring vasopressors to maintain mean arterial pressure ≥65 mmHg and having an increased serum lactate level >2 mmol/L despite adequate volume resuscitation (1, 2). The in-hospital mortality rate of sepsis exceeds 10%, and septic shock is even worse (1, 3). A meta-analysis of European and North American populations showed that the in-hospital mortality rate of septic

shock was as high as 39% (95% CI: 34.4–43.9%) (4). It is of far-reaching significance to clarify the independent risk factors related to mortality, which can further guide nursing and treatment, so as to achieve the purpose of reducing mortality especially in the intensive care unit (ICU).

Daily urine output (UO) is measured routinely in the ICU, and its prognostic value has already emerged. In 2013, Oh et al. (5) found that UO was significantly associated with the prognosis in critically ill patients with acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT). When the timing of CRRT initiation was stratified by 6 h UO, 28-day all-cause mortality rates were significantly lower in the non-oliguric group compared with the oliguric group. Huang et al. (6) found that reduced initial 24 h UO was associated with an increased risk in 7- and 30-day all-cause mortality and major adverse cardiovascular events (MACE) in ST-segment elevation myocardial infarction (STEMI) patients admitted without cardiogenic shock and renal dysfunction. Zhang et al. (7) investigated the relationship between UO on the first day of admission to the ICU and the in-hospital mortality of unselected critically ill patients and found that UO was an independent risk factor of mortality regardless of whether diuretics were used or not. Oliguria is one of the important signs of hypoperfusion in septic shock (8). However, due to the complexity of the composition of patients admitted to the ICU, it is not known whether the conclusion of Zhang et al. is applicable to septic shock. To date, no researches have confirmed the relationship between UO and mortality of patients with septic shock. This study is based on a well-known public database, Medical Information Mart for Intensive Care IV (MIMIC-IV) database, to investigate the relationship between UO on the first day of admission and the in-hospital mortality of intensive care patients with septic shock.

METHODS

Database

MIMIC-IV (https://mimic.mit.edu/) builds upon the MIMIC-III database (9) and has made many improvements. MIMIC-IV contains comprehensive information (laboratory measurements, medications administered, vital signs documented, etc.) of patients admitted to a Tertiary Academic Medical Center in Boston, MA, USA between 2008 and 2019. The database is designed to support a wide variety of healthcare research. An individual who passed the "Protecting Human Research Participants" exam on the National Institutes of Health website can access the database (certification number 37474354 for author Tianyang Hu).

All patients in the database are anonymous and no informed consent is required.

Study Population and Data Extraction

The patients diagnosed with "septic shock" in the MIMIC-IV database are divided into two categories: "septic shock" with International Classification of Disease (ICD) code 78552 (9th revision), and "severe sepsis with septic shock" with ICD code (10th revision). The inclusion criteria were: (1) aged≥18 years; (2) UO (ml/day) assessed within 24 h from admission; (3) UO (ml/kg/h) could be calculated within 24 h from admission. Post-procedural septic shock was excluded. Since the same patient may have multiple admission records, we only included the first ICU stay for each patient.

The following data was extracted from the MIMIC-IV database (version 1.0) by Navicat Premium software (version 15.0): age, gender, length of ICU stay, length of hospital stay, Charlson comorbidity index, Sequential Organ Failure Assessment (SOFA) score/ hemoglobin/white blood cells/platelets/creatinine/blood urea nitrogen/total bilirubin/heart rate/mean arterial pressure/respiratory rate/weight/urine output/ whether complicated with AKI/whether to take diuretics/whether treated with renal replacement therapy (RRT) on the first day of admission and hospital expire flag (a binary flag which indicates whether the patient died in hospital). Charlson comorbidity index (10) is a scoring system to quantify comorbidities (including myocardial infarct, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatic disease, peptic ulcer disease, liver disease, diabetes, paraplegia, renal disease, malignant cancer, metastatic solid tumor, and acquired immunodeficiency syndrome). Diuretics mainly include bumetanide, chlorothiazide, furosemide, hydrochlorothiazide, metolazone, and spironolactone. If a variable was assessed multiple times on the first day of admission, took the average value.

Statistical Analysis

Use the Kolmogorov-Smirnov test to evaluate whether the variables follow the normal distribution. If followed, then express the variable as mean ± standard deviation (M ± SD) and compare with independent sample *t*-test; if not follow, then express the variable as the median with interquartile range (IQR) and compare with Wilcoxon rank-sum test. Categorical variables were expressed as numbers and percentages, and compared by Chi-square test. Binomial Logistic regression analysis was conducted to evaluate the impact of UO on in-hospital mortality in patients with septic shock. Variables with a *P*-value <0.1 in the univariate analysis were included in the multivariate analysis. Z test was conducted following the method of Delong et al. (11) to compare the predictive value of UO, SOFA, and UO+SOFA by comparing the area under curves (AUC) of the receiver operating characteristic curves (ROC).

To reduce potential bias, propensity score matching (PSM) analysis was performed between the death group and survival

Abbreviations: UO, urine output; MIMIC-IV, Medical Information Mart for Intensive Care IV; AKI, acute kidney injury; CRRT, continuous renal replacement therapy; MACE, major adverse cardiovascular events; STEMI, ST-segment elevation myocardial infarction; ROC, receiver operating characteristic; AUC, areas under the receiver operating characteristic curves; PSM, propensity score matching; SOFA, Sequential Organ Failure Assessment; HR, hazard ratio; OR, Odds Ratio; CI, Confidence Interval; ICU, intensive care unit; ICD, International Classification of Disease; RRT, renal replacement therapy; M ± SD, mean ± standard deviation; IQR, interquartile range; LOS, Length of Stay; CCI, Charlson Comorbidity Index; Hb, Hemoglobin; WBC, White Blood Cells; PLT, Platelets; Cr, Creatinine; BUN, Blood Urea Nitrogen; TBil, Total Bilirubin; HR, Heart Rate; bpm, beat per minute; MAP, Mean Arterial Pressure; RR, Respiratory Rate; cpm, count per minute.

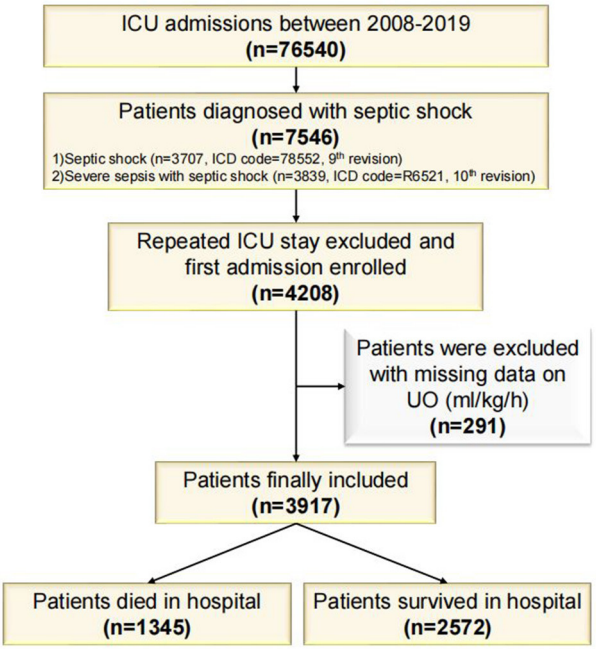

FIGURE 1 | Flowchart of study cohort. ICU, Intensive Care Unit; ICD, International Classification of Disease; UO, Urine Output.

group. All potential confounders were included in the PSM analysis: age, gender, Charlson comorbidity index, hemoglobin, white blood cells, platelets, creatinine, blood urea nitrogen, total bilirubin, heart rate, mean arterial pressure, respiratory rate on the first day of admission, whether complicated with AKI, whether to take diuretics, and whether treated with RRT on the first day of admission. The PSM analysis was performed by a 1:1 nearest neighbor matching algorithm (a caliper of 0.001) without replacement, and the propensity score was calculated by the logistic regression model.

In-hospital mortality is regarded as a time-to-event variable and the event is death during hospitalization. Patients were censored when they were discharged alive, and patients were followed during the hospital stay (7). UO was divided into high-UO group and low-UO group according to the optimal cut-off value indicated by the ROC curve, and Kaplan-Meier survival curves were drawn. The log-rank test was used to evaluate whether there was a difference in survival rate between the two groups.

All the analyses were performed using the software IBM SPSS Statistics (v26.0; IBM, Armonk, NY) and MedCalc (v19.6.1; MedCalc Software Ltd, Ostend, Belgium). A P-value < 0.05 was considered to be statistically significant.

RESULTS

Baseline Characteristics

MIMIC-IV database contains 76,540 ICU admissions. Finally, 3,917 patients were included in this study (of which 1,345 died and 2,572 survived in the hospital, **Figure 1**) and the in-hospital mortality rate was 34.34%. The length of hospital stay of the survival group was longer than that of the death group ($P < 0.001$). Age, Charlson Comorbidity Index, SOFA score, the level of creatinine, total bilirubin, blood urea nitrogen, heart rate and respiratory rate on the first day of admission in the death group were higher than in the survival group significantly ($P < 0.001$ for all). Mean arterial pressure, the level of hemoglobin and platelets in the survival group were higher than in the death group significantly ($P < 0.001$ for all). UO in the survival group was higher than in the death group significantly ($P < 0.001$). The proportions of complicated with AKI, treated with diuretics and RRT in the death group were higher than in the survival group ($P < 0.001$ for all). After PSM with age, gender, Charlson comorbidity index, the level of hemoglobin, white blood cells, platelets, creatinine, blood urea nitrogen, total bilirubin, heart rate, mean arterial pressure, respiratory rate, whether complicated with AKI, whether to take diuretics, and whether treated with RRT on the first day of admission, the matching variables were balanced and comparable between the two groups (of which 963 died and 963 survived, $P > 0.05$ for all, **Table 1**). After PSM, the length of hospital stay of the survival group was still longer than that of the death group ($P < 0.001$). SOFA score on the first day of admission in the death group was higher than in the survival group significantly ($P < 0.001$), and UO in the survival group was still significantly higher than in the death group ($P < 0.001$). The baseline characteristics were presented in **Table 1**.

Logistic Regression Analysis

Considering the collinearity between UO (ml/day) and UO (ml/kg/h), the latter was included in the binomial logistic regression analysis. UO was a risk factor for in-hospital mortality in patients with septic shock before (OR: 0.285, 95% CI: 0.247–0.330, $P < 0.001$) and after (OR: 0.507, 95% CI: 0.434–0.593, $P < 0.001$) adjustment (**Table 2**). After PSM, UO was still a risk factor for in-hospital mortality in patients with septic shock before (OR: 0.544, 95% CI: 0.465–0.638, $P < 0.001$) and after (OR: 0.678, 95% CI: 0.578–0.796, $P < 0.001$) adjustment (**Table 3**).

Comparison of ROC Curves

The ROC curves were drawn to clarify the predictive value of UO for in-hospital mortality of septic shock (**Figure 2**). Before PSM (**Figure 2A**), the AUCs of UO, SOFA, combining UO and SOFA (UO + SOFA) were 0.722, 0.725, and 0.753, respectively (**Table 4**). The AUC of UO was comparable to SOFA ($Z = 0.237$, $P = 0.8127$), while the AUC of UO+SOFA was greater than that of UO ($Z = 5.079$, $P < 0.0001$) and SOFA ($Z = 6.264$, $P < 0.0001$). UO+SOFA had the highest sensitivity (69.81%) and Youden's index (0.4007), while UO had the highest specificity (76.94%). After PSM (**Figure 2B**), the AUCs of UO, SOFA, and UO+SOFA were 0.637, 0.622, and 0.643, respectively (**Table 5**). The AUC of UO was still comparable to SOFA ($Z = 1.090$, $P = 0.2756$), while the AUC of UO + SOFA was also greater than that of SOFA ($Z = 3.034$, $P = 0.0024$), but comparable to UO ($Z = 0.640$, $P = 0.5219$). UO+SOFA had the Youden's index (0.2420), while UO

TABLE 1 | Demographic and clinical characteristics of the study population.

Characteristics	Before PSM			After PSM		
	Death (n = 2,572)	Survival (n = 1,345)	P	Death (n = 963)	Survival (n = 963)	P
*Age, year	71 (60–82)	68 (56–79)	0.000	72 (61–82)	72 (61–82)	0.961
*Gender, male	723 (53.8)	1398 (54.4)	0.720	521 (54.1)	512 (53.1)	0.681
LOS hospital, day	6.5 (2.0–14.5)	11.5 (6.6–20.6)	0.000	7.1 (2.5–14.6)	12.7 (7.1–21.6)	0.000
LOS ICU, day	3.7 (1.5–8.7)	3.4 (1.9–7.7)	0.243	3.9 (1.6–8.6)	3.8 (2.1–9.0)	0.005
*CCI	7 (5–9)	6 (4–8)	0.000	7 (5–9)	7 (5–9)	0.869
Laboratory tests						
*Hb, g/dL	9.8 (8.5–11.4)	10.3 (8.9–11.7)	0.000	9.9 (8.6–11.5)	10.2 (8.8–11.5)	0.147
*WBC, 10^9/L	14.0 (8.2–20.3)	13.8 (9.3–19.3)	0.854	14.1 (8.2–20.4)	13.7 (9.1–20.3)	0.776
*PLT, 10^9/L	156 (89–241)	183 (124–255)	0.000	169 (99–259)	163 (115–236)	0.668
*Cr, ng/dL	1.7 (1.2–2.7)	1.3 (0.9–2.0)	0.000	1.5 (1.1–2.5)	1.5 (1.0–2.4)	0.617
*BUN, mmol/L	36.0 (23.5–56.0)	26.0 (16.5–42.3)	0.000	33.5 (22.0–52.0)	32.5 (20.5–52.5)	0.318
*TBil, mg/dL	1.6 (0.6–2.9)	1.1 (0.5–2.7)	0.000	1.4 (0.6–2.7)	1.3 (0.6–2.7)	0.778
Vital signs						
*HR, bpm	96 (82–108)	90 (78–102)	0.000	94 (80–107)	94 (81–106)	0.899
*MAP, mmHg	71 (65–76)	72 (68–77)	0.000	71 (66–77)	72 (67–77)	0.207
*RR, cpm	22 (19–26)	21 (18–24)	0.000	22 (19–25)	22 (19–25)	0.519
SOFA score	12 (9–15)	8 (5–11)	0.000	11 (8–14)	8 (6–12)	0.000
Day 1 UO, ml/day	595 (186–1264)	1400 (825–2317)	0.000	715 (264–1400)	1170 (649–1950)	0.000
Day 1 UO, ml/kg/h	0.32 (0.10–0.67)	0.75 (0.41–1.25)	0.000	0.38 (0.13–0.73)	0.63 (0.33–1.05)	0.000
*Day 1 AKI	425 (31.6)	538 (20.9)	0.000	264 (27.4)	254 (26.3)	0.607
*Day 1 diuretic	269 (20.0)	318 (12.4)	0.000	166 (17.2)	161 (16.7)	0.762
*Day 1 RRT	366 (27.2)	271 (10.5)	0.000	181 (18.8)	182 (18.9)	0.954

Values are expressed as the median (IQR) or n (%).
PSM, Propensity Score Matching; LOS, Length of Stay; ICU, Intensive Care Unit; CCI, Charlson Comorbidity Index; Hb, Hemoglobin; WBC, White Blood Cells; PLT, Platelets; Cr, Creatinine; BUN, Blood Urea Nitrogen; TBil, Total Bilirubin; HR, Heart Rate; bpm, beat per minute; MAP, Mean Arterial Pressure; RR, Respiratory Rate; cpm, count per minute; SOFA, Sequential Organ Failure Assessment; UO, Urine Output; AKI, Acute Kidney Injury; RRT, Renal Replacement Therapy.
**Covariables included in the PSM.*

had the highest specificity (71.55%) and SOFA had the highest sensitivity (70.40%).

Comparison of Kaplan-Meier Curves

Before PSM, UO was divided into high-UO group and low-UO group with the optimal cut-off value of 0.39 ml/kg/h. The Kaplan-Meier curves are shown in **Figure 3**. The median survival time of the high-UO group was 42.097 days (95% CI: 37.842–52.060), while of the low-UO group was 14.470 days (95% CI: 12.726–16.674), and the difference was statistically significant ($P < 0.0001$). Compared with the high-UO group, the hazard ratio (HR) of the low-UO group was 2.6857 (95% CI: 2.3955–3.0112). After PSM, the optimal cut-off value was 0.38 ml/kg/h (**Figure 4**). The median survival time of the high-UO group was 23.632 days (95% CI: 21.448–27.116), while of the low-UO group was 11.449 days (95% CI: 9.955–12.926), and the difference was statistically significant ($P < 0.0001$). Compared with the high-UO group, the HR of the low-UO group was 1.7879 (95% CI: 1.5669–2.0401).

DISCUSSION

To the best of our knowledge, this study investigated the relationship between urine output on the first day of admission and in-hospital mortality of intensive care patients with septic shock for the first time. We confirmed that UO is an independent risk factor for septic shock. Before PSM, the optimal cut-off value determined by the ROC curve was 0.39 mg/kg/h. Compared with the high-UO group, the HR of the low-UO group was 2.6857, suggesting that the risk of in-hospital death in the low-UO group was 2.6857 times that of the high-UO group. The optimal cut-off value after PSM was 0.38 mg/kg/h, which was almost the same as before PSM. It was found that the risk of in-hospital death in the low-UO group was 1.7879 times that of the high-UO group. Therefore, the above results indicate that a low level of UO on the first day is significantly associated with an increase in in-hospital mortality.

The PSM is a "post-randomization" statistical analysis method, which reduces the influence of biases and confounding variables on the results in retrospective studies to a certain extent. AKI is the most frequent complication in septic shock and RRT is the standard of care for severe AKI (12). If patients with septic shock have oliguria or anuria on admission, they may progress to AKI at a later stage. A study showed that 3–5 h consecutive oliguria in patients with septic shock may be an indicator to measure the risk of AKI (13). Meanwhile, the most frequent indication for acute dialysis was oliguria (14). In

TABLE 2 | Binomial Logistic regression analysis of urine output for in-hospital mortality among intensive care patients with septic shock (before PSM).

Variable	Univariable		Multivariable	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.014 (1.010–1.018)	0.000	1.009 (1.002–1.016)	0.007
Gender (male)	0.976 (0.855–1.114)	0.720		
LOS hospital	0.976 (0.971–0.982)	0.000	0.966 (0.960–0.973)	0.000
LOS ICU	1.004 (0.996–1.013)	0.282		
CCI	1.161 (1.133–1.188)	0.000	1.143 (1.107–1.181)	0.000
Hemoglobin	0.925 (0.894–0.956)	0.000	0.942 (0.904–0.982)	0.005
WBC	1.005 (0.999–1.010)	0.088	0.998 (0.992–1.004)	0.532
Platelets	0.998 (0.998–0.999)	0.000	1.001 (1.000–1.001)	0.095
Creatinine	1.182 (1.131–1.234)	0.000	0.719 (0.661–0.781)	0.000
BUN	1.015 (1.012–1.017)	0.000	1.009 (1.005–1.014)	0.000
Total bilirubin	1.182 (1.131–1.234)	0.000	1.035 (1.017–1.054)	0.000
Heart rate	1.016 (1.013–1.020)	0.000	1.017 (1.012–1.022)	0.000
MAP	0.967 (0.959–0.975)	0.000	0.988 (0.979–0.998)	0.016
Respiratory rate	1.086 (1.069–1.102)	0.000	1.045 (1.025–1.066)	0.000
SOFA score	1.015 (1.012–1.017)	0.000	1.175 (1.147–1.203)	0.000
Day 1 UO, mg/kg/h	0.285 (0.247–0.330)	0.000	0.507 (0.434–0.593)	0.000
Day 1 AKI	1.747 (1.505–2.027)	0.000	1.030 (0.854–1.240)	0.760
Day 1 diuretic	1.772 (1.483–2.117)	0.000	1.590 (1.284–1.968)	0.000
Day 1 RRT	3.174 (2.667–3.777)	0.000	2.285 (1.779–2.935)	0.000

PSM, Propensity Score Matching; OR, Odds Ratio; CI, Confidence Interval; LOS, Length of Stay; ICU, Intensive Care Unit; CCI, Charlson Comorbidity Index; WBC, White Blood Cells; BUN, Blood Urea Nitrogen; MAP, Mean Arterial Pressure; SOFA, Sequential Organ Failure Assessment; UO, Urine Output; AKI, Acute Kidney Injury; RRT, Renal Replacement Therapy.

TABLE 3 | Binomial Logistic regression analysis of urine output for in-hospital mortality among intensive care patients with septic shock (after PSM).

Variable	Univariable		Multivariable	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.001 (0.995–1.007)	0.858		
Gender, male	1.038 (0.868–1.242)	0.681		
LOS hospital	0.981 (0.974–0.987)	0.000	0.979 (0.972–0.986)	0.000
LOS ICU	0.992 (0.982–1.002)	0.106		
CCI	1.006 (0.974–1.039)	0.715		
Hemoglobin	0.982 (0.939–1.027)	0.434		
WBC	0.999 (0.992–1.006)	0.792		
Platelets	1.001 (1.000–1.001)	0.099	1.002 (1.001–1.002)	0.000
Creatinine	1.005 (0.948–1.066)	0.871		
BUN	1.001 (0.998–1.004)	0.601		
Total bilirubin	0.999 (0.979–1.020)	0.913		
Heart rate	1.000 (0.994–1.005)	0.853		
MAP	0.999 (0.989–1.009)	0.801		
Respiratory Rate	0.993 (0.973–1.014)	0.513		
SOFA score	1.102 (1.078–1.127)	0.000	1.105 (1.079–1.133)	0.000
Day 1 UO, mg/kg/h	0.544 (0.465–0.638)	0.000	0.678 (0.578–0.796)	0.000
Day 1 AKI	1.054 (0.862–1.290)	0.607		
Day 1 diuretic	1.038 (0.818–1.316)	0.762		
Day 1 RRT	0.993 (0.790–1.248)	0.954		

PSM, Propensity Score Matching; OR, Odds Ratio; CI, Confidence Interval; LOS, Length of Stay; ICU, Intensive Care Unit; CCI, Charlson Comorbidity Index; WBC, White Blood Cells; BUN, Blood Urea Nitrogen; MAP, Mean Arterial Pressure; SOFA, Sequential Organ Failure Assessment; UO, Urine Output; AKI, Acute Kidney Injury; RRT, Renal Replacement Therapy.

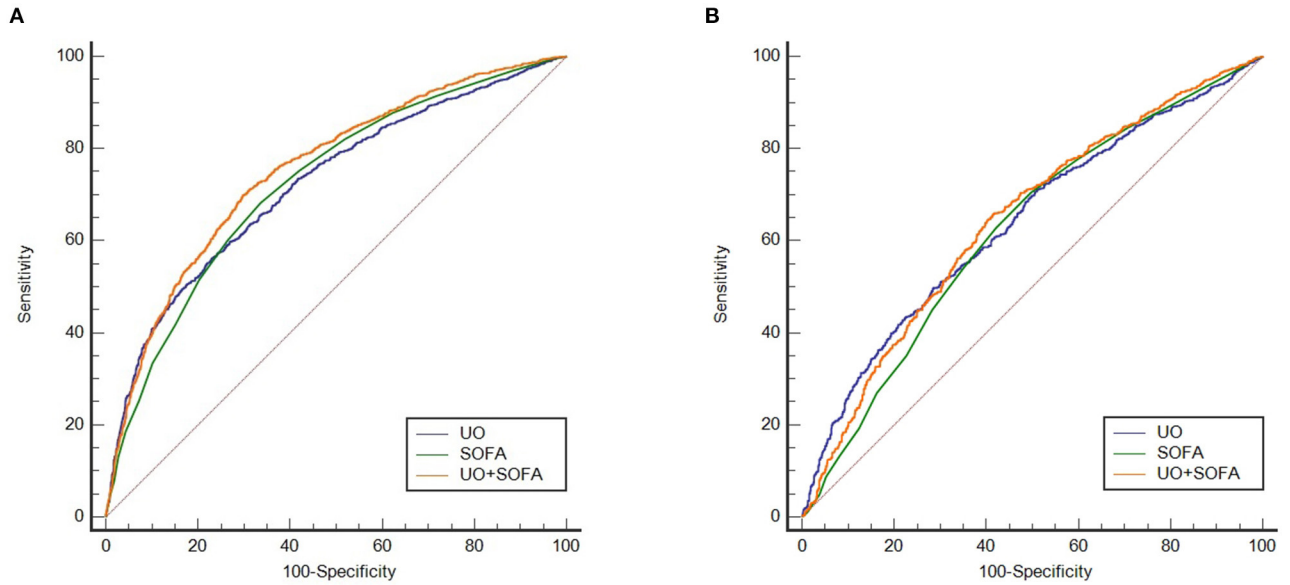

FIGURE 2 | (A) ROC curves of UO, SOFA, UO + SOFA (before propensity score matching); **(B)** ROC curves of UO, SOFA, UO + SOFA (after propensity score matching). UO, Urine Output (mg/kg/h); SOFA, Sequential Organ Failure Assessment.

TABLE 4 | Comparison of ROC curves (before PSM).

Factor	AUC	95%CI	Optimal cut-off	Sensitivity	Specificity	Youden's index
UO	0.722	0.708~0.736	0.39	56.21	76.94	0.3315
SOFA	0.725	0.710~0.739	9	68.18	66.45	0.3462
UO + SOFA	0.753	0.740~0.767	0.34265*	69.81	70.26	0.4007

UO, Urine Output (mg/kg/h); SOFA, Sequential Organ Failure Assessment.
**Prediction probability of logistic regression model for combining UO and SOFA, corresponding to UO = 0.94, SOFA = 11.*

TABLE 5 | Comparison of ROC curves (after PSM).

Factor	AUC	95%CI	Optimal cut-off	Sensitivity	Specificity	Youden's index
UO	0.637	0.615~0.659	0.38	49.64	71.55	0.2118
SOFA	0.622	0.600~0.644	8	70.40	50.36	0.2077
UO + SOFA	0.643	0.621~0.664	0.49034*	65.84	58.36	0.2420

UO, Urine Output (mg/kg/h); SOFA, Sequential Organ Failure Assessment.
**Prediction probability of logistic regression model for combining UO and SOFA, corresponding to UO = 0.58, SOFA = 9.*

general, whether patients are complicated with AKI and whether to undergo RRT or diuretic therapy are significantly related to UO theoretically. We have also balanced some important laboratory tests and vital signs. Among them, white blood cells count is related to infection and is often used to detect sepsis (15); creatinine and BUN can reflect renal function (16); platelets and total bilirubin can reflect coagulation function and liver function (17); respiratory rate is related to respiratory function; hemoglobin not only reflects the presence of anemia, but also is related to oxygenation (18). We were trying to evaluate indicators related to cardiac function, but the missing values of indicators such as troponin and BNP in the MIMIC-IV database are too many, and some even exceed 90%. Imaging data of cardiac function, such as ejection fraction, are currently not available in MIMIC-IV database. Thus, we finally used heart rate and mean arterial pressure as matching parameters to minimize the cardiac function bias between the two groups. After PSM, although the risk of in-hospital death in the low-UO group was lower than

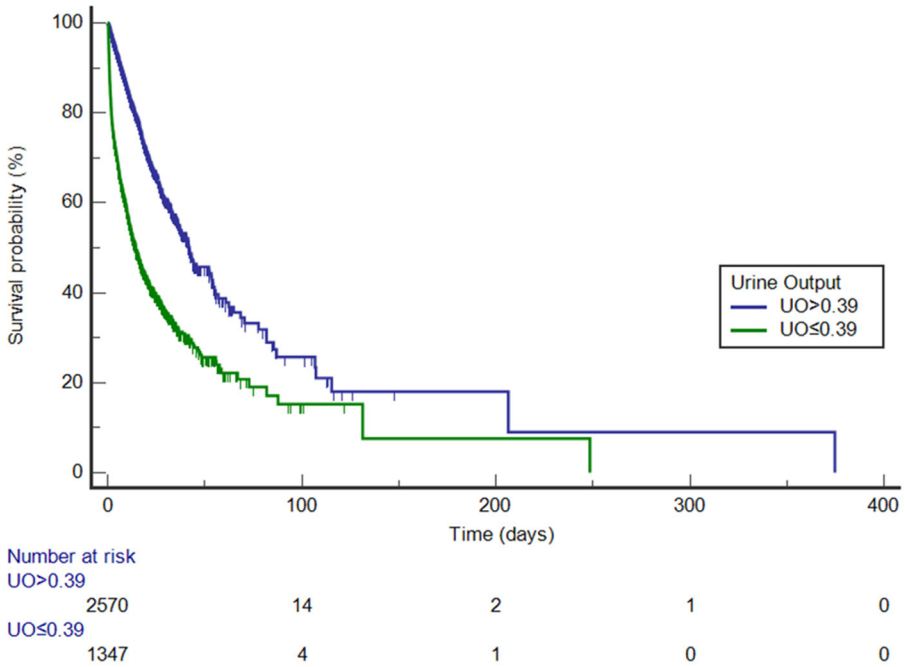

FIGURE 3 | Kaplan-Meier survival curves by urine output category (before propensity score matching, log-rank $P < 0.0001$). UO, Urine Output (mg/kg/h).

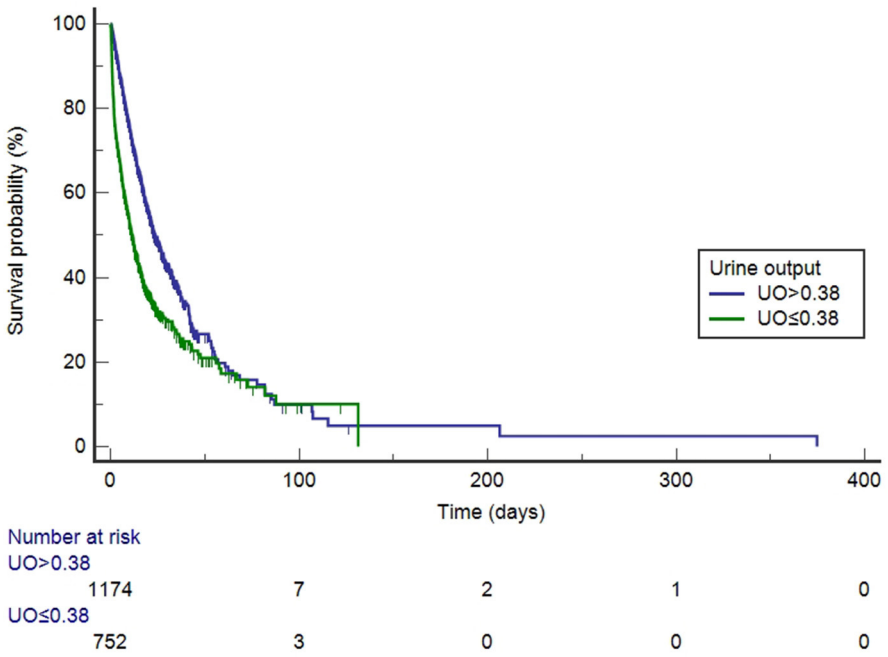

FIGURE 4 | Kaplan-Meier survival curves by urine output category (after propensity score matching, log-rank $P < 0.0001$). UO, Urine Output (mg/kg/h).

before, it still had approximately twice the risk of death (1.7879 times) compared to the high-UO group. It can be seen that the UO on the first day of admission does not depend on whether complicated with AKI and whether to undergo RRT or diuretic therapy, but is directly related to the prognosis of the patients, reflecting the independence of its predictive value.

Septic shock is a complex syndrome with severe hemodynamic changes, manifested by profound cardiovascular

derangements, redistribution of blood flow between organs, and microcirculatory alterations (2). Cardiovascular derangements and the redistribution of blood flow can seriously affect splanchnic circulation, and if the kidney is compromised, it will correspondingly lead to a decrease in UO. Meanwhile, sepsis, especially in severe patients, is almost invariably related to altered coagulation, which can easily lead to disseminated intravascular coagulation (19) and microvascular thrombosis (20). Thrombus formation leads to insufficient tissue perfusion. Since the septic shock is a subtype and severe type of sepsis, which is more prone to progress to coagulation dysfunction and eventually leads to insufficient tissue perfusion, resulting a decrease in UO. In addition, septic shock can cause a vasodilatory state due to excessive NO production, vasopressin deficiency and resistance (21, 22), and further aggravate tissue hypoperfusion. The above reasons are independent of AKI and lead a decrease in UO, therefore, even after adjusting for confounding factors such as AKI, UO is still an independent risk factor for in-hospital mortality in patients with septic shock.

The essence of sepsis and septic shock is organ dysfunction, and the severity of organ dysfunction has been assessed through various scoring systems. Currently, SOFA is the predominant scoring system used for sepsis and septic shock, and it is also one of the definitions of sepsis recognized by Sepsis-3 (1). Several studies have confirmed the value of SOFA in predicting the mortality of septic shock (23–28), but its performance is not satisfactory. In our study, before PSM, the AUC of UO exceeded 0.7, which was of moderate predictive value. After PSM, AUC dropped to 0.637, suggesting that the predictive value was limited. However, the predictive value of UO was always comparable to the SOFA score system. Even so, the application of UO alone in the prediction of in-hospital mortality for septic shock still lacks practical significance. The combination of predictors may improve prediction performance. As the task force of Sepsis-3 pointed out, there are many novel biomarkers that can identify renal dysfunction or coagulopathy earlier than the elements used in SOFA, but they need to be more extensively verified before they are incorporated into the clinical criteria for sepsis (1). In this study, the combined prediction efficiency of UO and SOFA was higher than that of SOFA alone. However, since the respective weights were not given, the corresponding cut-off values of the two were not suitable for predicting in-hospital mortality directly. Thus, it may be a better choice to consider creating a new scoring system, such as incorporating UO into SOFA as a factor, similar to the "UO-corrected SOFA scoring system."

Our findings emphasize the importance of monitoring UO in clinical practice in order to identify high-risk patients with septic shock early and intervene as soon as possible to achieve the goal of reducing in-hospital mortality. UO monitoring is easy to perform and inexpensive, and is especially suitable for promotion in countries with limited resources. We recommend using the weight-corrected UO, namely UO (ml/kg/h). Moreover, we also emphasize the importance of UO in the hemodynamic management of septic shock. Hemodynamic support for patients with septic shock is crucial (29), including the use of large amounts of fluids in combination with vasopressors, and in some cases with inotropic agents. The hemodynamic targets for resuscitation of septic shock often rely on macro-hemodynamic parameters, including heart rate, mean arterial pressure, and central venous pressure. However, despite the restoration of macro-hemodynamic parameters, persistent alterations in microcirculatory blood flow can still lead to organ failure (2, 30, 31). This dissociation between the macrocirculation and microcirculation is the so-called "a loss of hemodynamic coherence" (32). UO reflects renal perfusion and is also an effective indicator of microcirculation perfusion. Thus, monitoring UO may play a positive role in hemodynamic management for septic shock.

We must point out the limitations of this study: (1) The patients in the MIMIC-IV database are mainly white, and a large number of patients cannot be identified by ethnicity. Therefore, the variable ethnicity was not included in the PSM analysis, which has a potential impact on the results; (2) At present, it is difficult to identify the exact sites of infection and causative organisms of the patients in the database. The predictive value of UO for septic shock caused by different reasons or in different sites of infection (such as kidney vs. other sites) may be significantly different; (3) The daily fluid intake (including drinking water) of the patients will also affect the UO, but since the exact values of these variables cannot be obtained, the influence of these confounding factors on the results cannot be ignored. It should be pointed out that a large part of the patients in this study may not have been diagnosed with septic shock at the time of admission. Therefore, our findings may also be applicable to intensive care patients with the potential to develop septic shock. The advantage of this study lies in the large sample size, which allows us to have enough space for the PSM analysis and makes our conclusions more reliable.

CONCLUSIONS

UO is an independent risk factor for septic shock. Low levels of UO significantly increase the in-hospital mortality of intensive care patients with septic shock. The predictive value of UO for patients with septic shock is comparable to the SOFA scoring system, and the combined predictive value of the two surpasses SOFA alone. Since the above results are based on this retrospective study, rigorous prospective clinical trials are still needed to confirm.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation

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

AUTHOR CONTRIBUTIONS

TH and YM conceived and designed the study. TH and ZQ extracted the data, analyzed and interpreted the data, and drafted the work. YM participated in design of the study, assisted with revisions of the manuscript, and takes responsibility for the content of the manuscript including the data and analysis. All authors have approved the final version of the manuscript for submission and agree to be accountable for all aspects of the work.

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Association of Time–Varying Intensity of Ventilation With Mortality in Patients With COVID–19 ARDS: Secondary Analysis of the PRoVENT–COVID Study

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Background: High intensity of ventilation has an association with mortality in patients with acute respiratory failure. It is uncertain whether similar associations exist in patients with acute respiratory distress syndrome (ARDS) patients due to coronavirus disease 2019 (COVID–19). We investigated the association of exposure to different levels of driving pressure (ΔP) and mechanical power (MP) with mortality in these patients.

Methods: PRoVENT–COVID is a national, retrospective observational study, performed at 22 ICUs in the Netherlands, including COVID–19 patients under invasive ventilation for ARDS. Dynamic ΔP and MP were calculated at fixed time points during the first 4 calendar days of ventilation. The primary endpoint was 28–day mortality. To assess the effects of time–varying exposure, Bayesian joint models adjusted for confounders were used.

Results: Of 1,122 patients included in the PRoVENT–COVID study, 734 were eligible for this analysis. In the first 28 days, 29.2% of patients died. A significant increase in the hazard of death was found to be associated with each increment in ΔP (HR 1.04, 95% CrI 1.01–1.07) and in MP (HR 1.12, 95% CrI 1.01–1.36). In sensitivity analyses, cumulative exposure to higher levels of ΔP or MP resulted in increased risks for 28–day mortality.

Conclusion: Cumulative exposure to higher intensities of ventilation in COVID–19 patients with ARDS have an association with increased risk of 28–day mortality. Limiting exposure to high ΔP or MP has the potential to improve survival in these patients.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT04346342.

Keywords: coronavirus disease 2019, acute respiratory distress syndrome, driving pressure, mechanical power, mortality

INTRODUCTION

The coronavirus disease 2019 (COVID−19) is an infection caused by the highly contagious Severe Acute Respiratory Coronavirus−2 (SARS–CoV−2), of which the first outbreak was reported in Wuhan, China in the beginning of December, 2019 (1). Worldwide, healthcare workers are faced with surges of infected patients who need hospitalization and eventually admission to an intensive care unit (ICU) because of need for invasive ventilation. The care of coronavirus disease 2019 (COVID−19) patients receiving ventilation is challenging and the outcomes are poor (2). Mortality rates as high as 50% have been reported in invasively ventilated COVID−19 patients that develop acute respiratory distress syndrome (ARDS) (2).

Different ventilation strategies have been studied in the setting of acute respiratory failure, and evidence from randomized clinical trials supports the use of ventilation with a low tidal volume (V_T) and a low plateau pressure to decrease the risk of ventilator–induced lung injury (VILI) in patients with acute respiratory distress syndrome (ARDS) (3–5). There is increasing interest in limiting exposure to driving pressure (ΔP) and mechanical power (MP) in invasively ventilated patients (6–8). ΔP, the pressure applied by the ventilator to support the delivery of tidal volume, (7) is a surrogate for cyclic lung strain (9). MP, a parameter that combines several ventilatory variables, including respiratory rate (RR), V_T, flow and ΔP, represents the amount of energy transferred from the ventilator to the respiratory system (8, 10). Associations of ΔP and MP with outcomes in patients receiving mechanical ventilation is well described (7, 8, 11–19).

A potential limitation of earlier studies is the inclusion of patients with spontaneous efforts during ventilation (18, 20). Even if appropriately adjusted for resistance, flow, and chest wall elastance, any estimate of these variables during spontaneous efforts would reflect both the ventilator's contribution and respiratory muscle activity, and thus would not represent the total energy imparted during inflation (20). Also, mechanical ventilation is a dynamic process, and thus far only one investigation studied the cumulative effect of ΔP and MP beyond baseline (12). To date, there have been no studies that assessed the impact of intensity of ventilation on outcome of COVID−19 patients with ARDS. To ascertain the effect of time–varying exposure to different levels of ΔP and MP on 28–day mortality in COVID−19 patients with ARDS, we conducted a secondary analysis of a national multicenter investigation, named the "PRactive of VENTilation in COVID−19" (PRoVENT–COVID) study. Our objectives were to estimate the effects of different levels of ΔP and MP over the first 4 days of ventilation on 28–day mortality in COVID−19 patients with ARDS, and whether there was a cumulative effect of exposure over time. We hypothesized that intensity of ventilation has an association with mortality in patients with COVID−19 ARDS, in a similar way as has been described in patients with ARDS from another cause.

METHODS

Study Design and Participants

This is a preplanned secondary analysis of the "Practice of VENTilation in COVID−19" (PRoVENT–COVID) study, an investigator–initiated, multicenter, observational cohort study in patients with COVID−19 ARDS undertaken at 22 ICUs during the first 3 months of the pandemic in the Netherlands (21–23). The protocol of the PRoVENT–COVID study was prepublished, (21) and the statistical analysis plan for the current analysis, finalized before assessing the database, is available online (22). The institutional review boards of each participating center approved the study protocol, and need for individual patient informed consent was waived based on the observational nature of the study. Study sites were recruited through direct contact by members of the steering committee of the PRoVENT–COVID study. Study coordinators contacted the local doctors, trained and helped the data collectors, and monitored the study according to the International Conference on Harmonization Good Clinical Practice–guidelines. Integrity and timely completion of data collection was ensured by the study coordinators.

Consecutive patients aged 18 yr or older were eligible for participation if they were admitted to one of the participating ICUs and had received invasive mechanical ventilation for COVID−19 ARDS. The PRoVENT–COVID study itself had no exclusion criteria—for the current analysis we excluded patients if they had spontaneous breathing activity in more than half the observations, or when life status was unknown at day 28.

Procedures and Outcome

Demographics and data regarding premorbid diseases and home medication were collected at baseline. In the first hour of invasive ventilation, and every 8 h thereafter at fixed time points ventilator settings and parameters were collected up to day 4. Since plateau pressure was not recorded in the current study, all measurements of dynamic ΔP were calculated as peak inspiratory pressure (P_{peak}) minus positive end–expiratory pressure. Dynamic MP was calculated as 0.098 * RR * VT * [P_{peak} – (0.5 × dynamic ΔP)]. Both variables were calculated only considering moments without evidence of spontaneous breathing (additional information in **eMethods** in **Supplementary Information**). The Berlin definition for ARDS was used for classification of severity as mild, moderate and severe (24).

The primary outcome was 28–day mortality. In secondary analyses, we investigated whether the strength of association between intensity of ventilation and 28–day mortality changed over time. In addition, we quantified the effect of cumulative response, and we examined whether ARDS severity class changed the effects of time–varying ΔP and MP on 28–day mortality.

Analysis Plan

For the final assessment, patients receiving ventilation without evidence of spontaneous breathing at less than 50% of the available timepoints were identified and deselected

TABLE 1 | Baseline patient characteristics and outcomes.

	Participants (*n* = 734)
Age, years	65 (57–72)
Male gender–no (%)	540 (73.6)
Body mass index, kg/m^2	27.8 (25.4–31.1)
Transferred under invasive ventilation	109 (14.9)
Days between intubation and admission	0.0 (0.0–0.0)
Use of non–invasive ventilation prior to intubation–no (%)	66/661 (10.0)
Duration of non–invasive ventilation, hours	6 (2–14)
Chest CT scan performed–no (%)	254/715 (35.5)
Lung parenchyma affected–no (%)	
0%	9/254 (3.5)
25%	77/254 (30.3)
50%	80/254 (31.5)
75%	71/254 (28.0)
100%	17/254 (6.7)
Chest X–ray performed–no (%)	397/455 (87.3)
Quadrants affected–no (%)	
1	28/395 (7.1)
2	97/395 (24.6)
3	117/395 (29.6)
4	153/395 (38.7)
Severity of ARDS–no (%)	
Mild	65 (8.9)
Moderate	424 (57.8)
Severe	245 (33.4)
Co–existing disorders–no (%)	
Hypertension	247 (33.7)
Heart failure	27 (3.7)
Diabetes	163 (22.2)
Chronic kidney disease	29 (4.0)
Baseline creatinine, μmol/L*	78 (62–100)
Liver cirrhosis	2 (0.3)
Chronic obstructive pulmonary disease	62 (8.4)
Active hematological neoplasia	9 (1.2)
Active solid neoplasia	21 (2.9)
Neuromuscular disease	3 (0.4)
Immunosuppression	15 (2.0)
Previous medication–no (%)	
Systemic steroids	28 (3.8)
Inhalation steroids	83 (11.3)
Angiotensin converting enzyme inhibitor	126 (17.2)
Angiotensin II receptor blocker	81 (11.0)
Beta–blockers	131 (17.8)
Insulin	46 (6.3)
Metformin	116 (15.8)
Statins	217 (29.6)
Calcium channel blockers	137 (18.7)
Vital signs at day 01	
Heart rate, bpm**	84 (74–98)
Mean arterial pressure, mmHg**	80 (73–88)
Laboratory tests at day 01	
pH**	7.36 (7.30–7.41)
Worst PaO_2/FiO_2, mmHg***	117 (91–154)
$PaCO_2$, mmHg**	45 (39–51)
Lactate mmol/L**	1.2 (0.9–1.5)
Organ support at day 01–no (%)	
Continuous sedation	703/732 (96.0)
Inotropic or vasopressor	574/732 (78.4)
Vasopressor	573/732 (78.3)
Inotropic	37/732 (5.1)
Fluid balance, mL****	637 (77–1445)
Urine output, mL****	675 (360–1116)
Ventilation support at day 01	
Assisted ventilation–no (%)[a]	185/731 (25.3)
Volume controlled	125/731 (17.1)
Pressure controlled	421/731 (57.6)
Pressure support	12/731 (1.6)
Synchronized intermittent mandatory ventilation	68/731 (9.3)
Airway pressure release ventilation	3/731 (0.4)
INTELLiVENT-ASV	12/731 (1.6)
Other	90/731 (12.3)
Tidal volume, mL/kg PBW**[b]	6.3 (5.9–7.0)
Tidal volume ≤ 8 mL/kg PBW	609 (95.9)
PEEP, cmH_2O**[b]	13 (12–15)
Peak pressure, cmH_2O**[b]	27 (24–30)
Driving pressure, cmH_2O**[b]	14 (12–16)
Driving pressure > 15 cmH_2O	206/640 (32.2)
Mechanical power, J/min**[b]	18.9 (15.7–22.8)
Mechanical power > 17 J/min	381/640 (59.5)
Dynamic compliance, mL/cmH_2O**[b]	32 (27–40)
Total respiratory rate, mpm**[b]	22 (20–24)
Set respiratory rate, mpm**[b]	22 (20–24)
Minute ventilation, L/min**[b]	9.5 (8.4–11.0)
FiO_2**	0.60 (0.50–0.70)
$etCO_2$, mmHg**	37 (32–42)
Rescue therapy at day 01–no (%)	
Prone positioning	225/719 (31.3)
Duration, hours	8 (4–12)
Recruitment maneuver	15/590 (2.5)
ECMO	
Use of NMBA	212/731 (29.0)
Hours of use of use	0 (0–8)
Clinical outcome	
28–day mortality	214 (29.2)

Data are median (quartile 25%–quartile 75%) or No (%). Percentages may not total 100 because of rounding. CT, computed tomography; PEEP positive end expiratory pressure; ECMO, extracorporeal membrane oxygenation; FiO_2, inspired fraction of oxygen; PEEP, positive end–expiratory pressure; NMBA, neuromuscular blocking agent.

**Most recent measurement in 24 h before intubation, or at ICU admission under invasive ventilation.*

***Aggregate as the mean of a maximum of four values.*

****Worst value of four available.*

*****Collected in the period after intubation or ICU admission with ventilation until 24:00.*

[a]Assisted ventilation defined as any mode other than pressure or volume controlled. The mode of ventilation reported is the mode used 1 h after intubation.

[b]Only assessed in moments without spontaneous breathing activity.

(**Supplementary Information eMethods**). Continuous variables were reported as median (25th – 75th percentile) and compared with Wilcoxon rank–sum tests, and categorical variables as number and percentage, and compared with Fisher exact tests. The following variables were considered for adjustment in all models described below: age, gender, body mass index, PaO_2 to FiO_2 ratio, plasma creatinine, medical history of hypertension, heart failure, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, active hematological neoplasia and/or active solid tumor, use of angiotensin converting enzyme inhibitors, use of angiotensin II receptor blockers, use of a vasopressor or inotropes, fluid balance, arterial pH, mean arterial pressure, and heart rate. These baseline covariates were selected according to clinical relevance and as used in previous study (23). Finally, all continuous variables were standardized to interpret their effect on outcome in standard deviation units.

To estimate the association of subject–specific longitudinal profiles of either ΔP and MP with 28–day mortality, we used Bayesian joint models with shared random effects and adjusted for the covariates described above (25, 26). The repeated values of ΔP and MP (a maximum of 13 measurements) were included as time–varying exposure variables. Natural cubic splines were used in both the fixed–effects and random–effects models to account for the non-linearity of the longitudinal exposure profiles. To investigate whether the association between ΔP and MP and 28–day mortality changed over time, p–splines were included in an interaction term and presented in time–varying plots. Estimation of Bayesian joint model was done considering 28,000 iterations, 3,000 adapt, 3,000 burn–in, and 50 of thinning. Model diagnostics were done by visual inspection of the diagnostic plots. These results were presented as hazard ratios with corresponding 95% credible intervals (CrI).

To further expand the findings of the original model, 3 additional analyses were performed. First, the model described above was expanded to a multivariate joint model. In addition to the baseline covariates, the following time–varying covariates were included: daily use of prone positioning and daily use of inotropes or vasopressors. Second, the calculation of dynamic ΔP and MP were further restricted to moments when a neuromuscular blocking agent was administered, thereby decreasing the chance of including moments at which a patient was having spontaneous breathing activity. Third, patients with missing data in their 28–day vital status were not excluded but assessed in 2 different scenarios: i. best–case scenario (these patients were all considered alive at day 28); and ii. worst–case scenario (these patients were all considered to have died before or at day 28).

Three sensitivity analyses were added. First, to quantify the effect of cumulative exposure, we estimate the association between the percentage of moments with high ΔP and MP and 28–day mortality. The cut–off used to determine high ΔP was 15 cm H_2O; the cut–off used for high MP was 17 J/min (7, 8, 12, 27). Second, we investigated the relationship between cumulative dose and 28–day mortality using the area under the ΔP and MP time curve above the thresholds described above divided by the number of hours of exposure, as a measure of dose. Using this definition, 1 cm H_2O or 1 J/min of dose describes that a patient's average ΔP and MP were 1 cmH_2O or 1 J/min per mL/cm H_2O above the thresholds described for the duration of the exposure window. Third, we investigated the impact of time–weighted average ΔP and MP calculated as the area under the ΔP and MP time curve divided by the number of hours of exposure. For these 3 exposures, the impact on outcome was assessed using (shared–frailty) Cox proportional hazard models adjusted by the covariates described above.

The models were reassessed in a subgroup analysis according to ARDS severity at baseline (24). The models were repeated, considering an interaction between ΔP and MP and ARDS severity at baseline. The amount of missing data is $< 3\%$ for the variables of interest, as shown in the **Appendix** (**Supplementary Information Table 1**). For the final models, missing data in covariates were imputed by the median due to the low number of missing, and in the repeated measurements of ΔP and MP over the days a linear imputation was used (**Supplementary Information Figure 1**). All analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing), and a $p < 0.05$ was considered significant. *P* values for the Bayesian models were calculated as the tail probabilities using the formula 2 x min{P(θ > 0), P(θ < 0)}, with θ denoting the corresponding regression coefficient from the survival submodel.

RESULTS

From March 1, 2020, through June 1, 2020, 31 ICUs were invited for participation in the PRoVENT–COVID study, and 22 met inclusion criteria. A total of 1,340 individuals were screened. Of the 1,122 invasively ventilated COVID−19 patients, 734 (66.6%) were included in the current analysis (**Supplementary Information Figure 2**). Reasons for exclusion were evidence for presence of spontaneous breathing activity in more than 50% of available timepoints of data collection ($n = 368$), and unknown life status at day 28 ($n = 20$). Demographic characteristics and ventilation characteristics are presented in **Table 1**. The patients had a median age of 65 yr [IQR 57–72] and 194 (26%) of them were women. 8.9% of the patients had mild ARDS, and 57.8% and 33.4% moderate or severe ARDS, respectively. The most prevalent premorbid conditions were hypertension and diabetes. 29.2% of the patients died within the first 28 days of follow–up. Additional clinical outcomes and use of rescue therapies are presented in the **Appendix** (**Supplementary Information Table 2**).

In the first calendar day of ventilation, median ΔP was 14 (13–17) cm H_2O, and median MP was 18.9 (15.7–22.8) J/min (**Table 1**). ΔP was > 15 cm H_2O in 32.2% of the patients, and MP was > 17 J/min in 59.5% in 66.1% of the patients. ΔP and MP over the first 4 calendar days of ventilation are shown in **Figure 1**.

In the first 4 calendar days of ventilation, and after adjusting for confounders, both a high time–varying ΔP (HR, 1.04 [95% CrI, 1.01–1.07]) and a high time–varying MP (HR, 1.12 [95% CrI, 1.01–1.36]) had an association with increased risk of 28–day mortality (**Table 2**). The strength of the association of ΔP as well as of MP with 28–day

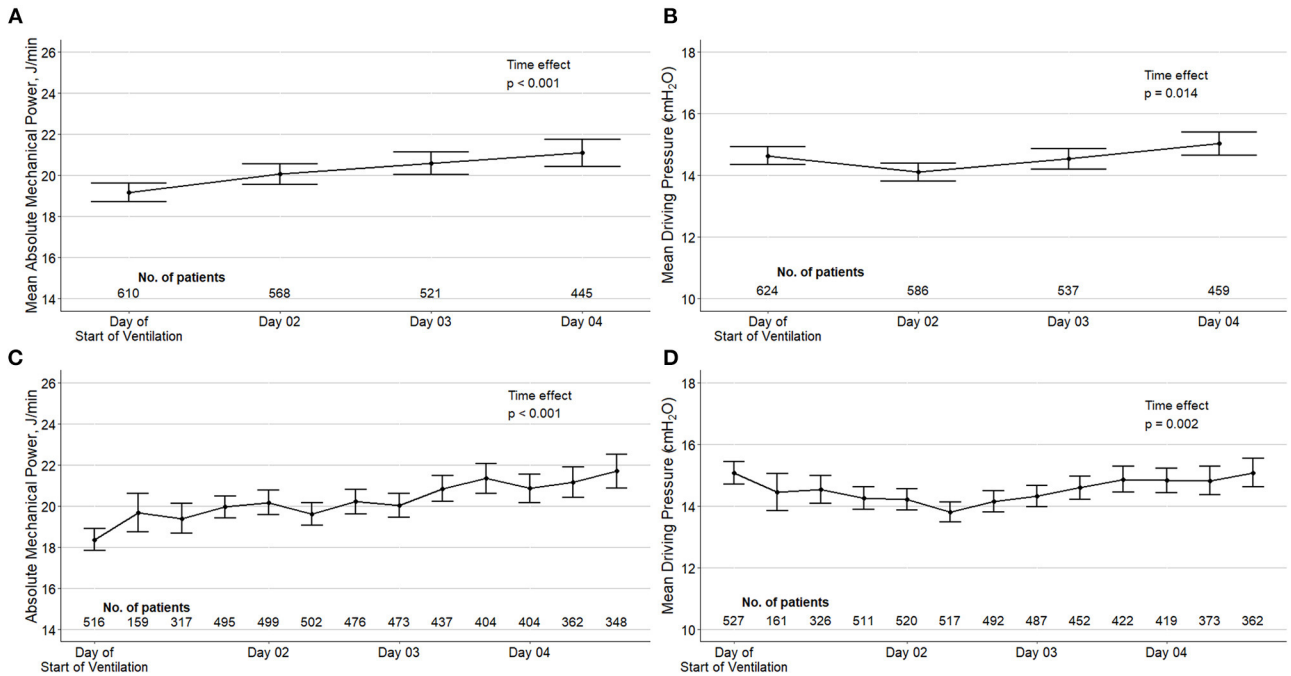

FIGURE 1 | Driving pressure and mechanical power over the first four days of ventilation in the included patients. Top panels **(A, B)**: mean daily values of ΔP and MP according to a maximum of four measurements in the day of start of ventilation and three measurements in the next days. Bottom panels **(C, D)**: ΔP and MP per time point of assessment. Circles are means and error bars are 95% confidence intervals. Both variables were calculated using only measurements without spontaneous breathing activity. *P* values from a mixed-effect model with time as fixed effect (as continuous variable) and patients as random effect to account for repeated measurements.

mortality decreased slightly over the first 4 days (**Figure 2**). As shown in the **Appendix**, the effect of time–varying ΔP was more pronounced in patients with ARDS classified as severe (**Supplementary Information Figure 3**). The full multivariable model is reported in **Supplementary Information Table 3**. After adjustment for the daily use of prone positioning and inotropes or vasopressors, both higher ΔP (HR, 1.04 [95% CrI, 1.00–1.09]) and higher MP (HR, 1.06 [95% CrI, 1.00–1.15]) had an association with increased risk of 28–day mortality (**Table 2**). When restricting the measurements to moment at which neuromuscular blocking agents were administered, both ΔP (HR, 1.38 [95% CrI, 1.14–1.61]) and MP (HR, 1.35 [95% CrI, 1.06–1.73]) had an association with 28–day mortality (**Table 2**). The findings of the best– and worst–case scenario confirmed the findings of the primary analysis (**Table 2**).

The number and percentage of measurements per patient with ΔP > 15 cm H_2O and MP > 17 J/min in the first 4 days of ventilation are shown in the **Appendix** (**Supplementary Information Figure 4**). A higher percentage of measurements with ΔP > 15 cm H_2O (HR, 1.61 [95% CI, 1.03–2.51] $p = 0.037$) or with MP > 17 J/min (HR, 2.42 [95% CI, 1.44–4.07] $p < 0.001$) had an association with increased risk of 28–day mortality (**Figure 3**). Comparison of baseline characteristics and outcomes according to the median ΔP and MP in the first 4 calendar days is shown in **Supplementary Information Table 4**.

Time–weighted average and cumulative dose of ΔP and MP are shown in the **Appendix** (**Supplementary Information Figure 5**). Cumulative dose of ΔP > 15 cm H_2O (HR, 1.09 [95% CI, 1.05–1.14]; $p < 0.001$) and MP > 17 J/min (HR, 1.06 [1.03–1.09]; $p < 0.001$) had an association with increased risk of 28–day mortality (**Figure 3**). In accordance, time–weighted average ΔP (HR, 1.06 [95% CI, 1.03–1.09]; $p < 0.001$) and time–weighted average MP (HR, 1.04 [95% CI, 1.02–1.06]; $p < 0.001$) also had an association with an increased risk on 28–day mortality.

DISCUSSION

The findings of this observational study assessing the impact of time–varying ΔP and MP on 28–day mortality in invasively ventilated COVID−19 patients with ARDS, can be summarized as follows: (1) exposure to both higher ΔP and higher MP during the first 4 calendar days of ventilation had associations with increased risk of 28–day mortality in joint models; and (2) a higher cumulative exposure to ΔP > 15 cm H_2O or MP > 17 J/min over the first 4 days of ventilation had associations with increased risk for 28–day mortality. These findings support the suggestion that limiting the intensity of ventilation in COVID−19 patients with ARDS by using strategies that result in a lower ΔP or MP could improve patient outcome, alike what has been suggested in patients with ARDS from another origin.

TABLE 2 | Multivariable model assessing the association of time-varying driving pressure and mechanical power over the first four days of ventilation with 28-day mortality.

	Time-varying models		Models of cumulative exposure	
	Hazard ratio (95% CrI)	***p* value***	**Hazard ratio (95% CI)**	***p* value**
Time-varying models (main models)				
Driving pressure	1.04 (1.01 to 1.07)	0.010	—	—
Mechanical power	1.12 (1.01 to 1.36)	0.018	—	—
Sensitivity time-varying models				
Multivariate joint model**				
Driving pressure	1.04 (1.00 to 1.09)	0.030	—	—
Mechanical power	1.06 (1.00 to 1.15)	0.049	—	—
Restricted to moments when NMBA was used				
Driving pressure	1.38 (1.14 to 1.61)	<0.001	—	—
Mechanical power	1.35 (1.06 to 1.73)	<0.001	—	—
Best-case scenario***				
Driving pressure	1.03 (1.01 to 1.07)	0.007		
Mechanical power	1.13 (1.02 to 1.32)	0.014		
Worst-case scenario***				
Driving pressure	1.03 (1.00 to 1.07)	0.018		
Mechanical power	1.09 (1.01 to 1.22)	0.014		
Models of cumulative exposure				
Percentage of moments with $\Delta P > 15$ cmH_2O	—	—	1.61 (1.03 to 2.51)	0.037
Percentage of moments with MP > 17 J/min	—	—	2.42 (1.44 to 4.07)	< 0.001
Cumulative dose of $\Delta P > 15$ cmH_2O	—	—	1.09 (1.05 to 1.14)	< 0.001
Cumulative dose of MP > 17 J/min	—	—	1.06 (1.03 to 1.09)	< 0.001
Time-weighted average ΔP	—	—	1.06 (1.03 to 1.09)	< 0.001
Time-weighted average MP	—	—	1.04 (1.02 to 1.06)	< 0.001

CrI, credible interval; CI, confidence interval; NMBA, neuromuscular blocking agents. Hazard ratios were the adjusted hazard ratios associated with a 1-standard deviation increment. Values higher than 1 indicate increased mortality.

**P values calculated as the tail probabilities using the formula 2 x min {$P(\theta > 0)$, $P(\theta < 0)$}, with θ denoting the corresponding regression coefficient from the survival submodel.*

***Expansion of the original joint models further adjusted by the following time-varying covariates: daily use of prone positioning and daily use of inotropes or vasopressor.*

****In the best-case scenario, 20 patients with missing in 28-day mortality were considered alive at day 28. In the worst-case scenario, 20 patients with missing in 28-day mortality were considered dead at day 28.*

The findings of our study are in line with previous investigations of ΔP and MP in patients with ARDS from another cause than COVID−19. Indeed, in patients with ARDS from another origin, a higher ΔP and higher MP at baseline have been shown to have an association with increased mortality (7–10, 12–16). A recent study showed the adverse effect of exposure to higher cumulative doses of ΔP and MP over the duration of ventilation in critically ill patients receiving ventilation due to respiratory failure (12). We also found that a high time–varying ΔP and MP, and a higher cumulative exposure to harmful levels of ΔP and MP have an association with increased 28–day mortality, and as in the previous study these effects were more pronounced in patients with more severe forms of ARDS at baseline.

Considering all the available evidence, monitoring intensity of ventilation seems an attractive approach, both in patients with ARDS due to COVID−19 and in patients with ARDS from another origin. Yet, it remains uncertain whether the found association only reflects the relationship between respiratory system compliance and patient outcome. It is also uncertain whether measures to lower the intensity will result in better outcomes. Nevertheless, our results, corrected for baseline covariates and time–varying confounding, could represent a causal effect. Thus, ventilation strategies aiming to lower the intensity of ventilation need to be studied, preferably in randomized clinical trials.

The present cohort of COVID−19 patients with ARDS is comparable to other COVID−19 cohorts in relation to baseline characteristics and clinical outcomes (28–31). Key variables of ventilation management, like V_T, RR and ΔP were comparable to what was described in previous studies in patients with COVID−19, and in line with recommendations for lung–protective ventilation in patients with ARDS (28–31). MP in our cohort was lower than what was reported in one study in COVID−19 patients, (30) suggesting a better adoption of lung–protective ventilation in our study.

Our study has strengths. We restricted the analysis to patients who were without spontaneous breathing activity for the majority of time, and ΔP and MP was only calculated for the moments a patient was passive. Calculation ΔP and MP in the presence of spontaneous breathing is not yet validated. For the main analyses we used joint models, which allows the examination of the effect of

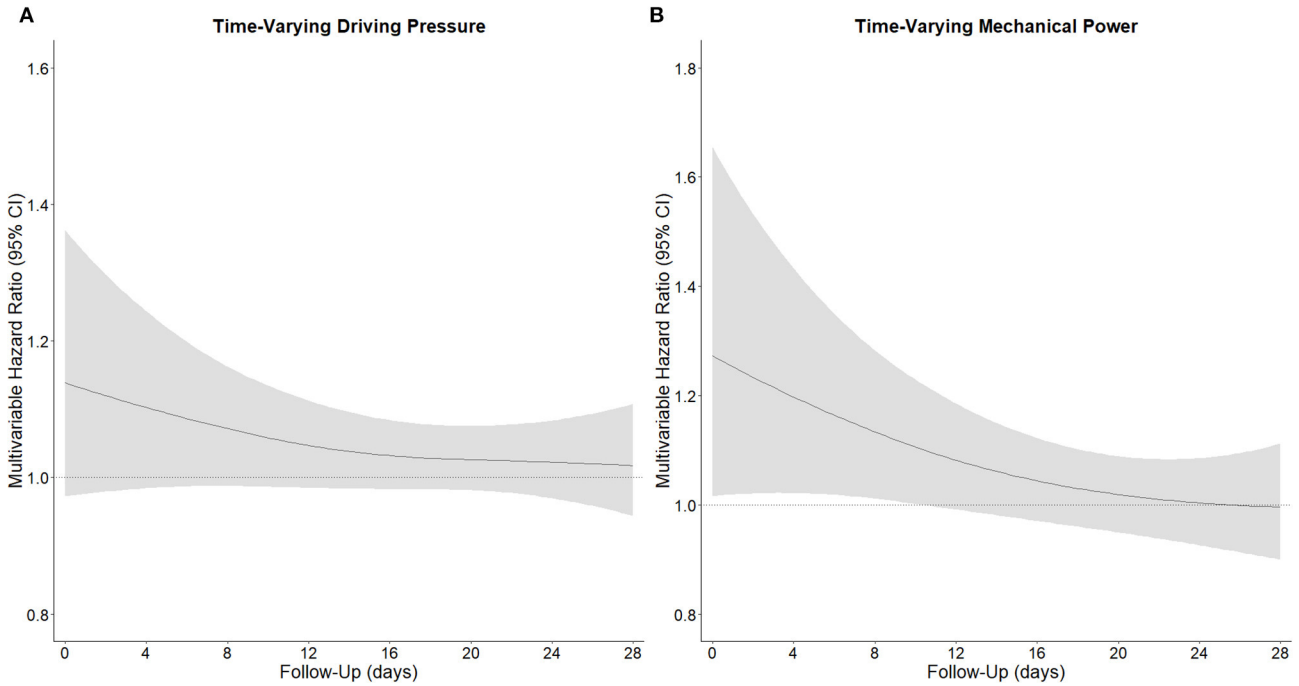

FIGURE 2 | Time-varying hazard ratio of driving pressure and mechanical power for 28-day mortality. **(A)**, Time-varying ΔP, and **(B)** time-varying MP. Time-varying hazard ratio obtained from a Bayesian joint model estimating the association between ΔP and MP and 28-day mortality, including p-splines in an interaction term. The strength of the association decreased over time. All models were adjusted for age, gender, body mass index, PaO_2 to FiO_2 ratio, plasma creatinine, medical history of hypertension, heart failure, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, active hematological neoplasia and/or active solid tumor, use of angiotensin converting enzyme inhibitors, use of angiotensin II receptor blockers, use of a vasopressor or inotropes, fluid balance, arterial pH, mean arterial pressure, and heart rate. Natural cubic splines were used in both the fixed-effects and random-effects models to account for the nonlinearity of the longitudinal exposure profiles.

a time–varying, endogenous covariate on a time–to–event outcome, accounting for non–random dropouts due to death during follow–up.

Our study also has limitations. First, our study was conducted very early in the local outbreak, and anti–inflammatory strategies, like steroids and other treatments such as anti–IL−6, were not yet extensively used. We did not collect data regarding the use of steroids or other anti–inflammatory drugs. Also, we did not collect D–dimer levels and other laboratory tests that may have predictive value, as these were not yet routinely performed so early in the local outbreak. However, the variables used for adjustment are in line with previous studies assessing the impact of ΔP and MP in patients receiving ventilation (12, 14, 15). Our models used several important clinical variables. Of note, we only had measurements of dynamic airway ΔP as plateau or transpulmonary pressures were not routinely connected. Airway ΔP does correlate with transpulmonary ΔP, but it represents a surrogate, which might be affected by other factors. However, recent data suggested that this calculation is reliable (32). Also, we could not adjust for all possible yet unmeasured confounders and due to the observational nature, no causal relationship can be inferred or determined. A high incidence of thromboembolic complications has been found in the present study, which is in line with other reports on COVID−19 patients (33). Pulmonary embolism could cause an increase in dead space, as such affecting the intensity of ventilation. Another limitation is that participating centers did not all use the same disease severity score. Indeed, some centers reported either APACHE II or APACHE IV scores, others used the SAPS II, and some only SOFA scores. These scores cannot be used interchangeably. However, several baseline covariates were used in our models, representing all organ systems, several supportive treatments and pre–existing comorbidities. The mortality rate in our study is lower than that reported in one recent cohort of COVID−19 patients, (2) but higher than in other reports (34, 35). It remains to be determined whether the associations found here are also present in cohorts with other mortality rates. In addition, the models used had some limitations. All baseline covariates were assumed to be measured without error. The joint models assume the correct specification of random effect structure, and all interdependencies between longitudinal and time–to–event outcomes should be explained by the latent, subject–specific random effects structure. Also, there is a risk of residual confounding.

CONCLUSIONS

In conclusion, in this cohort of COVID−19 patients with ARDS, exposure to higher ΔP or MP, or to a higher cumulative exposure

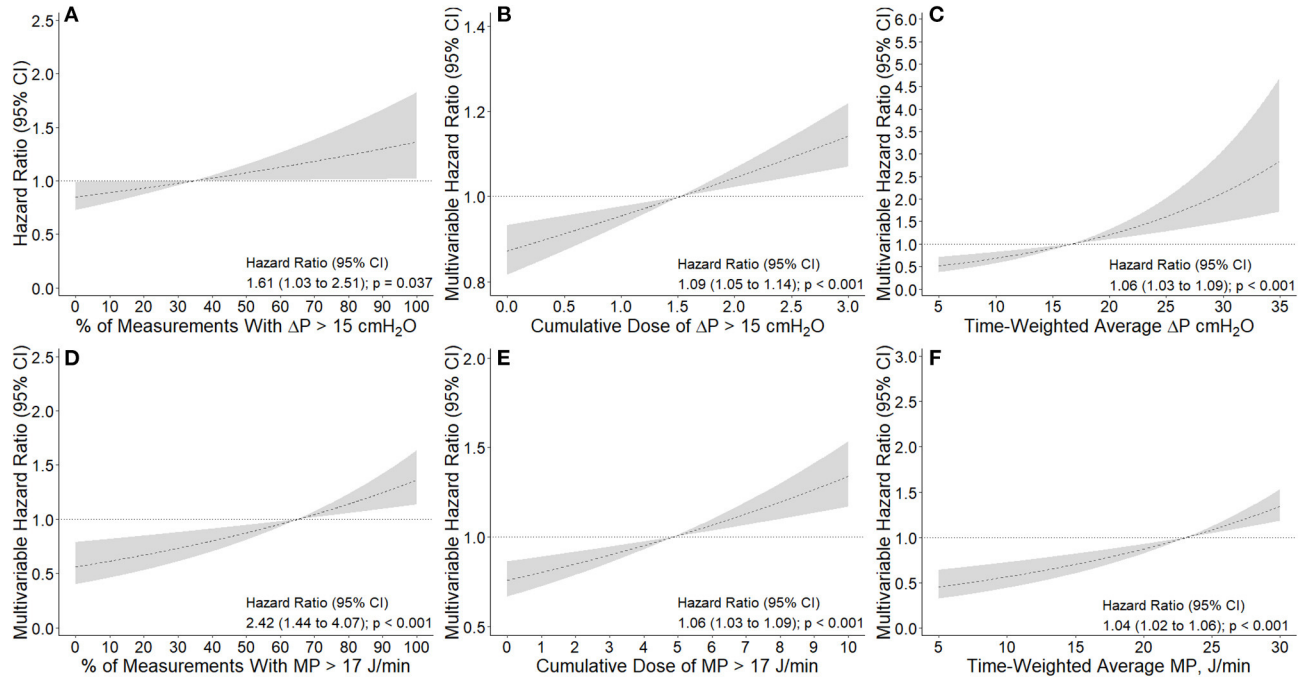

FIGURE 3 | Association between intensity of exposure to higher driving pressure and mechanical power and 28-day mortality. **(A, D):** Association between percentage of measurements with $\Delta P > 15$ cmH_2O and MP > 17 J/min and 28-day mortality. Percentage calculated from a maximum of 13 measurements extracted every 8 h. **(B, E):** Association between cumulative dose of $\Delta P > 15$ cmH_2O and MP > 17 J/min and 28-day mortality. Cumulative dose calculated as area under the ΔP and MP time curve above the thresholds described above divided by the number of hours of exposure, as a measure of dose. Using this definition, 1 cmH_2O or 1 J/min of dose describes that a patient's average ΔP or MP was 1 cmH_2O or 1 J/min above the thresholds described for the duration of the exposure window, respectively. **(C,F)** Association between time-weighted average ΔP and MP and 28-day mortality. Time-weighted average calculated as the area under the ΔP and MP time curve divided by the number of hours of exposure. All models were adjusted for age, gender, body mass index, PaO_2 to FiO_2 ratio, plasma creatinine, medical history of hypertension, heart failure, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, active hematological neoplasia and/or active solid tumor, use of angiotensin converting enzyme inhibitors, use of angiotensin II receptor blockers, use of a vasopressor or inotropes, fluid balance, arterial pH, mean arterial pressure, and heart rate. Dashed lines and gray areas represent hazard ratio and 95% confidence interval for increasing values the variable analyzed as a continuous variable and centralized in the mean of each variable. *ΔP is driving pressure and MP is mechanical power*.

to a $\Delta P > 15$ cm H_2O or a MP > 17 J/min over the first 4 calendar days of ventilation had associations with increased risk for 28–day mortality. To ascertain a causal relationship between ΔP and MP with mortality, randomized clinical trials are needed.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review boards of each participating center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

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AUTHOR CONTRIBUTIONS

MiS and AS had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. MiS, MaS, GM, FP, and AS concept and design. All authors contributed to acquisition, analysis or interpretation of data, drafting the manuscript, and critical revision of the manuscript for important intellectual content. MiS and AS statistical analysis. FP and MaS obtained funding. MaS, FP, and AS supervision. All authors contributed to acquisition, analysis or interpretation of data, drafting the manuscript, and critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.725265/full#supplementary-material

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BRIEF RESEARCH REPORT
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Extracorporeal Cytokine Removal in Critically Ill COVID-19 Patients: A Case Series

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Introduction: Extracorporeal hemoadsorption (HA) is a potential adjunctive therapy in severe cases of COVID-19 associated pneumonia. In this retrospective study we report data from critically ill patients treated with HA during the first and second wave of the pandemic.

Patients and Methods: All patients, who received HA therapy with CytoSorb within the first 96 h of intensive care unit (ICU) admission without hospital-acquired bacterial superinfection, were included. Clinical and laboratory data were collected: on admission, before (T_B) and after (T_A) HA therapy.

Results: Out of the 367 COVID-19 cases, 13 patients were treated with CytoSorb, also requiring mechanical ventilation and renal replacement therapy. All patients were alive at the end of HA, but only 3 survived hospital stay. From T_B-T_A there was a tendency of decreasing norepinephrine requirement: 193.7 [IQR: 34.8–270.4] to 50.2 [6.5–243.5] ug/kg/day and increasing PaO2/FiO2 ratio 127.8 (95% CI: 96.0–159.6) to 155.0 (115.3–194.6) mmHg but they did not reach statistical significance ($p = 0.14$ and 0.58, respectively). Treatment related adverse events were not reported.

Conclusion: The treatment was well-tolerated, and there was a tendency toward an improvement in vasopressor need and oxygenation during the course of HA. These observations render the need for prospective randomized trials.

Keywords: ARDS, cytokine storm, CRRT, COVID-19, hemoadsorption, Cytosorb

INTRODUCTION

Treating critically ill Coronavirus Disease 19 (COVID-19) patients has become the most significant challenge intensive care has ever faced. The vast number of patients requiring care simultaneously, and the novelty and unpredictability of the virus created an unprecedented environment for all involved. Hospitals were overwhelmed by the continuous influx of severely unwell patients, and

intensive care units (ICUs) expanded beyond their original footprint. Critical care mortality of COVID-19 patients significantly exceeded the mortality of any other viral pneumonia (37.9 vs. 22.0%) (1).

Due to the early introduction of lockdown during the first wave of the pandemic, Hungary experienced significantly less pressure on its healthcare system compared to most of the Western European countries. However, it was hit very hard by the next consecutive waves during the autumn and winter of 2020–2021, which resulted in one of the, if not the highest rates of COVID-19 mortality per capita in the world (304.33 deaths/100,000) (2).

Early reports of observations indicated that cytokines might play a considerable role in the development of severe COVID-19. Patients who required critical care admission had higher levels of cytokines. Furthermore, tumour necrosis factor-alfa (TNFa) levels correlated with disease severity, and high interleukin 6 (IL-6), C-reactive protein (CRP), D-Dimer and ferritin levels were found to be predictors of mortality (3–5).

There is some evidence that with extracorporeal cytokine adsorption, substantial IL-6 removal is achievable in severely ill patients with septic shock, acute respiratory distress syndrome (ARDS), and multi-organ failure (6). In a Hungarian proof of concept randomised, controlled pilot study, a significant reduction in vasopressor need and serum pro-calcitonin levels were found (7). Moreover, in a retrospective study of patients with septic shock, cytokine removal was associated with a decreased observed vs. expected 28-day all-cause mortality (8).

In our tertiary intensive care unit (ICU) we have also used hemoadsorption (HA) as an adjuvant therapy in selected patients with septic shock since 2016. During the COVID-19 pandemic our institute served as one of the biggest referral centres in the country, with 120 dedicated COVID-19 ICU beds during the peak of the pandemic (un-published data). As the situation proved desperate, we also reached out for adjunctive therapies such as cytokine adsorption in the most severely ill COVID-19 patients.

The aim of the current retrospective study is to summarise our experience with extracorporeal cytokine adsorption in critically ill COVID-19 patients admitted to our ICU during the first and second wave of the pandemic.

PATIENTS AND METHODS

For this retrospective case-series we screened the records of all patients admitted to the ICU of the Department of Anaesthesia and Intensive Care Medicine at Fejér County St. György University Teaching Hospital, Székesfehérvár, Hungary, between March 1st 2020 and January 31st, 2021. Approval from the local ethics committee was obtained (No: 18/2021.05.11.). Due to the retrospective and anonymized data collection process, patients' informed consent was not deemed necessary.

We identified patients, who had severe, life-threatening COVID-19 confirmed with either polymerase chain reaction (PCR) or rapid antigen testing.

All patients had severe respiratory failure requiring mechanical ventilation and received standard intensive monitoring and therapy based on international guidelines and recommendations.

All patients, who received HA therapy within the first 96 h of ICU admission were considered eligible for the analysis. Hemadsorption treatment was applied as an additional adsorbent cartridge (CytoSorb/CytoSorbents Europe GmbH, Berlin, Germany) integrated into a continuous renal replacement (CRRT) circuit. Patients who received HA more, than up to a maximum of 96 h after ICU admission were presumed to have septic shock due to hospital acquired secondary infections, hence they were excluded. Those, who died within 48 h of the application of the first HA were excluded.

The indication to start CRRT was based on our local protocol: CRRT was commenced in patients with acute renal injury (AKI) stage II according to Kidney Disease Improving Global Outcomes (KDIGO) criteria or severe refractory fluid overload, furthermore in patients without renal indication in order to facilitate HA therapy in hemoperfusion mode only (9).

Executing CRRT with or without HA was at the discretion of the attending senior intensive care physician. Nevertheless, by-and-large the following indications were taken into account: suspicion of hyper-inflammatory state based on elevated inflammatory markers such as CRP and granulocyte count combined with considerable hemodynamic instability necessitating increasing doses of catecholamines and/or severe ARDS defined by the Berlin definition and/or multiple organ failure (10).

Integration of the adsorber into the CRRT circuit (Prismaflex System/Baxter International Inc., Deerfield, IL, USA) was handled by trained intensive care physicians following the manufacturer's instructions. The cartridge was placed in pre-dialyzer position. CRRT was performed in continuous hemodiafiltration mode (CVVHDF), as our standard practise, at a blood flow rate of 100–250 ml/min with systemic unfractionated heparin or regional citrate anticoagulant as recommended by the KDIGO 2012 recommendations (11). Based on the attending physician's decision the cartridges were changed every 12 or 24 h. According to our standard operating procedures, hemoadsorption was discontinued in cases of clinical improvement as indicated by a reduction in the required catecholamine dose, increase in the PaO2/FiO2 ratio, or due to deterioration in the patient's overall condition or no improvement after 2 treatment sessions.

Data collection was undertaken retrospectively by review of our electronic medical records. We gathered pertinent information on demographic data and past medical his-tory. For risk stratification the 4C and sequential organ failure assessment scores (SOFA) were calculated. Relevant clinical data were assessed at three different time points: (1) on admission, (2) at the onset of the adsorbent therapy ("before," TB), and 3) after the completion of adsorbent therapy ("after," TA). We measured inflammatory markers such as leukocyte and granulocyte count, CRP, procalcitonin (PCT), hemodynamic parameters such as catecholamine requirement, serum lactate levels; respiratory function as indicated by the PaO2/FiO2 ratio, PaCO2, and

renal function assessed by blood urea nitrogen, creatinine and glomerular filtration rate. We analysed the association between the use of HA and changes in SOFA-score, in the PaO2/FiO2 ratio and in catecholamine dose, as well as days on mechanical ventilation, ICU length-of-stay and 28-day mortality.

Statistical Analysis

Data were collected in a preformatted, anonymized table, which had been used exclusively for all further analyses. All calculations were undertaken by means of descriptive statistics. Continuous variables were expressed as mean (confidence interval, CI) or median [interquartile range, IQR], as appropriate. Statistical analyses and graphs were performed with STATA 15 software using parametric and non-parametric methods for mean and median comparisons as appropriate.

RESULTS

Baseline Characteristics

Out of the 367 patients treated with COVID-19 in the ICU, invasive ventilation was necessary in 153 cases. Thirty-Seven patients were on CRRT, and 13 patients were included the current case series suffering from COVID-19 viral pneumonia, who received hemoadsorption therapy with CytoSorb. Three patients were admitted from a COVID-19 medical ward and one patient was transferred from another hospital's ICU. All four patients were admitted to our ICU within 24 h after the onset of symptoms, hence hospital acquired infection was highly unlikely.

Baseline characteristics, comorbidities and on-admission laboratory values of the included patients are presented in **Table 1**. All patients but one were male, with a mean age of 57 (±11) years. According to the 4C prognostic mortality score most patients (61.6%) had at high or very high risk on admission. Nine patients (69.2%) suffered from some sort of comorbidity, including hypertension, diabetes, or acute myocardial infarction. Regarding on-admission laboratory parameters, as indicated by the average values, granulocyte/lymphocyte ratio, CRP, lactate dehydrogenase, creatinine kinase, glutamic oxaloacetic transaminase, and gamma-glutamyl transferase, were elevated. The applied antiviral and adjunctive therapies are detailed in **Table 1**.

The mean time between the onset of symptoms and admission to the Emergency Department was 5.9 (95% CI: 4.11–7.55) days. The diagnosis of SARS-CoV2 infection was confirmed by PCR in ten and antigen testing in three cases. Two patients had chest computer tomography and eleven X-Ray, and except for one patient, bilateral pathology was observed. Nine patients were admitted from the Emergency Department to the ICU on the day of admission, three were transferred from the department of COVID-Internal Medicine and one from the ICU of another town.

TABLE 1 | Baseline characteristics.

	Patients (n = 13)
Age, years	62 [48–67]
Male	12 (92.3%)
Charlson comorbidity index	2.77 (1.1–4.4)
4C Mortality score for COVID-19	10.4 (7.6–13.2)
Low risk	1 (7.7%)
Intermediate risk	4 (30.8%)
High risk	5 (38.5%)
Very high risk	3 (23.1%)
Onset of symptoms before admission, days	5.8 (3.8–7.8)
Confirmed by COVID-19 antigen test	3 (23.1%)
Confirmed by PCR	10 (76.9%)
Comorbidities	
Hypertension	9 (69.2%)
Diabetes	6 (46.2%)
Gastroesophageal reflux disease	1 (7.7%)
Acute myocardial infarction	2 (15.4%)
Benign prostatic hyperplasia	2 (15.4%)
Other	6 (46.2%)
No comorbidities	4 (30.8%)
Laboratory values on admission	
Leukocytes, $\times 10^3/\mu L$	8.9 (6.6–11.2)
Lymphocytes, $\times 10^3/\mu L$	0.7 [0.58–1.04]
Granulocytes, $\times 10^3/\mu L$	7.4 (5.3–9.6)
Granulocyte/lymphocyte ratio	10.0 (6.9–13.1)*
C-reactive protein, mg/L	203 (116–290)*
Glutamic oxaloacetic transaminase, U/L	96 [42–113]*
Glutamate pyruvate transaminase, U/L	75 (40–109)*
Gamma-glutamyl transferase, U/L	131.1 (66.3–195.9)*
Alkaline phosphatase, U/L	100.1 (68.3–131.9)
Lactate dehydrogenase, U/L	1,164.8 (780.9–1,548.8)*
Blood urea nitrogen, mmol/L	6.4 [4.8–13.3]
Creatine kinase, U/L	160 [126–489]*
Initial antiviral therapy	**11 (84.6%)**
Oseltamivir	4 (30.8%)
Favipiravir	6 (46.2%)
Lopinavir	1 (7.7%)
Adjunctive therapy	
Dexamethasone	2 (15.4%)
Hydrocortisone	4 (30.8%)
Methyl-prednisolone	4 (30.8%)
Tocilizumab	4 (30.8%)
Anti-COVID-19 convalescent plasma	5 (38.5%)

*Data are presented as mean (95% CI), median [IQR], or n (%), unless otherwise specified. PCR, polymerase chain reaction. U/L, unit/litre; ICU, intensive care unit. *represents elevated values.*

Treatment Characteristics

In all cases, hemoadsorption was combined with CRRT. On average 1.6 days elapsed from admission to the application of the first adsorber. Regarding the number of treatments, 1 patient received only one treatment, 5 received two, 6 received three treatments and in 1 case hemoadsorption was repeated for four consecutive sessions.

All patients required mechanical ventilation due to acute hypoxemic respiratory failure. Vasopressor was also required in all patients. In the first 24 h the total dose of norepinephrine was

193.7 ug/kg/day [34.8–270.4] which decreased to 50.2 [6.5–243.5] ug/kg/day, by the end of the course of hemoadsorption, but did not reach statistical significance (**Figure 1A**). There was a similar tendency of an improvement in the PaO2/FiO2 ratio during the course of hemoadsorption from 127.8 (96.0–159.6) to 155.0 (115.3–194.6) mmHg (**Figure 1B**). Apart from the leukocyte count, all other recorded laboratory parameters and the SOFA score showed statistically non-significant changes from before to after the therapy (**Table 2**).

Fluid management and inotropic support are summarised in **Table 3**. The only significant change was a negative fluid balance (TB vs. TA) on the completion of hemoadsorption compared to the onset of implication of the first HA.

Overall Outcomes

All patients survived the course of hemoadsorption and were still alive 72 h after initiation of the treatment, hence therapy was not terminated due to deterioration and also no adverse

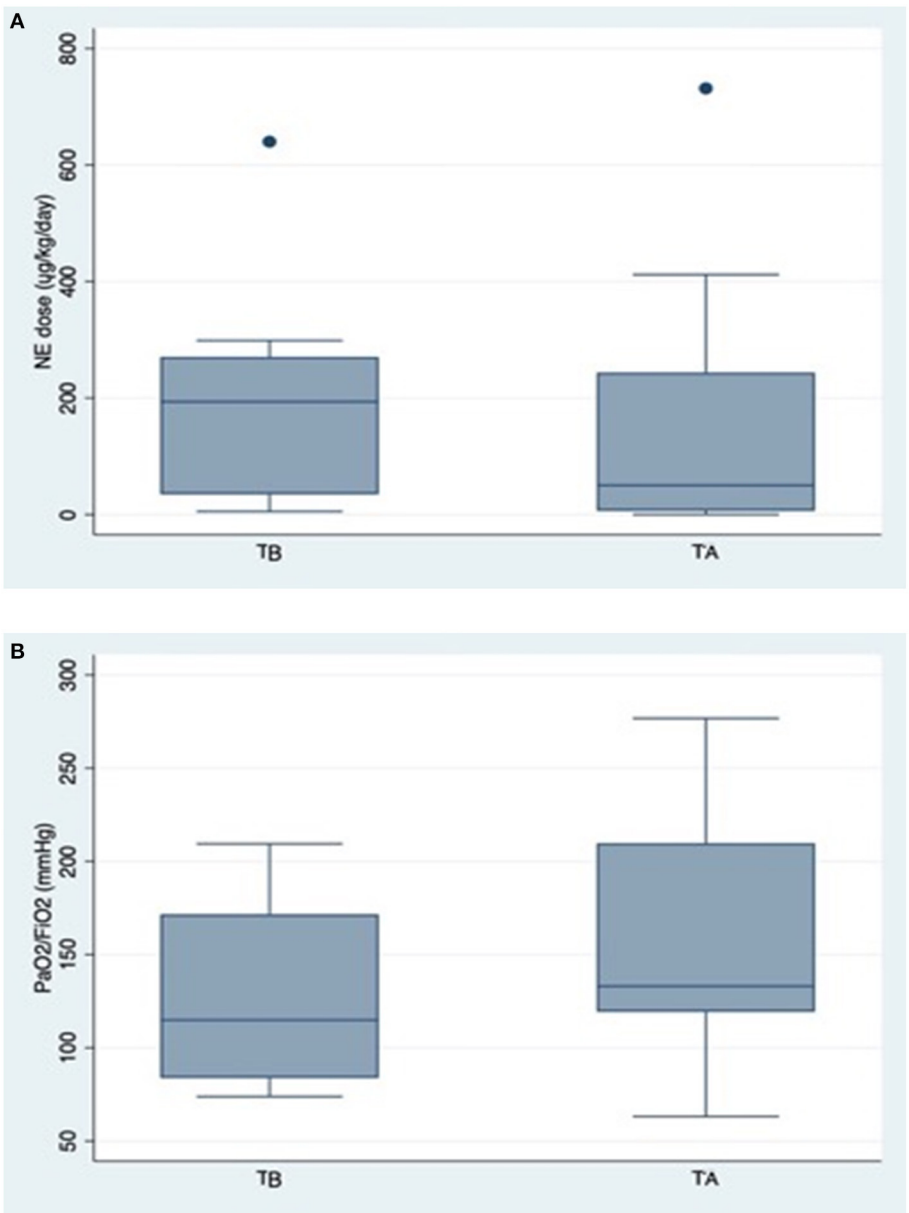

FIGURE 1 | (A) Change in norepinephrine requirement during hemoadsorption. NE, Norepinephrine; TB, data collected before the start of hemoadsorption; TA, data collected after the completion of hemoadsorption. Data are presented as box-plots. For statistical analysis Stata 15.1 was used. For explanation see main text. **(B)** Change in the PaO2/FiO2 ratio during hemoadsorption. TB, data collected before the start of hemoadsorption; TA, data collected after the completion of hemoadsorption. Data are presented as box-plots. For statistical analysis Stata 15.1 was used. For explanation see main text.

TABLE 2 | Before and after comparisons for all patients.

Laboratory values	T_B	T_A	*p*-value
Leukocytes, $\times 10^3/\mu L$	8.5 (6.5–10.4)	13.0 (9.0–16.9)	>0.01
Haemoglobin, g/L	119 (108–129)	115 (105–126)	0.33
Platelets, $\times 10^3/\mu L$	190 (140–241)	178 (130–225)	0.52
Creatinine, umol/L	109 [86–264]	104 [67–177]	0.05
Blood urea nitrogen, mmol/L	11.2 (6.9–15.6)	8.4 (5.7–11.2)	0.08
Glomerular filtration rate, mL/min/1.73 m^2	55.3 (34.1–76.5)	57.5 (38.4–76.6)	0.58
C-reactive protein, mg/L	266.1 (142.0–390.2)	206.4 (129.0–283.8)	0.28
Procalcitonin, ng/mL ($n = 5$)	0.64 [0.20–2.85]	0.49 [0.49–0.62]	0.59
Bilirubin, mmol/L ($n = 3$)	11.0 [10.0–333.0]	106.0 [30.0–182.0]	0.32
Arterial blood gases			
pH	7.35 (7.33–7.39)	7.31 (7.23–7.38)	0.28
PaO2, mm Hg	109 (82–135)	99 (85–112)	0.50
PaCO2, mm Hg	43 (37–48)	46 (37–55)	0.47
Plasma bicarbonate, mmol/L	23.3 (20.7–25.8)	22.6 (19.7–25.5)	0.65
Arterial lactate, mmol/L	1.68 [1.23–2.27]	1.58 [1.33–2.25]	0.74
SOFA score	15.0 [15.0–17.5]	14.5 [14.0–16.5]	0.03

Data are expressed as mean (95% CI) or median [IQR], unless otherwise specified. HA, hemoadsorption. n = number of patients when <13. PaO2, Partial pressure of oxygen. PaCO2, Partial pressure of carbon dioxide. PaO2/FiO2, Ratio of the partial pressure of oxygen and the fraction of inspired oxygen. SOFA, Sequential organ failure assessment.

TABLE 3 | Summary of daily changes in fluid balance and inotropic support requirement.

Fluid balance	T_B	T_A	*p*-value
Intake fluids per orally, ml	335 (141–529)	554 (150–958)	0.24
Intake fluids intravenously, ml	3,760 (3143–4377)	3,386 (2875–3897)	0.31
Spontaneous urinary output, ml	1,600 [750–2,300]	1,200 [0–3,850]	0.32
Other fluid loss, ml	200 [0–400]	0 [0–200]	0.24
Remove by continuous renal replacement, ml	0 [0–300]	953 [300–2,400]	0.20
Daily fluid balance, ml	1,947 (1,053–2,840)	−322 (−1,388–745)	>0.01
Vasopressor requirement			
Norepinephrine, μg/kg/day	193.7 [34.8–270.4]	50.2 [6.5–243.5]	0.48
Dobutamine, μg/kg/day ($n = 4$)	0.0 [0.0–1,005.7]	0.0 [0.0–5,437.5]	0.03

Data are expressed as mean (95% CI) or median [IQR], unless otherwise specified. HA, hemoadsorption.

events were detected. Four patients survived 28-days, but overall, only 3 patients (23.1%) were discharged from the hospital alive. ICU length of stay was 14 [5–30] days while patients spent a median 17 [6–30] days in the hospital in total.

DISCUSSION

To the best of our knowledge, this is the first and largest comprehensive case series on COVID-19 patients treated with hemoadsorption from Eastern Europe—i.e.: from the former socialist block. Although the results showed some improvement in both haemodynamics and oxygenation, likely due to small sample size statistical significance was not achieved. Nevertheless, all patients were alive when treatment was decided to be terminated by the attending medical team, and treatment-related adverse events were not re-ported. Death occurred due to multiple organ failure at least 2 weeks later in most cases, suggesting that hemoadsorption started within 96 h after admission to ICU was well-tolerated and helped to stabilise and overcome the initial critical phase.

The purpose of hemoadsorption in the critically ill in general is to remove excessive inflammatory mediators and by doing so, to attenuate the host immune response. Whether a dysregulated immune response is present or not in COVID-19 patients at all, hence whether hemoadsorption is indicated or not, remains a controversial topic despite extensive publications in the field over the last year. There are several reports indicating elevated cytokine levels in the critically ill forms of COVID-19, some even observed the presence of cytokine storm (12, 13). However, there are also reports contradicting this hypothesis (14, 15). Nevertheless, as the clinical phenotype of COVID-19 is highly variable, ranging from asymptomatic cases to multi organ failure, one would expect a similar variability for the immune response (16). In recent correspondence by Rieder et al. on 8 patients with severe COVID-19 requiring veno-venous-extracorporeal membrane oxygenation (the CYCOV study), patients who received extracorporeal cytokine adsorption had lower IL-6 levels after 72 h of treatment compared to patients without cytokine adsorption (17). However, after completion of the study the difference was not significant (18).

In our patients there are some signals of increased inflammatory activity as indicated by the elevated granulocyte/lymphocyte ratio, and CRP and PCT levels, but these are certainly less pronounced as seen in septic shock or severe ARDS (14). Regardless of the measured inflammatory biomarker levels, the clinical picture of hemodynamic instability requiring vasopressor support, severe ARDS and renal failure indicates the presence of a dysregulated immune response of some sort. Therefore, which biomarkers are the most appropriate to be measured in COVID-19 patients remains to be elucidated (19).

Regarding the clinical effects of hemoadsorption with CytoSorb, there are several, consistent reports of hemodynamic stabilisation before and after the therapy from several fields of critical care including cardiac surgery, septic shock and also recent reports in COVID-19 patients (7, 20, 21). However, the amount of published data on hemoadsorption in COVID-19 patients remains very limited. The largest patient cohort on COVID-19 patients treated with hemoadsorption to date, was published by Alharthy et al. (22). Fifty patients with life threatening COVID-19 and acute kidney injury were treated with CytoSorb. Before and after treatment laboratory and clinical values were compared in the 35 survivors to 15 non-survivors. Most organ functions, including vasopressor need, PaO2/FiO2, and inflammatory biomarker levels improved significantly amongst survivors, while al-most all of these

deteriorated in non-survivors. Due to the limited number of patients we could not compare the 10 non-survivors to the 3 survivors in our study. Nevertheless, the tendency was also similar in our cases, although the improvement did not reach statistical significance. This can be explained on the one hand by the small sample size, on the other hand there is some data suggesting the starting HA within 24 h as compared to the average 1.6 days found in our study, could have more profound effects (23).

Our patient cohort is similar to that presented in previous studies, as far as age, comorbidities and treatment modalities are concerned. However, the observed mortality was substantially higher (77%) than in other studies (22, 24, 25), apart from one recent randomised trial in patients requiring extracorporeal membrane oxygenation (ECMO) due to COVID-19 caused respiratory failure (18). In this study, Supady et al. (18) report a striking difference in 28-day survival of 18% in the cytokine adsorption treated vs. 76% in the controls. However, this was a small (17 vs. 17 patients) single centre study. There were also some baseline differences between the groups as far as baseline norepinephrine requirement, D-Dimer levels, and fluid balance were concerned, hence firm conclusions from this study cannot be drawn at present. Finally, this trial included patients on ECMO, which is different from our patient cohort. One most note, that the average 4C mortality score was 10.4, which indicates high risk and corresponds to 31.4–34.9% in-hospital mortality. Five patients were in the high risk and three in the very high risk category. However, these patients all required mechanical ventilation, therefore were of a selected subpopulation with even more severe conditions (26).

However, an important fact is that mortality rate during the 2nd and especially during the 3rd wave of COVID-19 was a lot higher in Eastern Europe in general than in West-ern Europe or in the United States (27–30). Unfortunately, we do not have detailed data on ICU/mechanical ventilation specific outcomes, hence we cannot give a clear explanation either for the differences between East and West Europe nor for the high mortality observed in our current case series.

However, it has long been reported by key opinion leaders of intensive care medicine in the region, that although we are more than 3 decades after the changing of the socialist system, several fundamental issues are still to be solved (31, 32). The most burning is the lack of human resources, including ICU nurses and doctors alike. Although our study did not focus on COVID-19 mortality in general, we cannot exclude that in the overall high ICU mortality, the low number of specialised ICU nurses and doctors and other logistic and organisational factors played a role. Unfortunately, we cannot refer to published data, but during the pandemic in our ICU, the specialised patient-to-nurse ratio often increased above 4:1. This is of utmost importance, as it has been shown by several studies that nurse/patient ratio has an exponential effect on mortality (33–35). In fact, if the patient-to-nurse ratio increases from 1:1 to >2.5:1 per shift, the adjusted risk ratio "shifts, with at least one death" increases from 1 to 3.5 (95% CI: 1.3–9.1) (31). In this region of Europe, we must take this opportunity to learn from the lessons of the pandemic that state-of-the art equipment and treatment strategies, such as CytoSorb, cannot make a difference if the human resources do not match the international/European standards.

Limitations

Our study has many more limitations than strengths. It is a retrospective, single centre, small sample size case series without a control group. However, there are very few studies in general available on this topic. Furthermore, our results are not negative as far as the treatment's immediate effects are concerned. Regarding long term outcomes, in comparison to international results, ours cannot be compared for the reasons detailed above. We could not present data on IL-6, and several other parameters.

Conclusions

In this small, singe centre cohort we present the results of COVID-19 patients treated with hemoadsorption during their life-threatening critical illness. The treatment was well-tolerated and there was a tendency toward improvement in both vasopressor need and oxygenation during the course of hemoadsorption. These observations render the need for prospective randomised trials.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Fejér County St. György University Teaching Hospital, Székesfehérvár, Hungary (No: 18/2021.05.11). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZM: conceptualisation, supervision, project administration, and funding acquisition. MR and MV: methodology. MV and BH: software. KO and MR: validation. AV and DB: formal analysis. MV: investigation, resources, and data curation. TL, MR, MV, and KO: writing—original draft preparation. ZM, LB, and ÁS: writing—review and editing. BH: visualisation. All authors have read and agreed to the published version of the manuscript.

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REVIEW
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PPV May Be a Starting Point to Achieve Circulatory Protective Mechanical Ventilation

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Pulse pressure variation (PPV) is a mandatory index for hemodynamic monitoring during mechanical ventilation. The changes in pleural pressure (P_{pl}) and transpulmonary pressure (P_L) caused by mechanical ventilation are the basis for PPV and lead to the effect of blood flow. If the state of hypovolemia exists, the effect of the increased P_{pl} during mechanical ventilation on the right ventricular preload will mainly affect the cardiac output, resulting in a positive PPV. However, P_L is more influenced by the change in alveolar pressure, which produces an increase in right heart overload, resulting in high PPV. In particular, if spontaneous breathing is strong, the transvascular pressure will be extremely high, which may lead to the promotion of alveolar flooding and increased RV flow. Asynchronous breathing and mediastinal swing may damage the pulmonary circulation and right heart function. Therefore, according to the principle of PPV, a high PPV can be incorporated into the whole respiratory treatment process to monitor the mechanical ventilation cycle damage/protection regardless of the controlled ventilation or spontaneous breathing. Through the monitoring of PPV, the circulation-protective ventilation can be guided at bedside in real time by PPV.

Keywords: pulse pressure variation, mechanical ventilation, cardiopulmonary interactions, circulatory-protection, blood flow

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HIGHLIGHTS

- Pulse pressure variation (PPV) is a mandatory index for hemodynamic monitoring during mechanical ventilation.
- PPV originates from increased pleural pressure (P_{pl}) and transpulmonary pressure (PL).
- P_{pl} mainly affects the right ventricular preload, while PL is more influenced by right heart overload.
- The role of PPV in right heart overload should be emphasized in lung and circulatory protection during mechanical ventilation, reducing the occurrence of ventilator-induced lung injury (VILI), and patient self-inflicted lung injury (P-SILI).
- PPV is a real-time monitoring index for the mechanical ventilation that can promptly alert for abnormal breathing and circulation at the bedside.

Hemodynamic monitoring is essential to improve gas exchange, optimize organ and tissue perfusion, and avoid ventilation-related lung injury (VILI) in the mechanical ventilation patients. Therefore, the low-tidal volume lung protection strategies are widely used in clinical practice. However, in the process of mechanical ventilation for the patients with acute respiratory distress syndrome (ARDS) or even coronavirus disease 2019 (COVID-19), we have increasingly realized that only low-tidal volume lung protective ventilation is far from sufficient. Many patients still die from acute circulatory failure caused by ventilation-related lung injury (VILI) or patient self-inflicted lung injury (P-SILI) (1, 2). In clinical work, we are always thinking about whether we can use a direct indicator to reflect the mechanical ventilation management to achieve circulatory protection on the basis of lung protection at the same time. The cardiopulmonary interaction produced by positive pressure ventilation leads to the changes in pleural pressure (P_{pl}) and transpulmonary pressure (P_L), and the accompanying hemodynamic effects provide clues for us, which make it possible to monitor the hemodynamic changes under mechanical ventilation (3–5). Specific indicators can prompt the hemodynamic situation of a patient to the doctor at the bedside and provide precise treatment directions. In the intensive care unit (ICU), it is necessary to establish the central venous catheters and peripheral arterial catheters for critically ill patients. The analysis of the central venous pressure, arterial blood pressure, pressure waveform, and blood gas of arteries and veins can enable doctors to perform more precise and individualized strategies in circulation and respiratory management for the patients (6). When the peripheral arterial catheter is indwelled for arterial blood pressure measurement, the arterial pressure waveform has a periodic change with the respiratory cycle that can be displayed in the monitor, which we call pulse pressure variation (PPV). The previous studies have shown that during strictly controlled mechanical ventilation, a PPV value >13% indicates that the patients have fluid responsiveness when high tidal volume or lung compliance exists (7, 8). However, sometimes we find that the PPV value is more than 13%, but the mechanical ventilation patients have no fluid responsiveness. At this time, the decline in patient cardiac output is often due to high right ventricle afterload. Therefore, expanding the usage of PPV can identify and evaluate the hemodynamic dysfunction of a patient early during mechanical ventilation and better implement circulatory and lung protection ventilation therapy.

MECHANISM OF PPV

Positive-pressure ventilation changes the normal respiratory physiology of a patient, leading to the changes in P_{pl} and P_L. The changed cardiopulmonary interaction produced by P_{pl} and P_L can result in the changes in preload and afterload of the two ventricles, which in turn causes the blood flow changes (3, 5). In controlled ventilation mode, inhalation can increase P_{pl} and reduce venous return, reducing the preload of the right ventricle. After the gas is inhaled, the lung tissue stress during tidal inhalation is the pressure distending the lung, called P_L, the difference between airway pressure and P_{pl}. P_L causes an increase in afterload to the right ventricle due to the increased pulmonary vascular resistance during inhalation (9). The result is that the output of the right ventricle is the lowest at the end of inspiration (4, 9). For the left ventricle, the increase in P_{pl} during inhalation can lead to a decrease in the afterload of the left ventricle due

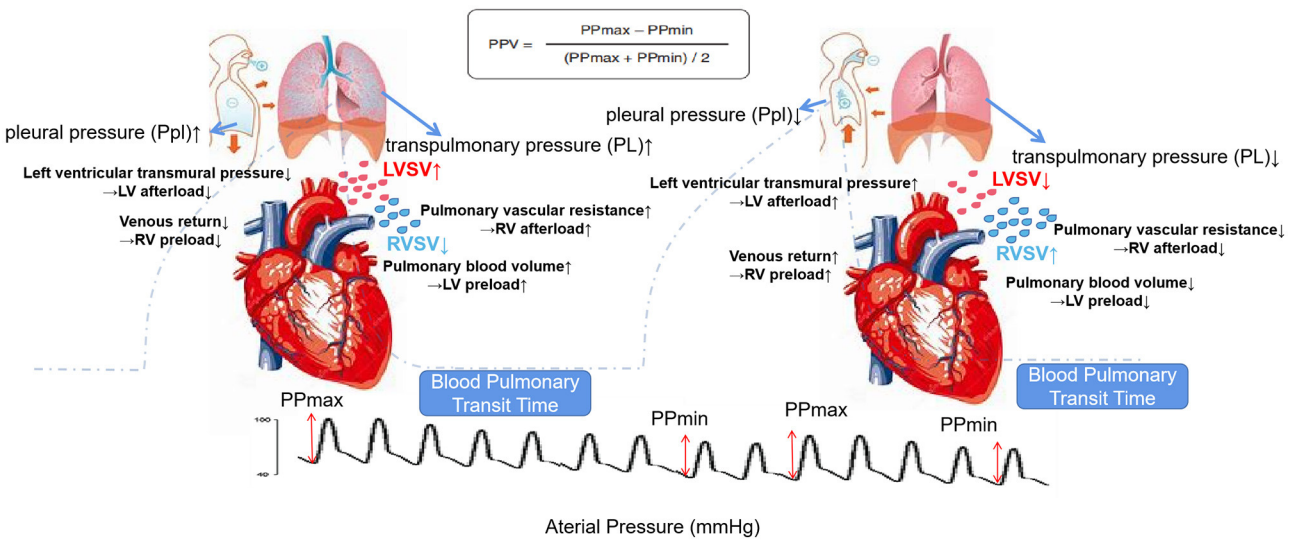

FIGURE 1 | Schematic effects of high transpulmonary pressure (P_L) and pleural pressure (P_{pl}) on the right ventricle (RV) vs. left ventricle (LV) to explain the mechanisms of pulse pressure variation (PPV). Blood pulmonary transit time means 2–4 heartbeat cycles for this blood flow effect conducting from the right heart to left heart. When exhaling, the effect of the drop in the stroke volume (SV) of the right heart during inhalation will affect the left heart. Top: calculation formula of PPV; background dotted line: airway pressure tracing; bottom: arterial pressure tracing. The left side is the inspiratory phase, and the right side is the expiratory phase.

to the decrease in left ventricular transmural pressure. At the same time, the P_L causes an increase in the preload of the left ventricle. Therefore, the result for left ventricle output can be the largest at the end of inhalation (10). However, as the left and right hearts are connected, the influence of inhalation on the reduction of right ventricle output will result in the reduction of left ventricle preload. There are 2–4 heartbeat cycles for this blood flow effect conducting from the right heart to the left heart, which is called the blood pulmonary transit time. At this time, the inhalation changes to exhalation, and eventually, the left ventricle output decreases during exhalation. The different cardiac outputs of inhalation and exhalation create the basis for the existence of PPV. The maximum pulse pressure difference appears during inhalation, and the minimum pulse pressure difference appears during exhalation. The ratio of the difference between the two values to the average of the two values is defined as PPV. **Figure 1** shows the schematic effects of the mechanisms of pulse pressure variation.

PPV AND BLOOD FLOW DURING MECHANICAL VENTILATION

P_{pl} and P_L can affect the pulmonary blood flow during mechanical ventilation. The P_{pl} causes a change in the right ventricle inflow and the left ventricle outflow. P_L causes the changes in right ventricle outflow and left ventricle inflow. From the above, we know that the P_{pl} during mechanical ventilation causes a decrease in the right ventricle preload, and the P_L causes an increase in the right ventricle afterload, both of which are responsible for the decrease in global cardiac output. However, we need to determine which pressure constitutes the bulk of decreased cardiac output so that we can better determine whether fluid resuscitation or P_L should be reduced. The wrong judgments will lead to the wrong treatments and ultimately cause patient injury.

In general, we focused on the hemodynamic effect of capacity status on right ventricle preload. When both the left and right ventricles have fluid responsiveness, the global cardiac output will increase as the capacity increases. At this time, the increased PPV can represent whether the patients have fluid responsiveness. If any one of the ventricles has no fluid responsiveness, the global cardiac output will ultimately not increase due to fluid resuscitation (10). When pulmonary compliance is normal or slightly decreased, a high tidal volume can cause P_{pl} to affect the change in alveolar pressure by 50% or more. At this time, if combined with a hypovolemic state, the influence of increased P_{pl} on right ventricle preload will constitute the bulk of cardiac output, resulting in a positive PPV (11). Therefore, when using PPV to predict the fluid responsiveness during mechanical ventilation, the doctors need to consider pulmonary compliance and tidal volume (7, 12).

In addition, PPV can be used to predict the hemodynamic effect of positive end-expiratory pressure (PEEP) on the right ventricle (13). High PEEP can cause decreased cardiac output, resulting in increased PPV, but not all the patients have fluid responsiveness. Research has suggested that the higher the PPV before using mechanical ventilation, the more obvious the decrease in cardiac output caused by PEEP (13). However, for the patients without fluid responsiveness, if the high PPV does not represent reduced right ventricle preload, we need to consider whether it is due to the increased right ventricle afterload that causes the decrease in cardiac output. This suggests that the high PPV in the patients with ARDS may not mean hypovolemia but a significant increase in P_L, which causes an increase in right ventricle afterload and a decreased left ventricle inflow. At this time, we should not only take note of cardiac output decreases but also be alerted of the incidence of acute cor pulmonale (ACP).

PPV AND RIGHT VENTRICLE AFTERLOAD

Positive pressure ventilation affects the right ventricle afterload, which in turn affects pulmonary blood flow, resulting in a decrease in cardiac output and an increase in PPV. At this time, the patients usually have no fluid responsiveness. P_L rises during mechanical ventilation, which can compress the alveolar capillaries. Since right heart failure is more sensitive to afterload than preload, the decrease in right ventricular outflow during inhalation has a greater relationship with right ventricle afterload. Two clinical studies have reported that in the case of right heart failure, the patients with high PPV have no fluid responsiveness. Moritz et al. found that in the early stage after cardiac surgery or during septic shock, the patients with increased pulmonary artery pressure had a poor response to fluid resuscitation, and PPV could not be used to predict the fluid responsiveness (14). Yazine Mahjoub et al. (15) found that right heart failure can lead to false-positive PPV. Such patients can be combined with the tricuspid annular systolic velocity (Sta) to identify whether the patients have increased right ventricular afterload. Therefore, we emphasize that when the capacity of a patient is sufficient but lung compliance is significantly reduced or when the ventilator support conditions are high, P_L can significantly increase the right ventricular afterload or cause ACP, resulting in a decrease in cardiac output (3). As mentioned before, the effects of mechanical ventilation on preload and afterload of the right ventricle can cause cardiac output and blood pressure to decrease, leading to an increase in PPV, but different effects will have completely different treatments. When decreased right ventricular preload contributes to the bulk of decreased cardiac output, it indicates the need for fluid resuscitation; when the right ventricular afterload effect is dominant, fluid resuscitation is not recommended or may even be harmful. In this case, limiting P_L is the first choice, that is, the essence and connotation of circulation-protective ventilation.

PPV AND SPONTANEOUS BREATHING

Many studies have recommended reducing the dose of sedatives during mechanical ventilation and allowing the patients to maintain spontaneous breathing. However, the abnormal spontaneous breathing of the mechanically ventilated patients, defined here as excessive spontaneous breathing, can cause hemodynamic disturbances. Excessive spontaneous breathing

causes negative changes in the Ppl and increases the P_L, resulting in excessive alveolar expansion, increased functional residual capacity (FRC), increased pulmonary vascular resistance (PVR), decreased pulmonary blood flow, increased V/Q ratio, and increased dead space ventilation (16). The injury caused by spontaneous breathing not only affects the alveoli but also affects pulmonary blood flow. Transvascular pressure is the difference between intravascular pressure and pressure outside the vessels. The more negative P_{pl} generated during spontaneous breathing will increase transvascular pressure and aggravate pulmonary edema due to promoting alveolar flooding and increased RV flow (17). In the healthy lung, the changes in local P_{pl} are evenly transmitted across the lung surface; this phenomenon is called "fluid-like" behavior. However, the injured lungs exhibit "solid-like" behavior, where a non-aerated lung region impedes the rapid generalization of a local change in P_L. In such cases, the lung expansion is heterogeneous. Therefore, there will be different regional P_{Ls} and different degrees of lung inflation. At this time, if spontaneous breathing is superimposed, it will undoubtedly cause more changes in P_{pl} and uneven conduction, resulting in uneven lung expansion or even excessive expansion (18, 19). Eventually, lack of synchronization between the reserved spontaneous breathing and mechanical ventilation of a patient as well as mediastinal swing may impair pulmonary circulation and right heart function (20, 21). The hemodynamic effects caused by the above conditions can also be predicted by PPV.

PPV AND COVID-19 AND ARDS

In COVID-19 pneumonia, a large proportion of the patients have L-type ARDS. Their lung gas volume is usually high, lung recruitability is minimal, and hypoxemia likely results from the loss of hypoxic pulmonary vasoconstriction and impaired regulation of pulmonary blood flow (22). During hypoxia, the patients are usually forced to increase their tidal volume and thus increase minute ventilation volume (23). At this time, if the patients maintain spontaneous breathing, there will be an increase in the work of spontaneous breathing, which will cause the P_{pl} negative changes and increase the transvascular pressure. In the case of increased pulmonary vessel permeability caused by inflammation, increased transvascular pressure will extremely increase the risk of pulmonary edema resulting from vascular leakage (24), which will progress L-type ARDS toward H-type ARDS. Manifestation of H-type is similar to typical ARDS. The lung lesions are distributed in a dependent area. The movement of the diaphragm during spontaneous breathing is mainly in the dependent area, which is conducive to the recruitment maneuvers and increased pulmonary blood flow in the dependent area (25–27). When mild and moderate ARDS occurs, the patients have a relatively low P_L, and spontaneous breathing can increase transpulmonary vascular pressure, reduce pulmonary vascular resistance, increase pulmonary blood flow, and improve ventilation while improving oxygenation. However, in severe ARDS, the patients have higher P_L, spontaneous breathing can cause uneven blood flow and airflow distribution, alveoli easily expand excessively, pulmonary vascular resistance increases and decreases pulmonary blood flow in normal ventilation areas, and transpulmonary vascular pressure increases and exacerbates pulmonary edema in dependent areas. Unfortunately, we have not yet seen the publication of studies on the treatment or prognosis of PPV in the patients with COVID-19 until now. However, according to the principle of PPV, a high PPV plays an important role in the monitoring of circulation damage/protection during mechanical ventilation. We hypothesize that the PPV abnormalities in the patients with COVID-19 may be a common phenomenon. Through PPV monitoring, the circulation management of mechanical ventilation can be incorporated into the entire mechanical ventilation and weaning process.

LIMITATIONS OF PPV

When PPV is used as an index to predict fluid responsiveness, we need to clarify whether PPV is positive. We must also be

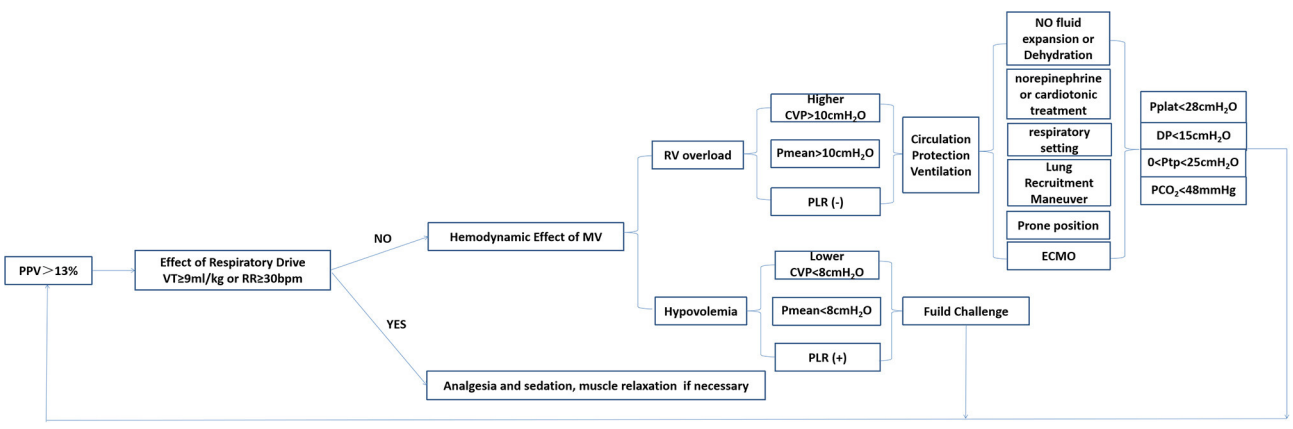

FIGURE 2 | Management and intervention process based on PPV monitoring. PPV, pulse pressure variation; MV, mechanical ventilation; RV, right ventricle; CVP, central venous pressure; P_{mean}, mean airway pressure; PLR, passive leg raising test; ECMO, extracorporeal membrane oxygenation; DP, driving pressure; Ptp, transpulmonary pressure.

alert to the possibility of false negatives and positives and the corresponding reasons. Multiple studies have shown that the threshold index for PPV to assess fluid responsiveness is 13% (8, 28). In the clinical applications, it should be noted that when the tidal volume is >8 ml/kg tidal volume (VT) mechanical ventilation, the PPV fluctuates at "9% < PPV ≤ 13%" and may also prompt the possibility of fluid responsiveness (29). Myatra et al. proposed that PPV is poor in predicting fluid responsiveness at 6 ml/kg VT (AUROC curve, 0.69). When VT increased to 8 ml/kg, PPV more reliably predicted fluid responsiveness (AUROC curve, 0.91). The study also found that during the increase in VT, if the absolute value of PPV increases by ≥3.5%, the fluid responsiveness can be predicted quite accurately (AUROC curve, 0.99) (30). Messina et al. (31) conducted a tidal volume challenge (TVC) in the prone position patients, that is, increasing the tidal volume from 6 to 8 ml/kg in a short period of time, and attempting to use pulse pressure variability and stroke volume variability to assess the fluid responsiveness of a patient. In addition, the studies have pointed out that when the PPV of a patient is >13%, the passive leg raising tests are performed. If the PPV decreases, it indicates that the patient has fluid responsiveness. If the PPV is unchanged, it indicates that the right ventricle afterload of a patient increases (32). Therefore, we must pay attention to the premise of ensuring tidal volume (8 ml/kg PBW) when we use PPV to judge potential fluid responsiveness. Additionally, we can combine or use more indicators for judging fluid responsiveness, for example, transmural central vascular pressure, to further improve the accuracy of PPV (33, 34). However, we may not need to emphasize whether the patient is in a fully controlled ventilation state or whether the tidal volume needs to be increased to 8 ml/kg when we use PPV as an indicator of right ventricle afterload. What we need to pay attention to is whether P_L affects the pulmonary circulation and right heart function. However, the only concern is to identify whether the patient has arrhythmia. Arrhythmia has become the most important obstacle that affects the accurate determination of PPV. In addition, we should also notice the effect of increased intra-abdominal pressure on P_{pl} and P_L. In general, the clinical significance of positive PPV has a new extension and significance than before.

CIRCULATION-PROTECTIVE VENTILATION STRATEGY

At present, the lung-protection ventilation strategies have been affirmed and widely used, such as low tidal volume, limiting plateau pressure (<27 cmH_2O), and driving pressure (<15 cmH_2O), to reduce lung stress injury caused by mechanical ventilation. Recruitment maneuver and perform reasonable PEEP titration can also be considered. Applying P_L monitoring achieves the end-expiratory $P_L > 0$ cmH_2O and end-inspiratory $P_L < 25$ cmH_2O. As high $PaCO_2$ can aggravate pulmonary vasoconstriction, treatment should be used to limit hypercapnia ($PaCO_2 < 48$ mmHg). Prone position ventilation is used to reduce pulmonary circulation resistance, improve ventilation and blood flow matching, and improve right heart function. Therefore, based on the above treatment to prevent secondary injury (35), what else can we do? A new understanding of PPV will help us to protect the circulation during mechanical ventilation. After the relevant circulation-protective ventilation treatment, the criterion for whether we successfully protect circulation is whether the PPV returns to normal. Therefore, in the process of related lung protection ventilation, the following circulation-protective procedures can be considered to guide our mechanical ventilation to achieve lower PPV, such as evaluating and treating the spontaneous breathing effort and respiratory support conditions and monitoring and optimizing the flow status and volume status of a patient. Combined with our previous studies (36, 37), we propose a possible processing procedure for the PPV abnormalities, as shown in **Figure 2**.

In summary, we should pay attention to the role of P_{pl} and P_L in the cardiopulmonary interaction and their effect on blood flow. We should be sufficiently aware of the universality of increased right ventricle afterload caused by mechanical ventilation. PPV is an indicator that can help us distinguish the causes of decreased cardiac output and has become a necessary indicator for monitoring circulatory damage in ARDS during mechanical ventilation. At the bedside, we can adjust our mechanical ventilation parameter by monitoring the PPV to achieve circulation-protective mechanical ventilation. Therefore, we believe that PPV is the starting point to achieve circulation-protective mechanical ventilation. During mechanical ventilation, PPV should be continuously monitored, and its abnormalities should be treated clinically and dealt with immediately instead of shelving.

AUTHOR CONTRIBUTIONS

PP, LS, and HH wrote the manuscript. DL and YL responsible to this manuscript. All authors contributed to the article and approved the submitted version.

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Effects of Dexmedetomidine on Postoperative Nausea and Vomiting in Adult Patients Undergoing Ambulatory Thyroidectomy: A Randomized Clinical Trial

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Background: Postoperative nausea and vomiting (PONV) is a common and disturbing problem in patients undergoing ambulatory thyroidectomy. This prospective trial aimed to explore whether dexmedetomidine (DEX) combined with azasetron (AZA) can further drop the incidence of PONV in patients undergoing ambulatory thyroidectomy compared with AZA.

Methods: This single-center, randomized, double-blind trial involved 172 adult patients undergoing ambulatory thyroidectomy. The individuals were randomized to DEX + AZA group and AZA group. In the DEX + AZA group, patients received dexmedetomidine 0.5 $\mu g\ kg^{-1}$ for 10 min and then the infusion rate was held at 0.1 $\mu g\ kg^{-1}\ h^{-1}$ until the completion of the operation, while the same amount of 0.9% saline in the AZA group. At the completion of the surgery, 10 mg azasetron was administered to every patient in both groups. The primary outcome was the incidence of 24 h PONV after ambulatory thyroidectomy. The secondary outcomes included residence time in recovery room, pain scores, severity of nausea, and adverse events.

Results: No significant difference was found in the incidence of 24-h PONV between the DEX + AZA group and the AZA group [36% (30 of 84) vs. 38% (32 of 84); relative risk, 0.94; 95% confidence interval (CI), 0.63–1.40; $P = 0.749$]. The incidence of severe nausea was similar between the DEX + AZA group and the AZA group [57% (12 of 21) vs. 43% (9 of 21); relative risk, 1.33; 95% CI, 0.72–2.50; $P = 0.355$].

Conclusions: Intraoperative dexmedetomidine combined with azasetron failed to drop the incidence of 24-h PONV compared with azasetron alone in patients undergoing ambulatory thyroidectomy.

Keywords: dexmedetomidine, postoperative nausea and vomiting, ambulatory, thyroidectomy, anesthesia

INTRODUCTION

At present, thyroid tumors and thyroid disease are on the rise worldwide. In China, people with thyroid tumors are increasing rapidly, and the incidence rate is 3 times more common in women than in men (1, 2). Surgery is the main treatment for thyroid cancer. Guided by the concept of enhanced recovery after surgery, ambulatory thyroidectomy has become a new trend, and more and more thyroidectomies have been performed in the ambulatory operating room (3, 4).

Postoperative nausea and vomiting (PONV) refer to any nausea, retching, and vomiting that happen after the anesthesia and operation (5). Studies have reported that, if not treated, ∼35% of patients will develop nausea, vomiting, or both symptoms after surgery, which is a very painful experience, even more serious than postoperative pain (6). However, ∼60–80% of patients develop nausea and vomiting after thyroidectomy without antiemetic prophylaxis, which may result in severe complications, such as aspiration pneumonia caused by accidental inhalation of vomitus or postoperative neck hematoma and even airway obstruction caused by hematoma (7). PONV often prolongs the patient's stay in the recovery room or even the discharge time, which is the main reason for accidental readmission after elective surgery (8). However, prophylactic antiemetic agents can improve the condition.

The latest guidelines for the management of PONV advised that the combination of different prophylactic drugs can drop the rate of PONV (9). Dexmedetomidine (DEX) is a novel α2-adrenoreceptor agonist that has good sedative, analgesic, and sympatholytic effects. Because of its advantages of reducing intraoperative opioid dose and accelerating postoperative recovery, dexmedetomidine is increasingly used during anesthesia. Recently, several researches have suggested that intraoperative DEX may have an antiemetic effect and can decrease the incidence of PONV (10, 11). Azasetron (AZA) as a selective serotonin receptor antagonist that is recommended by the guidelines as a first-line antiemetic agent in high-risk patients. However, there have been no studies of dexmedetomidine in combination with azasetron for the prevention of PONV after ambulatory thyroidectomy.

We designed this trial to explore the effect of dexmedetomidine on nausea and vomiting after ambulatory thyroidectomy. We hypothesized that dexmedetomidine combined with azasetron can further decrease the incidence of nausea and vomiting 24 h after ambulatory thyroidectomy compared with azasetron.

METHODS

Ethics

Ethical approval for this study (PJ2020-13-21) was provided by the Ethics Committee of The First Affiliated Hospital of Anhui Medical University, Hefei China (Chairperson Prof Heng Wang) on 29 October 2020. This randomized, double-blind trial was registered at the Chinese Clinical Trial Registry (ChiCTR2000039603, Principal Investigator: Yao Lu, MD, PhD, November 03, 2020). Individuals scheduled to undergo ambulatory thyroidectomy were enrolled. An informed consent was obtained from each patient.

Study Design

Eligible patients were 18–60 years old of any sex with American Society of Anaesthesiologists classification I–II and body mass index of 18–30 kg/m^2. The exclusion criteria included allergy to dexmedetomidine, antiemetic or analgesic medication intake within 24 h before surgery, use of antipsychotic drugs or corticosteroids, previous heart failure or arrhythmia and diabetes mellitus with poor glycemia control, uncontrolled hypertension, liver and kidney dysfunction, gastric dynamic disorder, and vestibular disease.

Randomization and Masking

The investigators, independent of the blind study, prepared the randomized schedule and study drugs. During the study, patients, investigators, anaesthesiologists, nurses in the post-anesthesia care unit (PACU) or ward, and the recorders of each observation index were all unaware of the group assignment. The SPSS version 23.0 generated random numbers, which were delivered in sealed, opaque envelopes. And the envelopes were opened after consent was obtained. The study drugs included dexmedetomidine (100 μg ml^{-1}, diluted with 0.9% saline to 4 μg ml^{-1}), azasetron, and 0.9% saline, all prepared according to random numbers. Patients were randomly allocated into the DEX + AZA group or the AZA group in a 1:1 ratio.

Study Treatments

The patient received routine pre-operative preparation, and no premedication was administered. Standard monitoring was done after arriving at the operating room. Before anesthesia induction, 0.5 μg kg^{-1} for 10 min of dexmedetomidine was administered intravenously and then the infusion rate reduced to 0.1 μg kg^{-1} h^{-1} until the end of surgery in the DEX + AZA group or the same amount of 0.9% saline in the AZA group. General anesthesia was induced i.v. with propofol (2 mg kg^{-1}) and sufentanil (0.4 μg kg^{-1}). To facilitate endotracheal intubation, cisatracurium (0.2 mg kg^{-1}) was administered. Anesthesia was maintained with remifentanil (0.1–0.2 μg kg^{-1} min^{-1}) and propofol (4–6 mg kg^{-1} h^{-1}), with their infusion rate adjusted to keep the bispectral index from 40 to 60. Sufentanil (5–10 μg) was administered to keep the heart rate and blood pressure fluctuations within 20% of baseline. In addition, to maintain adequate muscle relaxation, intermittent doses of cisatracurium were administered. At the end of surgery, every patient in each group was treated with 10 mg azasetron and 50 mg flurbiprofen axetil. Hypotension (mean arterial pressure of <60 mmHg or <80% of the baseline) was treated with 6 mg ephedrine and bradycardia (heart rate of <45 beats min^{-1}) with atropine 0.5 mg. After the operation, when autonomous breathing was regular, tidal volume was >6 mL kg^{-1}, SpO2 could be maintained at more than 95% during air intake, the trachea catheter was withdrawn, patients were transported to PACU. No analgesia was routinely applied.

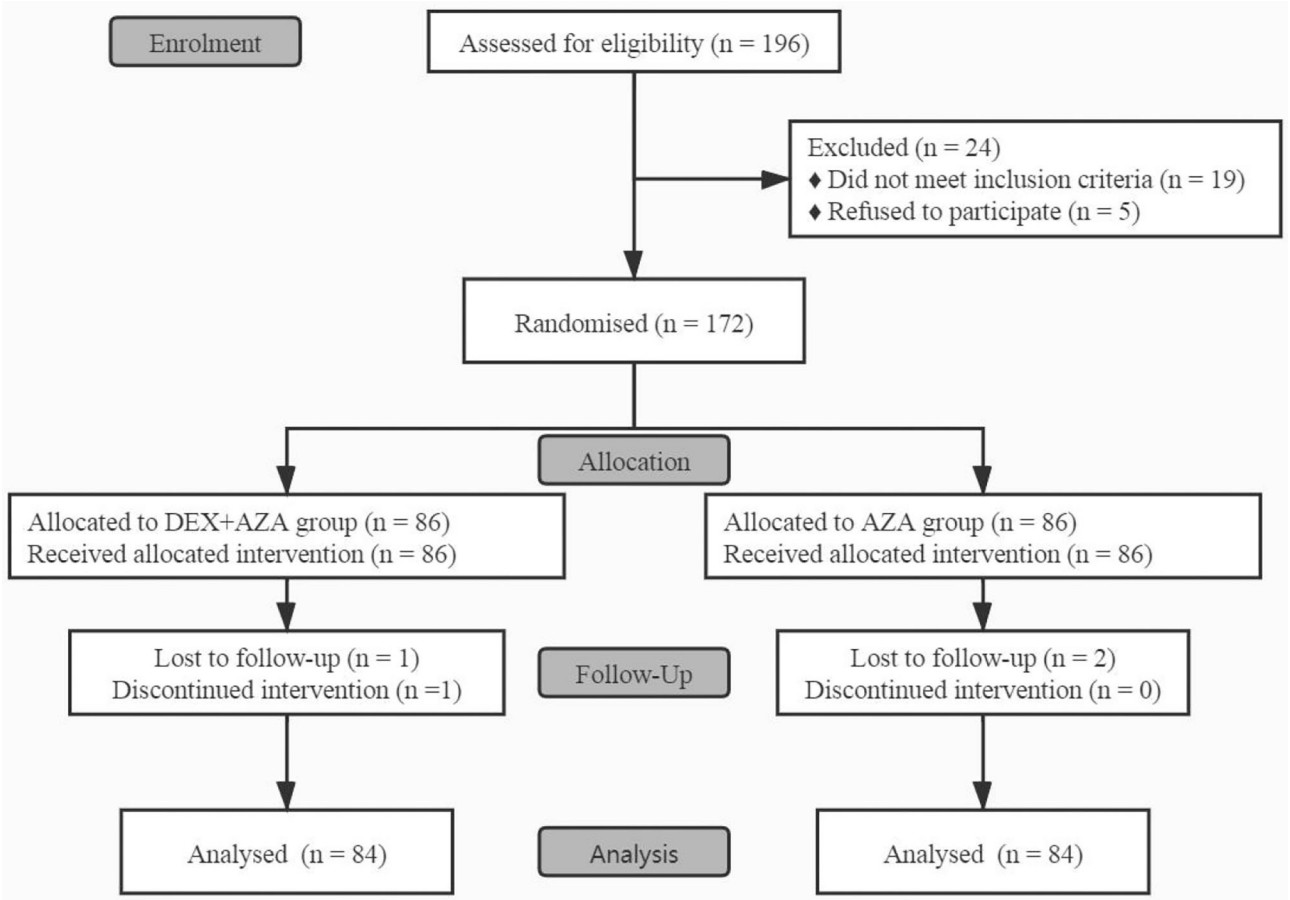

FIGURE 1 | CONSORT flow diagram for this study. This flow diagram shows this single-center, double-blind, randomized trial conducted on adult patients undergoing ambulatory thyroidectomy from November 2020 to January 2021. In the DEX + AZA group, patients received dexmedetomidine 0.5 $\mu g\ kg^{-1}$ for 10 min before anesthesia, and then the infusion rate was held at 0.1 $\mu g\ kg^{-1}\ h^{-1}$ until the completion of operation. In the AZA group, patients received an equal volume of saline with same pattern as the DEX + AZA group. Patients in both groups were given 10 mg azasetron before the end of surgery. DEX + AZA, dexmedetomidine + azasetron; AZA, azasetron.

Outcomes

Data were collected by investigators who were unaware of randomization and did not participate in the whole procedure. Relevant baseline characteristics and perioperative data of patients were carefully recorded.

The primary outcome was the incidence of 24 h PONV after ambulatory thyroidectomy. PONV was defined as at least 1 incident of nausea, vomiting, or retching or a combination of these symptoms. The investigators assessed the incidence of PONV at the time of leaving PACU, 6 and 24 h postoperatively in the ambulatory surgical ward by asking patients if they had experienced nausea and vomiting. Only a "YES" or "NO" answer can be accepted.

The secondary outcomes included severity of nausea, residence time in PACU, visual analog scale (VAS) pain scores at the time of leaving PACU, 6 and 24 h postoperatively, and adverse events.

Moreover, 11-point numerical rating scale (NRS) was used to assess nausea severity (0, no nausea; 10, worst nausea imaginable). Mild nausea is defined as NRS score from 1 to 3, whereas severe nausea is >3 (12). Rescue antiemetic agents will be administered if patients have experienced severe nausea or 2 or more times of vomiting or retching or patients request an antiemetic agent at any time. Intramuscular injection of 10 mg metoclopramide hydrochloride was used as the rescue antiemetic agent. Morphine is not allowed to be used after surgery. Parecoxib sodium 40 mg will be administered for VAS pain score of >3. In the PACU, Ramsay sedation scale (RSS) was used to assess sedation levels (1, agitated; 2, cooperative and oriented; 3, can respond to simple questions; 4, asleep, but with a quick reaction to stimulus; 5, asleep, arousable; 6, asleep, unarousable) (13). Oversedation was defined as RSS value of >4.

Statistical Analysis

Based on our unpublished pilot trial, we observed that, despite 10 mg azasetron being given intraoperatively, ∼40% of patients develop PONV after thyroidectomy. A 20% decline in the occurrence of PONV is considered clinically significant; hence,

TABLE 1 | Patient baseline characteristics and intraoperative variables.

Characteristics/intraoperative variables	DEX + AZA ($n = 84$)	AZA ($n = 84$)	*P*-value
Mean age ± SD, yr	44 ± 11	41 ± 10	0.157[a]
Sex, no. (%)			0.717[b]
Female	63 (75)	65 (77)	
Male	21 (25)	19 (23)	
Mean BMI ± SD, kg/m^2	23.5 ± 2.6	23.2 ± 2.7	0.432[a]
ASA classification, no. (%)			0.070[b]
I	25 (30)	15 (18)	
II	59 (70)	69 (82)	
Smoking, no. (%)	7 (8)	5 (6)	0.549[b]
Hypertension, no. (%)	16 (19)	9 (11)	0.129[b]
History of motion sickness, no. (%)	34 (40)	23 (27)	0.073[b]
Median duration of anesthesia (IQR), min	97 (84, 117)	105 (85, 128)	0.059[c]
Median duration of surgery (IQR), min	79 (67, 98)	89 (72, 110)	0.100[c]
Medication administered during surgery			
Median propofol (IQR), mg	595 (500, 675)	558 (482, 656)	0.553
Median remifentanil (IQR), μg	580 (466, 700)	768 (600, 1,019)	<0.001
Median sufentanil (IQR), μg	35 (30, 35)	35 (30, 35)	0.497

DEX, dexmedetomidine; AZA, azasetron; IQR, interquartile range; ASA, American Society of Anesthesiologists; BMI, body mass index.
[a]*Analyzed using Independent-sample t test.*
[b]*Analyzed using χ^2 test.*
[c]*Analyzed using Mann–Whitney U-test.*

TABLE 2 | Nausea and vomiting outcomes and postoperative characteristics.

Outcomes/postoperative characteristics	DEX + AZA ($n = 84$)	AZA ($n = 84$)	*P*-value
PONV, no. (%)			
PACU	0	0	–
PACU-6 h	15 (18)	16 (19)	0.842[a]
6–24 h	20 (24)	24 (29)	0.483[a]
0–24 h	30 (36)	32 (38)	0.749[a]
Nausea, no. (%)			
PACU	0	0	–
PACU-6 h	9 (11)	12 (14)	0.484[a]
6–24 h	13 (15)	12 (14)	0.828[a]
Vomiting, no. (%)			
PACU	0	0	–
PACU-6 h	9 (11)	5 (6)	0.264[a]
6–24 h	12 (14)	18 (21)	0.227[a]
Use of rescue antiemetics, no. (%)	19 (23)	23 (27)	0.476[a]
Use of rescue analgesics, no. (%)	5 (6)	4 (5)	0.732[a]
Median residence time in PACU (IQR), min	45 (40, 55)	45 (40, 55)	0.391[b]

DEX, dexmedetomidine; AZA, azasetron; IQR, interquartile range; PONV, postoperative nausea and/or vomiting; PACU, Post-anesthesia Care Unit.
[a]*Analyzed using χ^2 test.*
[b]*Analyzed using Mann–Whitney U test.*

80 patients are required per group with an alpha level of 0.05 and power of 80%. Finally, we planned to enroll 196 patients to allow for a 20% dropout. Categorical data are presented as number (percentage) and were analyzed by χ^2 test or Fisher's exact test. Independent *t*-test or Mann-Whitney *U*-test was applied for analyzing continuous variables, which are presented as mean ± standard deviation or medians (interquartile range). χ^2 test or Fisher's exact test was used to analyze the incidences of PONV, nausea severity, and adverse events presented as number (percentage). Postoperative pain scores are presented as median (interquartile range) and were analyzed with the linear mixed model. Two-tailed $P < 0.05$ was considered statistically significant. SPSS 23.0 was used for data analysis.

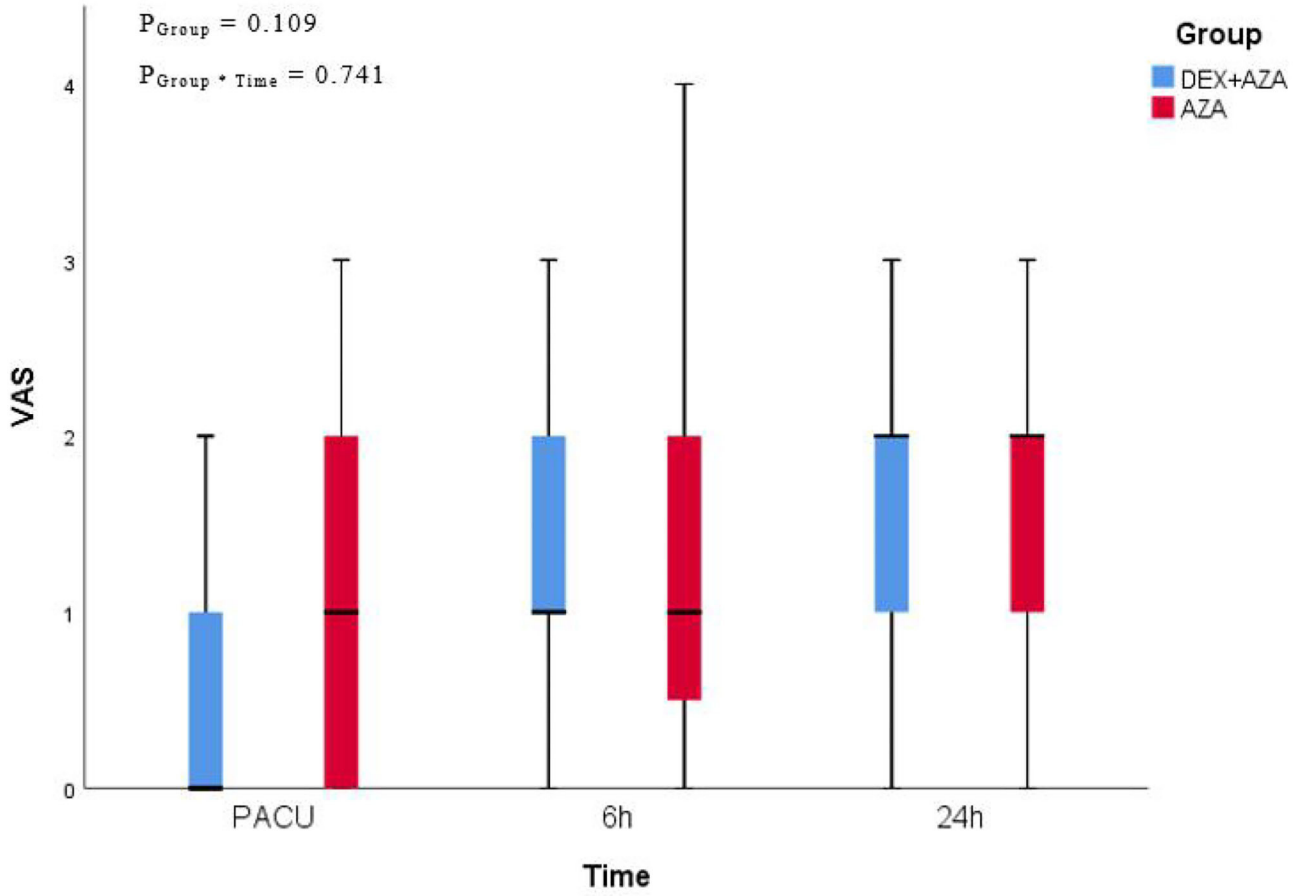

FIGURE 2 | Postoperative pain assessment. We adopted 11-point visual analog scale (VAS) for pain assessment at the time of leaving PACU, 6 and 24 hours after thyroidectomy. VAS presented as median (interquartile range). In the DEX + AZA group ($n = 84$), patients received dexmedetomidine 0.5 μg kg^{-1} for 10 min before anesthesia, and then the infusion rate was held at 0.1 μg kg^{-1} h^{-1} until the completion of operation. In the AZA group ($n = 84$), patients received an equal volume of saline with same pattern as the DEX + AZA group. Patients in both groups were given 10 mg azasetron before the end of surgery. $P_{Group*Time}$ = *P*-value of the group and time interaction obtained by the linear mixed model. DEX + AZA, dexmedetomidine + azasetron; AZA, azasetron.

RESULTS

Before the study began, 24 patients were excluded because they did not meet the inclusion criteria or withdrew their consent, so we enrolled a total of 172 patients between November 2020 to January 2021 (**Figure 1**). In the DEX+AZA group, 1 patient was excluded from the analysis because of failure to pump dexmedetomidine at the beginning of surgery. Because of missing data, 2 patients in each group were excluded from the analysis after being treated with study drugs. In total, 168 patients finished the study and were evaluated for all study outcomes: 84 in the DEX + AZA group and 84 in the AZA group.

No significant differences were found in baseline characteristics, smoking status, or history of motion sickness between two the groups. No significant differences were found in intraoperative variables between groups except the consumption of remifentanil (**Table 1**).

Outcomes

The incidence of 24-h PONV was not significantly different between the two groups (**Table 2**). In the DEX+AZA group, 30 of 84 patients (36%) experienced at least 1 episode of retching, vomiting, or both during the first 24-h postoperative period compared with 32 of 84 patients (38%) in the AZA group (relative risk, 0.94; 95% CI, 0.63–1.40; $P = 0.749$). The postoperative characteristics such as the residence time in PACU and the use of analgesic and antiemetic drugs were not significantly different in the two groups.

Throughout the study period, the VAS scores were not significantly different at any time points between the two groups (**Figure 2**). Postoperative nausea occurred in 21 patients in each group. The severity of nausea was similar in both groups 24 h after surgery; severe nausea (NRS score, >3) occurred in 9 patients (43%) in the AZA group and 12 patients (57%) in the DEX+AZA group (relative risk, 1.33; 95% CI, 0.72–2.50; $P = 0.355$) (**Figure 3**). No serious adverse events were observed during the study. The main intraoperative adverse events were

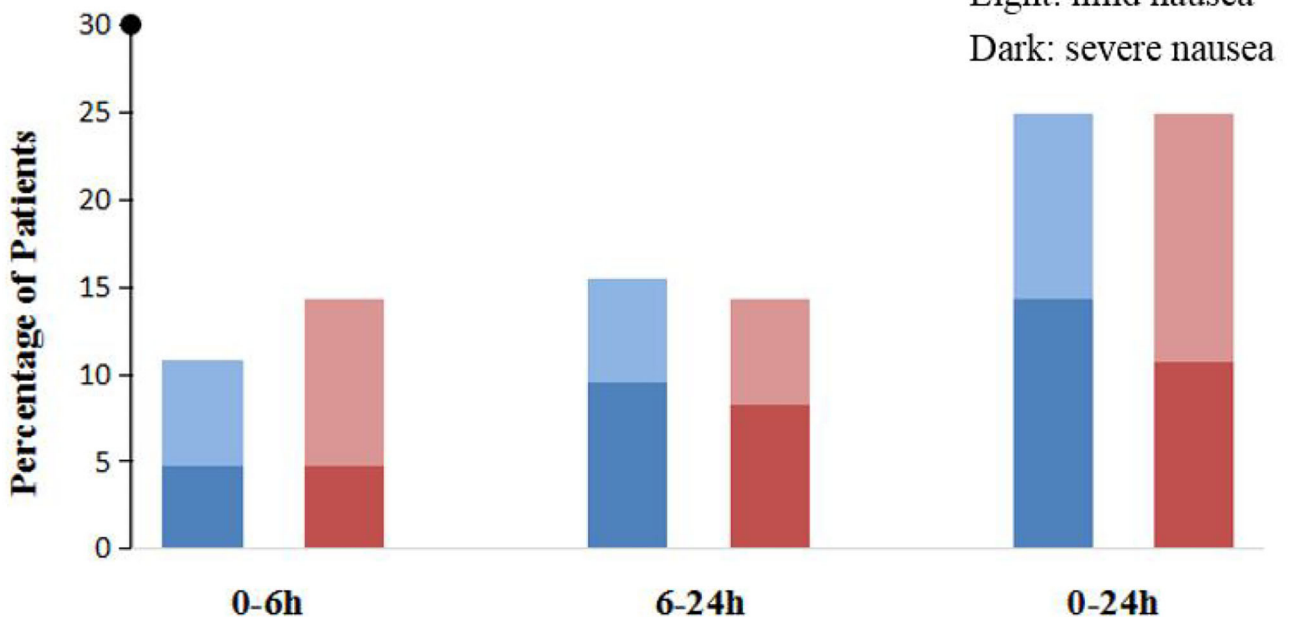

FIGURE 3 | Postoperative nausea intensity assessment. We adopted 11-point numerical rating scale (NRS) to assess nausea severity (0, no nausea; 10, worst nausea imaginable). Mild nausea is defined as NRS score from 1 to 3, whereas severe nausea is >3. In the DEX + AZA group ($n = 84$), patients received dexmedetomidine 0.5 μg kg^{-1} for 10 min before anesthesia, and then the infusion rate was held at 0.1 μg kg^{-1} h^{-1} until the completion of operation. In the AZA group ($n = 84$), patients received an equal volume of saline with same pattern as the DEX + AZA group. Patients in both groups were given 10 mg azasetron before the end of surgery. DEX + AZA, dexmedetomidine+ azasetron; AZA, azasetron; Light, mild nausea; Dark, severe nausea.

hypotension and bradycardia, and the rate did not differ between the two groups (**Table 3**).

DISCUSSION

The results in this randomized, double-blind trial suggest that intra-operative dexmedetomidine combined with azasetron did not significantly decrease the rate of 24-h PONV compared with azasetron alone.

The mechanism of PONV is very complex, and the trauma caused by surgery, inflammation, visceral stimulation, pain, hypoxia, and hypotension are the main stimulating factors (14). When the stimulus reaches the vomiting center *via* the afferent nerve, dopamine, histamine, serotonin type 3 (5-HT3), tachykinin 1 (NK1), or muscarinic receptors are activated to trigger the vomiting response (15). Moreover, relevant studies have reported that female sex, non-smoker, history of PONV, and use of postoperative opioids are high-risk factors for PONV, and the occurrence of PONV is also related to the type of surgery and intraoperative anesthesia management (16). The incidence of PONV varies greatly among different types of surgery, such as otolaryngology, gynecological, and endoscopic surgeries with relatively high incidence of PONV. In the maintenance of intraoperative anesthesia, inhalation of anesthetic drugs can easily cause PONV compared with total intravenous anesthesia (TIVA) (17, 18).

Geng et al. (13) conducted a study to investigate the effect of adjuvant dexmedetomidine on PONV during gynecological laparoscopic surgery. The results indicate that intraoperative dexmedetomidine (0.5 μg kg^{-1} loading dose followed by 0.1 μg kg^{-1} h^{-1} infusion until the end of surgery) can reduce the incidence of postoperative nausea 2 h after surgery. Song et al. (19) found that, in orthopedic surgery, adding 10 μg kg^{-1} dexmedetomidine to a fentanyl-based PCA drug mixture improved the frequency and intensity of severe postoperative nausea in patients with high-risk factors of PONV. However, the mechanism of the antiemetic function of dexmedetomidine is still unknown. Possible mechanisms to reduce PONV by dexmedetomidine include the following: dexmedetomidine has sedative and analgesic effects and can reduce the dose of narcotic drugs and opioids. It can also inhibit the excitability of the sympathetic nerve and reduce the release of catecholamine, because catecholamine may be a contributing factor of PONV (5).

This trial aimed to clarify the effect of dexmedetomidine combined with azasetron on PONV. Unfortunately, it fails to

TABLE 3 | Comparison of perioperative adverse events.

Variables	DEX + AZA ($n = 84$)	AZA ($n = 84$)	*P*-value
Adverse events during operation			
Hypotension, no. (%)	2 (2)	2 (2)	–
Bradycardia, no. (%)	6 (7)	8 (10)	0.577[a]
Adverse events in the PACU			
Agitation, no. (%)	1 (1)	3 (4)	0.613[b]
Over sedation, no. (%)	6 (7)	3 (4)	0.493[b]

DEX, dexmedetomidine; AZA, azasetron; PACU, Post-anesthesia Care Unit.
[a]*Analyzed using* χ^2 *test.*
[b]*Analyzed using continuity correction* χ^2 *test.*

further drop the occurrence of PONV, but the gross incidence of PONV in the 2 groups was not as high as reported in previous articles. This may be caused by the fact that we have adopted a multimodal prophylactic approach to prevent PONV, including TIVA with propofol, no inhalation anesthesia was used and no postoperative opioid used, which reduces the baseline risk of PONV.

There may be several reasons for the differences in the outcomes between the present study and the previous articles. First, a multimodal prophylactic treatment was used to accelerate the patient's recovery after surgery, but too many antiemetic factors may have masked the effect of dexmedetomidine on PONV. Second, due to the sedative and analgesic properties of dexmedetomidine, the amount of intraoperative remifentanil in the experimental group was significantly reduced compared with the control group. However, many studies have reported that remifentanil has a short half-life and quick elimination, and there is no effect on PONV (20–22). Therefore, although dexmedetomidine reduced the dose of intraoperative remifentanil, it fails to drop the occurrence of PONV. Third, Aouad et al. reported that 1, 0.5, and 0.25 $\mu g\ kg^{-1}$ dose of dexmedetomidine had no significant difference in the occurrence of PONV, but they cause dose-dependent hypotension (23). For decreasing the incidence of perioperative adverse events and promoting postoperative recovery, the selected dose of dexmedetomidine (0.5 $\mu g\ kg^{-1}$ loading dose followed by 0.1 $\mu g\ kg^{-1}\ h^{-1}$ infusion) may be relatively small, which may affect the antiemetic effect of dexmedetomidine. Finally, dexmedetomidine may not decrease the incidence of PONV.

There are several limitations in this study. First, our trial did not adopt a multicenter study method, which may make our results not universal. Second, only one dose of dexmedetomidine was adopted in this trial, and this dose of dexmedetomidine combined with azasetron did not produce significant effects in patients undergoing ambulatory thyroidectomy. Third, we included patients with ambulatory thyroidectomy in our study, and traditional procedures such as cholecystectomy and endoscopic surgery with a high incidence of PONV were not selected. In future studies, we can choose different types of surgery to compare the incidence of PONV.

In summary, we find that intraoperative dexmedetomidine combined with azasetron did not significantly decrease the incidence of 24-h PONV compared with azasetron alone in adult patients undergoing ambulatory thyroidectomy.

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 human participants were reviewed and approved by Ethics Committee of The First Affiliated Hospital of Anhui Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CX and YL: study design. CX: ethics approval and registration. CX, CZ, and HS: patient recruitment. CX and CZ: data collection. CX and HS: data analysis. CX, CZ, and YL: drafting. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.781689/full#supplementary-material

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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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SYSTEMATIC REVIEW
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The Effect of Dexmedetomidine as a Sedative Agent for Mechanically Ventilated Patients With Sepsis: A Systematic Review and Meta-Analysis

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Purpose: Dexmedetomidine has been shown to improve clinical outcomes in critically ill patients. However, its effect on septic patients remains controversial. Therefore, the purpose of this meta-analysis was to assess the effect of dexmedetomidine as a sedative agent for mechanically ventilated patients with sepsis.

Methods: We searched PubMed, Embase, Scopus, and Cochrane Library from inception through May 2021 for randomized controlled trials that enrolled mechanically ventilated, adult septic patients comparing dexmedetomidine with other sedatives or placebo.

Results: A total of nine studies involving 1,134 patients were included in our meta-analysis. The overall mortality (RR 0.97, 95%CI 0.82 to 1.13, $P = 0.67$, $I^2 = 25\%$), length of intensive care unit stay (MD −1.12, 95%CI −2.89 to 0.64, $P = 0.21$, $I^2 = 71\%$), incidence of delirium (RR 0.95, 95%CI 0.72 to 1.25, $P = 0.70$, $I^2 = 0\%$), and delirium free days (MD 1.76, 95%CI –0.94 to 4.47, $P = 0.20$, $I^2 = 80\%$) were not significantly different between dexmedetomidine and other sedative agents. Alternatively, the use of dexmedetomidine was associated with a significant reduction in the duration of mechanical ventilation (MD –0.53, 95%CI −0.85 to −0.21, $P = 0.001$, $I^2 = 0\%$) and inflammatory response (TNF-α: MD −5.27, 95%CI −7.99 to −2.54, $P < 0.001$, $I^2 = 0\%$; IL-1β: MD −1.25, 95%CI −1.91 to –0.59, $P < 0.001$, $I^2 = 0\%$).

Conclusions: For patients with sepsis, the use of dexmedetomidine as compared with other sedative agents does not affect all-cause mortality, length of intensive care unit stay, the incidence of delirium, and delirium-free days. But the dexmedetomidine was associated with the reduced duration of mechanical ventilation and inflammatory response.

Keywords: sepsis, anesthesiology, meta–analysis, dexmedetomidine, mechanical ventilation, sedation

INTRODUCTION

Sepsis, defined as a life-threatening organ dysfunction due to a dysregulated immune response to infection, affects millions of patients per year and carries a high risk of mortality, becoming a major global health problem (1, 2). According to the Global Burden of Diseases Study, in 2017, an estimated 48.9 million incident cases of sepsis were reported worldwide with nearly 11.0 million patients dying, accounting for 19.7% of all global deaths (3).On the other hand, sepsis is often complicated with acute respiratory distress syndrome that requires mechanical ventilatory support (4, 5), research showed that more than 20% of septic patients needed invasive ventilation in the USA (6).

Sedation is an integral component of care for mechanically ventilated patients to reduce the anxiety and stress associated with tracheal intubation and other invasive interventions (7, 8). However, the appropriate choice of a preferred sedative agent for patients with sepsis remains controversial. For decades, γ-aminobutyric acid (GABA) receptor agonists (such as propofol and midazolam) were widely used as sedative drugs in the intensive care units (ICU) (9–11). Recently, dexmedetomidine, a highly selective α-2 adrenergic receptor agonist, is a unique alternative sedative compared with GABA receptor agonists considering its analgesic properties with wider safety margin due to the lack of suppressive effects on respiration (12). Using dexmedetomidine to induce sedation while preserving a degree of arousability for critically ill patients. Limited but increasing evidence suggests that dexmedetomidine has a promising future as a sedative agent in the ICU, its use resulted in a shorter duration of mechanical ventilation (MV) or ICU length of stay (13–17), a reduced incidence of coma or delirium (15, 17–19).

Recently, Hughes and coworkers conducted the MENDS II trial concerning the effect of dexmedetomidine vs. propofol on the short-term and long-term outcomes of mechanically ventilated adults with sepsis. In this pragmatic randomized controlled trial (RCT) involving more than 400 patients, there was no significant difference in the delirium or ventilator-free days, ICU length of stay, and 90-day mortality between the patients receiving dexmedetomidine or propofol. Therefore, the effects of dexmedetomidine in septic patients receiving MV remains controversial. We aimed to assess the effects of dexmedetomidine on clinical outcomes in mechanically ventilated patients with sepsis.

METHODS

Our study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (20) (**Supplementary Material 1**). The study protocol was registered in PROSPERO (CRD42019145061). A literature search was performed in PubMed, Embase, Scopus, and Cochrane Library for eligible RCTs in English from inception through May 2021. The search used broad search terms containing "sepsis," "ventilation," "dexmedetomidine," and "randomized." In addition, full details on our search terms and strategy detailed were recorded in **Supplementary Material 2**.

Eligibility Criteria

Study inclusion criteria were as follows: (1). Population: adult (≥18 years old) patients with sepsis receiving mechanical ventilation and intravenous sedation; (2). Intervention: the use of IV dexmedetomidine regardless of dose, start time, and duration; (3). Comparison: the use of other IV sedative drugs or placebo regardless of dose, start time, and duration; (4). Outcomes: the primary outcome was overall mortality (including ICU, hospital, 28/30-day mortality). The secondary outcomes were duration of mechanical ventilation, ICU length of stay, and inflammatory responses (serum levels of inflammatory markers after 24 h). (5). Design: RCT.

The following studies would be excluded: (1). If the study evaluated obstetrical patients because sedation practices and mechanical ventilation strategies are different in the patient population (21); (2). Patients did not receive IV sedatives or mechanical ventilation; (3). Studies published only in abstract form.

Data Extraction and Quality Assessment

Two authors independently retrieved relevant studies and extracted data from included studies. The characteristics of studies (first author, years of publication, study design, population, sedation goal, intervention, and control sedative agents, outcomes) were recorded in **Table 1**. Further information (study design, number of participants, sex ratio, mean age, inclusion, and exclusion criteria) was recorded in **Supplementary Material 3.**

Two authors independently assessed the methodological quality of the included studies by using the Cochrane risk of bias tool (22).

Statistical Synthesis and Analysis

We combined the data from the included studies to estimate the pooled relative ratio (RR) with a 95% CI for the primary outcome, and the secondary outcomes were pooled as mean difference (MD) with 95%CI.

The heterogeneity between studies was tested by the Chi-squared test with significance set at a *P*-value of 0.1, and quantitatively by inconsistency (I^2) statistics (23). Substantial heterogeneity was identified when $I^2 > 30\%$ and we employed a random-effects model to perform the analysis, otherwise, a fixed-effects model would be used. In addition, we used the funnel plot and Egger's regression test to assess the publication bias (24).

A predefined subgroup analysis was stratified by population (sepsis or septic shock) and control drug (propofol or others) to investigate the potential source of heterogeneity. Furthermore, we performed a sensitivity analysis to explore the effect of individual studies by omitting each one at a time.

RESULTS

Study Identification and Characteristics

A total of nine studies (25–33) involving 1,134 patients were included (search process in **Figure 1**). Among the nine included studies, two studies were subgroup analyses of patients with

TABLE 1 | Characteristics of the included studies.

First author, year	Design	Patients	Interventions	Sedation goals	Outcomes
Hughes et al. (33)	Multicenter, double-blind	422 patients with sepsis	Intervention group: DEX for 0.15 to 1.5 μg/kg·h; Control group: propofol for 5 to 50 μg/kg·h	RASS score of −2 to 0	30-day mortality, 90-day mortality, delirium free days
Cioccari et al. (31)	Multicenter, open-label	83 patients with septic shock	Intervention group: DEX start at 1 μg/kg·h, followed at adjusted dose (maximunm at 1.5 μg/kg·h); Control group: propofol directed by the treating physician	RASS score of −2 to +1	ICU mortality, in-hospital mortality, 90-day mortality, duration of mechanical ventilation, ICU length of stay, incidence of delirium
Liu et al. (32)	Single-center, open-label	200 patients with septic shock	Intervention group: DEX start at a loading dose of 1 μg/kg·h, followed at 0.2 to 0.3 μg/kg·h; Control group: propofol start at a loading dose of 1 mg/kg, followed at 1 to 3 mg/kg·h	RASS score of −2 to 0	28-day mortality, duration of mechanical ventilation, ICU length of stay
Kawazoe et al. (30)	Multicenter, open-label	201 patients with sepsis	Intervention group: DEX start at start at 0.1 μg/kg·h then at 0.1–0.7 μg/kg·h; Control group: propofol at 0–3 mg/kg/h or midazolam at 0–0.15 mg/kg/h	RASS score of −2 to 0	28-day mortality, duration of mechanical ventilation, ICU length of stay, incidence of delirium
Guo et al. (29)	Single-center, open-label	45 patients with septic shock	Intervention group: DEX for 0.2 to 0.7 μg/kg·h; Control group 1: propofol; Control group 2: midazolam;	RASS score of −2 to −1	In-hospital mortality, duration of mechanical ventilation, ICU length of stay
Pandharipande et al. (28)	Multicenter, double-blind	63 patients with sepsis	Intervention group: DEX at a median rate of 0.74 μg/kg·h, max does at 1.5 μg/kg·h; Control group: lorazepam at a median rate of 3 mg/h, max does at 10 mg/h	RASS score of −2 to +1	28-day mortality, ICU length of stay, delirium free days
Tasdogan et al. (27)	Single-center, open-label	40 patients with sepsis	Intervention group: DEX at a loading does of 1 μg/kg for 10 min, followed by 0.2–2.5 μg/kg·h; Control group: propofpl at a loading does of 1 mg/kg·h for 15 min, followed by 1–3 mg/kg·h	RSS≤2	28-day mortality, duration of mechanical ventilation, ICU length of stay, levels of TNF-α and IL-1β
Memis et al. (27)	Single-center, open-label	40 patients with septic shock	Intervention group: DEX at a loading does of 1 μg/kg for 10min, followed by 0.2–2.5 μg/kg·h; Control group: propofpl at a loading does of 1 mg/kg·h for 15 min, followed by 1–3 mg/kg·h	RSS≤2	ICU mortality, ICU length of stay
Memis et al. (27)	Single-center, open-label	40 patients with sepsis	Intervention group: DEX at a loading does of 1 μg/kg for 10 min, followed by 0.2–2.5 μg/kg·h; Control group: midazolam at a loading does of 0.2 mg/kg·h for 10 min, followed by 0.1–0.5 mg/kg·h	RSS < 2	ICU mortality, levels of TNF-α and IL-1β

DEX, dexmedetomidine; RASS, Richmond agitation sedation scale; RSS, Ramsay sedation score.

sepsis from previous RCTs [Cioccari et al. (31) performed a subgroup analysis of the SPICE III trial (34), Pandharipande et al. (28) performed a subgroup analysis of the MENDS trial (35)]. **Table 1** shows the characteristics of the included studies. Five trials used dexmedetomidine in patients with sepsis (25, 27, 28, 30, 33), and four in patients with septic shock (26, 29, 31, 32). Different control drugs were also identified: propofol in six arms (26, 27, 29, 31–33), midazolam in two arms (25, 29), lorazepam in

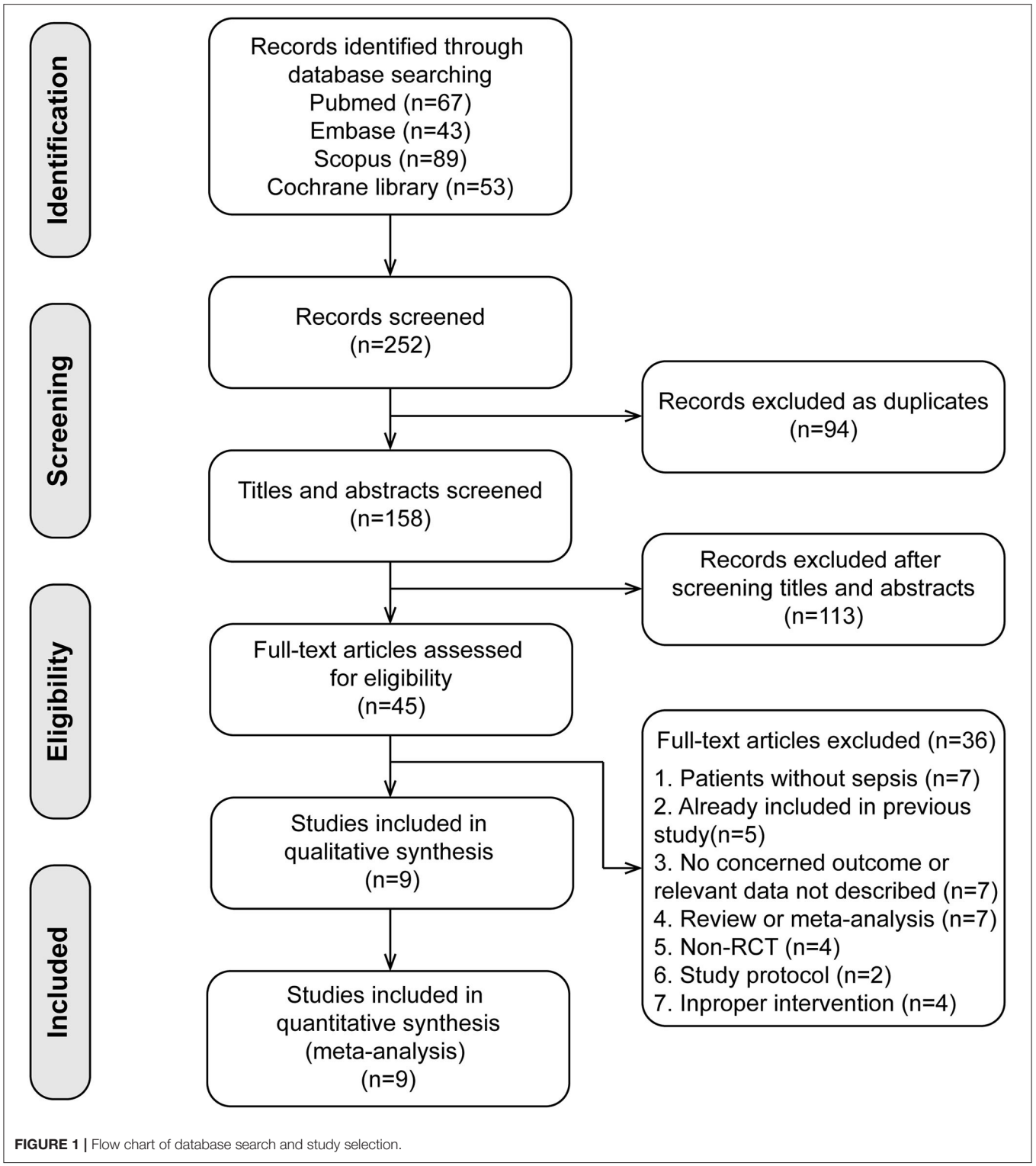

FIGURE 1 | Flow chart of database search and study selection.

one arm (28), and propofol plus midazolam in one arm (30). Five studies (25–27, 31, 32) used a loading dose of dexmedetomidine at 1 μg/kg·h, the other four studies (28–30, 33) used infusion dose of dexmedetomidine between 0.1 to 1.5 μg/kg·h. The target sedation goals were Richmond Agitation Sedation Scale of −2 to 0 in three trials (30, 32, 33), −2 to 1 in two trials (28, 31), −2 to −1 in one trial (29), and Ramsay Sedation Score <2 in three trials (25–27).

Other detailed information of included studies (e.g., study design, sex ratio, age, APACHE II and SOFA score at trial enrollment, definitions of sepsis or septic shock) were reported in **Supplementary Material 3**.

Quality Assessment

The quality assessment by the Cochrane risk of bias tool was summarized in **Figure 2**. Seven studies (25–27, 29–32) had a high risk of bias because they were open-label trials. Three studies (25, 29, 32) did not report the details of random sequence generation and allocation concealment. The blinding method for outcome assessment was not reported in five studies (25, 26, 29, 31, 32), which would either underestimate or overestimate the size of the effect. Moreover, in the trial by Guo et al. (29), the doses of control sedative drugs were not reported. The details for quality assessment and reason for judgment were recorded in **Supplementary Material 3**.

We used the funnel plot and Egger's test to assess the publication bias (**Supplementary Material 4**), the results showed there was a potential risk of publication bias for the overall mortality and length of ICU stay (Egger's test, $P < 0.01$). Thus, we performed an additional analysis by using the trim and fill method. The imputed studies produced a symmetrical funnel plot and the analysis after imputing continued to show no association between the use of dexmedetomidine and overall mortality (RR 1.05, 95%CI 0.91 to 1.22 $P = 0.67$, $I^2 = 32\%$), but a shortened length of ICU stay was observed (MD −2.42, 95%CI −4.15 to –0.68, $P < 0.01$, $I^2 = 75\%$).

Primary Outcome

All studies involving 1,134 patients reported mortality (two studies reported the ICU mortality, four studies reported the 28/30-day mortality, one study reported the in-hospital mortality, one study reported the 30-day and 90-day mortality, one study reported the ICU, in-hospital, and 90-day mortality). The risk for overall mortality was similar between dexmedetomidine and control regimens without significant heterogeneity (RR 0.97; 95%CI 0.82 to 1.13, $P = 0.67$, $I^2 = 25\%$; **Table 2**, **Figure 3**). Similarly, the use of dexmedetomidine had no significant effect on the ICU mortality, 28/30-day mortality, in-hospital mortality, and 90-day mortality (**Table 2**, **Supplementary Material 5**).

We performed predefined subgroup analyses stratified by population (sepsis or septic shock) and control drug (propofol or others). Compared with propofol, the use of dexmedetomidine does not affect the overall mortality (RR 1.01, 95%CI 0.86 to 1.20, $P = 0.87$, $I^2 = 4\%$; **Figure 4A**). However, a trend toward the reduction of all-cause mortality by dexmedetomidine when compared with other sedations (RR 052, 95%CI 0.25 to 1.06, $P = 0.07$, $I^2 = 0\%$; **Figure 4A**) was observed, although it was not statistically significant. The use of dexmedetomidine had no different effect on mortality for patients with sepsis or septic shock (**Figure 4B**). Moreover, the sensitivity analysis by removing each trial showed similar results to the overall analysis, indicating good robustness (**Supplementary Material 5**).

Secondary Outcomes

A total of seven studies with eight cohorts reported the length of ICU stay and there was no significant difference between patients who received dexmedetomidine and other sedative drugs (MD −1.12, 95%CI −2.89 to 0.64, $P = 0.21$, $I^2 = 71\%$; **Table 2**, **Figure 5A**). The overall analysis from five studies (six cohorts) showed that the use of dexmedetomidine was associated with a slight reduction in the duration of MV (MD −0.53, 95%CI −0.85 to −0.21, $P = 0.001$, $I^2 = 0\%$; **Table 2**, **Figure 5B**). However, since the significant heterogeneity, this result should be interpreted prudently.

In the subgroup analysis, compared with propofol, the use of dexmedetomidine was associated with the shortened duration of MV, but no significant difference in the length of ICU stay. Furthermore, the population (sepsis or septic shock) has no significant effect on the length of ICU stay. The positive effect of dexmedetomidine in reducing the duration of MV became not significant in the sepsis subgroup (**Table 2**, **Supplementary Material 5**). The sensitivity analysis by removing each trial showed no significant difference in the length of ICU stay, indicating good robustness. The positive effect of dexmedetomidine in reducing the duration of MV became not significant after omitting the study by Liu et al. (32) (**Supplementary Material 5**).

Two studies reported the serum levels of inflammatory markers including the tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) after 24 h of treatment. In the group of patients receiving dexmedetomidine, the serum levels of TNF-α and IL-1β after 24 h of treatment were significantly lower than that in the control group (TNF-α: MD −5.27, 95%CI −7.99 to −2.54, $P < 0.001$, $I^2 = 0\%$; IL-1β: MD −1.25, 95%CI −1.91 to −0.59, $P < 0.001$, $I^2 = 0\%$; **Table 2**, **Figure 5C**).

In addition, four studies reported the incidence of delirium or the delirium-free days. The results indicated that the use of dexmedetomidine had no significant effect on the incidence of delirium (RR 0.95, 95%CI 0.72 to 1.25, $P = 0.70$, $I^2 = 0\%$; **Table 2**, **Figure 6A**) and delirium free days (MD 1.76, 95%CI −0.94 to 4.47, $P = 0.20$, $I^2 = 80\%$; **Table 2**, **Figure 6B**).

Furthermore, there were no differences in the use of vasopressor, the incidence of hypotensive, and mean arterial pressure during the study period between the dexmedetomidine and control groups (**Supplementary Material 6**).

DISCUSSION

In this meta-analysis, we systematically and comprehensively reviewed nine studies with 1,134 patients to evaluate the effect of dexmedetomidine for mechanically ventilated patients with sepsis. Overall, compared with other sedation, the use of dexmedetomidine does not affect the overall mortality, length of ICU stay, incidence of delirium, or the delirium-free days. We found that patients with sepsis receiving dexmedetomidine had a shortened duration of MV, limited evidence suggested that the use of dexmedetomidine was associated with a lower level of TNF-α and IL-1β.

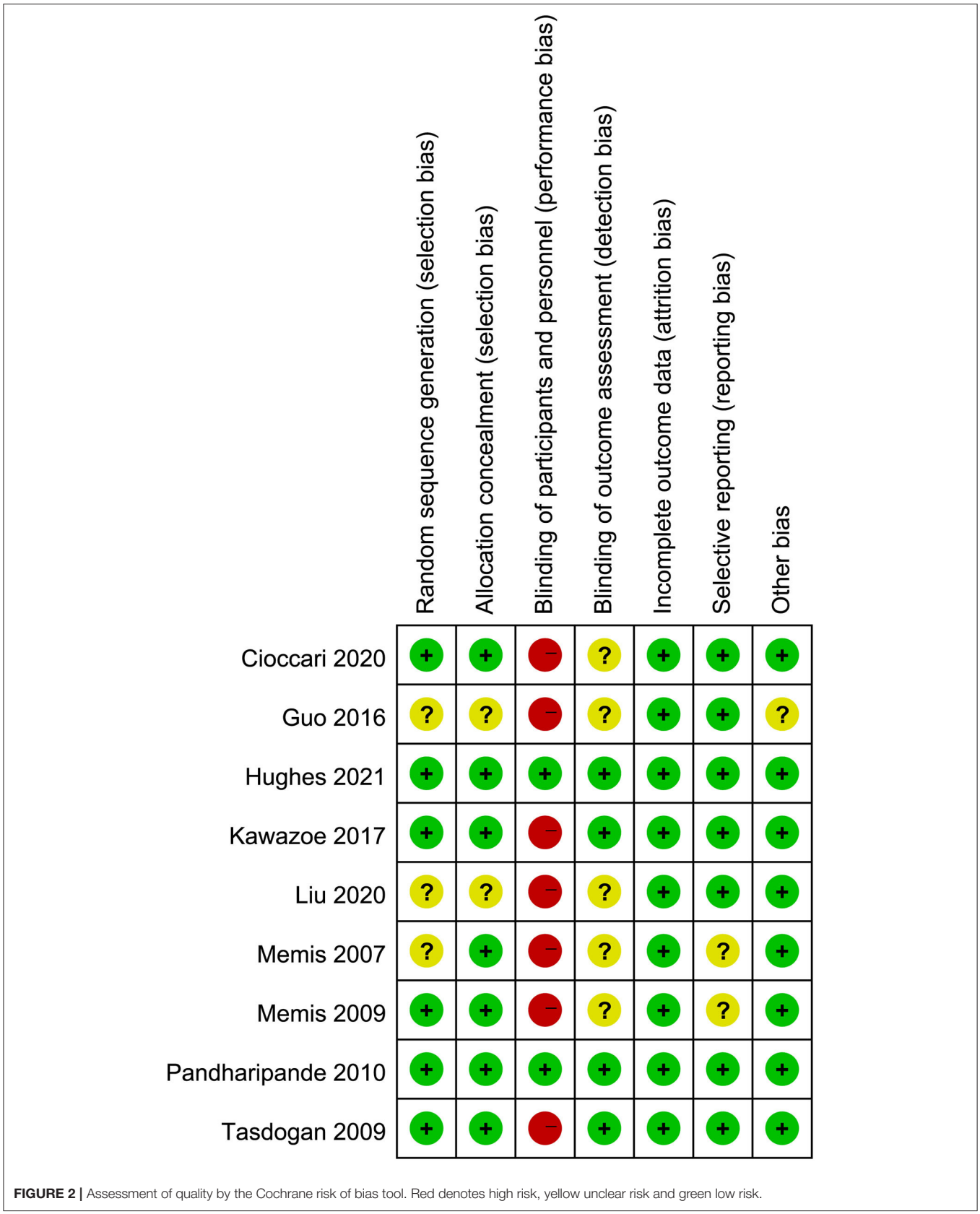

FIGURE 2 | Assessment of quality by the Cochrane risk of bias tool. Red denotes high risk, yellow unclear risk and green low risk.

TABLE 2 | Primary and secondary outcomes of this meta-analysis.

Outcome	N	Result
Overall mortality	9	RR 0.97, 95%CI 0.82 to 1.13, $P = 0.67$, $I^2 = 25\%$
ICU mortality	3	RR 0.60, 95%CI 0.30 to 1.21, $P = 0.15$, $I^2 = 0\%$
28/30-day mortality	5	RR 1.00, 95%CI 0.84 to 1.18, $P = 0.98$, $I^2 = 56\%$
In-hospital mortality	2	RR 0.74, 95%CI 0.38 to 1.45, $P = 0.37$, $I^2 = 0\%$
90-day mortality	2	RR 0.94, 95%CI 0.75 to 1.18, $P = 0.67$, $I^2 = 29\%$
Length of ICU stay	8	MD −1.12, 95%CI −2.89 to 0.64, $P = 0.21$, $I^2 = 71\%$
Duration of MV	6	MD −0.53, 95%CI −0.85 to −0.21, $P = 0.001$, $I^2 = 0\%$
Inflammatory response		
TNF-α	2	MD −5.27, 95%CI −7.99 to −2.54, $P < 0.001$, $I^2 = 0\%$
IL-1β	2	MD −1.25, 95%CI −1.91 to −0.59, $P < 0.001$, $I^2 = 0\%$
Incidence of delirium	2	RR 0.95, 95%CI 0.72 to 1.25, $P = 0.70$, $I^2 = 0\%$
Delirium free days	2	MD 1.76, 95%CI −0.94 to 4.47, $P = 0.20$, $I^2 = 80\%$

N, number of studies; ICU, intensive care unit; RR, risk ratio; CI, confidence interval; MV, mechanical ventilation; MD, mean difference; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β.

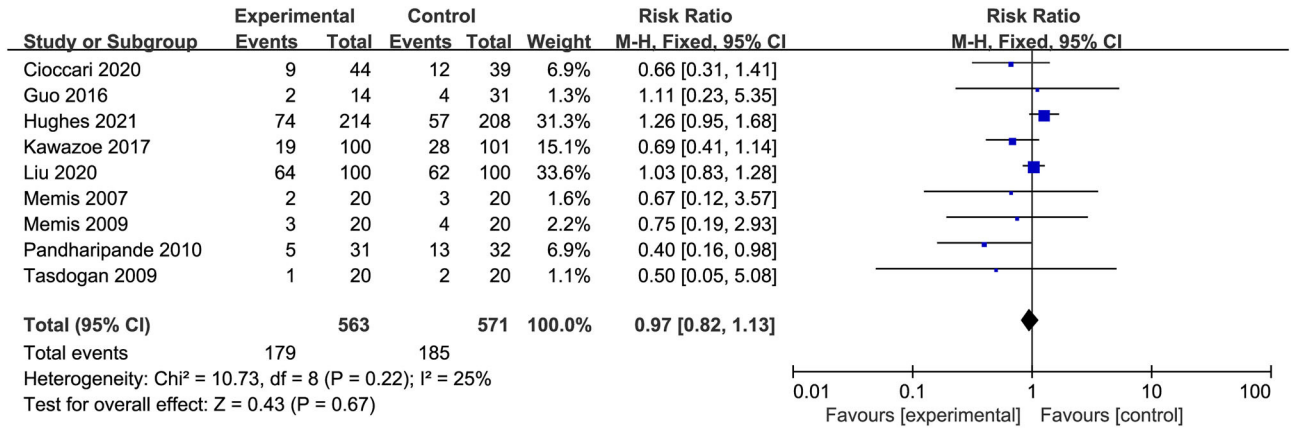

FIGURE 3 | Forest plot comparing the effect of DEX on mortality.

Moreover, we found survival benefits in comparison with other sedations including lorazepam and midazolam, but not with propofol.

There are three previous systematic reviews and meta-analyses (36–38) comparing the effect of dexmedetomidine vs. other sedative agents on clinical outcomes of patients with sepsis. The results of previous studies showed that compared with other sedatives, the use of dexmedetomidine had beneficial effects on 28-day mortality, but there were no significant differences in the length of ICU stay and duration of MV (36–38). Our meta-analysis indicated that the use of dexmedetomidine was associated with the shortened duration of MV for mechanically ventilated patients with sepsis, but no significant differences in the overall mortality. These differences may result from several newly published RCTs. In contrast to previous meta-analyses, our research updated the findings by including recently published RCTs (31–33), especially the MENDS II trial. In this pragmatic RCT involving 400 patients from 13 medical centers, there are many important methodologic advances including a higher degree of trial drug allocation concealment and blinding, better separation between groups concerning sedative exposure, and stricter adherence to light sedation approaches (33). The increased trials and number of patients could provide more robust results. Moreover, in the subgroup analyses, we found survival benefits in comparison with lorazepam, not with propofol.

Dexmedetomidine could promote macrophage phagocytosis and bactericidal killing further enhancing the mucosal immunity and bacterial clearance, which are of great importance for patients with sepsis and septic shock. Previous research has shown that dexmedetomidine had potential anti-inflammatory effects in both animal and human studies (39). Results of our meta-analysis also suggest that after 24 h of receiving dexmedetomidine, the levels of TNF-α and IL-1β were significantly lower than the control group. This anti-inflammatory effect of dexmedetomidine can suppress the exaggerated production of inflammatory cytokines in septic shock (4, 40, 41).

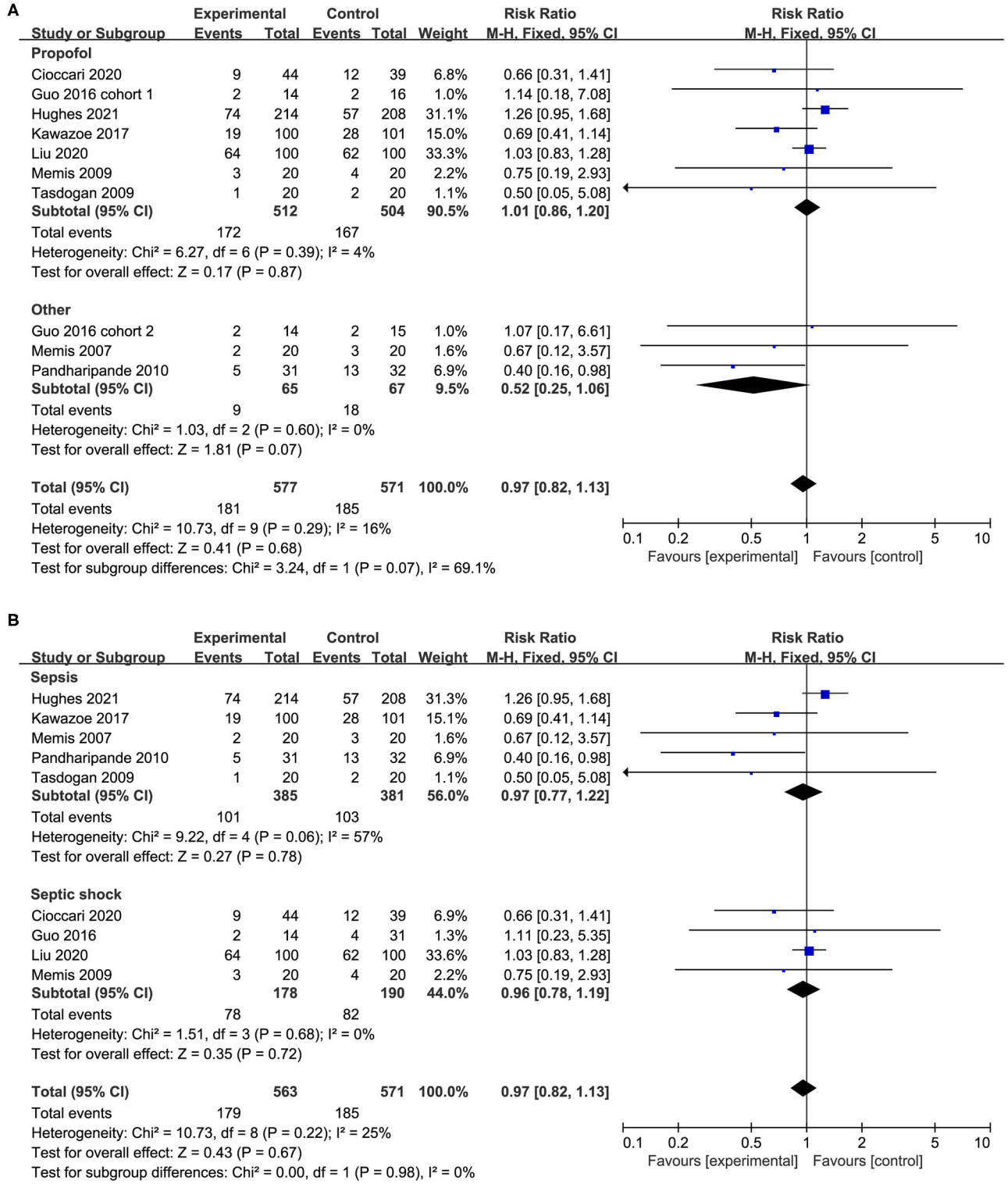

FIGURE 4 | Subgroup analysis for mortality **(A)** propofol vs. other sedation, **(B)** sepsis vs. septic shock.

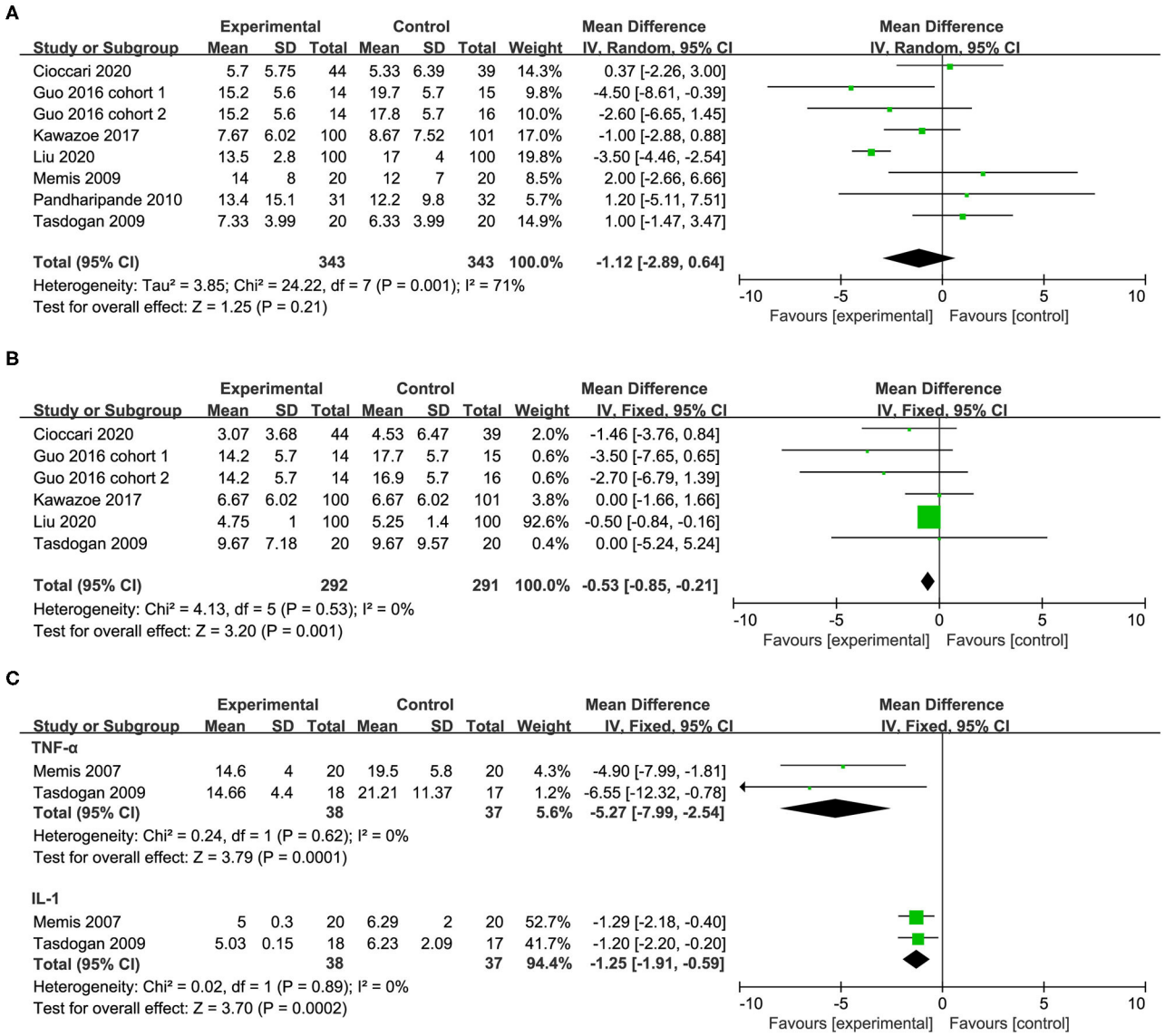

FIGURE 5 | Forest plot comparing the effect of DEX on **(A)** length of ICU stay, **(B)** duration of MV, **(C)** inflammatory response.

The strengths of our study include the comprehensive and up-to-date search strategies, specific and targeted inclusion criteria, comprehensive and rigorous analytical methods. Three studies included in our meta-analysis are recently published RCTs (31–33) with large populations. The predefined subgroup analysis found survival benefits of dexmedetomidine in comparison with other sedations including lorazepam and midazolam.

However, our meta-analysis also had several limitations. First of all, the main limitation is the limited number of included studies and the small sample size. Six of the included trials are typically characterized as small studies because the sample size was smaller than 100 patients, which may lead to small study effect bias (42). Second, the diagnostic criteria for sepsis or septic shock, the dose of the sedative drug, and target sedation goals were varied among included studies. These factors may cause clinical heterogeneity. Third, the follow-up duration in most included studies was relatively short, only two studies reported 90-day mortality as the long-term outcome. More RCTs with long follow-ups were necessary to demonstrate the effects of dexmedetomidine on long-term outcomes. Last but not the least, in some included studies, the dexmedetomidine was combined with other sedative agents including opioids or benzodiazepines in the intervention

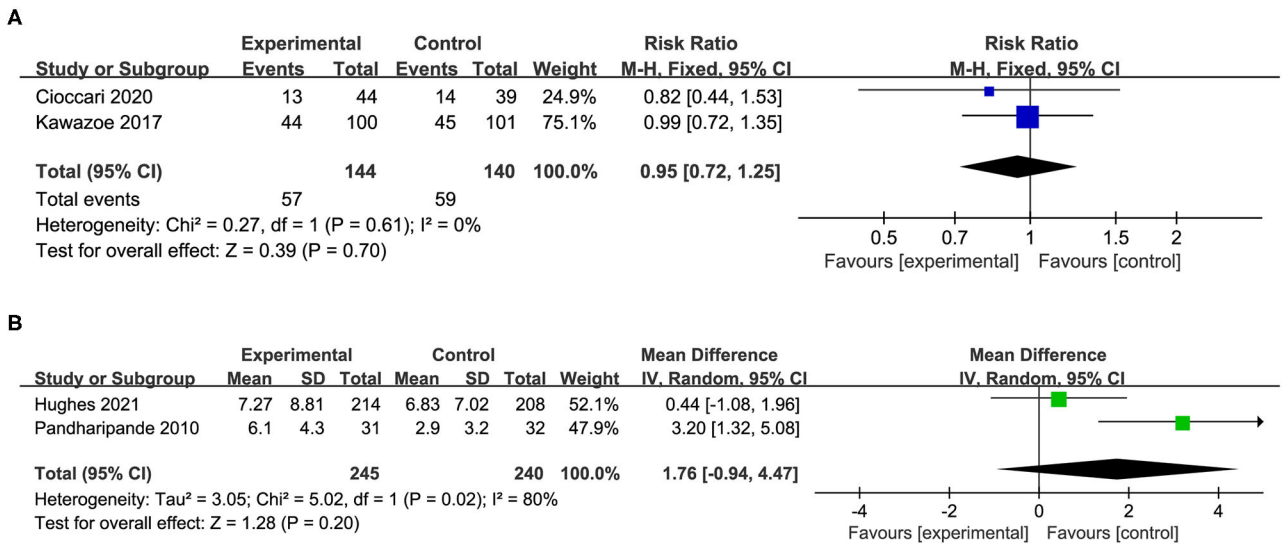

FIGURE 6 | Forest plot comparing the effect of DEX on **(A)** incidence of delirium, **(B)** delirium free days.

group for sedation. Therefore, the actual efficacy of single administration with dexmedetomidine for patients with sepsis requires further validation.

CONCLUSION

In conclusion, our meta-analysis suggests that for patients with sepsis or septic shock, the use of dexmedetomidine has no effect on all-cause mortality and length of ICU stay, but may offer advantages in terms of reducing the duration of mechanical ventilation and inflammatory response. However, considering the significant heterogeneity and the limited number of included studies with small sample sizes. Well-designed, multicenter RCTs with a large sample size are needed to further evaluate the effect of dexmedetomidine on short-term and long-term clinical outcomes for patients with sepsis and to compare its effects with other sedative agents.

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

CW contributed to the acquisition and analysis of the data, the initial draft writing of this paper, and the final approval of the version to be published. QC, PW, and WJ contributed to the collection and interpretation of data. CZ, ZG, and KX contributed to the concept of the review and the revision of this paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.776882/full#supplementary-material

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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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Using RNA-Seq to Investigate Immune-Metabolism Features in Immunocompromised Patients With Sepsis

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Objective: Sepsis is life threatening and leads to complex inflammation in patients with immunocompromised conditions, such as cancer, and receiving immunosuppressants for autoimmune diseases and organ transplant recipients. Increasing evidence has shown that RNA-Sequencing (RNA-Seq) can be used to define subendotype in patients with sepsis; therefore, we aim to use RNA-Seq to identify transcriptomic features among immunocompromised patients with sepsis.

Methods: We enrolled patients who were admitted to medical intensive care units (ICUs) for sepsis at a tertiary referral centre in central Taiwan. Whole blood on day-1 and day-8 was obtained for RNA-Seq. We used Gene Set Enrichment Analysis (GSEA) to identify the enriched pathway of day-8/day-1 differentially expressed genes and MiXCR to determine the diversity of T cell repertoire.

Results: A total of 18 immunocompromised subjects with sepsis and 18 sequential organ failure assessment (SOFA) score-matched immunocompetent control subjects were enrolled. The ventilator-day, ICU-stay, and hospital-day were similar between the two groups, whereas the hospital mortality was higher in immunocompromised patients than those in immunocompetent patients (50.0 vs. 5.6%, $p < 0.01$). We found that the top day-8/day-1 upregulated genes in the immunocompetent group were mainly innate immunity and inflammation relevant genes, namely, *PRSS33, HDC, ALOX15, FCER1A*, and *OLR1*, whereas a blunted day-8/day-1 dynamic transcriptome was found among immunocompromised patients with septic. Functional pathway analyses of day-8/day-1 differentially expressed genes identified the upregulated functional

biogenesis and T cell-associated pathways in immunocompetent patients recovered from sepsis, whereas merely downregulated metabolism-associated pathways were found in immunocompromised patients with septic. Moreover, we used MiXCR to identify a higher diversity of T cell receptor (TCR) in immunocompetent patients both on day-1 and on day-8 than those in immunocompromised patients.

Conclusions: Using RNA-Seq, we found compromised T cell function, altered metabolic signalling, and decreased T cell diversity among immunocompromised patients with septic, and more mechanistic studies are warranted to elucidate the underlying mechanism.

Keywords: RNA-Seq, sepsis, immunocompromised, immune, metabolism, pathway analyses

BACKGROUND

Sepsis, characterised by a dysregulated host-pathogen inflammation, is a leading cause of death worldwide, and approximately 5.3 million people died from sepsis annually (1). There is a global increase of patients with distinct immunocompromised conditions due to the advance of cancer treatment, increasing biologics, and immunosuppressants for autoimmune diseases and growing organ transplant recipients (2), and it has been estimated that patients with immunocompromised conditions account for approximately 35% of all intensive care unit (ICU) admissions (3). Therefore, there is a crucial need to explore the alternation of immunological responses in sepsis among patients with immunocompromised conditions.

Immunoparalysis, characterised by not only immunologic but also by metabolic dysregulation after sepsis, has been increasingly recognised and attributed to be one of the key biological bases of prolonged impact on long-term mortality in patients with sepsis (4, 5). Recent advances in sequencing technology, such as RNA-Sequencing (RNA-Seq), immune repertoire sequencing, and single-cell RNA-Seq (sc-RNA-Seq), allow for addressing immunological and metabolic features in patients with sepsis and coronavirus disease 2019 (COVID-19) infection (6–8). Several studies have used MicroArray and RNA-Seq to identify the transcriptomic signature, so-called subendotype, in patients with sepsis, and the currently identified sepsis-associated subendotypes included early improvement of organ dysfunction after sepsis and the responsiveness of steroids in patients with a septic shock (6, 9, 10). Additionally, analytic tools, namely, MiXCR, have been recently developed to investigate the diversity of T cell receptor (TCR) using bulk RNA-Seq data (11, 12). Despite increasing studies to explore transcriptomic features of sepsis, the transcriptomic signature of sepsis among immunocompromised patients remains unclear. In the present study, we used RNA-Seq to identify distinct transcriptomic features and MiXCR to explore the diversity of TCR between immunocompromised and severity-matched immunocompetent patients with sepsis.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Institutional Review Board approval of the Taichung Veterans General Hospital (CE20069B). Informed consent was obtained from all participants prior to the enrolment in the study and collection of blood samples.

Definition of Patients With Immunocompromised Conditions

Patients were defined as immunocompromised if they had at least one of the following conditions, such as patients receiving immunosuppressive therapy due to autoimmune disease, organ transplant recipient, and patients with active malignant disease consisting of active haematological disease, or solid tumour under therapy (13).

Enrolment of Subjects

We enrolled 18 immunocompromised patients and 18 severity-matched (day-1 sequential organ failure assessment (SOFA) score ± 1) immunocompetent patients who were admitted to the medical ICUs between March 2020 and February 2021 at Taichung Veterans General Hospital, a tertiary referral centre in central Taiwan, for sepsis and extracted blood samples on day-1 and day-8 after the ICU admission. Patients who did not survive until day-8 were not included for analyses; therefore, each enrolled patient for analyses had paired day-1 and day-8 samples in this study. In the present study, we used PAXgene Blood RNA Tube to collect blood samples from enrolled subjects. Clinical variables, such as comorbidities, severity scores (day-1 SOFA score, day-3 SOFA score, day-7 SOFA score, and Acute Physiology and Chronic Health Evaluation (APACHE) II score), laboratory data, and outcomes (ventilator-day, ICU-stay, hospital-stay, and overall mortality) were recorded.

RNA Preparation and Sequencing

We used PAXgene Blood RNA Kit to extract RNA, and the average RNA integrity number (RIN) was 8.31 ± 0.58 in the present study. The library was constructed in accordance with

the manufacturer's instruction, and 800–1,000 ng fragmented RNA was used for further experiments. The RNA-Seq was conducted on the NovaSeq platform (Illumina, San Diego, CA, USA), with at least 50–60 million 150-bp pair-end reads per sample.

Bioinformatics Analyses

The quality of sequencing was good, and Phred scores 30 was applied, and sequence reads were mapped to the human reference genome GRCh38 by HISAT2 (14). Read counts were calculated by featureCounts (15), and the differentially expressed genes were identified by R package DEseq2 (16). The average mapped rate and read counts were 82.3 ± 7.8% and 104.0 ± 43.6 million reads, respectively. Gene Set Enrichment Analysis (GSEA) was used for functional annotation of the whole differentially expressed genes (17), and the visualised enrichment map was performed by using Cytoscape 3.8.2 (18). In brief, we used an enrichment map, which organise gene sets into a similarity network, to visualise GSEA results, and the node, link, and node colour represent gene-set, the overlap of member gene, and enrichment score, respectively (19).

Diversity of TCR Analyses

Raw sequences from RNAseq were conducted into MiXCR v3.0.13 (11, 12) to quantitate the clonotypes of patients with sepsis. After obtaining the quantitated clonotypes, VDJTools v 1.2.1 (20) was used to calculate the sample diversity and counts of complementarity-determining region-3 (CDR3).

Statistical Analyses

Continuous variables were presented as median (interquartile range), and data for categorical variables were shown as numbers (percentages). The differences between the immunocompromised and immunocompetent groups were analysed by the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. Statistical analyses were two-sided, and the level of significance was set at 0.05 for clinical data. Data analyses were conducted using R version 4.0.2.

RESULTS

Patient Characteristics

A total of 36 patients with sepsis were enrolled, consisting of 18 immunocompromised patients and 18 day-1 SOFA score-matched immunocompetent control subjects. Of the 18 immunocompromised patients, the number of subjects with solid tumour receiving therapy, haematological cancer, those with autoimmune disease underwent immunosuppressants, and renal transplant recipients were 3, 6, 7, and 2, respectively. The comorbidities and laboratory data were comparable between the two patient groups. With respect to the outcome, the ventilator-day, ICU-stay, and hospital-day were similar between the two groups, whereas the hospital mortality was much higher in immunocompromised patients compared to those in immunocompetent patients (50.0 vs. 5.6%, $p < 0.01$; **Table 1**; refer to **Supplemental Table 1** for details with regards to pathogens and infection sites).

TABLE 1 | Characteristics of the enrolled immunocompromised and severity-matched immunocompetent critically ill patients with septic.

	Immunocompromised $n = 18$	Immunocompetent $n = 18$	*p*-value
Demographic data			
Age (years)	58.0 (50.3–68.3)	71.0 (57.5–81.5)	0.06
Sex (female)	9 (50.0%)	6 (33.3%)	0.50
Immunocompromised factors			NA
Solid tumour receiving therapy	4 (22.2%)	NA	
Haematological cancer	5 (27.8%)	NA	
Autoimmune disease	7 (38.9%)	NA	
Renal transplant recipient	2 (11.1%)	NA	
Severity scores			
APACHE II score	28.0 (23.0–31.3)	26.0 (22.5–29.5)	0.32
SOFA score, day-1	11.0 (8.5–15.0)	12.0 (8.0–13.3)	1.00
SOFA score, day-3	11.0 (7.0–12.3)	10.0 (5.5–13.0)	0.66
SOFA score, day-7	6.5 (3.8–11.0)	7.0 (3.0–12.0)	0.91
Comorbidities			
Diabetes mellitus	5 (27.8%)	10 (55.6%)	0.18
Congestive heart failure	1 (5.6%)	1 (5.6%)	1.00
COPD	2 (11.1%)	1 (5.6%)	0.50
End-stage renal disease	4 (22.2%)	1 (5.6%)	0.34
Cerebral vascular disease	0 (0%)	4 (22.2%)	0.10
Laboratory data			
White blood cell counts (/ml)	11,445.0 (7640.0–13077.5)	11,175.0 (9342.5–12872.5)	0.89
Platelet count (1000/ml)	41.0 (16.5–145.3)	77.5 (44.5–150.0)	0.12
Creatinine (mg/dl)	1.3 (0.8–4.7)	2.1 (0.8–3.1)	0.91
Albumin (mg/dl)	2.8 (2.5–3.2)	3.1 (2.9–3.4)	0.14
Lactate (mg/dl)	29.0 (19.4–51.0)	39.6 (21.1–73.6)	0.30
C-reactive protein (mg/dl)	11.7 (5.1–26.9)	8.2 (3.4–21.1)	0.56
Outcome			
Ventilator-day, days	12.0 (8.8–25.3)	12.5 (7.8–18.0)	0.54
ICU-stay, days	14.0 (9.5–25.3)	14.5 (11.8–27.5)	0.74
Hospital day	33.5 (14.8–47.8)	28.5 (22.8–41.3)	0.96
Mortality	8 (44.4%)	2 (11.1%)	0.03

Data were presented as median (interquartile range) and number (percentage). APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit.

Distinct Dynamic Transcriptome in Immunocompetent and Immunocompromised Patients With Sepsis

Principal component analysis was performed on the top 500 variable genes across samples, and we found that the transcriptome was similar on day-1 among immunocompromised and immunocompetent patients (**Figure 1A**). We found that the separation between day-1 and day-8 was apparently better in the immunocompetent group than those in the immunocompromised group, suggesting the regulated recovery from sepsis in the immunocompetent group (**Figures 1B,C**). We then compared gene-expression profiles between day-8 and day-1 in the immunocompromised and immunocompetent groups through using the criteria with $p < 0.01$ and log fold change >0.25 or <-0.25 to define differential expression genes (DEGs). In the immunocompetent group, there were 978 DEGs (362 upregulated and 616 downregulated genes), whereas merely 368 DEGs (200 upregulated and 168 downregulated genes) in the immunocompromised group. We found that the top upregulated genes in the immunocompetent group were mainly innate immunity and inflammation relevant genes, namely, *PRSS33, HDC, ALOX15, FCER1A*, and *OLR1*, whereas a significant downregulation was found in *PCSK9*, which has been implicated with increased clearance of endogenous lipids and bacteria among patients with septic with day-3 functional improvement after resuscitation (6) (**Figure 1D**). In contrast with the highly dynamic transcriptome in immunocompetent patients with septic, we found a relatively blunted dynamic transcriptome among immunocompromised patients with septic (**Figure 1E**).

Enriched Pathway of the Dynamic Transcriptome in Immunocompetent Patients With Sepsis

To illustrate the alteration of biological pathways in the immunocompetent group, we used GSEA and a functional map to illustrate the enriched pathway. In brief, the identified gene ontology (GO) terms were mapped as a network of gene sets (nodes) related by mutual overlap (edges), and the red and blue nodes represent up and downregulated pathways, respectively. We identified a total of 11 clusters with significant over-represented GO terms using normalised $p < 0.005$ (**Figure 2**; **Supplemental Table 2**). We noted that ribosome RNA (rRNA) relevant pathways, such as rRNA biogenesis and metabolic process, were activated, indicating the potential cellular proliferation and tissue repair among immunocompetent patients with septic in the recovery from sepsis. In contrast, autophagy-related pathways, such as macroautophagy, autophagosome organisation, and vesicle budding from the membrane, were downregulated among immunocompetent patients with septic, implicating the resolution of inflammation result from sepsis. Notably, we identified the enriched upregulation of T cell-related pathways, such as T cell selection, positive alpha/beta T cell lineage commitment, and T cell lineage commitment, and the finding indicated the functional T cell immunity in immunocompetent patients was recovered from sepsis. We further used the Reactome to validate our finding in T cell-associated pathway and found an enriched interferon-gamma signalling pathway in which the leading edge genes consisted of *HLA-DRB1*, *HLA-DPB1, HLA-DPA1, HLA-DRB5, HLA-DQB1*, and *HLA-DRA* (**Supplemental Table 3**). Collectively, the functional pathway analyses identified the upregulated biogenesis and functional T cell signalling in immunocompetent patients recovered from sepsis.

Enriched Pathway of the Dynamic Transcriptome in Immunocompromised Patients With Sepsis

We then explored the alteration of the pathway in immunocompromised patients with septic. Similar to the aforementioned limited differentially expressed genes, we identified merely four downregulated pathways, and no upregulated pathway can be enriched. In contrast with the aforementioned upregulated T cell and metabolic process, we found downregulated antigen processing, antigen presentation, and metabolism-associated pathways in immunocompromised patients clinically recovered from sepsis on day-8 (**Figure 3**; **Supplemental Tables 4, 5**). In detail, the enriched downregulated metabolism-associated pathways included the tricarboxylic acid (TCA) cycle, aerobic respiration, respiratory electron transport chain, and cellular respiration pathway. These results showed an aberrantly blunted immunological pathways and impaired metabolism status among immunocompromised patients clinically recovered from sepsis.

MiXCR to Assess the Diversity of TCR

Given that we found the distinct T cell-associated pathway between immunocompetent and immunocompromised patients recovered from sepsis, we then employed MiXCR to determine the diversity of TCR through using the number of unique CDR3, Shannon's diversity index, and inverse Simpson index (**Figure 4**). We found the day-1 number of unique CDR3 tended to be higher in immunocompetent patients than that in immunocompromised patients. Unlike the mildly increased number of unique CDR3 on day-8 among immunocompetent patients, the number of unique CDR3 in immunocompromised patients remained low (**Figure 4A**). We then used Shannon's diversity index to quantify the diversity of unique CDR3 and found a significantly higher diversity of TCR in immunocompetent patients both on day-1 and on day-8 (**Figure 4B**). We also used the inverse Simpson index, which reflects abundant clonotypes to assess the diversity of unique CDR3 and found a similar trend with a significantly decreased diversity of TCR in immunocompromised, particularly on day-8 (**Figure 4C**).

DISCUSSION

In the present study, we used RNA-Seq, analysed by functional pathway analyses and MiXCR, to identify immunological

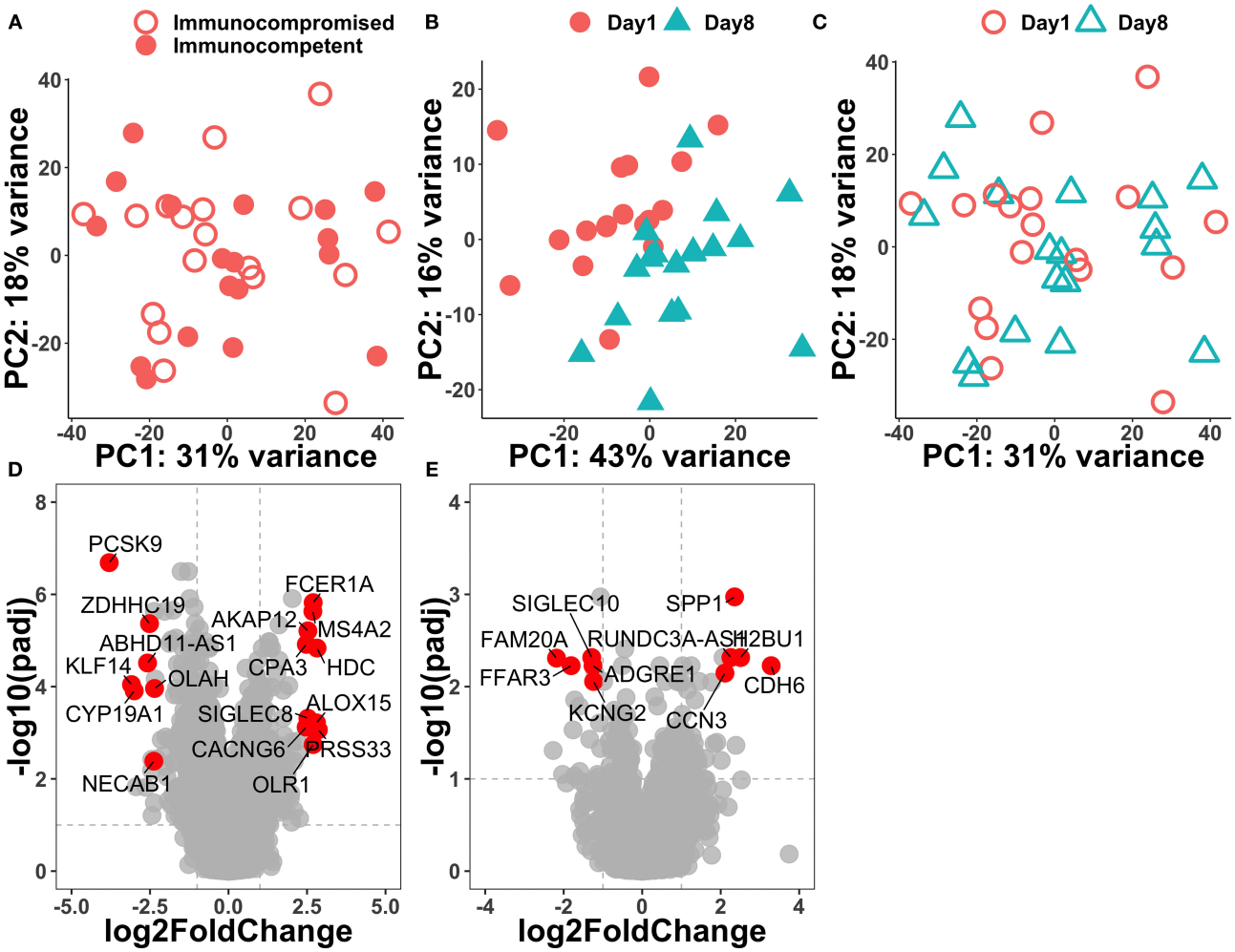

FIGURE 1 | Principal component analysis of day-1 transcriptome among all of the enrolled patients with septic **(A)** and volcano plots of differentially expressed genes between day-1 and day-8 in immunocompetent **(B,D)** and immunocompromised **(C,E)** patients with sepsis.

and metabolic features in immunocompromised patients with sepsis. We found impaired T cell-associated signalling, altered metabolic signalling, and decreased diversity of TCR among immunocompromised patients with septic. The aforementioned immunological and metabolic features indicate a dysregulated recovery in patients with septic with immunocompromised conditions, such as solid cancer underwent therapy, active haematological cancer, and receiving immunosuppressants, due to autoimmune disease or renal transplant recipient. These findings demonstrate the application of RNA-Seq to characterise the subendotype with immunoparalysis in patients with septic, particularly among immunocompromised patients.

The WHO recognises sepsis as a global health priority in 2017 (21), and it is estimated that that sepsis affects 48.9 million patients (38.9–62.9) worldwide and accounts for 19.7% (18.2–21.4) of all global deaths in 2017 (22). Immunocompromised conditions have been found to affect the outcome of sepsis substantially; however, the underlying mechanism remains elusive due to the complexity and high heterogeneity of sepsis (23, 24). The RNA-Seq has been increasingly used to address complex diseases, namely, sepsis, which disrupts the pathophysiological homeostasis result from complex, numerous, intertwined, and subcellular biological events (25). Several studies have used RNA-Seq to identify the subendotype in sepsis, and the identification of subendotype should be essential for individualised medicine in patients with sepsis (26). Antcliffe et al. recently conducted a *post-hoc* transcriptomic analysis among 176 patients in VANISH trial which aimed to explore the mortality impact of vasopressors and corticosteroid in patients with a septic shock and reported two transcriptomic sepsis response signatures (SRSs), such as relatively immunosuppressed signature (SRS-1) and relatively immunocompetent signature (SRS-2) (9). Notably, Antcliffe et al. found that the use of corticosteroids was associated with increased 28-day mortality in patients with septic with SRS-2, whereas the mortality was independent of the use of corticosteroids in patients with septic with SRS-1 (9).

In the present study, the upregulated T cell and Human Leukocyte Antigen (HLA)-associated pathway in immunocompetent patients with septic should be consistent

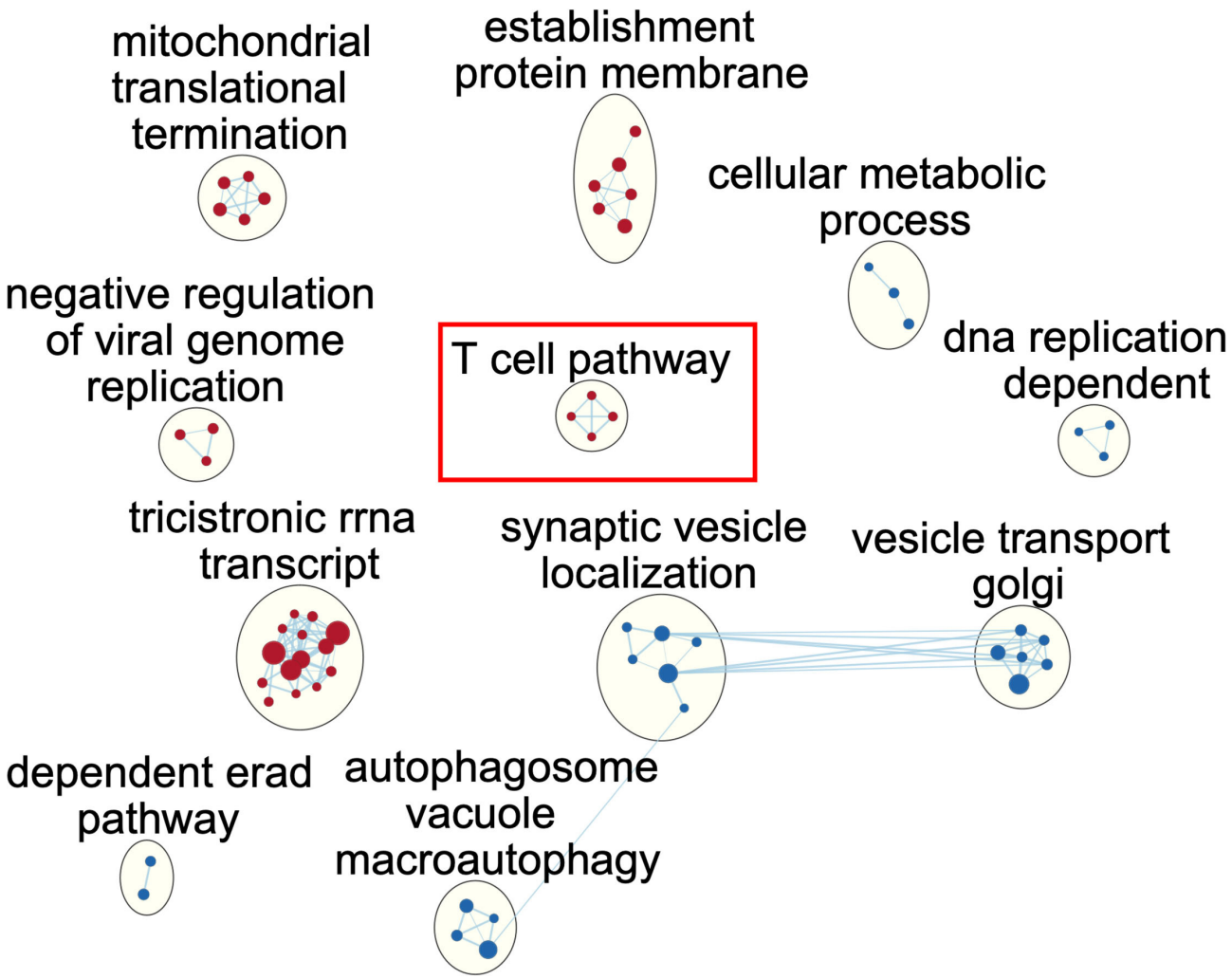

FIGURE 2 | Network of enriched gene ontology term clusters in immunocompetent patients with sepsis. Red circles represent the upregulated pathways, and blue circles represent the downregulated pathways.

with the aforementioned SRS-2 (**Figure 2**). The potentially harmful effect of corticosteroid among patients with septic with specific endotype was recently corroborated using the same dataset by Wong et al., whose team has developed a 100-gene-expression array, which was designed to assess adaptive immunity and glucocorticoid receptor signalling in paediatric septic shock (27). In brief, Wong et al. found that among the 44 patients with endotype A, a phenotype with relatively suppressed adaptive immunity, the mortality tended to be higher among the 26 patients receiving corticosteroid compared with 18 patients without corticosteroid (12/26, 46% vs. 4/18, 22%, $p = 0.105$) (27). Accordingly, the aforementioned evidence and our data demonstrated that the transcriptomic signature should have a crucial role in precision medicine among patients with sepsis.

Sepsis is a complex disease with a robust innate response and dysregulated inflammation, which in turn leads to organ dysfunction (28). In the present study, we used over-representation analysis (ORA) to identify several DEGs in immunocompetent patients, and the identified DEGs, namely, *PRSS33, HDC, ALOX15, FCER1A, OLR1*, and *PCSK9*, were mainly innate immunity and cellular damage-associated genes (7, 29–33). For example, *ALOX15* and *FCER1A* were monocyte-associated genes, which have been found to play a pivotal role in the pathogenesis of bacterial sepsis (7, 31). Moreover, we also used CIBERSORT to estimate cellular types and found the relative abundance of monocyte in enrolled patients, and this finding was in line with the increasing data to show the crucial role of monocyte in the pathogenesis of sepsis (**Supplemental Figure 1**) (7, 31). Notably, the transcriptome was similar on day-1 among immunocompromised and immunocompetent patients (**Figure 1A**), and the findings highlight the crucial need of using paired day-1 and day-8 samples to address the dynamic transcriptome in patients with septic with high heterogeneity as shown in this study and the previous study (6). Furthermore, we employed functional pathway analysis to demonstrate the essential role of T cell

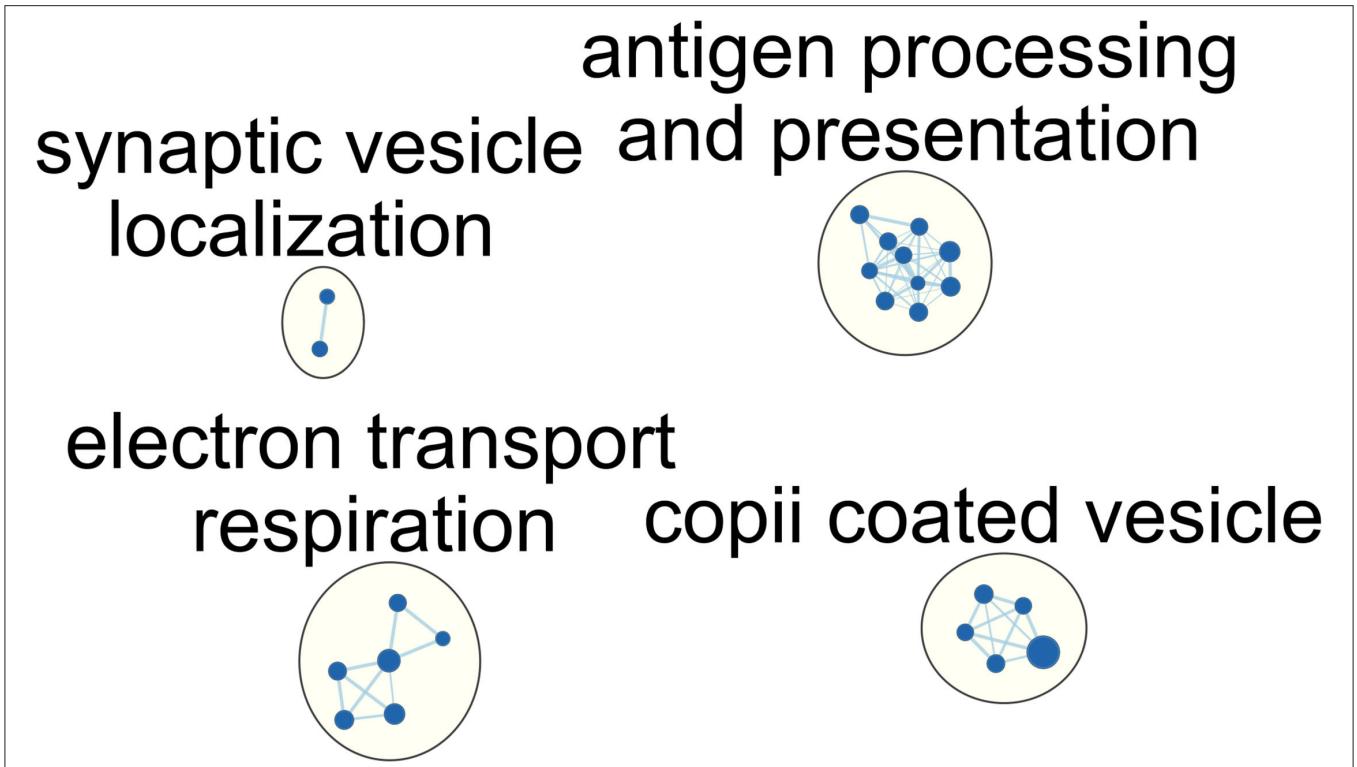

FIGURE 3 | Network of enriched gene ontology term clusters in immunocompromised patients with sepsis. Blue circles represent the downregulated pathways.

immunity-relevant pathways among immunocompromised patients with sepsis. The aforementioned findings highlight that ORA-based analysis alone may not be adequate to address the complex disease with a high number of differentially expressed genes given that the top DEGs may represent the robust dysregulated immunological and cellular function (34). In contrast, functional pathway-based analyses enable us to identify the distinct T cell-associated pathways as we have shown in the present study (**Figure 2**).

Several transcriptomic studies have been used to address the T cell immunity in sepsis, and distinct terms were used, such as T cell exhaustion, immunoparalysis, and immune-suppressive signatures (4, 9, 26). Cheng et al. reported an *in vitro* model of innate immunotolerance/immunoparalysis defined by diminished production of lactate and pro-inflammatory cytokines in lipopolysaccharide (LPS) or *Candida albicans*-treated monocytes isolated from patients with sepsis and found not only compromised innate immunity but also impaired energy metabolism in patients with septic with immunoparalysis (4, 26). Increasing evidence has further validated the key role of metabolic abnormality in sepsis (35, 36), and our data also found impaired metabolic pathways, such as TCA cycle and cellular respiration, in immunocompromised patients with sepsis (**Figure 3**). Additionally, alteration in the metabolic state of T cells has been implicated with the impaired capacity of T cell expansion and effector function of T cells (37, 38). The impaired metabolic signalling as we have shown in this clinical study may hence be associated with the T cell paralysis in the immunocompromised patient with septic, although more mechanistic studies are warranted to elucidate the complex immune-metabolism interaction (4).

Increasing studies have shown the role of T cell exhaustion in patients with sepsis; however, few studies addressed the T cell diversity in patients with septic, particularly immunocompromised patients. Venet et al. conducted a pilot study to explore day-1 and day-7 diversity of TCR in 41 patients with a septic shock through using genomic DNA and multiplex PCR (39). Venet et al. reported that a reduced day-7 TCR β-chain diversity tended to be associated with an increased risk of mortality in the univariable analysis (39). Furthermore, patients who developed a nosocomial infection after the sepsis episode appeared to have a lower day-1 TCR β-chain diversity compared with those without nosocomial infection (39). Recently, there are crucial advances, such as 5′ rapid amplification of cDNA ends (RACE) and measurement of TCR in RNA-level (40). Genomic DNA was widely used to measure the diversity of TCR due to high stability and fixed copy number per cell; however, PCR-associated error result from unused segments and introns remains a major limitation of the DNA-based approach for TCR repertoire (40). In contrast, using RNA with a simplified PCR amplification strategy enables the comprehensive identification of TCR variants and is increasingly applicated (41). MiXCR is a framework that allows for analysing raw sequences of RNA-Seq to determine clonotypes of TCR. Therefore, the

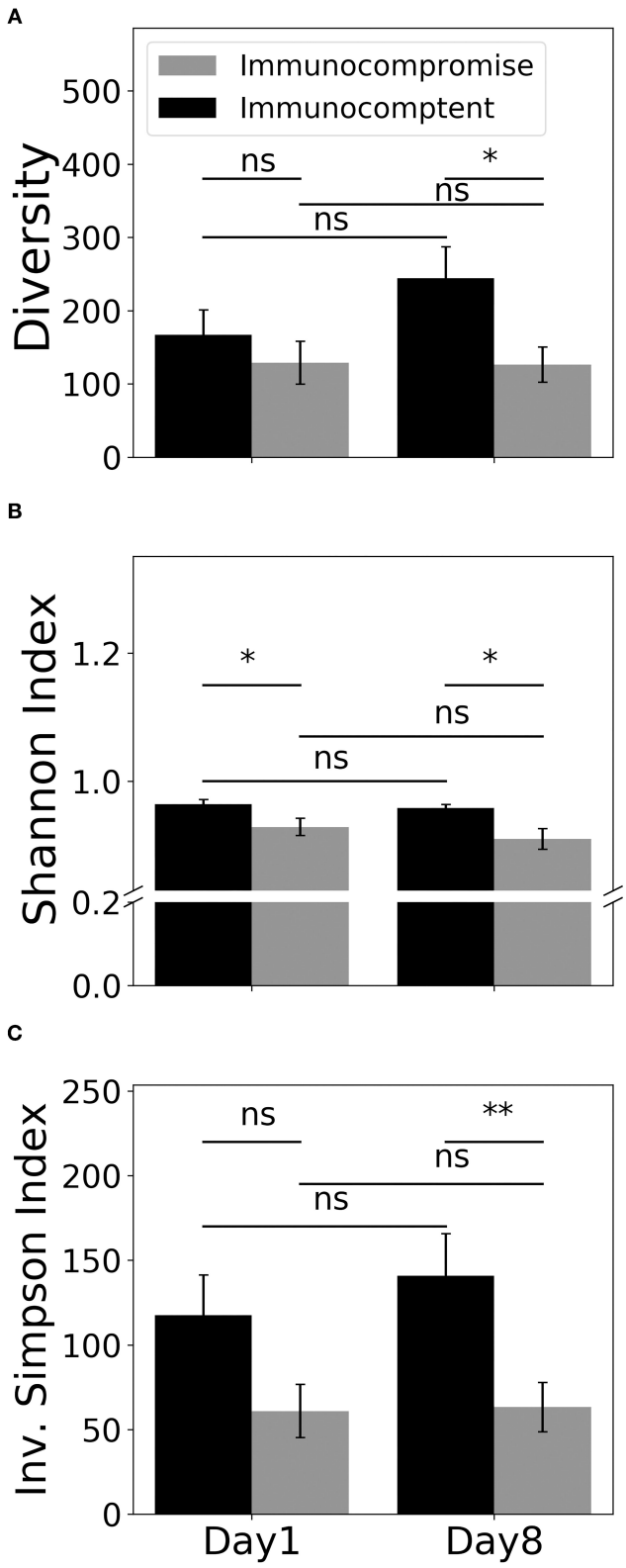

FIGURE 4 | Diversity of the T cell receptor. Counts **(A)**, Shannon's diversity index **(B)**, and inverse Simpson index **(C)** of unique CDR3. (CDR3, complementarity-determining region-3). * < 0.05, ** < 0.005.

approach with MiXCR enables us to simultaneously conduct whole transcriptome and diversity of TCR (11). Similar to our approach, Zhigalova et al. recently demonstrated the utility of using MiXCR and RNA-Seq data to determine the dynamic diversity of TCR among tumour-infiltrating T cells treated with programmed death receptor 1 (PD-1) antibody in mouse melanoma model (42). In the present study, we used RNA-Seq to identify the impaired T cell signalling and MiXCR to reveal the decreased diversity of TCR among immunocompromised patients with septic, and the consistent findings indicate the immunocompromised condition-associated T cell paralysis in sepsis. Similar to our results in immunocompromised patients with septic, Cabrera-Perez et al., conducting qualitative and quantitative analyses of Ag-specific CD4 T cell populations in cecal ligation puncture-treated mice, found a prolonged alteration of Ag-specific T cell repertoire after sepsis despite the recovered number of T cells (43). Collectively, this evidence highlights the feasibility of using data of RNA-Seq and MiXCR to address the diversity of TCR in patients with sepsis and point out the crucial role of reduced diversity of TCR in immunocompromised patients with sepsis.

There are limitations to this study. First, the relatively small number of immunocompromised patients with septic. However, we enrolled severity-matched controls, and the highly comparable controls should allow us the identification of distinct pathways between the two groups. Second, the use of bulk RNA-Seq and future sc-RNA-Seq and functional experiments are warranted for validation at the cellular level. Third, we used RNA-Seq data to assess the diversity of TCR, and future T cell repertoire using 5′RACE should be needed to clarify the issue of TCR in patients with sepsis.

CONCLUSION

The increased number of immunocompromised patients with sepsis is a growing issue worldwide, and the RNA-Seq can be used to address the complex immunologic and metabolic features. In the present study, we enrolled immunocompromised patients with septic with severity-matched controls and conducted RNA-Seq with functional pathway and MiXCR analyses. We found that not only dysregulated innate immunity but also compromised T cell function, altered metabolic signalling, and decreased T cell diversity among immunocompromised patients with septic. These data highlight the feasibility of using RNA-Seq to characterise immunologic and metabolic features in immunocompromised patients with septic, and studies using sc-RNA-Seq and 5′RACE are warranted to elucidate the underlying cellular pathway.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE182522.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board approval of the Taichung Veterans General Hospital (CE20069B). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

P-LC, H-HC, C-YW, C-LW, T-MK, and W-CC: conceived and designed the experiments. P-LC, Y-HJ, and W-CC: acquired data. P-LC, T-HH, and W-CC: contributed materials/analysis tools. P-LC and W-CC: wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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CASE REPORT
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Case Series: Video-Assisted Minimally Invasive Cardiac Surgery During Pregnancy

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Surgical intervention is expected to improve maternal outcomes in pregnant patients with heart disease once the conservative treatment fails. For pregnant patients with heart disease, the risk of cardiac surgery under cardiopulmonary bypass (CPB) must be balanced due to the high fetal loss. The video-assisted minimally invasive cardiac surgery (MICS) has been progressively applied and shows advantages in non-pregnant patients over the years. We present five cases of pregnant women who underwent a video-assisted minimally invasive surgical approach for cardiac surgery and the management strategies. In conclusion, the video-assisted MICS is feasible and safe to pregnant patients, with good maternal and fetal outcomes under the multidisciplinary assessment and management.

Keywords: minimally invasive cardiac surgery (MICS), video-assisted, pregnancy, cardiopulmonary bypass, perioperative management

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INTRODUCTION

Heart diseases complicate 2–4% of pregnancies but account for up to 15% of maternal deaths (1). The cardiac potential to adapt hemodynamic change is impaired in women with structural heart disease, presented with reduced systolic and diastolic function (2). Once cardiac decompensation happens, cardiac surgery might be a solution for pregnant patients with structural heart diseases and compromised cardiac function. Maternal mortality after cardiac surgery during pregnancy is reported to be comparable to non-pregnant patients for about 11.2%, but the high fetal loss (33.1%) cannot be ignored (1). The management of these patients should be made with adequate multidisciplinary discussions, including cardiologists, anesthetists, and obstetricians, with aims to improve maternal and fetal outcomes.

The minimally invasive cardiac surgery (MICS) has been progressively applied in non-pregnant patients over the years and showed advantages, such as less transfusion rate and shorter postoperative ventilation support time as compared to that of mid-sternotomy approach thus, resulting to shorter ICU time and length of stay (3, 4). However, few MICS during pregnancy has been reported. This article presents a case series of five pregnant women who underwent a video-assisted MICS cardiac surgery during pregnancy in a tertiary medical center.

CASE SERIES

We retrospectively reviewed the records of all pregnancies with cardiac surgery in our hospital between 2019 and 2021. Only patients who underwent a video-assisted MICS ($n = 5$) were included. Informed consent has been obtained from all the patients. Baseline characteristics of all patients are

shown in **Table 1**, and intraoperative and postoperative information of all patients are shown in **Tables 2**, **3**.

PERIOPERATIVE MANAGEMENT STRATEGY

Perioperative management was discussed by the multidisciplinary team that included cardiologists, cardiac surgeons, anesthesiologists, perfusionists, and gynecologists. The operative procedure was conducted under general anesthesia with 35 F left double-lumen intubation to allow single lung ventilation. A central venous catheter was placed on the right internal jugular vein. A 16 Fr venous cannula was placed in the superior cava vein through the right jugular vein for cardiopulmonary bypass (CPB) venous return. Transesophageal echocardiography was routinely set up for intraoperative monitoring. The patient was placed in a supine position with elevated right chest. Propofol and rocuronium were used for anesthetic induction. Sevoflurane, propofol, dexmedetomidine, and rocuronium were used for anesthetic maintenance with certain level of Nacrotrend values between 40 and 60. Sufentanil was intermittently given to ensure enough analgesia. Magnesium sulfate was used to inhibit uterine contraction. The fetal heart rate and uterine contraction were monitored by TEE and tocodynamometer. After heparinization, venous cannula and arterial cannula were placed in the right femoral vein and artery. A right anterolateral 4th intercostal 3.5 cm incision was made and the thoracoscopy was inserted *via* the 4th or 5th intercostal space. The high-flow, high-pressure normothermic CPB was then started. Vacuum-assisted venous drainage was also utilized (maximum negative pressure 20–40 mmHg). Cold Del Nido cardioplegia solution (blood and crystalloid mixed formula) was used as anterograde. During CPB, the hematocrit was maintained between 25 and 29%, as well as normothermia. Post-bypass transesophageal echocardiography has ensured a satisfying surgical outcome and fetal survival. An intercostal nerve block with 0.5% ropivacaine combined with intravenous analgesia, was used for postoperative multimodal analgesia.

The patient was transferred to the intensive care unit temporarily for monitoring. Fetal status was ensured by Doppler echography and uterine contraction was monitored by the tocodynamometer after the surgery. Atosiban was used postoperatively to inhibit uterine contraction. Warfarin and/or low molecular weight heparin were administrated for anticoagulation for those patients who underwent mechanical valve replacement.

DISCUSSION

MICS During Pregnancy

Compared to standard sternotomy, the minimally invasive approach through thoracoscopy has been utilized in the past decades. However, there were few reports about occurrence of MICS with thoracoscopy during pregnancy. In one previous study, Nguyen et al. (5) reported a case of acute papillary muscle rupture during pregnancy. The minimally invasive mitral valve repair *via* the right thoracotomy was conducted, with main consideration on how morbidly obese this patient was. In our

TABLE 1 | Baseline characteristics of five patients undergoing minimally invasive cardiac surgery (MICS) during pregnancy.

Case no.	Age (years)	Gravida	Para	Weight (kg)	GA when cardiac surgery (weeks)	Diagnosis	NYHA grade	Preoperative transthoracic echocardiography	LVEF (%)	Pulmonary arterial systolic pressure (mmHg)	ECG
1	35	5	2	60	18	Rheumatic heart disease	II	Severe mitral stenosis with moderate regurgitation, and mild tricuspid and aortic regurgitation	62	45	Normal
2	27	2	1	40	22	Infective endocarditis	II	Moderate mitral stenosis and severe mitral regurgitation with abnormal vegetation echo	60	80	Sinus tachycardia
3	38	2	0	56	18	Rheumatic heart disease	II	Moderate-severe mitral valve stenosis with moderate-severe regurgitation, moderate tricuspid regurgitation	73	62	Normal
4	34	2	1	58	31	Rheumatic heart disease	III	Severe mitral stenosis and mild mitral regurgitation	76	60	Normal
5	32	5	2	54	18	Left atrial myxoma	II	A medium-echo 19 mm × 10 mm irregular mass with good mobility in the left atrium, with good mobility and the stalk adherent to the fossa ovalis, considered as a myxoma	66	<40	Normal

MICS, minimally invasive cardiac surgery; NYHA, New York Heart Association (NYHA) Classification; LVEF, left ventricular ejection fraction; ECG, electrocardiogram.

TABLE 2 | Intraoperative information of five patients undergoing MICS during pregnancy.

Case no.	Intervention	Intubation	Total operation time (minutes)	CPB time (minutes)	Aortic cross-clamp time (minutes)	Lowest core temperature (°C)	Pulsatile perfusion
1	Mitral valve replacement	Double lumen tube	145	75	47	36.0	Yes
2	Mitral valve replacement	Single lumen tube	165	92	64	36.0	Yes
3	Mitral valvuloplasty	Double lumen tube	170	88	68	36.0	Yes
4	Mitral valve replacement	Double lumen tube	133	67	43	36.0	Yes
5	Left atrial myxoma excision	Double lumen tube	135	46	21	35.7	No

TABLE 3 | Postoperative information of five patients undergoing MICS during pregnancy.

Case no.	Extubation time after surgery (hours)	Complication	Blood transfusion	Length of stay (days)	Maternal mortality	Gestational age when pregnancy termination (weeks)	Fetal outcomes
1	7	No	No	11	No	20	Abortion
2	5	No	2U RBC	13	No	35	Abortion due to fetal cerebral anomaly
3	5	No	No	18	No	37	Normal Term Infant
4	10	Atrial fibrillation*	No	22	No	37	Normal Term Infant
5	1	No	2U RBC	13	No	26	Abortion due to fetal chromosomal abnormality

**Four days after the surgery, the patient had an episode of acute atrial fibrillation with heart rate of 171 bpm. The sinus rhythm was returned with a heart rate of 92 bpm after the Valsava maneuver twice. One day after the first episode, the patients felt palpation with no reason and the ECG revealed a rapid onset of atrial fibrillation with a heart rate of 175 bpm. Antiarrhythmic drugs (12.5 mg beta-blocker and 0.2 mg deslanoside) were given and the episode was terminated. Beta-blocker was used to maintain the sinus rhythm.*

center, we conducted the MICS *via* video-assisted thoracoscopy, with the advantage of smaller operative incision, rather than the right thoracotomy. Also, favorable outcomes after the minimally invasive approach can be obtained more than that of standard sternotomy with less postoperative pain, faster recovery, less postoperative complication, and shorter length of stay in the hospital (6, 7). Qiu et al. (3) demonstrated that a full sternotomy was an independent risk factor for postoperative ventilation support. It was known that prolonged mechanical ventilation affects fetus morbidity and mortality in cardiac surgery during pregnancy (8). In our study, all fetuses remained alive after the cardiac surgery, supporting our supposition that the minimally invasive approach has its benefits to fetal survivals in pregnant women who underwent cardiac surgery under CPB. Also, sternal complications following a median sternotomy, including infection, sternal instability, and non-union, were reported by 1–8% worldwide (9). Sternal precautions were recommended for prevention of complication, which consisted of weight restrictions on the use of the upper limbs immediately after surgery for 6–12 weeks (10). This may interfere with normal maternal-infant bonding because motion restriction after median sternotomy may affect the mother in holding her child and in breastfeeding (5, 11). However, longer operation duration in the MICS should come into consideration for pregnant women as CPB time is reported as a risk factor for fetal mortality (12), but this may be solved by experienced surgeons. During MICS, single lung ventilation technique is required for a satisfactory field exposure. In our cases, we applied double lumen tube in four patients and single lumen tube in one patient. All surgical field remained satisfactory to the surgeons, including the one with single lumen intubation. Hypoxemia in the lung isolation after cardiopulmonary bypass (CPB) surgery might impair fetal oxygenation, and whether single lumen tube in MICS benefits to these patients still needs further investigation.

Cardiopulmonary Bypass During Pregnancy

Cardiopulmonary bypass can pose significant effects on both the fetus and the mother. Sustained uterine contraction during CPB is regarded as a risk factor to fetus survival. The cooling and rewarming process during CPB induces uterine contraction, especially after maternal hypothermia, which induces placental hypoperfusion and, consequently, fetal hypoxia. Hemodilution of progesterone during CPB also enhances uterine contraction (12, 13). In our study, we performed high perfusion pressure and normothermic CPB to ensure placental perfusion. It is thought that pulsatile perfusion can release endothelium-derived growth factors from the vascular endothelium and reduce uterine contractions, which may result to good fetal outcomes in pregnant women who undergo cardiac surgery (12, 14). In our study, non-pulsatile CPB was performed in two cases and both fetuses were alive after cardiac surgery, though 1 patient eventually has terminated pregnancy due to fetal chromosomal abnormality. Pulsatile perfusion was performed in three patients and one patient has terminated pregnancy due to fetal cerebral

anomaly. In fact, there are few clinical data to support the advantage of pulsatile perfusion over non-pulsatile perfusion in pregnant women. In the cohort study of John et al. (15), there was a reported three fetal deaths among the 21 non-pulsatile CPB cases. Most of them (two fetal deaths) happened in women with other comorbidities. Further clinical research evidence is required to determine the beneficial application of pulsatile or non-pulsatile perfusion in cardiac surgery of pregnant women.

In the study of Jha et al. (1), the pooled rate of maternal complications was at 15%, maternal heart failure at 5.8%, and arrhythmia at 2.1%, respectively. In our study, only 1 patient experienced cardiovascular complication of acute onset of atrial fibrillation that requires treatment. Maternal mortality is comparable to that of CPB in non-pregnant women in the previous studies, with the estimated rate of 11% in the meta-analysis of Jha et al. (1). Maternal status with worse NYHA and emergency surgery contributed to unfavorable maternal outcomes in these patients. In our cases, all women survived and may benefit from good maternal NYHA status and semi-urgent surgery.

Management of Cardiac Surgery During Pregnancy

The decision to perform cardiac surgery during pregnancy should be thoroughly discussed within a multidisciplinary team of obstetricians, cardiologists, cardiac surgeons, anesthetists, and gynecologists. The Modified World Health Organization Classification of Cardiovascular Disease in Pregnancy is used as reference for risk stratification of maternal and neonatal complication (16). Compared to non-pregnant women, pregnant women are at higher risk of aspiration, difficult intubation, and thromboembolism, which made them require more attention in preoperative preparation (17). Once the patient is supine,

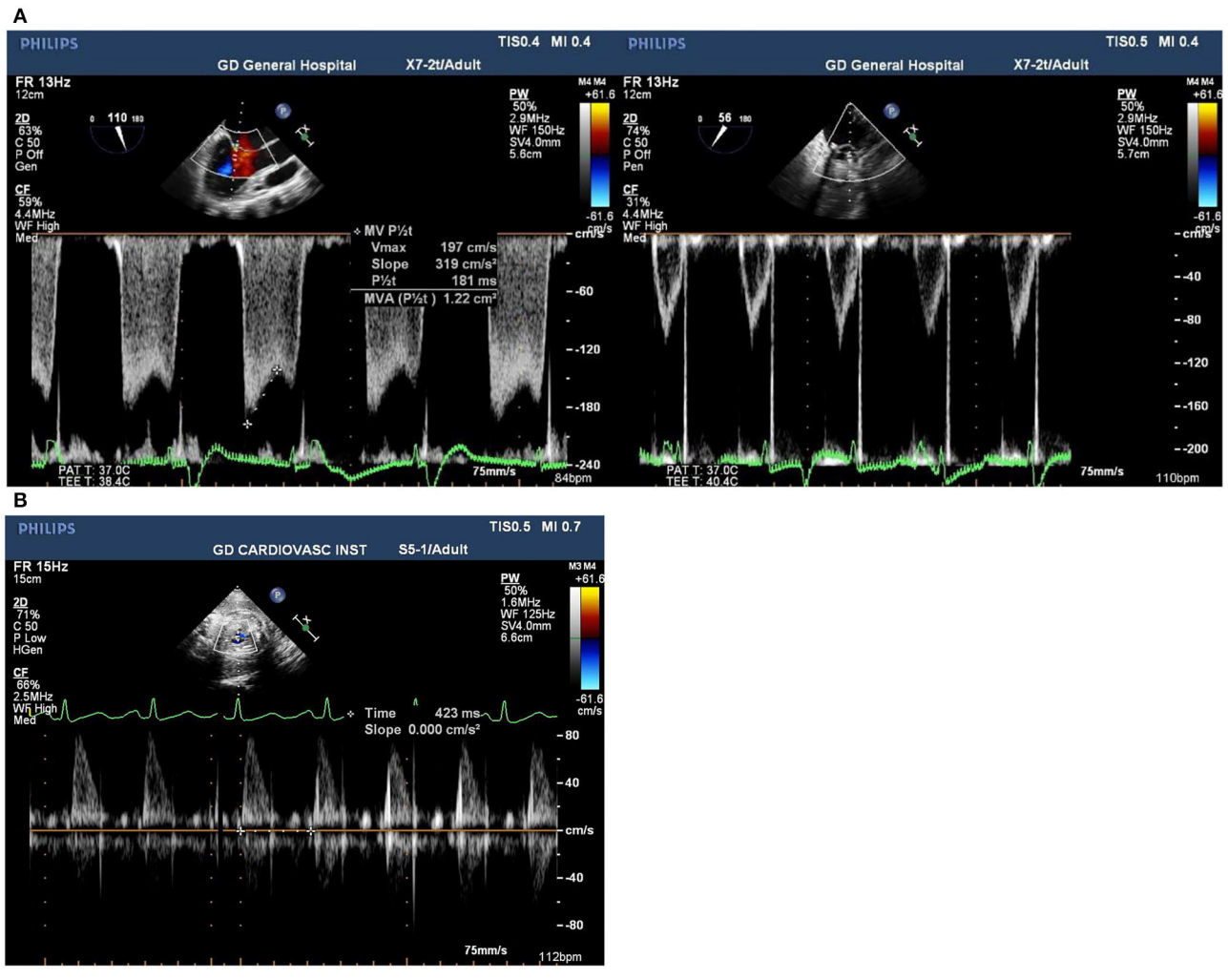

FIGURE 1 | (A) Pre and post-surgical mitral valve view of transesophageal echocardiogram images of mitral valve stenosis in Case 1. **(B)** Postoperative transesophageal echocardiogram images of Doppler of fetal blood flow in Case 3 presenting the fetal heart rate at 141 bpm.

the 15° position of left uterine displacement should be applied to avoid aortocaval compression after 18–20 weeks of gestation (18).

Pregnant patients are more sensitive to IV and inhalational medications. Propofol seems to be the preferred medication for induction in healthy pregnant patients. Mongardon et al. (19) demonstrated that the dose of propofol required in pregnant women for loss of consciousness is 8% less than in non-pregnant patients. Inhalation medication, such as desflurane and sevoflurane, inhibits myometrial contractions during the operation, which may be beneficial to pregnant women undergoing cardiac surgery (20). It suggested a more rapid onset of neuromuscular block with vecuronium and rocuronium in pregnant women (18).

Sympathomimetic agents, such as phenylephrine and norepinephrine, are safe to maintain blood pressure. In comparison with phenylephrine, ephedrine may act on fetal metabolism and be associated with neonatal acidosis and, therefore, should be considered as secondary choice of vasopressor in pregnancy (16, 21).

Intraoperative monitoring for both the mother and the fetus is critically significant to favorable maternal and fetal outcomes. If uterine contractions are detected, increase maternal intravascular volume may be helpful and tocolytic treatment can be administrated (22). Fetal bradycardia is an important indicator of fetal distress during CPB, which usually occurs at the initiation of CPB caused by a decrease in systemic vascular resistance, thereby affected by hemodilution, and the release of vasoactive substances. It has been reported that fetal heart rate (FHR) monitoring with an external cardiotocography reduces fetal mortality to 7.5% in cardiac surgeries with CPB (23). In our cases, the tocodynamometer combined with TEE were used to monitor uterine contraction and fetal heart rate (**Figure 1**). FHR was measured intraoperatively by Doppler echocardiography across the fetal blood flow, which was available in all pregnant women in our cases whose gestational age ranged from 18 to 31 weeks.

Commonly tocolytic drugs include magnesium, beta-adrenergic drugs, nitroglycerin, and prostaglandin inhibitors (22). In our case, we chose magnesium sulfate, which is mainly used for pre-eclampsia control, to inhibit uterine contraction by decreasing acetylcholine transmission in motor nerve terminals (24). Also, it was reported that antenatal usage of magnesium sulfate may contribute to fetal neuroprotection and may reduce the risk of cerebral palsy or even death (25). However, magnesium may potentiate the activity of both depolarizing and non-depolarizing neuromuscular blocking agents (NMBA). Consequently, the dose of NMBA should be reduced (24). In a case of left atrial myxoma resection reported by Alexis et al. (26), a low dose of nicardipine, a calcium channel blocker, was used to inhibit uterine contractility and may show an advantage to restore FHR.

For these patients, postoperative monitoring is pivotal along with the assessment of fetus by using Doppler ultrasound, as well checking of uterine contraction with a tocodynamometer. If necessary, tocolytic drugs should be administrated in case of a preterm labor. The left lateral position should be maintained to prevent aortocaval compression (18). Furthermore, postoperative analgesia is important for pain control and can reduce the risk of premature labor. NSAIDs should be avoided in women as prenatal exposure to NSAIDs after 30 weeks gestational age is associated with an increased risk of premature closure of the fetal ductus arteriosus and oligohydramnios (27). In our center, multimodal analgesia was performed in every patient including intercostal nerve block with 0.5% ropivacaine and intravenous analgesia with opioids, resulting all patients to report a pain score <3 on a numeric rating scale (NRS).

CONCLUSION

The video-assisted MICS is feasible and safe with good maternal and fetal outcomes, which may be progressively applied in patients in the need for cardiac surgery during pregnancy. The multidisciplinary team for decision in the management of these patients is of vital importance to favorable outcome for both the mother and the fetus.

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 Guangdong Provincial People's Hospital Ethics Committee. The patients/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

NW, YY, and JH collected and organized the information of patients. AL wrote the first draft of the manuscript. JW, YY, JH, BL, and NW wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Effectiveness of Combined Strategies for the Prevention of Hypothermia Measured by Noninvasive Zero-Heat Flux Thermometer During Cesarean Section

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Background: Perioperative hypothermia (body temperature <36°C) is a common complication of anesthesia increasing the risk for maternal cardiovascular events and coagulative disorders, and can also influence neonatal health. The aim of our work was to evaluate the impact of combined warming strategies on maternal core temperature, measured with the SpotOn. We hypothesized that combined modalities of active warming prevent hypothermia in pregnant women undergoing cesarean delivery with spinal anesthesia.

Methods: Seventy-eight pregnant women were randomly allocated into three study groups receiving warmed IV fluids and forced-air warming (AW), warmed IV fluids (WF), or no warming (NW). Noninvasive core temperature device (SpotOn) measured maternal core temperature intraoperatively and for 30 min after surgery. Maternal mean arterial pressure, incidence of shivering, thermal comfort and newborn's APGAR, axillary temperature, weight, and blood gas analysis were also recorded.

Results: Incidence of hypothermia was of 0% in AW, 4% in WF, and 47% in NW. Core temperature in AW was constantly higher than WF and NW groups. Incidence of shivering in perioperative time was significantly lower in AW and WF groups compared with the NW group ($p < 0.04$). Thermal comfort was higher in both AW and WF groups compared with NW group ($p = 0.02$ and $p = 0.008$, respectively). There were no significant differences among groups for the other evaluated parameters.

Conclusion: Combined modalities of active warming are effective in preventing perioperative hypothermia. The routine uses of combined AW are suggested in the setting of cesarean delivery.

Keywords: spinal anesthesia, core temperature, perioperative hypothermia, SpotOn, cesarean delivery (CD)

INTRODUCTION

The inadvertent perioperative hypothermia (PH) is the unintentional cooling of a patient's core temperature (CT°) >36°C (1).

Perioperative hypothermia is estimated to occur in >60% of patients receiving spinal anesthesia for cesarean delivery (2–4), in whom it significantly impairs thermal autoregulation by inhibiting the vasomotor and shivering responses even above the level of the sensory block and causes a thermal redistribution of heat from core to peripheral tissues (5–9).

In absence of strategies for preserving normothermia, patients become hypothermic in the early 30–40 min of surgery, and they remain hypothermic during postoperative time (10).

Hypothermia increases the risk of cardiovascular events, such as myocardial ischemia, arrhythmias, and coagulative disorders, greater blood loss with a need of transfusions, wounds infection with delayed healing due to decreased antibody- and cell-mediated immune responses, and also oxygen availability in the peripheral wound tissues. PH changes the kinetics and action of various anesthetic and paralyzing agents, increases thermal discomfort, and is associated with delayed postanesthetic recovery (2, 11–14).

Moreover, PH can increase oxygen consumption and catecholamines release, which are responsible of intraoperative complications, such as hypoxia and increased pulmonary vascular resistances, and postoperative shivering can increase the metabolic rate leading to an increased incidence of early postoperative myocardial ischemia, especially in high risk-patients (15, 16).

Maternal hypothermia during cesarean delivery can also influence the APGAR score of newborns, neonatal temperature, umbilical vein blood gas analysis (BGA), and blood coagulation (13). Those consequences are particularly relevant in light of the fact that failure to identify and treat perioperative hypothermia is starting to be acknowledged as a potential cause of malpractice lawsuits, in case adverse outcomes arise. It is worth bearing in mind that if a claim should be filed based on alleged medical malpractice stemming from negligence, courts (particularly under tort law statutes) are likely to hold doctors and facilities liable, if the patient medical records and informed consent documentation process turn out to be inaccurate or lacking in any measure. Such inconsistencies may be viewed by the court as resulting from negligent practices rather than unavoidable clinical complications. Overall, any failure to comply with surgical safety protocols or to produce documentation proving adherence to guidelines issued by recognized scientific societies and bodies, and therefore deemed reliable, will likely result in unfavorable rulings for doctors and hospitals (17–19).

Abbreviations: ASA, American society of anesthesiologists; AW, warmed IV fluids and forced-air warming; BGA, blood gas analysis; BMI, body mass index; BSAS, bedside shivering assessment scale; CD, cesarean delivery; CT°, core temperature (°C); MAP, mean arterial pressure; NW, no warming; OR, operating room; P, p-value; PAC, pulmonary artery catheter; PH, perioperative hypothermia; SD, standard deviation; T° OR, temperature in the operating room; T5-15-20-25-35-40-45-55, time at 5-15-20-25-35-40-45-55 min from baseline; TCS, thermal comfort score; WF, warmed IV fluids.

Core temperature is the best single indicator of thermal status in humans. There are several methods to detect the body CT°, including: noninvasive, such as tympanic thermometer and rectal probe; and invasive, such as pulmonary artery catheter (PAC), esophageal and nasopharyngeal probes. All the studies on perioperative hypothermia used noninvasive CT° monitoring devices, especially tympanic thermometers.

A new skin temperature monitoring system, the SpotOn, measures the heat flux in a temporal artery and is a very reliable device in comparison with PAC, which is the gold standard.

The warming systems currently used to prevent and treat hypothermia, are classified into passive and active. Among the active warming methods, the most used are heated infusion fluids and forced air-warming systems; the latter can be applied on superior and/or lower extremities.

The multimodal approach of combined warming modalities (intravenous fluid infusion and forced air warming), temperature measurements, type of neuraxial anesthesia, duration, and time of warming (preoperative, preanesthetic, intraoperative) (2, 20, 21) have provided a series of interesting studies, but with no total consensus on the best management of perioperative hypothermia (2, 11, 12, 22).

Multiple studies used a single-modality warming intervention (warmed IV fluids (WF) or forced-air warming alone) and have shown little efficacy in preventing perioperative hypothermia during cesarean delivery (4, 20, 21, 23–27).

The aim of our prospective randomized controlled study is to evaluate the impact of combined strategies of warming in pregnant women undergoing cesarean delivery in terms of maternal CT° using the SpotOn.

METHODS

Study Design and Patient Population

After ethical approval, our prospective randomized, controlled study was performed at the Obstetrics and Gynecology Department of the University Hospital of Foggia, Italy (ClinicalTrials.gov registration number: NCT03473470).

Consecutive patients with the following inclusion criteria were considered for enrollment: healthy pregnant women (ASA 1-2), age from 18 to 40 years, at term gestation (≥ 37 weeks), and elective cesarean delivery with spinal anesthesia. Exclusion criteria included conditions, such as fever, diabetes mellitus, BMI > 40 kg/m, coagulative disorders, preeclampsia, and all those factors that can cause intraoperative bleeding (placental abruption or history of placenta previa). Before data collection, the purpose of the work was carefully explained and a written informed consent was obtained from each participant, according to the Helsinki declaration. From the original sample of 93 pregnant women, only 78 were enrolled in the work.

Women were randomly assigned, by a computer-generated randomization, to three study groups (26 patients each) to receive WF alone, warmed IV fluids and forced-air warming (AW), or IV fluids at room temperature with further no warming (NW).

Study Protocol

Demographic data, vital signs (heart rate, mean arterial pressure, and pulse oximetry), and CT° were obtained in the preoperative holding area. CT° was obtained using the 3M™SpotOn™ monitoring System (Model 370, 3M Science, St.Paul, MN, USA), where the single-use sensor was placed on the patient's forehead before surgery which provided a continuous noninvasive CT° monitoring. In this study, we refer to maternal hypothermia as core body temperature <36°C, according to the 2017 SIAARTI Best Clinical Practice on Perioperative Normothermia (28). Spinal anesthesia was performed with intrathecal isobaric 8 mg levobupivacaine and 20 μg fentanyl, administered *via* a 27-gauge Whitacre needle, inserted at the L2–L3 or L3–L4 intervertebral space. After the spinal anesthesia, patients were immediately positioned supine, and the uterus was manually displaced to the left. Intravenous crystalloid preloading (Ringer's lactate solution, 10 ml/kg) was infused 10 min before the lumbar puncture. Thereafter, a coload of 10 ml/kg/h Ringer's lactate solution was administered.

The WF group received the IV fluids coload warmed to 41°C through a 3M Ranger™ Fluid Warmer (Model 245, Arizant Healthcare, Maplewood, MN, USA). The AW group received WF, and a thermal blanket (forced-air warming system) was placed after spinal anesthesia all over the upper extremities and attached to the convective temperature management system 3M Bair Hugger™ (Model 505); the device was set to "high" (43°C) in the AW group and "ambient" in the WF and NW groups. NW group received IV fluid at room temperature (fluid warmer set to "off"), no active warming was administered, but routine care with blankets was provided.

Room Temperature Was Measured in the Operative Room (OR) on the Arrival of Patients

Core temperature ° and mean arterial pressure (MAP) were recorded on the arrival of patient in the operating room (baseline), 5 min postspinal anesthesia (T5), every 10 min during surgery until the end of the cesarean delivery, and 30 min after the surgery (discharge).

Patients were also asked to report the severity of shivering using the Bedside Shivering Assessment Scale (BSAS) from 0 to 3 (0 = no shivering, 1 = shivering localized to the core and neck, 2 = shivering including the upper extremities, 3 = total body shivering) and the thermal comfort scores (TCS) obtained by using a visual analog scale from 0 to 100 (0 mm= worst imaginable cold, 50 mm = thermos-neutrality, and 100 mm = insufferably hot); these data were taken at 20 and 40 min (T20, T40) from baseline and at discharge from the OR.

Total volume of estimated blood losses during surgery was recorded at the end of surgery.

Apgar scores and axillary neonatal temperature were recorded at 1 and 5 min after birth, and umbilical vein BGA was obtained for analysis from a double-clamped umbilical cord.

Statistical Analysis

The power analysis suggested that a sample size of 21 parturients/group was required to detect a 0.5°C difference among groups in maternal CT (assuming $\alpha = 0.01$ and power = 0.95) (29). The number was then increased to 26 per group to allow for a 20% patients drop-out rate.

The normality of distribution was assessed by Shapiro–Wilkinson test. Since we found almost all of the data normally distributed, the data were expressed as mean ± SD values or number as appropriate. Data were analyzed using repeated measurements analysis of variance (RANOVA). Differences between the groups at each time point were examined *post hoc* using an independent sample *t*-test. A paired sample *t*-test was used to detect changes within the groups. Categorical data were analyzed by Chi-squared test (χ^2). Level of statistical significance was chosen to be at $p < 0.05$. Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 15.0 for Windows.

RESULTS

We recruited 93 pregnant women of whom 78 were enrolled in the study and divided them into three groups of 26 patients each (**Figure 1**). There were no significant differences among the three groups as regards age, height, weight, BMI, gestational age, baseline temperature, and surgery duration (from surgical incision to skin closure) (**Table 1**).

At the arrival of the patients, the operative room temperature (T°OR) was 23.4°C ± 1.4°C in the NW, 24.25°C ± 1.8°C in the WF group, and 22.5°C ± 0.4°C in the AW group, with no significant difference between the study groups.

Intergroup analysis showed that at baseline, the patient CT° was 36.9°C ± 0.4°C in the NW group; 37°C ± 0.5°C in the WF group, and 37°C ± 0.5°C in the AW group ($p = 0.80$).

At T25, the CT° of NW, WF, and AW groups were respectively 36.4°C ± 0.4°C, 36.6°C ± 0.3°C, and 36.8°C ± 0.3°C; CT° in AW group was significantly higher than WF group and NW group (AW vs. WF: $p = 0.023$; AW vs. NW: $p = 0.001$).

At T35, the CT° of NW, WF, and AW groups were respectively 36.3°C ± 0.5°C, 36.5°C ± 0.3°C, and 36.7°C ± 0.3°C; CT° in AW group was significantly higher than WF group and NW group (AW vs. WF: $p = 0.025$; AW vs. NW: $p < 0.001$). The CT° in WF group was also statistically higher than the NW group ($p = 0.047$).

At T45, the CT° of NW, WF, and AW groups were respectively 36.2°C ± 0.3°C, 36.5°C ±0.3°C, and 36.7°C ± 0.3; CT° in AW and WF groups was significantly higher than the NW group (AW vs. NW: $p < 0.001$; WF vs. NW: $p = 0.004$).

At discharge, the CT° of NW, WF, and AW groups were respectively 36.2°C ± 0.3°C, 36.4°C ± 0.3°C, and 36.7°C ± 0.3°C; CT° in the AW group was significantly higher than WF group and NW group (AW vs. WF: $P = 0.001$; AW vs. NW: $p < 0.00$).

Intragroup analysis showed that the CT° gradually decreased in perioperative time in all the three groups and with significant difference from baseline to discharge (NW and WF: $p < 0.001$;

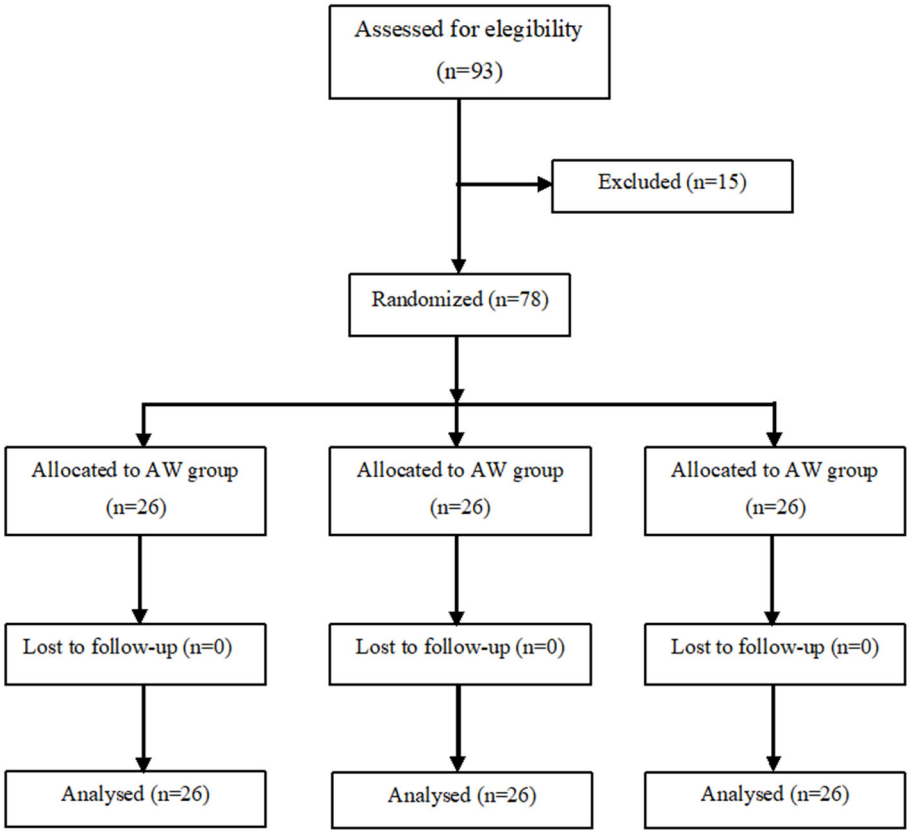

FIGURE 1 | Flowchart of patient's enrolment.

AW: $p = 0.045$). CT° in our study was never < 36°C in the AW group (**Table 2**, **Figure 2**).

As regards the MAP, no differences were observed among the three groups during the study period.

Blood loss in perioperative time was 258 ± 80 ml in the NW, 218 ± 90 ml in the WF group, and 227 ± 55 ml in the AW group, with no significant difference between these groups.

The other two statistically relevant outcomes were the BSAS (**Table 3**) and the maternal TCS (**Figure 3**).

At T20 and T40, the incidence of shivering was significantly higher in the NW than in the AW and WF groups (respectively, at T20: $p = 0.04$; at T40: $p < 0.001$).

At discharge, no shivering was observed. No patient had severe shivering (BSAS 3) in either group.

As far as thermal comfort is concerned, at T20, the TCS in the NW, WF, and AW groups were respectively 49 ± 6 mm, 51.5 ± 4 mm, and 51 ± 3 mm; TCS in NW group was lower only with respect to the WF group (NW vs. WF: $p = 0.04$).

At T40, the TCS in the NW, WF, and AW groups were respectively 46 ± 7 mm, 52 ± 5 mm, and 50 ± 2 mm; TCS in NW group was lower than WF and AW groups (NW vs WF: $p < 0.001$; NW vs. AW: $p = 0.007$).

At discharge, the TCS in the NW, WF, and AW groups were respectively 47 ± 7 mm, 50 ± 2 mm, and 50 ± 0 mm; TCS in NW group was lower than WF and AW groups (NW vs. WF: $p = 0.008$; NW vs. AW: $p = 0.02$). No patient reported insufferable hot during warming.

There were no significant differences among the three groups in terms of APGAR score and neonatal body temperature at 1 and 5 min from birth, birth weight, and umbilical cord vein BGA (**Table 4**).

No infant had complications or died.

DISCUSSION

The main results of our study demonstrated that the combination of AW is effective in reducing the loss of maternal CT° and the incidence of shivering during cesarean delivery (CD) in patients undergoing spinal anesthesia.

Warmed IV fluids, when used alone, are useful in maintaining a higher temperature during surgery and in postoperative time compared with no use of warming systems (NW), but they provided the worst results, in terms of incidence of shivering, compared with AW.

Perioperative hypothermia (PH), defined as a CT below 36°C, is one of the most common phenomena in surgical patients (30). Hypothermia during surgery can be generated by various factors,

TABLE 1 | Demographic and obstetric data of the study population.

	NW group ($n = 26$)	WF group ($n = 26$)	AW group ($n = 26$)	*P*
Age (years)	34 ± 5	33 ± 7	36 ± 63	0.756
Height (cm)	163 ± 6	162 ± 6	163 ± 5	0.926
Weight (kg)	73 ± 11	75 ± 16	79± 14	0.227
BMI (kg/m²)	27 ± 3	28 ± 6	29 ± 5	0.194
Gestational age (weeks)	38 ± 7	38 ± 1	38 ± 1	0.205
Baseline CT° (°C)	36.9 ± 0.4	37 ± 0.4	37 ± 0.4	0.807
Duration of surgery (min)	52 ± 10	52 ± 10	48 ± 6	0.207
T°OR (°C)	23 ± 1	24 ± 2	23 ± 0.4	0.103

Data are expressed in terms of mean ± standard deviation.
NW, IV fluids at room temperature and no forced-air warming; WF, warmed IV fluids; AW, warmed IV fluids and forced-air warming; P, p-value; CT, core temperature (°C); T°OR, temperature in the operating room (°C).

TABLE 2 | Maternal PH incidence.

Outcome	NW group ($N = 26$) *n* (%)	WF group ($N = 26$) *n* (%)	AW group ($N = 26$) *n* (%)	*P*
Peri-operative hypothermia (PH)	(47%)* ◦	1 (4%)* #	0 (0%)#◦	0.04*
				0.31#
				0.009◦

Data are expressed in terms of % of women with PH for each study group.
PH, peri-operative hypothermia; NW, IV fluids at room temperature and no forced-air warming; WF, warmed IV fluids; AW, warmed IV fluids and forced-air warming; P, p-value.
**, NW vs WF (P = 0.04); #, WF vs AW (P = 0.31); ◦, NW vs AW (P = 0.009).*

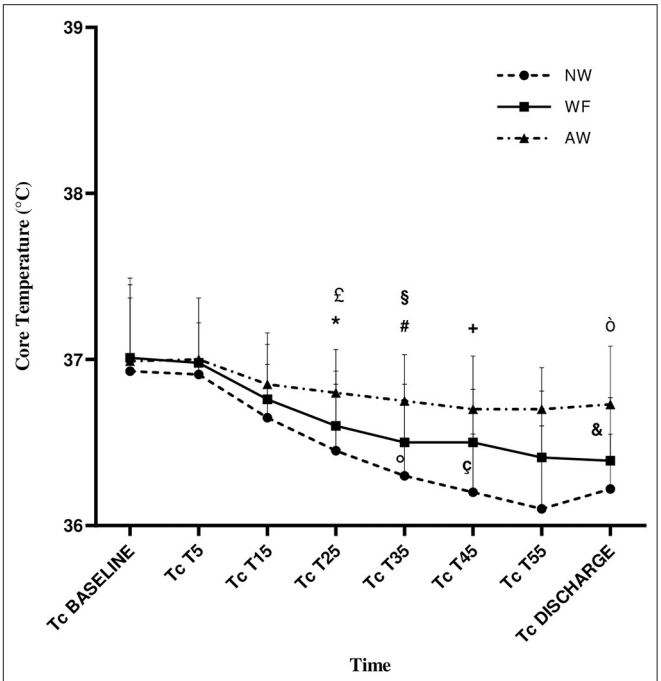

FIGURE 2 | Core temperature (CT°) variation during the perioperative time. Data are expressed in terms of mean ± standard deviation. NW, IV fluids at room temperature and no forced-air warming; WF, warmed IV fluids; AW, warmed IV fluids and forced-air warming; P, p-value; CT°, core temperature, expressed in °C; T°OR, temperature in the operating room, expressed in °C; CT°, core temperature; T5, T15, T25, T35, T45, and T55: 5, 15, 25, 35, 45, and −55 min from baseline, respectively. £, AW vs. WF (p = 0.023); *, AW vs. NW (p = 0.001); #, AW vs WF (p = 0.025); §, AW vs NW (p < 0.001); °, WF vs NW (p = 0.047); +, AW vs NW (p < 0.001); ç, WF vs NW (p = 0.004); ò, AW vs. NW (p < 0.001); &, AW vs. WF (p = 0.001).

such as exposure to the surgical environment, thermoregulatory dysfunction, and medications.

The primary mechanism of perioperative hypothermia during CD, after neuraxial anesthesia, is the redistribution of intravascular volume from the core to the peripheral compartment. Moreover, after a drop of CT, hypothermia triggers vasoconstriction and shivering in unblocked regions.

Perioperative hypothermia leads to intraoperative blood loss, cardiac events, coagulopathy, an increase in hospital stay and associated costs (31). Excluding therapeutic uses of hypothermia, such as in cardiac arrest patients and neonatal hypoxic-ischemic encephalopathy, perioperative PH should be avoided (32, 33).

Currently, a plethora of patient warming devices is utilized to reduce the incidence of intraoperative hypothermia; the most used are forced-air warming systems, which warm the patient from the outside, and WF, which may prevent a decrease in body temperature in the setting of redistribution hypothermia (3). Several studies have shown that WF, as a single modality of warming, are effective in minimizing maternal PH, but did not reduce the incidence of shivering (23, 31, 34, 35).

In other studies, forced-air warming was used as the only warming method. Butwick et al. applied the forced-air warming on the lower extremities to warm the peripheral compartment below the level of sympathetic inhibition, but they found no improvements in preventing PH and shivering (4). Fallis and Horn et al. used forced-air warming on the upper extremities, but they obtained contrasting results in terms of maternal CT° and shivering (20, 21). Therefore, the optimal area of application (upper or lower) for forced-air warming still needs be determined.

In the last few years, many groups focused their study on the effects of combining AW on maternal CT° and shivering incidence.

Cobb et al. used combined intraoperative WF and lower body forced-air warming, and they found good results compared with no warmed control group in reducing the trend of maternal CT° decline and greater maternal thermal comfort, but they still observed high incidence of PH and no decrease in shivering incidence (2).

In contrast with our study, patients were hypothermic in AW group. A potential explanation could be that our surgeons were faster in performing CD.

In regards to the timing of application of warming systems, some studies demonstrated that prewarming is effective in preventing redistribution hypothermia after both neuraxial and general anesthesia (20, 36).

TABLE 3 | Bedside shivering assessment scale evaluation (BSAS) during the perioperative time.

BSAS time	score	NW group ($N = 26$) *n* (%)	WF group ($N = 26$) *n* (%)	AW group ($N = 26$) *n* (%)	*P*
BSAS T20	0	22 (85%)*+	26 (100%)*	26 (100% +	0.04*+
	1	1 (4%)	0 (0%)	0 (0%)	
	2	3 (11%)	0 (0%)	0 (0%)	
BSAS T40	0	12 (46%)#ç	24 (92%)#	26 (100%) ç	<0.001#ç
	1	10 (38%)	2 (8%)	0 (0%)	
	2	4 (16%)	0 (0%)	0 (0%)	
BSAS discharge	0	23 (88%)	25 (96%)	26 (100%)	0.383
	1	3 (12%)	1 (4%)	0 (0%)	
	2	0 (0%)	0 (0%)	0 (0%)	

Bedside shivering assessment scale evaluation. Data are expressed in terms of mean ± standard deviation.

BSAS, bed shivering assessment scale; NW, IV fluids at room temperature and no forced-air warming; WF, warmed IV fluids; AW, warmed IV fluids and forced-air warming; T20, at 20 min from baseline; T40, at 40 min from baseline.

**, NW vs. WF ($p = 0.04$); +, NW vs. AW ($p = 0.04$); #, NW vs. WF ($p < 0.001$); ç, NW vs. AW ($p < 0.001$).*

With the aim of reducing redistribution hypothermia and core-to-periphery temperature gradient, Jun et al. treated patients with forced-air warming placed over the entire body 15 min before starting anesthesia and WF coload during surgery (11). This strategy yielded only little efficacy in preventing perioperative CT° decline and shivering in the warmed group with respect to no warmed control. Our work demonstrated that the combination of AW was effective when active warming interventions are applied during the intraoperative time, when forced-air warming started soon after the spinal anesthesia and continued for the whole time of surgery.

Horn et al. applied forced-air warming on the upper extremities 15 min before anesthesia, showing an additional efficacy when it was combined with WF in the setting of epidural anesthesia and CD (20). Differently, we applied forced-air warming after spinal anesthesia.

Furthermore, Meghana et al. compared the use of combined lower extremities AW to only forced-air warming modality. They observed that combined warming methods preserved maternal CT° and reduced shivering in patients undergoing CD and spinal anesthesia, recommending the use of active warming methods in the operation theater (22).

In our work, the combined AW interventions dampened the decline in CT° in perioperative time and decreased the incidence of shivering more than WF group, although the difference was not significant, and we cannot make a strong suggestion for AW strategy as routine care during CD.

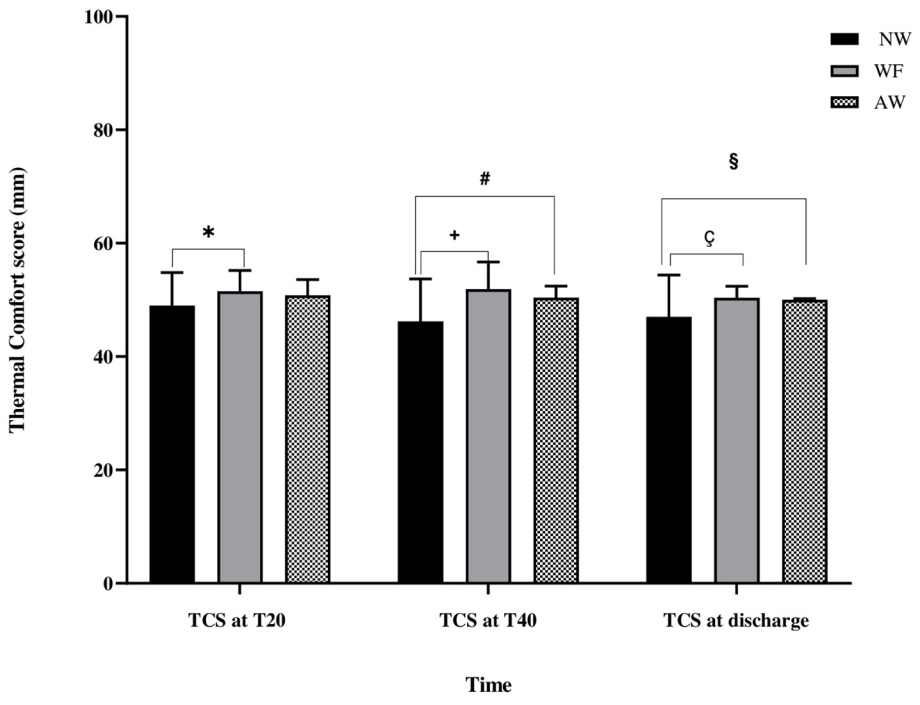

FIGURE 3 | Thermal comfort score evaluation (TCS) during the perioperative time. Thermal comfort score evaluation. Data are expressed in terms of mean ± standard deviation. TCS, thermal comfort score (mm); NW, IV fluids at room temperature and no forced-air warming; WF, warmed IV fluids; AW, warmed IV fluids and forced-air warming; T20, at 20 min from baseline; T40, at 40 min from baseline. *, NW vs. WF ($p = 0.04$); #, NW vs. AW ($p = 0.007$); +, NW vs. WF ($p < 0.001$); §, NW vs. AW ($p = 0.02$); ç, NW vs. WF ($p = 0.008$).

TABLE 4 | Neonatal demographics, APGAR scores, umbilical cord vein BGA, and temperature data.

	NW group (*N* = 26)	WF group (*N* = 26)	AW group (*N* = 26)	*P*
APGAR score 1 m	8 ± 8	8 ± 5	8 ± 3	0.948
APGAR score 5 m	8.9 ± 2	8.9 ± 3	9 ± 1	0.099
Temperature (°C) 1 m	35.9 ± 4	35 ± 7	36.6 ± 2	0.430
Temperature (°C) 5 m	35.9 ± 3	34.9 ± 7	36.6 ± 2	0.364
Birth weight (g)	2,966 ± 433	3,245 ± 597	3,087 ± 314	0.104
Umbilical cord vein BGA	7.32 ± 0.05	7.34 ± 0.05	7.33 ± 0.06	0.454

Data are expressed in terms of mean ± standard deviation.
NW, IV fluids at room temperature and no forced-air warming; WF, warmed IV fluids; AW, warmed IV fluids and forced-air warming; p, p-value; BGA, blood gas analysis.

As far as concern blood losses during surgery, a recent metaanalysis indicates that even mild hypothermia (CT° 34–36°C) increases blood loss and the relative risk of transfusion by small but significant amounts compared with normothermic surgical patients (37, 38). In our work, we did not observe a significant difference in blood loss among the three study groups.

Previous studies used infrared tympanic thermometer or oral probes whose reliability has been questioned (39). In our work, we used the SpotOn which is a new noninvasive, skin temperature monitoring system that measures the heat-flux in temporal artery by using a single-use sensor placed on the patient's forehead. Indeed, the SpotOn is much more sensitive to temperature changes when compared with other noninvasive devices.

In this work, our primary goals were the evaluation of maternal CT°, the incidence of shivering, and the maternal thermal comfort in perioperative period for women undergoing CD.

Secondary goals included blood loss evaluation and neonatal outcomes (Apgar score, axillary temperature, birth weight, and umbilical cord vein BGA), neither of them showing significant variation; these latter results are not surprising given our limited measures of neonatal well-being.

One potential limitation of our study consisted of the use of a nonvalidated visual analog scale for thermal comfort quantification. We used the same scale devised by Horn et al. (20) (0 mm = worst imaginable cold, 50 mm = thermoneutrality, and 100 mm = insufferable hot), which was not validated and was a little confusing to patients. Nevertheless, this scale can well differentiate between the hot and cold spectrum of discomfort compared with the one used by other studies that indicate with a verbal numerical scale the thermal comfort in terms of maternal satisfaction (0 = completely unsatisfied and 100 = completely satisfied) (2, 11).

Another limitation consisted of newborn CT° measured with axillary thermometer rather than the SpotOn system, which has a low level of accuracy as a method of monitoring body CT° in pediatric patients (<10 kg) (40).

CONCLUSIONS

In summary, our study showed that the combination of AW systems for elective CD under spinal anesthesia increases maternal CT°, prevents PH, and reduces the overall perioperative CT° decline and the incidence of shivering compared with the use of only WF or no interventions.

Based on the study findings, identification of women at risk of developing perioperative hypothermia may allow for more targeted AW strategies.

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 Comitato Etico Area 1-Azienda Ospedaliera Riuniti di Foggia (DDG n. 363 del 25.10.2016 e s.m.i. DDG n. 318 del 14.6.2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AC was responsible for the conception and design of the study, analyzed the data of the study, and wrote the manuscript. PM analyzed the data and wrote the manuscript. CF organized the database. PD performed the study. RB and SZ wrote a section of the manuscript. GC revised the final manuscript. AC, PM, CF, PD, RB, SZ, and GC approved the final manuscript. All authors contributed to the article and approved the submitted version.

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The Prognostic Capacity of the Radiographic Assessment for Lung Edema Score in Patients With COVID-19 Acute Respiratory Distress Syndrome—An International Multicenter Observational Study

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Background: The radiographic assessment for lung edema (RALE) score has an association with mortality in patients with acute respiratory distress syndrome (ARDS). It is uncertain whether the RALE scores at the start of invasive ventilation or changes thereof in the next days have prognostic capacities in patients with COVID-19 ARDS.

Aims and Objectives: To determine the prognostic capacity of the RALE score for mortality and duration of invasive ventilation in patients with COVID-19 ARDS.

Methods: An international multicenter observational study included consecutive patients from 6 ICUs. Trained observers scored the first available chest X-ray (CXR) obtained within 48 h after the start of invasive ventilation ("baseline CXR") and each CXRs thereafter up to day 14 ("follow-up CXR"). The primary endpoint was mortality at day 90. The secondary endpoint was the number of days free from the ventilator and alive at day 28 (VFD-28).

Results: A total of 350 CXRs were scored in 139 patients with COVID-19 ARDS. The RALE score of the baseline CXR was high and was not different between survivors and non-survivors (33 [24–38] vs. 30 [25–38], $P = 0.602$). The RALE score of the baseline CXR had no association with mortality (hazard ratio [HR], 1.24 [95% CI 0.88–1.76]; $P = 0.222$; area under the receiver operating characteristic curve (AUROC) 0.50 [0.40–0.60]). A change in the RALE score over the first 14 days of invasive ventilation, however, had an independent association with mortality (HR, 1.03 [95% CI 1.01–1.05]; $P < 0.001$). When

the event of death was considered, there was no significant association between the RALE score of the baseline CXR and the probability of being liberated from the ventilator (HR 1.02 [95% CI 0.99–1.04]; $P = 0.08$).

Conclusion: In this cohort of patients with COVID-19 ARDS, with high RALE scores of the baseline CXR, the RALE score of the baseline CXR had no prognostic capacity, but an increase in the RALE score in the next days had an association with higher mortality.

Keywords: intensive and critical care, ARDS, corona virus (COVID-19), mechanical ventilated, chest X-ray (CXR), RALE score, prognostication, radiograph (X-ray)

INTRODUCTION

Patients with coronavirus disease 2019 (COVID-19) frequently develop acute respiratory distress syndrome (ARDS), mandating intensive care unit (ICU) admission, usually for invasive ventilation (1, 2). Outcome prediction in these patients could use the classification based on the severity of oxygenation problems (3), albeit that this approach has been shown to be not so successful, at least not in patients with ARDS due to another cause (4). The chest radiograph (CXR) is a routine imaging tool for critically ill patients that receive invasive ventilation (5, 6) and could contribute to defining severity, progression, and complications and maybe also predict outcomes from COVID-19 (7, 8). One important drawback of the CXR, however, is the poor interobserver reliability in qualitative visual scoring of pulmonary opacifications (9).

The radiographic assessment for lung edema (RALE) score is a numeric scoring system, recently introduced in an attempt to improve the quantification of pulmonary abnormalities on the CXR. For this score, each quadrant of the chest at the CXR is scored for the extent of consolidations and density of opacities to define the extent and severity of lung parenchymal abnormalities. The RALE score has not only been found to have excellent diagnostic accuracy (10–12) but also to have the prognostic capacity in patients with ARDS due to COVID-19 (13, 14).

We hypothesized that the RALE score has the prognostic capacity in patients with COVID-19 ARDS (15, 16). In this international study, we determined the prognostic capacity of the RALE score of the first available CXRs that was obtained under invasive ventilation for COVID-19 ARDS. We also wished to determine the prognostic capacity of changes in the RALE score over the first 14 days after initiation of invasive ventilation.

METHODS

Study Design

This is an international, multicenter, retrospective observational study in invasively ventilated patients with COVID-19 admitted to participating ICUs between December 1, 2019, and May 31, 2020. The study enrolled ICU patients in the Amsterdam UMC, location AMC, Amsterdam, The Netherlands; University of Bari Policlinic Hospital, Bari, Italy; Miulli Regional Hospital, Acquaviva Delle Fonti, Italy; Centre Hospitalier Universitaire Brussels, Brussels, Belgium; Mahidol University Hospital in Bangkok, Thailand; and Chonburi Hospital, Chonburi, Thailand. The study protocol was initially approved by the institutional review board of the Amsterdam UMC, location AMC, Amsterdam, The Netherlands (approval letter W20_311 # 20.346). Thereafter, the protocol was approved in other hospitals. The need for individual patient informed consent was waived because of the observational nature of the study. The study is registered at clinicaltrials.gov (trial identification number NCT 04485338).

Inclusion and Exclusion Criteria

Consecutive patients were included if (1) admitted to one of the participating ICUs, (2) received invasive ventilation; and (3) with ARDS due to COVID-19 that was confirmed by reverse transcriptase-PCR. Patients were excluded if aged <18 years of age, when COVID-19 was not the reason for invasive ventilation, or if there was no CXR within 48 h after starting intubation.

Data Collection

An online case report form (www.castoredc.com) was used to collect and store the study data. Baseline and demographic characteristics included age, gender, body mass index (BMI); severity indexes, such as the acute physiology and chronic health evaluation (APACHE) II and IV score and the Sequential Organ Failure Assessment (SOFA); and ventilation characteristics at the moment of the CXR, such as FiO_2, positive end-expiratory pressure (PEEP), maximum airway pressure (Pmax), respiratory rate, tidal volume, and the nearest blood gas analysis results.

We collected all CXRs that were taken within the first 14 days after the start of invasive ventilation from each electronic imaging system and uploaded de-identified CXRs in JPEG format into the database.

RALE Scoring

The RALE score was calculated as described before (11, 13). In short, the chest at the CXR was divided into 4 quadrants by a vertical line over the spine and a horizontal line at the level of the first branch of the left main bronchus; each quadrant was then scored for the extent of alveolar opacities (*consolidation* score, from 0 to 4) and the corresponding density of alveolar opacities (*density* score, from 1 to 3) (**Supplementary Figure 1**). In case no consolidations were visible, the consolidation score was "0," and density was not scored. The final score is the sum of the product of the consolidation and density scores for each quadrant. The RALE score ranges from 0 (no abnormalities) to 48 (maximum abnormalities), where in a recent study, patients with ARDS have

RALE scores that range from 15 to as high as 26 (16). Among patients with ARDS, the baseline RALE score is not associated with the ARDS severity groups by P/F ratio (16).

Every CXR was scored by at least two independent scorers that were extensively educated in calculating RALE scores. For this, each scorer was trained in the RALE score by one of the investigators (CZ), who was trained during a 1-month focused period by the team that developed the RALE score (11). An interclass correlation coefficient (ICC) > 0.8 between the trainer and other scorers on a training sample of 22 CXRs from another set of CXRs of patients with ARDS was a prerequisite for scoring CXRs in the study dataset. A third scorer was involved only if the difference in numeric RALE score between two scorers was >25%, to reach a final consensus.

Endpoints

The primary endpoint was 90-day mortality; the secondary endpoint was the number of days free from the ventilator and alive at day 28 (VFD-28).

The ventilator and alive at day 28 was calculated as the number of days that a patient was alive and free of invasive ventilation if the period of unassisted breathing lasted > 24 consecutive hours. Patients who died or received invasive ventilation for more than 28 days had the lowest number of VFD-28, i.e., 0 days.

Statistical Analysis

We did not perform a formal sample size calculation—instead, the available patients served as the sample size for this study.

Demographic data and outcomes are summarized as mean (SD) or medians (interquartile range) for continuous variables and as frequencies (percentage) for categorical variables. In the case of normally distributed, continuous variables were compared between groups with a *t*-test or ANOVA. When not considered normally distributed, continuous variables were compared between groups with Mann–Whitney U test or Kruskal–Wallis test, as appropriate. Categorical variables were compared between groups by chi-square analysis. Missing data imputations were performed by random forest whenever any variable included in the analysis showed a missing data percentage of >10%.

Interobserver variability was assessed using ICC with a two-way mixed agreement model. Bland–Altman plots were used to visualize the aggregate agreement between the two scorers initially assessing a CXR.

The first CXR was labeled "baseline CXR." The association between the RALE score of the baseline CXR with mortality as a time-to-event was analyzed with a Cox regression model, reporting the hazard ratio (HR) with a 95% CI. Herein, baseline RALE score was used as a continuous numerical variable, while age, gender, pH, and lactate were entered as covariates. In an additional Cox model, the RALE score was categorized into quartiles. Herein, proportionality assumptions were checked by Schoenfeld and martingale residuals and influential observations. The predictive accuracy of the RALE score of the baseline CXR for 90-day mortality was also described by the area under the receiver operating characteristics curve (AUROC) with 95% CI.

The association of the baseline RALE score with VFDs was tested using a competing risk model with extubation and death as the events of interest. The results are described with the use of cumulative incidence function and reported as sub-distribution HR with 95% CI estimated from a Fine–Gray model (12).

To assess the association of changes in the RALE score overtime in the first 14 days from onset of mechanical ventilation of consecutive CXRs with mortality, we use a joint model fitting repeated RALE scores with a mixed model and mortality as a time-to-event variable using the same covariates specified in the previous models. The joint model combines Cox regression and linear mixed-effects (LME) models, where the LME part of the models estimates the linear change pattern of the RALE score over follow-up time.

All analyses were performed using a two-sided superiority hypothesis test, with a significance level of 0.05, and presented with a two-sided 95% CI. No corrections were performed for multiple comparisons across secondary clinical outcomes, thus, these findings should be considered exploratory. Analyses were performed using software R (version 4.0.2, R Core Team, 2016, Vienna, Austria).

RESULTS

Patients

From December 1, 2019, to May 31, 2020, 178 patients were screened in 6 ICUs. We excluded 36 patients from the analysis because a baseline CXR was missing and 3 other patients because of incomplete data (**Supplementary Figure 2**). In the remaining 139 patients, 350 CXRs were available within the first 14 days of invasive ventilation.

Baseline characteristics, ventilation characteristics, and outcomes are presented in **Tables 1**, **2**. The median age was 65 [59–74] years; the most common comorbidities were hypertension and diabetes. The majority of patients had moderate-to-severe ARDS with low lung compliance. Patients who did not survive had no improvement in lung compliance nor the RALE score compared to survivors during the first 14 days (**Supplementary Figure 3**). Non-survivors did receive a higher FiO_2, PEEP, and peak pressure than survivors. The crude 90-day mortality was high, 61.2%.

RALE Scoring

The interobserver agreement was high (ICC, 0.95 [95% CI 0.93–0.96]). In 14 of 350 (4%) CXRs, a third scorer was needed to reach the final consensus. Bland–Altman plots are shown in **Supplementary Figure 4.** The RALE scores of baseline CXRs were high, with a median RALE score of 32 [24–38], but comparable between survivors and non-survivors (**Figure 1A**). The RALE score of the baseline CXR was increased with worsening of ARDS severity (**Figure 1B**). Linear regression analysis showed how the RALE had no significant association with the dynamic compliance, with an R^2 of 0.0001.

TABLE 1 | Baseline characteristics of the patients.

	Overall ($n = 139$)	Alive* ($n = 54$)	Dead ($n = 85$)	*P*-value	SMD
Demographics					
Age, years (median [IQR])	65 [59–74]	61.0 [55–71]	69 [60–75]	0.002	0.505
Male gender—no (%)	65 (46.8)	27 (50.0)	38 (44.7)	0.663	0.106
Body mass index, kg·m^2 (median [IQR])	27.3 [24.7–30.0]	26.9 [24.2–29.4]	27.5 [25.4–30.0]	0.595	0.086
Comorbidities and severity					
Co-existing disorders—no (%)					
Hypertension—no (%)	70 (50.4)	25 (46.3)	45 (52.9)	0.555	0.133
Diabetes—no (%)	41 (29.5)	13 (24.1)	28 (32.9)	0.354	0.197
Chronic obstructive pulmonary disease—no (%)	23 (16.5)	7 (13.0)	16 (18.8)	0.502	0.161
Cardiovascular disease—no (%)	17 (12.2)	3 (5.6)	14 (16.5)	0.099	0.354
None—no (%)	17 (12.2)	9 (16.7)	8 (9.4)	0.314	0.217
Other—no (%)	60 (43.2)	14 (25.9)	46 (54.1)	0.002	0.601
APACHE II (median [IQR])	15 [12–20]	15 [12–18]	15 [12–20]	0.132	0.318
SOFA score (median [IQR])	6 [4–8]	6 [4–8]	5 [4–8]	0.481	0.033
Outcomes					
Survival time, days (median [IQR])	26 [19–90]	90 [90–90]	11 [7–21]	<0.001	4.525
ICU length of stay, days (median [IQR])	12 [70–20]	12 [6–21]	11 [7–20]	0.416	0.285
Hospital length of stay, days (median [IQR])	15 [8–29]	26 [15 −38]	11 [7–21]	<0.001	0.214

Data are median (quartile 25%–quartile 75%), mean (±SD) or No (%). Percentages may not total 100 because of rounding.
ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment.
**At day 90.*

TABLE 2 | Ventilation parameters measured with the baseline chest radiography.

	Overall ($n = 139$)	Alive* ($n = 54$)	Dead ($n = 85$)	*P*-value	SMD
Ventilatory parameters					
Ventilation mode—*n* (%)				0.252	0.413
Pressure controlled	36 (28.1%)	11 (21.6%)	25 (32.5%)		
Pressure support	7 (5.5%)	5 (9.8%)	2 (2.6%)		
Volume controlled	53 (41.4%)	20 (39.2%)	33 (42.9%)		
ASV/Intellivent	8 (6.2)	3 (5.9%)	5 (6.5%)		
Spontaneous	24 (18.8)	12 (23.5%)	12 (15.6%)		
FiO_2, % (median [IQR])	70 [50–90]	55 [40–80]	80 [60–100]	0.001	0.663
PaO_2/FiO_2 (median [IQR])	130 [88–175]	138 [96–184]	112 [86–152]	0.082	0.294
Tidal volume set, ml (median [IQR])	450 [410–500]	475 [403–500]	450 [415–495]	0.532	0.236
Tidal volume measured, ml (median [IQR])	450 [400–500]	458 [378–512]	450 [408–500]	0.852	0.069
Tidal volume, ml/kg PBW (median [IQR])	7.0 [6.3–7.5]	6.6 [6.0–7.6]	7.2 [6.5–7.6]	0.065	0.298
Respiratory rate, breaths/min (median [IQR])	20 [17–26]	24 [18–26]	20 [16–25]	0.134	0.225
Peak pressure, cmH_2O (median [IQR])	25 [18–29]	23 [17–26]	26 [21–30]	0.017	0.464
PEEP, cmH_2O (median [IQR])	10 [8–12]	9 [8–10]	10 [8–12]	0.013	0.378
Plateau pressure, cmH_2O (median [IQR])	25 [23–28]	25 [21–26]	26 [24–30]	0.281	0.578
Dynamic compliance, ml/cmH_2O (median [IQR])	29 [21–51]	34 [23–47]	26 [20–61]	0.491	0.113

Data are median (quartile 25%–quartile 75%), mean (±SD) or No (%). Percentages may not total 100 because of rounding.
PEEP, positive end-expiratory pressure; ASV, adaptive support ventilation; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of oxygen.
**At day 90.*

The Prognostic Capacity of the RALE Score of the Baseline CXR

The RALE score of the baseline CXR had no association with mortality (HR, 1.24 [95% CI 0.88–1.76]; $P = 0.222$). Estimates of 90 days survival in patients stratified by quartiles of the baseline RALE score is shown in **Figure 2**. There was no difference in survival between the quartiles after adjusting for age, gender, arterial pH, and plasma lactate. The baseline RALE score had no prognostic capacity for mortality (**Figure 3**).

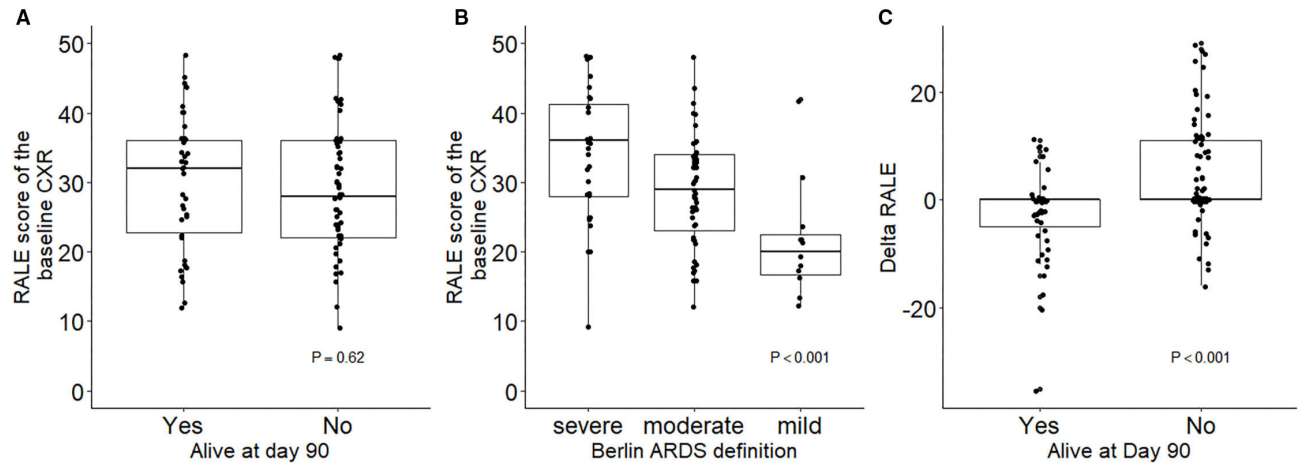

FIGURE 1 | Baseline RALE scores in survivors vs. non-survivors **(A)** and patients with different ARDS severity **(B)**. Changes in RALE score across the first 14 days after onset of invasive ventilation in survivors vs. non-survivors **(C)**. RALE, radiographic assessment for lung edema; ARDS, acute respiratory distress syndrome.

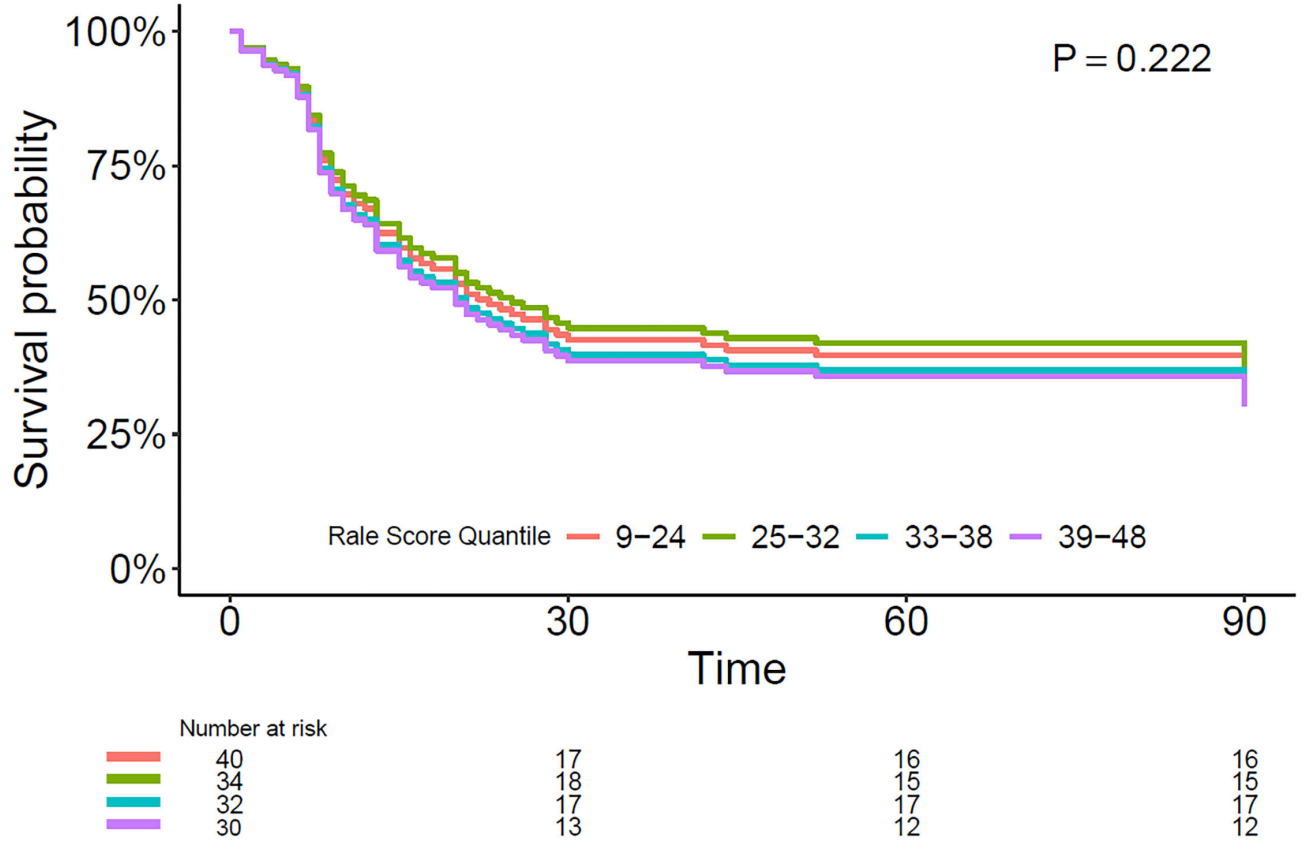

FIGURE 2 | Multivariable Cox model with survival estimates during the 90-day follow-up period in patients stratified by baseline RALE score quartiles. RALE, radiographic assessment for lung edema.

The Prognostic Capacity of Changes in RALE Score

The change in the RALE score over time was different between survivors and non-survivors (**Figure 1C**). An increase in the RALE score until day 14 had an independent association with mortality (HR, 1.03 [95% CI 1.01–1.05]; $P < 0.001$). In other words, for every point increase in the RALE score over time the risk of death increased by 3% [95% CI 1–5%].

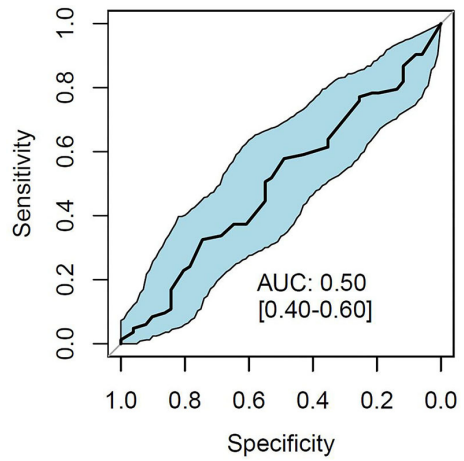

FIGURE 3 | Discriminative capacity of the baseline RALE score for 90-day mortality. AUC, area under the curve; RALE, radiographic assessment for lung edema.

Supplementary Figure 5 shows how the gradual worsening in RALE score over time increases the probability of death at 28 days.

Association Between the RALE Score and VFD-28

Overall patients had 0 [0–14] VFD-28, where survivors had 18 [11–23] VFD-28 and a duration of invasive mechanical ventilation of 10 [6–16] days. When the event of death was considered, there was no significant association between the RALE score of the baseline CXR and the probability of being liberated from the ventilator (HR 1.02 [95% CI 0.99–1.04]; $P = 0.08$).

DISCUSSION

The findings of this international multicenter study in patients with COVID-19 ARDS can be summarized as follows: (i) the extent and severity of parenchymal damage quantified by the RALE score were very high in survivors and non-survivors; (ii) the RALE score of the baseline CXR was neither associated with mortality in the first 90 days nor with successful liberation from invasive ventilation; and (iii) a worsening of the RALE score over the first 14 days of invasive ventilation was associated with an increased risk of death.

This study has several strengths. The study was designed to minimize bias by strictly adhering to a predefined statistical analysis plan and training of scorers. There was a minimal loss to follow-up. We had a low interobserver variability between the scorers, confirming the feasibility and reliability of the RALE score (11, 12, 16, 17). Finally, patients were enrolled in 6 hospitals in 4 different countries and included patients in university hospitals, teaching and non-teaching hospitals, contributing to the generalizability of the findings.

Counter to our hypothesis, we did not find an association of the RALE score of the baseline CXR with mortality or liberation from mechanical ventilation. We could even not find an association with mortality in the quartile with the highest RALE scores. The findings of our study are in line with those from a recent study that could not establish an association between RALE and mortality in ICU patients with COVID-19 (18). Contrasting, the baseline RALE score in patients with COVID-19 presenting to the emergency department did predict adverse outcomes (19) and also in patients with less severe COVID-19 ARDS located outside of an ICU (15). However, another study with a lower median RALE score in both survivors and non-survivors confirmed the capacity of the RALE score to predict adverse outcomes, defined as death or need for invasive ventilation (10). Our findings ultimately suggest that, when baseline RALE scores are high, the RALE score may not be helpful in predicting mortality and the chance of liberation from the ventilator.

Studies that assessed the prognostic capacity of the RALE score in patients with ARDS due to another cause than COVID-19 had conflicting results. The baseline RALE score did not predict outcome in these patients (16). Another study did find an association of baseline RALE with mortality (14). However, patients in this latter study showed very similar baseline RALE between survivors and non-survivors, just as in our cohort. In another RALE study, there was an association between the baseline RALE score and 28-day mortality, but without an association with VFDs (13). The median RALE score in our cohort of patients with COVID-19 ARDS was much higher than the RALE score reported in patients with ARDS due to another cause (12, 14–16, 20). For instance—in patients with ARDS included in the original study that reported on the RALE score, the RALE score was 27 [18–35] (13). Moreover, patients with a RALE score >30, frequently seen in our cohort, were in the highest quartile in a recent secondary analysis of another non-COVID ARDS trial (14). Whether COVID-19 ARDS is characterized by important pathophysiological differences compared to classical ARDS is still debated (21–24). In our cohort, median values of compliance were consistently low and in line with findings in previous studies (25, 26). Furthermore, our study confirmed the association between the change in RALE score over time and outcome, already identified in a recent study conducted on ARDS patients due to other etiologies (14). The change of RALE score over time was independently associated with outcome. This confirms recent findings in patients with COVID-19 in which the RALE score predicted mortality and the need for invasive ventilation (10). Furthermore, an increase in the RALE score was found to be associated with a prolonged need for invasive ventilation and with a lower number of VFDs in ICU patients with ARDS (16). In addition, the prognostic effect of early changes in RALE score in moderate-to-severe ARDS has been confirmed by another study in 135 ICU patients (14). The consistency of these findings, added to the feasibility of repeated bedside CXRs in patients with COVID-19, allows for the changes in RALE score to be used as an increasingly established prediction tool.

The RALE score is an easy reproducible tool that can be easily computed after a CXR is made. The interobserver variability we found is comparable to one of the original studies (11) and subsequent investigations (12–14, 16). Although dedicated CXR apparatuses are still not ubiquitous in ICUs in some low- and middle-income countries (27), it is considered a routine imaging tool for patients who receive invasive ventilation (5, 6). Conversely, CT is a more costly and less available imaging technique, with feasibility issues in patients with COVID-19 and with a radiation load that is much higher than that of a CXR (28). The findings of this and previous studies suggest that the RALE score is an attractive visual metric, especially in settings with low resources.

This study has several limitations. The retrospective design limits the inclusion of all potential confounders. The sample size of this study was relatively small. However, the narrow CI suggests that repeating the study on a larger sample is unlikely to change the result of a significant association between change in RALE score and outcome. Similarly, due to the retrospective collection of study CXRs, time points for CXRs could not be strictly predefined. The third scorer was not blinded for the previous scores, and this could have generated scoring bias. However, high interobserver variability between first and second scorers was only found in <5% of CXRs.

CONCLUSIONS

In this cohort of patients with COVID-19 ARDS, the RALE score of the baseline CXR was neither associated with 90-day mortality nor with the probability of being liberated from the ventilator. However, an increase in the RALE score over the next days had an association with higher mortality.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Board AMC. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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.2021.772056/full#supplementary-material

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Bioactivity of Inhaled Methane and Interactions With Other Biological Gases

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A number of studies have demonstrated explicit bioactivity for exogenous methane (CH_4), even though it is conventionally considered as physiologically inert. Other reports cited in this review have demonstrated that inhaled, normoxic air-CH_4 mixtures can modulate the in vivo pathways involved in oxidative and nitrosative stress responses and key events of mitochondrial respiration and apoptosis. The overview is divided into two parts, the first being devoted to a brief review of the effects of biologically important gases in the context of hypoxia, while the second part deals with CH_4 bioactivity. Finally, the consequence of exogenous, normoxic CH_4 administration is discussed under experimental hypoxia- or ischaemia-linked conditions and in interactions between CH_4 and other biological gases, with a special emphasis on its versatile effects demonstrated in pulmonary pathologies.

Keywords: normoxia, hypoxia, bioactive gases, methane, mitochondria

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INTRODUCTION

Respiration From the Atmosphere to the Cells

In the Earth's atmosphere, where oxygen (O_2) accounts for ~ 21% of the environmental gases, reduction-oxidation reactions provide the energy which makes complex organisms capable of sustaining life (Schmidt-Rohr, 2020). Heterotrophs, such as humans, consume organic compounds for energy production by burning O_2, with carbon dioxide (CO_2) and water as the ultimate end products. Through this process, the inspired O_2 level in the lungs is reduced to about 14.5% by the presence of alveolar water vapour and CO_2, and then the O_2 levels range from 3.4 to 6.8% by the time it reaches the peripheral tissues (Carreau et al., 2011). Thus, "normoxia" corresponds to the atmospheric O_2 pressure, and much lower but still physiological ("normal") levels of O_2 are found in different tissues within the organs (Carreau et al., 2011). The evolution of aerobic cells has created a range of control mechanisms for the optimal utilization of O_2 for subcellular, mitochondrial respiration, where multiprotein complexes of the electron transport system (ETS) are dedicated to accepting electrons from reduced carriers and delivering them to accessible molecular O_2. Three of these complexes (Complex I, III and IV) are also H^+ channels, responsible for a transmembrane electrochemical gradient between the surfaces of the inner membrane and the resulting driving force for ATP synthase (Complex V), which transforms adenosine diphosphate (ADP) into adenosine triphosphate (ATP).

In this substrate-level oxidative phosphorylation (OxPhos) reaction, availability of O_2 is the most critical issue. However, many other gases, oxidative or reductive metabolic by-products of this aerobic system can also influence the intra- and extramitochondrial responses. In addition, it is highly likely that the many ways in which the gases combine both in physical and biochemical ways

determine the nature of organ responses in clinical conditions associated with hypoxia. The first aim of this review is to summarize the knowledge of certain possibilities through which mitochondrial activity may be modulated by exogenous biological gases, with special emphasis on pulmonary reactions. Indeed, in terms of clinical applications, pulmonary gas delivery is an attractive idea, since applying a bioactive agent either prophylactically or at the time of an operation allows for prompt, specific and local interventions at the barrier sites of the respiratory tract. In this sense, a medically important gas should be easy to apply, have the appropriate chemical and physical properties and kinetics (e.g., be dissolved in plasma), and be nontoxic and biocompatible to achieve the expected biological results. The research on bioactive gases and derivatives has been intense, leading to the listing of four essential characteristics (simplicity, availability, volatility and effectiveness) and the definition of six criteria that make a gas physiologically important or irreplaceable (Wang, 2014). To date, nitrogen monoxide (NO), hydrogen sulphide (H_2S) and carbon monoxide (CO) are "officially" recognized as signalling substances and referred to as gasotransmitters (Wang, 2014). Against this background, many attempts have concentrated on the therapeutic outcomes of gas deliveries of individual gasotransmitters in various pathological conditions. Nevertheless, the consequences of a more complex interplay of intrapulmonary O_2 with NO, CO or H_2S have not yet been investigated systematically.

Further, it should also be taken into account that there are many other gas molecules present in the cellular environment that do not fully meet the gas mediator criteria under the current classifications. Although methane (CH_4) is conventionally believed to be physiologically inert, studies cited in this review demonstrate that it can modulate the pathways involved in key events of inflammation and influence the interactions of other biological gases. Therefore, the second part deals with CH_4 bioactivity, a consequence of exogenous, normoxic CH_4 administration in experimental hypoxic conditions and the implications of its interactions with other gases in respiratory pathologies.

Subcellular Hypoxia

Hypoxic air induces a number of compensatory responses in the microenvironment of the lung. As the cells become less oxygenated, pulmonary mitochondria have less access to substrates (O_2 and acetyl-CoA), and the uncontrolled calcium (Ca^{2+}) influx is accompanied by reactive oxygen species (ROS) formation (Lukyanova and Kirova, 2015). More importantly, a rapid compensatory mechanism prevents or reverses acute hypoxia-induced disturbances, while a delayed mechanism is responsible for a reversible reprogramming of the regulation of mitochondrial complexes so that the mitochondrial respiratory chain switches from oxidation of NAD-related substrates (Complex I) to succinate oxidation (Complex II), thus providing proper ATP synthesis. Indeed, Complex I contributes to roughly 80% of mitochondrial respiration in normoxia, whereas, during an impeded or deficient O_2 supply, this is significantly reduced in favour of Complex II [also causing mitochondrial fission through GPR91 signalling (Lu et al., 2018)], which then contributes to nearly 75–90% of the total respiration. Therefore, these hypoxic cells are able to respond in a regulated manner to reduced O_2 supply; compensatory mechanisms will ensure adequate ATP synthesis until the cellular PO_2 reaches a critically low (<1%) level. It follows that a mechanism that allows cells to sense even a minimal change in O_2 supply activates signalling pathways responsible for triggering adaptive responses. Cytochrome c oxidase (Complex IV) is the main enzyme that transfers electrons and binds O_2 in the ETS, and thus it was proposed that cells should have O_2-sensing mechanisms regardless of their bioenergetic state (Bell et al., 2005; Guzy and Schumacker, 2006; Kierans and Taylor, 2021).

The transcriptional activator hypoxia-inducible factor (HIF-1) is responsible for regulating oxygenation and is required for the increased expression of more than 60 genes under hypoxia. In aerobic conditions, cells express the COX4-1 regulatory subunit of Complex IV under HIF-1 regulation but switch to the COX4-2 subunit in hypoxic conditions (Semenza, 2011). The stability, subcellular localization and transcriptional activity of HIF-1α are also strongly affected by changes in O_2 levels. In normoxia, the transcriptional activity of HIF-1α is inhibited by ubiquitous proteases **(Figure 1)**. In this process, HIF-1α can bind to the von Hippel–Lindau tumour suppressor protein (pVHL) after hydroxylation of prolyl (PHD 1,3) (with 2 oxoglurate and Fe^{2+} as cofactors), which promotes ubiquitin-mediated degradation (Bell et al., 2005). In O_2-deficient states, hydroxylation does not occur, so pVHL cannot bind to HIF-1α, leading to a decrease in degradation processes. In the normoxic state, the binding of the transcription cofactors p300 and CBP to HIF-1α is inhibited so that, in contrast to the hypoxic state, further transcription processes are also prevented (Bell et al., 2005).

HIF-1α is regulated by mitochondria in two different ways. Firstly, if the respiration is inhibited, an intracellular O_2 level of around 1% is still sufficient for the hydroxylation of HIF-1α. Hydroxylation is only reduced when O_2 tensions are below 1% so that OxPhos or the ability to respire is not related to the regulation of hypoxic stabilization of HIF-1α. The second is that ROS production during hypoxia is required for HIF-1α protein stabilization (Brunelle et al., 2005). It would logically follow that in hypoxic conditions, ROS formation is reduced in the absence of O_2, but the levels paradoxically increase during hypoxia (Guzy and Schumacker, 2006). It has been shown that ROS generated at Complex III stabilize HIF-1α during hypoxia (Solaini et al., 2010) and that HIF-1α expression is reduced when Complex V is inhibited with oligomycin (Gong and Agani, 2005). Taken together, the available data do not allow us to clearly establish the exact role of mitochondrial ROS in the regulation of HIF-1α, but the pathway that stabilizes HIF-1α can undoubtedly be considered mitochondria-dependent. According to some authors, mitochondrial ROS can also stabilize HIF-1α under hypoxic conditions (*via* a transcriptional regulatory cascade) *via* the nuclear factor E2-related factor 2 (Nrf2) pathway (Lacher et al., 2018; Potteti et al., 2021).

Together with Complex III and IV, the Complex I (NADH: ubiquinone oxidoreductase) is involved in proton pumping from the matrix to the intermembrane space (extruding four hydrogen

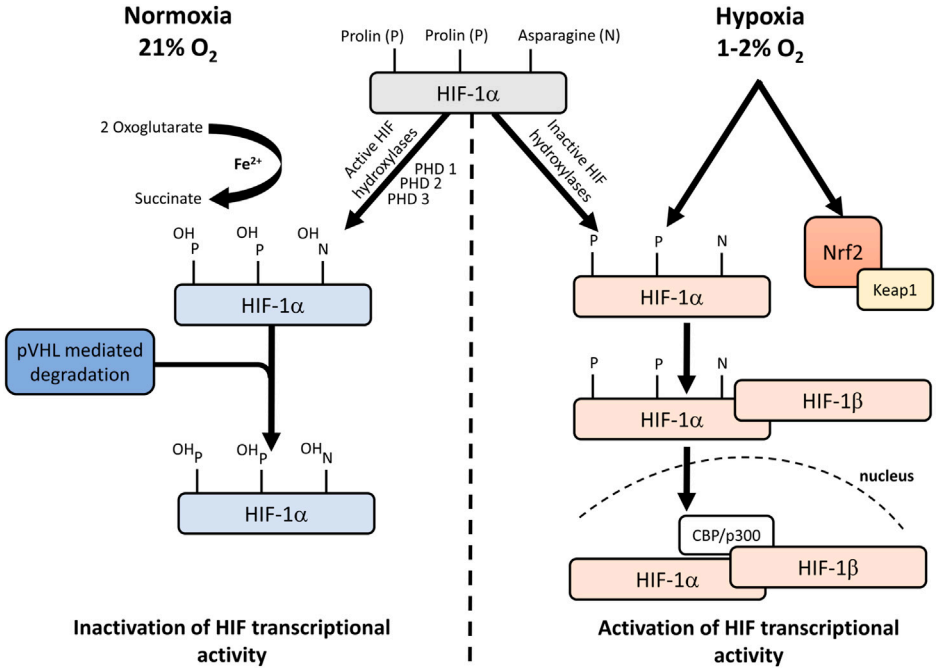

FIGURE 1 | Hypoxic regulation of HIF-1. Under normoxic conditions, NO impairs the activity of HIF-1α prolyl hydroxylases and inhibits HIF-1α ubiquitination and interaction with pVHL. Under hypoxic conditions, NO induces Keap1 signalling and suppresses HIF-1α hypoxic stabilization. CH_4 increases the expression of Nrf2. HIF: hypoxia-inducible factor; pVHL: von Hippel–Lindau protein; Ub: ubiquitin; NO: nitrogen monoxide; PHD: prolin hydroxylases; Keap1: kelch-like ECH-associated protein-1; Nrf2: nuclear factor-erythroid factor 2-related factor 2.

ions per NADH). Through this action, Complex I collects the Krebs cycle-derived reducing equivalents and participates in redox energy conversion, and the proton gradient across the membrane is then used for energy production by the ATP synthase during OxPhos (Ramsay, 2019). More importantly, considerable amounts of ROS can be generated by Complex I in the mitochondrial matrix when electrons flow both in the forward (forward electron transport; FET) or reverse (reverse electron transport; RET) direction. To date, flavin mononucleotide (FMN), Q-binding site and the iron–sulphur cluster N2 have been identified in mitochondrial superoxide generation. Although RET was long considered as an *in vitro* phenomenon, the *in vivo* role in ROS generation has recently been demonstrated (Scialò et al., 2017).

Complex I is one of the largest membrane-bound enzymes (1 MDa MW), with a FMN-containing protein and a number of (eight) iron–sulphur centres. The L-shaped structure consists of two major parts, with the components embedded in the inner membrane and the peripheral arm located in the cytoplasm or mitochondrial matrix (Martin and Matyushov, 2017). When a hydride ion is transferred from NADH to FMN at the peripheral arm, two electrons pass through the iron–sulphur clusters (chain of electron transfer cofactors; Fe2S2 and seven Fe4S2; terminal cofactor N2) to ubiquinone (membrane domain), where the proton extrusion is carried out through the membrane. A unique characteristic of Complex I has recently gained much attention as it has been demonstrated that the limited *in vivo* O_2 availability deactivates Complex I, which is required for the catalytic activity of ETS enzymes (Maklashina et al., 2002; Hernansanz-Agustín et al., 2017). Given a lack of available substrate, Complex I spontaneously forms a deactive (D) form that can be re-activated by exogenous NADH and ubiquinone administration (Galkin and Moncada, 2017; Blaza et al., 2018). The active (A) state catalyses the rapid NADH oxidation at a linear rate, while a lag phase is present during the D→A transition. The lag phase is prolonged at alkaline pH or in the presence of divalent cations, such as Ca^{2+} or Mg^{2+}. Most notably, the transition from catalytically active to dormant D form also occurs during acute hypoxia or ischaemia. The biological consequences of the conformational change are not fully mapped, but is has been shown that it fine-tunes ETS, may reduce oxidative/nitrosative stress and switches the NADH: ubiquinone oxidoreductase activity to a sodium-proton (Na^+/H^+) antiporter through its hydrophobic membrane-bound domains (ND2, ND4 and ND5 subunits) (Roberts and Hirst, 2012; Babot et al., 2014; Hernansanz-Agustín et al., 2017). In addition, the D-form is more sensitive to ischaemia/reperfusion (IR)-mediated oxidative injury than the A-form. Therefore, modulation of the dormant form may also be a protective strategy during ischaemia/hypoxia (Chouchani et al., 2013; Gorenkova et al., 2013).

Hypoxia and Inhaled Bioactive Gases

Although inhaled NO has been successfully tested in neonates and adult patients with acute respiratory distress syndrome (Feng et al., 2021; Lotz et al., 2021; Safaee Fakhr et al., 2021), the clinical

benefit of intrapulmonary administration is still subject to much debate (Sokol et al., 2016; Vieira et al., 2021). In this line, the oxygenation of the tissues is a main factor when the rather controversial results of gasotransmitter reactions are discussed. For example, the most important physiological mechanism linked to NO metabolism requires proper O_2 concentrations; under normal or higher O_2 tension, the half-life of NO is shorter, while in hypoxic environments NO will be eliminated after a significantly longer time with a number of prolonged effects (Kuschman et al., 2021). Likewise, a combination of low O_2 tension with mitochondria-derived ROS and higher NO flows leads to peroxynitrite formation (Thomas et al., 2008) with nitroxidative stress and post-translational protein modification (Campolo et al., 2020). Further, like other inhibitors of mitochondrial respiration, NO prevents the stabilization of HIF-1α. A recent key finding has revealed a novel role for Complex I in this process, as prior A/D conversion is necessary for S-nitrosothiols and peroxynitrite to interfere with the respiratory activity of mitochondria (Babot et al., 2014; Galkin and Moncada, 2017). Therefore, the hypoxia-linked mitochondrial duality may explain, at least partly, the controversial clinical results and the narrow range of effectiveness of NO inhalation.

H_2S is the next gas mediator, with Janus-faced characteristics being clearly present at the mitochondrial level. It is toxic when inhaled in high concentrations, while it is anti-inflammatory and cytoprotective at low partial pressure (Elrod et al., 2007; Cui et al., 2016; Scheid et al., 2021). Inhalation of 80–150 ppm H_2S induces a suspended animation state with reduced metabolic rate, which leads to an increased resistance to severe hypoxia (5% FiO_2) (Blackstone and Roth, 2007). In an oxygenated environment and in low (less than 1 μM) H_2S concentrations, the regular substrates of the respiratory chain are used for biological oxidation. However, as soon as H_2S content is increased (to less than 10 μM) an active sulphide quinone reductase (as the immediate electron acceptor) is available, H_2S acts as an alternative electron source for the respiratory chain. A H_2S concentration of over 10 μM impairs the mitochondrial function with the inhibition of Complex IV (Bouillaud and Blachier, 2011). Here it should be added that cancer cells may utilize this phenomenon by up-regulating H_2S, thus producing enzymes to stimulate mitochondrial ATP synthesis and maintain mitochondrial function (Szabo, 2021).

Carbon monoxide (CO) is the third gas in the sequence of gasotransmitters, again with dual properties: low levels exert cyto- and tissue protective effects, but in higher concentrations systemic toxicity comes to the fore. Due to its affinity to bind to the haem iron centre of haemoglobin, carboxyhaemoglobin (CO-Hb) formation ensues with cellular hypoxia. A number of signal transduction pathways have been recognized as potential targets of low concentrations of inhaled CO *via* its anti-inflammatory (Otterbein et al., 2000), anti-apoptotic (Ryter et al., 2018), anti-oxidative (Parfenova et al., 2012) and anti-proliferative effects. CO binds primarily to haem iron and may activate soluble guanylate cyclase, although with lower efficacy than NO (Sharma and Magde, 1999). Through the modulation of the mitogen-activated protein kinase pathway, CO inhibits the expression of several pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), and increases the expression of the anti-inflammatory cytokine interleukin-10 (IL-10) (Otterbein et al., 2000). The mitochondrium is also one of the recognized cellular targets for CO, with physiological concentrations of CO increasing mitochondrial ROS generation, which activates cellular endogenous mechanisms of defence involved in preconditioning and cytoprotection (Bilban et al., 2008). Furthermore, CO prevents apoptotic cell death by limiting mitochondrial membrane permeabilization, which inhibits the release of pro-apoptotic factors into the cytosol; both events are ROS-dependent (R. Oliveira et al., 2016). The protective effects of low concentrations (of up to 500 ppm) of inhaled CO have been observed in a number of lung injury models (Otterbein et al., 1999; Morse et al., 2003; Kohmoto et al., 2006). However, most of the clinical studies in various disease trials have been terminated because the expected primary outcomes had not been met.

Inhaled CH_4

The bioactivity of all recognized gas mediators is related to their tendency to react chemically with biologically important target molecules. Therefore, and precisely due to this characteristic, these compounds are also categorized as toxic asphyxiants in environmental chemistry. For example, and according to current knowledge on NO, CO and H_2S biochemistry, these gaseous substances all readily inhibit mitochondrial O_2 consumption by Complex IV (**Figure 2**). It is therefore important to consider that physiologically important gases that trigger vital functional changes will have profound adverse effects in any cellular system given sufficient exposure, and many of the unfavourable consequences are directly linked to inhibition of mitochondrial function.

On the other hand, simple asphyxiants, such as CH_4, act by physically limiting the utilization of O_2, without producing cytotoxic effects (Boros et al., 2018). Tissue hypoxia may indeed occur when CH_4 displaces the air and hence O_2 in a restricted space. However, in such cases, respiratory distress is not due to the chemical specificity of the gas, but to the decreased O_2 content (CH_4 in the inspired air should be present at about 14% or 140,000 parts per million by volume (ppmv) to reduce O_2 to 18%) (Boros and Keppler, 2019). Information on the respiratory effects of CH_4 is sparse but the inhalation of normoxic artificial air containing 21% O_2 and 2.5% CH_4 had no side-effects on the blood gas chemistry and mean arterial blood pressure in normotensive unstressed animals (Boros et al., 2012; Zaorska et al., 2021). Likewise, the administration of CH_4-enriched saline did not affect cytochrome c release in rats (Wang et al., 2017).

Under standard conditions for temperature and pressure, the solubility of CH_4 in blood is rather low (a blood:air partition coefficient of 0.066) but significantly higher in membrane bilayers (a partition coefficient of 0.20) [as reviewed by Boros and Keppler (2018)]. Therefore, the concentration of CH_4 in the tissues rapidly reaches equilibrium with that in the inspired air, and this equilibrium remains stable even with prolonged exposure time (Watanabe and Morita, 1998). It follows that inhaled CH_4 will move readily from the alveoli into the circulation, throughout

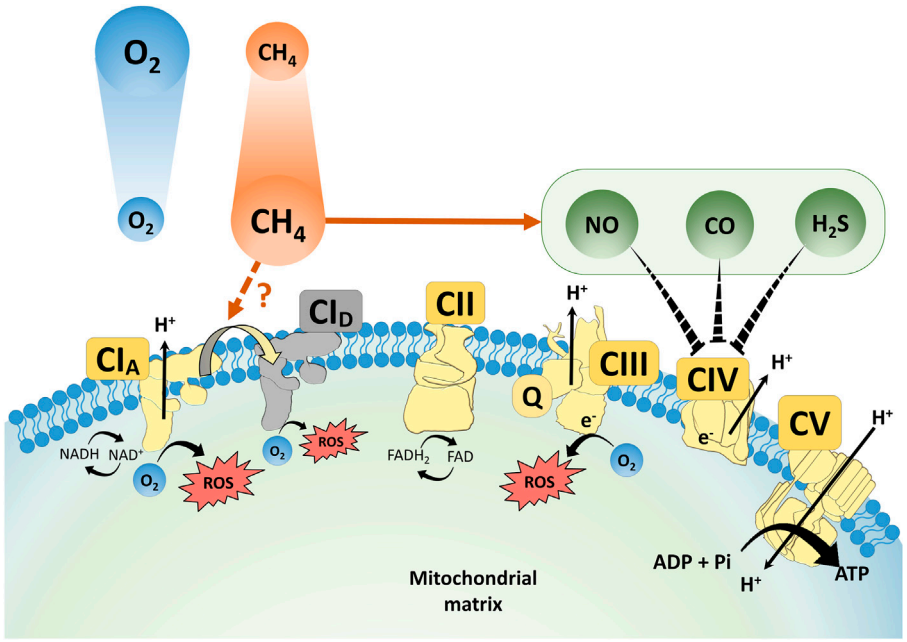

FIGURE 2 | A scheme for the proposed interaction of CH_4, NO, CO and H_2S at mitochondrial respiratory complexes. ROS: reactive oxygen species; NO: nitrogen monoxide; CO: carbon monoxide; NAD/NADH: nicotinamide adenine dinucleotide/dihydronicotinamide adenine dinucleotide; CI–V: Complex I–V; TCA cycle: tricarboxylic acid cycle; FAD/$FADH_2$: flavin adenine dinucleotide/dihydroflavine-adenine dinucleotide; Q: ubiquinone.

which it is distributed rapidly and may accumulate transiently at cell membrane interfaces, thereby changing the relationship between gases and the *in situ* functionality within this environment. Without a new exogenous supply, CH_4 will enter the circulation again and then be excreted through the lungs if its partial pressure is higher than that in the atmosphere.

The outcome of exogenous CH_4 respiration in the human body under stress conditions has not yet been evaluated. Nevertheless, a wealth of data is available in plants and animals in such situations and also on the links between CH_4 and gas messengers. The effects of CH_4 supplementation to CO, H_2S and NO biology were repeatedly shown during the adaptation to abiotic stress and germination inhibition in plant species, which confirmed that CO, NO and H_2S signalling mechanisms are involved in the molecular basis of CH_4-induced stress tolerance (Cui et al., 2015; Qi et al., 2017; Kou et al., 2018). Apart from plant pathophysiology, several series of *in vivo* analyses have demonstrated that CH_4-containing normoxic artificial air has anti-inflammatory effects by decreasing the biochemical, functional and structural consequences of nitroxidative stress [(Boros et al., 2012), (Mészáros et al., 2017a), (Poles et al., 2018)]. Data show that NO can directly inhibit mitochondrial functions *via* several pathways and that NO-influenced or mediated inhibition can be reversed with 2.2–2.5 %v/v CH_4-containing gas mixtures. Notably, it has been demonstrated that normoxic CH_4 ventilation decreases tyrosine nitrosylation after IR injury, a process which involved NO and peroxynitrite formation. In addition, exogenous CH_4 administration reduced the xanthine oxidoreductase (XOR)-linked nitrate reductase activity, the generation of nitrogen-centred radicals and the damage to nitrergic neurons during a standardized IR challenge (Poles et al., 2018). Along these lines, it has been shown that higher concentrations of exogenous CH_4 can lead to direct anti-cytokine effects *via* master switches, such as Nrf2/Keap1 and NF-κB (Mészáros et al., 2017b). More recently, the addition of 2.5% v/v CH_4-normoxic air mixture to the oxygenator sweep gas reduced the systemic inflammatory response to extracorporeal circulation in a clinically relevant large animal model. In this study, the inotropic demand was significantly lower, the renal arterial flow was significantly higher, and the hour diuresis remained in the low normal range as compared to the oliguria in the non-treated animals (Bari et al., 2019) (**Supplementary Table S1**).

In this line, many studies have also explored the relationship among CH_4 actions in the context of mitochondrial biology. Inhaled CH_4 reduced cytochrome c release and preserved the mitochondrial respiratory capacity *in vivo* and in transient anoxia-treated cell cultures as well (Strifler et al., 2016; Jász et al., 2021). Recently, we carried out a sequential study with exogenous normoxic CH_4 in simulated IR environments using a high-resolution respirometry system to quantify the ETS responses (Jász et al., 2021). In this protocol, CH_4 treatment restricted the forward electron transfer within Complex I in control mitochondria while effectively restricting RET in post-anoxic mitochondria, thus it could be concluded that interaction with Complex I occupies a key position in the protective mechanism of CH_4 against a hypoxia/reoxygenation injury (Jász et al., 2021). Parallel *in vivo* studies have also shown that the CH_4 content of an organ preservation solution effectively

influenced several components of the endoplasmic reticulum (ER) stress-mitochondria-related pro-apoptotic signalling pathways (Benke et al., 2021). The myocardial OxPhos capacity was more preserved and cytochrome c release was decreased as a result of CH_4-enriched storage, with the relative mRNA expression for hypoxia- and ER stress-associated genes (including HIF-1α) also being significantly reduced (Benke et al., 2021). Indeed, several previous studies demonstrated that exogenous CH_4 modulates the intrinsic, mitochondrial pathway of pro-apoptotic activation in model experiments (Ye et al., 2015; Chen et al., 2016; Liu et al., 2016; Jia et al., 2018) and CH_4 administration exhibited anti-apoptotic effects and protected the pulmonary epithelial cells in a murine model of ovalbumin-induced allergic asthma as well (Zhang et al., 2019). More importantly, the anti-apoptotic properties of CH_4 inhalation were associated with improved pulmonary compliance and surfactant production in a rodent model of lung IR injury (Zhang et al., 2021). In summary, a possible indirect way in which CH_4 supplementation modulates apoptosis is by reducing cytochrome c release from the inner mitochondrial membrane, which has already been demonstrated in several tissues (Chen et al., 2016; Strifler et al., 2016; Wang et al., 2017). It seems that further knowledge of inhaled CH_4 and other gaseous molecular species with their mitochondrial targets, most importantly of Complex I, has the potential to increase the understanding of the mechanism of pathological processes at work in the pulmonary alveoli and capillaries (**Figure 2**).

DISCUSSION AND CONCLUSION

Beyond O_2 and CO_2, many gases are biologically active. Signalling roles were demonstrated for NO, CO and H_2S and it has become clear that these simple, volatile molecules can influence the cellular biology in various ways. Likewise, the human diagnostic relevance of detection of exhaled gases, as signatures of oxido-reductive stress responses, is emerging as well (Paardekooper et al., 2017). Several aspects of mitochondrial respiration, such as energy production, Ca^{2+} homeostasis and intrinsic apoptosis, may also be targets of intertwined gaseous pathways but it is less clear how mitochondrial functions are altered if the membership of this molecular club changes and how the signals of a mixed gaseous input are translated to downstream manifestations of cellular reactions.

As the examples illustrate, bioactivity is not limited to those gases that have inherited the textbook characteristics of gasotransmitters. There is ample evidence that other, less prominent components of the endogenous gaseous network, such as molecular hydrogen (H_2) or CH_4, are also able to modulate mitochondrial respiration [(Mészáros et al., 2017b), (Hirano et al., 2021)]. As an analogy, other gaseous compounds, such as NO, H_2S and CO, were previously thought to be toxic pollutants without any physiologic effects in eukaryotes. CH_4 has a long evolutionary history on Earth (Hancock, 2017). It is permanent part of the gaseous environment, a nontoxic asphyxiant, which can change the symbiosis with other gas molecules within the internal milieu of aerobic cells. In this scheme, the recognized bioactivity suggests a role for exogenous CH_4 to modulate the hypoxia-linked pro-inflammatory signals towards resting conditions.

AUTHOR CONTRIBUTIONS

The manuscript concept was designed by MB, LJ, ST, AN, GV, DÉ, and MB wrote the article. All the authors discussed and commented on the manuscript at all stages and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.824749/full#supplementary-material

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A Thrombomodulin Promoter Gene Polymorphism, rs2239562, Influences Both Susceptibility to and Outcome of Sepsis

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Objective: Disseminated intravascular coagulation plays a key role in the pathophysiology of sepsis. Thrombomodulin is essential in the protein C system of coagulation cascade, and functional polymorphisms influence the human thrombomodulin gene (*THBD*). Therefore, we conducted a multicenter study to evaluate the influence of such polymorphisms on the pathophysiology of sepsis.

Methods: A collaborative case-control study in the intensive care unit (ICU) of each of five tertiary emergency centers. The study included 259 patients (of whom 125 displayed severe sepsis), who were admitted to the ICU of Chiba University Hospital, Chiba, Japan between October 2001 and September 2008 (discovery cohort) and 793 patients (of whom 271 patients displayed severe sepsis), who were admitted to the five ICUs between October 2008 and September 2012 (multicenter validation cohort). To assess the susceptibility to severe sepsis, we further selected 222 critically ill patients from the validation cohort matched for age, gender, morbidity, and severity with the patients with severe sepsis, but without any evidence of sepsis.

Results: We examined whether the eight *THBD* single nucleotide polymorphisms (SNPs) were associated with susceptibility to and/or mortality of sepsis. Higher mortality on severe sepsis in the discovery and combined cohorts was significantly associated with the CC genotype in a *THBD* promoter SNP (−1920*C/G; rs2239562) [odds ratio [*OR*] 2.709 (1.067–6.877), $P = 0.033$ and *OR* 1.768 (1.060–2.949), $P = 0.028$]. Furthermore, rs2239562 SNP was associated with susceptibility to severe sepsis [*OR* 1.593 (1.086–2.338), $P = 0.017$].

Conclusions: The data demonstrate that rs2239562, the *THBD* promoter SNP influences both the outcome and susceptibility to severe sepsis.

Keywords: genetic predisposition to disease, genetic testing, multicenter studies, disseminated intravascular coagulation, single nucleotide polymorphisms

INTRODUCTIONS

Sepsis is a global public death emergency, affecting millions of people worldwide, and representing one of the greatest global causes of mortality (1). Currently, numerous genetic polymorphisms are suggested to be associated with susceptibility to and/or outcome of sepsis, and we discovered several polymorphisms related to sepsis pathophysiology (2). One of the purposes of investigating the effects of genetic polymorphisms on the clinical course of diseases is to examine the association of particular molecular pathways, that is, cytokine networks (3, 4), cell death (5, 6), and coagulation/fibrinolysis systems among others (7–9).

Recently, precision medicine has gained attention, particularly for multifactorial diseases in the critical care field, and disseminated intravascular coagulation (DIC) in the pathophysiology of sepsis has of late been increasingly recognized to play a key role as well as to be a therapeutic target (10). Thrombomodulin is an integral membrane protein expressed on the surface of endothelial cells and serves as a cofactor for thrombin, having a pivotal role in the protein C system through the extracellular domain, which binds to thrombin. Thereby, thrombomodulin activates protein C and prevents excessive coagulation (11). Therefore, the recombinant agent is thought to be a promising drug for sepsis-induced coagulopathy (12–14). A recent clinical trial using recombinant human thrombomodulin (ART-123) still showed a tendency of survival benefit in phase three multicenter study (14). Activated protein C (APC) decomposes the coagulation factors Va and VIIIa, thus exerting the anti-coagulative properties (15). The pathophysiology of sepsis-induced DIC is recognized as a perfusion abnormality by fibrin clotting, against which APC has therapeutic potential. Additionally, APC acts both by activating the endothelial receptors, such as protease-activated receptor-1and endothelial protein C receptor, as well as by degrading histones (16). Although the polymorphisms of protein C genes, for example, PROC(−1641), are already demonstrated to be associated with the mortality and organ failures of sepsis (9), there is only limited investigation of the thrombomodulin gene *THBD*.

Accordingly, we postulated that some of the *THBD* single nucleotide polymorphisms (SNPs) are associated with susceptibility to and/or mortality of sepsis. In addition, the present study aimed to evaluate the role of thrombomodulin in the pathophysiology of sepsis through a genetic association study with Japanese multicenter cohorts, focusing on the *THBD* gene polymorphisms.

MATERIALS AND METHODS

Patient Selection

The subjects were recruited as part of a prospective, observational study of adults admitted during 2001–2012 into a network of Japanese intensive care units (ICUs). The study using these subjects has been reported elsewhere (6). The protocol was approved by the institutional Ethics Committees at all the five participating institutes [the Ethics Committee of Chiba University School of Medicine (permission number 205), the Ethical Committee of Kurume University (bioethics permission number 49), the Medical Research Ethics Committee of Tokyo Medical University, the Ethics Review Board of Hyogo College of Medicine (permission number 208), and the Ethics Committee of Kimitsu Chuo Hospital (permission number 120)]. Following approval by the institutional ethics committees, a written informed consent was obtained from the patients or their next of kin. **Figure 1** summarizes the patient inclusion process.

Discovery Cohort

In total, 259 critically ill patients admitted to the ICU of Chiba university hospital in Chiba, Japan, between October 2001 and September 2008 were included (**Table 1**). The inclusion criteria were as follows: admission to the ICU, aged 20 years or older, and the patients able to provide informed written consent or obtainable from a family member or the legal representative. The exclusion criteria are as follows: pregnancy, treatment for hematologic malignancies, the patients receiving radiation treatment and chemotherapy, a history of genetic therapy, and being outside the scope of active treatment. The blood samples were obtained immediately after admission to the ICU. The genomic DNA was extracted from the whole blood cells.

Multicenter Validation Cohort

In the multicenter validation cohort, 793 critically ill patients admitted to the general ICU of each of the five tertiary emergency centers of Kurume University Hospital, Tokyo Medical University Hachioji Medical Center, Hyogo College of Medicine, Kimitsu Chuo Hospital, and Chiba University Hospital (updated permission number 457) from October 2008 to September 2012 were included (**Table 1**). The inclusion and exclusion criteria were the same as for the discovery cohort. The blood cells were refrigerated and transferred to the Chiba University Hospital, where the genomic DNA was subsequently extracted.

Propensity Score Matching in Multicenter Cohort

We conducted a case-cohort study to compare the *THBD* genotypic distributions in whole blood genomic DNA from the critically ill patients with severe sepsis vs. non-sepsis with similar age, gender, severity of illness, and mortality to assess the genetic association for susceptibility to sepsis between the similar severity of critically ill patients. The patients with sepsis tend to die than the patients with non-sepsis do. Therefore, propensity score matching was implemented to compare *THBD* genotypic distributions between the severe sepsis and non-sepsis having similar severity, such as mortality. Controls were matched as follows: the participating centers submitted similar blood samples from critically ill patients who were not known to have sepsis. All the patients who did not meet severe sepsis criteria were placed into the matching pool, and those who had any evidence of sepsis were then excluded. Each patient with sepsis was tentatively matched

TABLE 1 | Baseline characteristics of the study population.

	Discovery cohort				Multicenter validation cohort			
	All ***n* = 259**	**Non-sepsis patients** ***n* = 104**	**SS patients** ***n* = 125**	***P***	**All** ***n* = 793**	**Non-sepsis patients** ***n* = 454**	**SS patients** ***n* = 271**	***P***
Age (years), mean ± SD	56.8 ± 17.4	56.5 ± 17.5	57.6 ± 17.3	0.492*	63.9 ± 17	62.5 ± 17.9	66.6 ± 15.0	0.0008*
Male/female gender, *n*	146/113	60/44	69/56	0.659**	517/276	296/158	176/95	0.915**
Length of ICU stay (days), median (IQR)	6 (3–15)	3 (1–7)	13 (7–15)	<0.0001*	9 (4–19)	7 (4–14)	15 (7–29.3)	<0.0001*
SOFA score, median (IQR)	6 (3–11)	3 (1–6)	10 (7–13)	<0.0001*	6 (3–9)	4 (2–6)	9 (6–12)	<0.0001*
APACHE II score, median (IQR)	15 (9.3–22)	10 (6–14)	21 (17–27)	<0.0001*	16 (10–23)	13 (8–19.8)	21 (16–26)	<0.0001*
Severe sepsis morbidity (%)	48.3				33.8			
Mortality (%)	17	5.71	29.6	<0.0001**	12.2	5.4	25.3	<0.0001**
Post-surgical operation								
Post-cardiovascular surgery, *n* (%)	28 (10.8)	24 (23.0)	4 (3.2)	<0.0001**	21 (2.6)	5 (1.1)	12 (4.4)	0.009**
Post-gastrointestinal surgery, *n* (%)	33 (12.7)	11 (10.6)	15 (12.0)	0.835**	79 (10.0)	25 (5.5)	43 (15.9)	<0.0001**
Others, *n* (%)	20 (7.7)	12 (11.5)	6 (4.8)	0.083**	6 (0.8)	3 (0.7)	3 (1.1)	0.677**
Intracranial disease (ICH/CI), *n* (%)	5 (2.0)	0 (0)	4 (3.2)	0.128**	68 (8.6)	62 (13.7)	3 (1.1)	<0.0001**
Respiratory failure, *n* (%)	28 (10.8)	4 (3.8)	22 (17.6)	0.001**	77 (9.7)	15 (3.3)	51 (18.8)	<0.0001**
Heart failure, *n* (%)	10 (3.9)	4 (3.8)	3 (2.4)	0.705**	72 (9.1)	61 (13.4)	8 (3.0)	<0.0001**
Endogenous abdominal disease								
Acute pancreatitis, *n* (%)	22 (8.5)	16 (15.4)	6 (4.8)	0.012**	39 (4.9)	22 (4.8)	14 (5.2)	0.861**
Gastrointestinal bleeding, *n* (%)	5 (2.0)	2 (1.9)	3 (2.4)	>0.9999**	36 (4.5)	33 (7.3)	2 (0.7)	<0.0001**
Hepatic failure, *n* (%)	7 (2.7)	3 (2.9)	4 (3.2)	>0.9999**	19 (2.4)	7 (1.5)	9 (3.3)	0.124**
Others, *n* (%)	13 (5.0)	1 (1.0)	9 (7.2)	0.024**	34 (4.3)	10 (2.2)	21 (7.7)	0.001**
CPAOA, *n* (%)	4 (1.5)	2 (1.9)	2 (1.6)	>0.9999**	39 (4.9)	32 (7.0)	4 (1.5)	0.001**
Trauma, *n* (%)	11 (4.3)	9 (8.7)	0 (0)	0.001**	119 (15.0)	105 (23.1)	8 (3.0)	<0.0001**
Intoxication, *n* (%)	8 (3.1)	4 (3.8)	4 (3.2)	>0.9999**	23 (2.9)	17 (3.7)	6 (2.2)	0.283**
Burn, *n* (%)	2 (0.8)	1 (1.0)	0 (0)	0.454**	12 (1.5)	7 (1.5)	4 (1.5)	>0.9999**
Others, *n* (%)	63 (24.3)	11 (10.6)	43 (34.4)	<0.0001**	149 (18.8)	50 (11.0)	83 (30.6)	<0.0001**

P values (non-sepsis patients vs. severe sepsis/septic shock patients) were calculated with Student's t-test or Mann-Whitney U-test and Fisher's exact test**.*
ICH, intracranial hemorrhage; CI, cerebral infarction; CPAOA, cardiopulmonary arrest on arrival; SS, severe sepsis/septic shock; SOFA, sequential organ failure assessment; APACHE II, the acute physiology and chronic health evaluation; SD, standard deviation, IQR, interquartile range. Discovery and validation cohorts include 30 and 68 mild sepsis patients (non-severe), respectively.

with all the patients in the pool of the same gender and hospital discharge status (alive vs. dead). Then, each patient with severe sepsis was matched with one patient from its set of potential non-sepsis matches. The patient selection above was performed employing the propensity score matching method with a Greedy 5-to-1 digit-matching algorithm for the clinical factors, that is, age, gender, severity scores, and hospital discharge status. Once all the propensity-score matching was performed, we compared the baseline covariates between the two groups. Ultimately, 444 patients (222 with severe sepsis/septic shock (SS) while 222 displayed non-sepsis) were selected (**Table 2**).

TABLE 2 | Propensity score-matched patients with severe sepsis and without any evidence of sepsis in the validation cohort (post-matching results).

	Non-sepsis patients ***n* = 222**	**SS patients** ***n* = 222**
Age (years), mean ± SD	66.7 ± 16.4	66.2 ± 14.9
Male/female gender, *n*	194/222	191/222
SOFA score, mean ± SD	6.6 ± 3.7	9.1 ± 4.1
APACHE II score, mean ± SD	21.0 ± 7.7	20.8 ± 7.6
Mortality (%)	35.4	35.9

SS, severe sepsis/septic shock; SOFA, sequential organ failure assessment; APACHE II, the acute physiology and chronic health evaluation; SD, standard deviation.

Data Collection

The baseline characteristics (age and gender) and clinical data, such as length of ICU stay, Sequential Organ Failure Assessment (SOFA) scores (17), Acute Physiology and Chronic Health Evaluation (APACHE) II scores (18), morbidity of severe sepsis and septic shock, and ICU mortality, were obtained after the patients were documented at study entry. The APACHE II scores

and SOFA scores were calculated during the first 24 h after admission. The diagnosis of sepsis, severe sepsis, and septic shock was based on the criteria presented at the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference in 1992 (19). In the present study, both the severe sepsis and septic shock are expressed together as the SS group.

SNP Selection and Genotyping

Genomic DNA was isolated from the banked whole blood specimens collected on ICU admission. We genotyped eight markers from the region surrounding *THBD* (**Supplementary Table 1**). Genotyping of SNPs was performed using the APEX testing methods, an outsourced service provided by Asper Biogene (Tartu, Estonia, https://www.asperbio.com/, accessed on 2021/5/25). Eight SNPs distributed in *THBD* and its promoter region (rs2239562, rs3216183, rs121918667, rs1800577, rs1042579, rs41348347, rs1042580, and rs3176123) were investigated. We were able to assign the genotype in >95% of typed samples. To verify the genotypes of the SNPs, some were tested in a set of earlier Japanese samples (6). The allelic statuses were determined employing the PCR with sequence-specific primers, using the TaqMan® probe (Applied Biosystems, Foster City, CA, USA). The genotyped SNPs can be found at dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). The SNP genotyping matching rate was 100%, according to the earlier results (6).

Statistical Analysis

The primary endpoint was a mortality of severe sepsis, and the secondary endpoint was susceptibility to severe sepsis concerning the *THBD* SNP. Hardy–Weinberg equilibrium (HWE) for the population distribution of the variant alleles was determined according to the approach described by Guo and Thompson (20). The allelic *chi*-square tests were applied for each SNP. The statistical analyses for the genetic association tests were performed using the SNP & Variation Suite 8.8.3 software (Golden Helix, Bozeman, MT, USA). Significant differences in the mean ± SD or median (interquartile range; IQR) values between the two groups were evaluated by using the Student's *t*-test or Mann–Whitney *U*-test, depending on the variables. The statistical analyses were performed using the GraphPad PRISM 8, version 8.3.0 (GraphPad Software, San Diego, CA, USA) for Windows. We considered differences to be significant at $P < .05$.

RESULTS

The Baseline Characteristics of the Discovery and Multicenter Validation Cohorts

Table 1 summarizes the baseline characteristics of the discovery cohort ($n = 259$) and the multicenter validation cohort ($n = 793$). Mortality and the SOFA and APACHE II scores were significantly higher in the SS group than those in the non-sepsis group in both the cohorts ($P < 0.0001$, **Table 1**). In the validation cohort, the SS patients were significantly older than the non-septic controls ($P = 0.0008$, **Table 1**). By contrast, no significant difference in the SOFA or APACHE II scores was detectable between the SS groups of the two cohorts ($P = 0.1126$, 0.8714, respectively), which indicated that there was no marked difference in severity in the SS patients between the two cohorts. In the validation cohort, trauma, heart failure, and intracranial diseases were overrepresented in the non-sepsis group.

Genotype Distributions of Eight *THBD* SNPs Related to the Outcome of Severe Sepsis

To determine the frequencies of eight SNPs of *THBD*, which are associated with the mortality of severe sepsis, genotyping of these polymorphisms was performed in the discovery cohort. The observed allele frequencies and genotypic distributions for the investigated polymorphisms are summarized in **Supplementary Table 2** (discovery cohort), **3** (validation cohort), and **4** (combined cohort). The distributions of genotypes for all the investigated polymorphisms conformed to the HWE test ($P > 0.01$).

The genotype call rate of the eight SNPs was 96.3–100%, although the genotypic distributions in rs1042579 diverged from the HWE in the studied subjects ($P = 0.005$, **Supplementary Table 4**). Among the eight SNPs, a *THBD* promoter SNP (−1920*C/G; rs2239562) was found to be most significantly associated with mortality of SS group in the discovery cohort [odds ratio (*OR*) 2.709 (range of lower and upper confidence bound; 1.067–6.877), $P = 0.033$ in a dominant model with the trend test, **Figure 2A**] and the trend was maintained in the validation cohort [*OR* 1.446 (0.782–2.675), $P = 0.249$, **Figure 2A**]. As a result, the association was statistically strengthened in the combined cohort [*OR* 1.768 (1.060–2.949), $P = 0.028$ in a dominant model with the trend test, **Figure 2A**]. Consequently, the carriage of the CC genotype was significantly associated with a worse outcome of SS group. All the genotypic distributions in relation to a mortality of SS of both the cohorts are presented in **Supplementary Tables 2–4**.

Genotypic Distributions of Eight *THBD* SNPs Related to Susceptibility to Severe Sepsis

Because of the retrospective nature of the study, the baseline imbalances between the SS and non-sepsis existed; therefore, we identified 222 subjects from each of the SS and non-sepsis groups from the validation cohort by propensity score matching (17) to equalize morbidity of severe sepsis and severity of illness of both the cohorts (**Table 2**). **Figure 1** summarizes the patient inclusion process. By matching with the Propensity Score in the multicenter validation cohort, 222 patients with severe sepsis and the same number of controls with a similar number of ICU deaths, the severity of illness (APACHEII), and age and gender-matched without evidence of any infection also admitted to the ICUs were included (**Table 2**). The genotypic distributions of the *THBD* promoter SNP (rs2239562) and the *THBD* exon 1 SNP (rs41348347) were significantly different between the SS and non-sepsis groups with similar severity of illness [*OR* 1.593 (1.086–2.338), $P = 0.017$ and *OR* 0.107 (0.013–0.853), $P = 0.011$, respectively in a dominant model with the trend test, **Figure 2B**]. By contrast, the minor allele

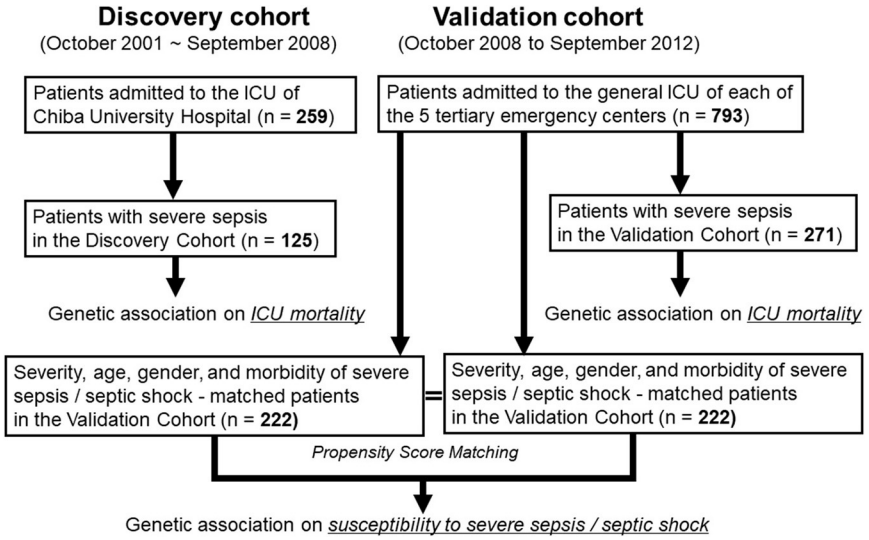

FIGURE 1 | Flow diagram of patient inclusion.

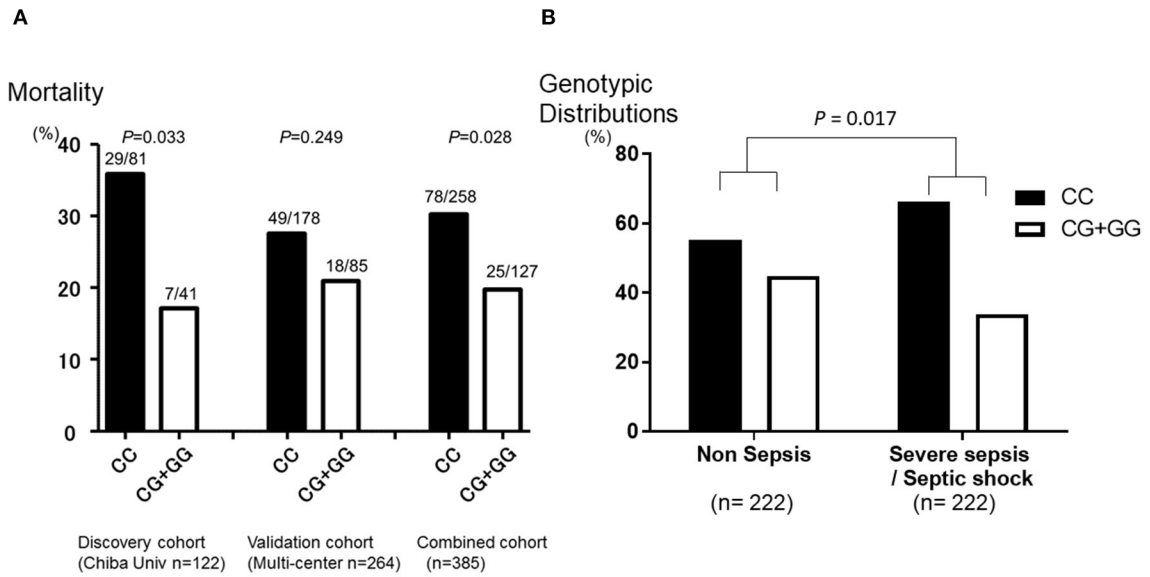

FIGURE 2 | Mortality and morbidity of severe sepsis in genotype categories of *thrombomodulin gene (THBD)* single nucleotide polymorphisms (SNPs) rs2239562. **(A)** The *Y*-axis of the graph shows the mortality of severe sepsis in the SNPs that is in the promoter region of *THBD* (−1920°C/G; rs2239562). The patients with the CC genotype of rs2239562 were significantly associated with worse outcome of severe sepsis than the CG + GG genotype of the SNP in the discovery cohort ($P = 0.033$). The trend was maintained in the validation cohort ($P = 0.249$), and the association was strengthened in the combined cohort ($P = 0.028$). **(B)** The *Y*-axis of the graph shows the genotypic distributions of the SNP that is in the promoter region of *THBD* (−1920°C/G; rs2239562) in the patients with severe sepsis and without any evidence of sepsis (non-sepsis). The percentage of patients with the CC genotype of rs2239562 was significantly higher in the SS group than those in the non-sepsis group ($P = 0.017$). *The P* values for the SNP were evaluated with the chi-square test on the dominant model analysis with the correlation/trend test.

frequency of the exon SNP rs41348347 was too low to reveal the clinical implications (1.14%), and this SNP was found not to be related to the outcome of SS patients in the previous analysis (**Supplementary Tables 2–4**). Therefore, the association between rs2239562 and susceptibility to SS was confirmed, even though both the groups were similar for severity and the other background characteristics. The genotype call rate of the eight SNPs was 93.9–100%, and the genotypic distributions in all the eight SNPs did not diverge from the HWE in the studied subjects (**Supplementary Table 5**).

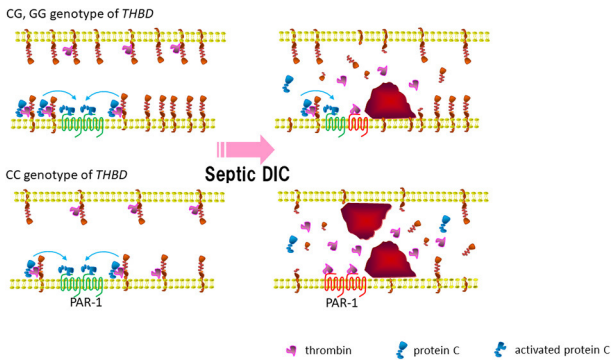

FIGURE 3 | *Thrombomodulin gene* promotor may influence thrombomodulin guided activated protein C (APC) function and limit endothelial cell damage in sepsis. In the blood stream, the thrombomodulin levels influence APC and antiinflammatory PAR-1 signaling. Bottom right: restricted thrombomodulin (due to carriage of CC genotype of rs2239562) promote thrombin induced inflammation and endothelial cell damage. APC, activated protein C; PAR-1, protease-activated receptor-1; DIC, disseminated intravascular coagulation.

DISCUSSIONS

The coagulation disorders are common in sepsis, and the patients frequently progress to develop DIC. The present study indicated that a coagulopathy-associated *THBD* promoter SNP, rs2239562, had a significant influence on the outcome as well as the progress of severe sepsis/septic shock. First, we determined the worse outcome of severe sepsis to be associated with the CC (major) allele homozygotes of rs2239562. Intriguingly, the frequency of the CC carriers was also augmented in the SS in a propensity-matched patient cohort. In the pathophysiology of sepsis, *THBD* promotor may influence the thrombomodulin-guided APC function and limit endothelial cell damage (**Figure 3**). In the bloodstream, the thrombomodulin levels influence APC and antiinflammatory protease-activated receptor-1 (PAR-1) signaling. The restricted thrombomodulin (due to carriage of CC genotype of rs2239562) promotes thrombin-induced inflammation and endothelial cell damage.

Some drugs in development aim to regulate sepsis-induced coagulopathy or when inflammation has been initiated. One of the promising drugs is ART-123, a recombinant form of the anticoagulant protein thrombomodulin from Asahi Kasei Pharma that is currently in a global clinical trial (14, 21). Emerging evidence is accumulating, which demonstrates the therapeutic efficacy of ART-123 (22, 23), and the mechanisms of thrombomodulin of not only anticoagulation but also anti-inflammation through the adsorption of high-mobility group-B1 (HMG-B1) have been advocated (24). In the recently published Japanese Guidelines, ART-123 has been weakly recommended for the patients with sepsis with DIC (25). From a practical point, the proper use of ART-123, such as target-illness severity and dosage for the patients with sepsis-induced DIC, remains unclear, particularly for those with renal impairment (26) as well as for those on continuous hemodiafiltration (27). Because of the anticoagulant properties of ART-123, the most critical concern in treating the patients with DIC, who are susceptible to bleeding, is a severe hemorrhage due to abrupt increases in plasma concentration of this drug. The present study results should shed light on the precision medicine for sepsis-induced DIC utilizing the pharmacogenetics associated with the coagulation system.

An earlier study reported soluble thrombomodulin concentrations and deep venous thrombosis associated with 2729A>C and A455V missense mutations in the Japanese (28). The *THBD* 1418T allele in rs1042579 SNP was associated with the lower soluble thrombomodulin levels, both in plasma and in HUVEC-conditioned medium, and with an increase in functional membrane-bound thrombomodulin in HUVEC, explaining the increased APC levels and the reduced venous thromboembolism risk (29). Interestingly, *THBD* is also recognized as a pathogenic gene of the atypical hemolytic uremic syndrome (aHUS) (30), caused by complement dysregulation and may occasionally be triggered by a septic insult. Therefore, the present data indicating that a *THBD* polymorphism influenced the susceptibility, as well as the outcome of sepsis, might be key in the pathogenesis of aHUS. To counteract sepsis-triggered aHUS, the continued accumulation of knowledge regarding the patterns of disease onset and response to the treatments under different genetic backgrounds, such as *THBD,* will be essential for developing future treatment strategies (31). Further, a recent study demonstrated that one of the *THBD* SNPs, rs1962, was related to the risk of death in the patients with sepsis (32). The above reports support the deep association between the *THBD* SNPs and the pathophysiology of sepsis-induced organ dysfunctions.

Our work has several limitations. First, we used the criteria of the 2013 surviving sepsis campaign guidelines to include patients because this work was initiated before introducing the SEPSIS-3 diagnostic criteria (33). Second, the statistical significance of the results of the *THBD* genetic association was relatively weak because the patient characteristics were very heterogeneous, such that it was challenging to show the influence on an outcome with the present sample volume. Interestingly, a recent study demonstrates that phenotype γ and δ reveals the characteristics of hematologic dysfunctions, such as coagulopathy (34). A survey in Japan conducted by the Japanese Association for Acute Medicine reported that the incidence of DIC is high and exceeded 50% in sepsis (35). At least in part, these may explain the lack of significance concerning the association between the *THBD* SNP and the incidence of SS in "the less severe" validation cohort. More studies along these lines will clarify these questions. Third, neither the gene expression nor biomarker values can be recruited since we performed piggyback evaluation using the DNA samples from an earlier genetic association study of sepsis (6). Ideally, we might have examined the concentrations of soluble thrombomodulin as a phenotype parameter of the rs2239652 SNP promotor influence. However, it is also true that the concentrations do not always correlate with sepsis severity (36). Finally, any data on site of infection, administered antimicrobials, microbiology, and coagulopathy markers were not provided. Although we used a prospective registry of the genetic association study for the critically ill patients, the data

were not mandatory in the long-continued cohorts. Even in such miscellaneous populations, the rs2239562 *THBD* SNP was associated with susceptibility to and outcome of SS. This suggests the crucial role of the coagulation system in the pathophysiology of sepsis.

CONCLUSIONS

Our mid-scale population association study supports the hypothesis that the genetic predispositions to severe sepsis as well as to the worse outcome of sepsis exist. Consequently, a variation in the promoter region of the *THBD* appears to explain, in part, the susceptibility to severe sepsis/septic shock in the Japanese multicenter ICU. Whole-genome sequencing targeting *THBD* with a more extensive study population is warranted to be able to transfer the present data to the clinical settings.

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

The studies involving human participants were reviewed and approved by the Ethics Committee of Chiba University School of Medicine (permission number 205), the Ethical Committee of Kurume University (bioethics permission number 49), the Medical Research Ethics Committee of Tokyo Medical University, the Ethics Review Board of Hyogo College of Medicine (permission number 208), and the Ethics Committee of Kimitsu Chuo Hospital (permission number 120). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EW, MH, and SO: conceived and designed the experiments. YT, MO, and AT: performed the experiments. EW, YT, MO, BM, and MH: analyzed the data. OT, TS, TI, JK, NK, and EMS: contributed reagents, materials, and analysis tools. EW and GH: wrote the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.762198/full#supplementary-material

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Lidocaine and Bupivacaine Downregulate MYB and *DANCR* lncRNA by Upregulating *miR-187-5p* in MCF-7 Cells

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Background: Breast cancer is the most common malignancy and a leading cause of death among women. The majority of patients require surgery, and retrospective studies have revealed an association between anaesthetic techniques during surgery and clinical outcomes. Local anaesthetics (LAs) influence carcinogenesis by interacting with non-coding RNAs (ncRNAs). However, the detailed mechanisms underlying the association between LAs and ncRNAs remain unclear.

Methods: In this study, the effects of two commonly used LAs, lidocaine and bupivacaine, on the malignancy of MCF-7 breast cancer cells were investigated. The expression profiles of the microRNAs (miRNAs) that responded to treatment with LAs were determined through next-generation sequencing.

Results: Data from the functional assay revealed that the LAs suppressed the proliferation of MCF-7 cells. The result of next-generation sequencing revealed that 131 miRNAs were upregulated, following treatment with the LAs. Validation using polymerase chain reaction (PCR) identified *miR-187-5p* as a potential biomarker, and it was selected for further analyses. Prediction with bioinformatics tools and luciferase reporter assays revealed that *MYB* is a direct target gene of *miR-187-5p*. Based on the hypothesis that lncRNAs acts as miRNA sponges, the target lncRNA, *DANCR,* of *miR-187-5p* was predicted using DIANA-LncBase v2 and validated using luciferase reporter assays. In addition, the reciprocal suppressive effect between *DANCR* and *miR-187-5p* was determined.

Conclusions: This study suggests that one of the anti-tumour mechanisms of lidocaine and bupivacaine is mediated through the *DANCR-miR-187-5p-MYB* axis. This may provide a novel molecular mechanism of tumour suppression in breast cancer.

Keywords: *miR-187-5p*, *MYB*, *DANCR*, bupivacaine, lidocaine

INTRODUCTION

Breast cancer is one of the most common malignancies and a leading cause of death among women (https://gco.iarc.fr/). Most patients require surgery; however, residual disease from scattered micro-metastases and tumour cells related to surgery is usually inevitable (1). Experimental and clinical retrospective studies indicate an association between the types of anaesthetic techniques used during the cancer resection surgery and outcomes (1, 2). There are conflicting results on the administration of volatile anaesthetics and opioids and cancer development, metastasis, and recurrence (3, 4). Regional anaesthesia is hypothesised to attenuate immunosuppression and surgical stress by minimising the requirement for opioids and volatile anaesthetics, therefore improving the long-term postoperative outcomes (2). The overall survival for different local anaesthetics (LAs) varies among the clinical studies; however, laboratory studies suggest that LAs have direct inhibitory effects on tumour cells (5, 6).

Lidocaine and bupivacaine are commonly used amide-type LAs for regional anaesthesia and peripheral nerve blockade, providing excellent perioperative pain relief, especially in breast cancer surgery (7). The mechanisms underlying the inhibitory effect of LAs on cancer cell proliferation, migration, and metastasis, are elucidated (5, 8, 9). However, the precise mechanism remains unclear; it could involve sodium channel blockade, DNA demethylation (10, 11), or interactions between LAs and non-coding RNAs (ncRNAs). Lidocaine inhibits the growth and invasion of gastric carcinoma cells by upregulating miR-145 (12). It inhibits proliferation and induces apoptosis in colorectal cancer cells by upregulating miR-520a-3p and targeting EGFR (13).

Dysregulated gene expression is a major hallmark of cancer. ncRNAs play a critical role in tumourigenesis, growth, and progression (14–16). NcRNAs are categorised as small ncRNAs (sncRNAs) and long ncRNAs (lncRNAs) with a cut-off length of 200 bp; they are directly or indirectly involved in diverse biological processes through epigenetic, transcriptional, and post-transcriptional mechanisms (17). MicroRNAs (miRNAs) are a type of short ncRNAs that regulate cellular proliferation, differentiation, apoptosis, adhesion, epithelial-mesenchymal transition (EMT), and metastasis in various cancers (18). The competing endogenous RNA (ceRNA) theory (19) states that miRNAs recognise miRNA response elements (MREs) in different RNA molecules, and induce target repression through miRNA-RNA-induced silencing complex (RISC)-mediated degradation. Therefore, the transcriptional regulation of diverse RNAs, including mRNAs and ncRNAs, can regulate biological processes through a novel MRE-mediated mechanism (19).

The tumour-suppressive effects of lidocaine and bupivacaine were studied in MCF-7 cells. The effects of these LAs on the function of *miR-187-5p*, Differentiation Antagonising Non-Protein Coding RNA (*DANCR)*, and *MYB* were investigated for elucidating the underlying mechanism. Our findings provide novel insights into the process of tumour suppression for future breast cancer therapy.

METHODS

Cell Culture

MCF-7 cells were purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan), and HEK-293T cells were provided by Dr. Shau-Ping Lin (Institute of Biotechnology, National Taiwan University, Taiwan). All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Thermo Fisher, CA, USA) containing 1% penicillin-streptomycin (Gibco) and 10% foetal bovine serum (FBS) (HyClone, Logan, UT, USA). The culture plates were maintained at 37°C in a humidified atmosphere of 5% CO_2 and were routinely tested for Mycoplasma sp. using Mycoplasma polymerase chain reaction (PCR) Detection Kit (ABM Inc., Vancouver, BC, Canada).

Cell Viability Assay

MCF-7 cells were seeded at 5×10^3 cell into 96-well plates 1 day before lidocaine or bupivacaine (Sigma, St. Louis, MO, USA) treatment at the indicated concentrations. LAs and the controls were diluted in 200 μL DMEM for each well. For the thiazolyl blue tetrazolium bromide (MTT) colorimetric assay, 5 mg/mL of MTT (Sigma) were added to each well and incubated at 37°C for 4 h. The supernatants were removed and replaced with dimethyl sulfoxide (DMSO) (Sigma). The absorbance was measured using a microtiter plate reader (BioTek, Winooski, VT, USA) at 570 nm.

Wound Healing Assays

MCF-7 cells were seeded at a density of 2.5×10^4 cells/well in a medium containing 10% FBS and incubated overnight. The culture inserts were removed and an image of the gap area at 0 h was captured. The cells were further incubated at 37°C in a 5% CO_2 incubator, and the images were captured at 12, 24, and 36 h, respectively. The cell migration ability in the gap area was quantified using ImageJ v1.8.0 (National Institutes of Health, USA).

Cell Migration

Migration assays were performed using SPLInsert™ Hanging plate (SPL Life Sciences, Pocheon, South Korea). The upper chamber of the transwell unit was seeded at 4×10^4 cells/well in 0.2 mL serum-free DMEM. The lower chambers were loaded with 0.6 mL DMEM containing 10% FBS as chemo-attractant. The MCF-7 cells were incubated at 37°C for 36 h. A methanol: acetic acid (3:1) mixture was added to the lower chamber for fixing the cells by incubating at 25°C for 10 min. The non-migrating cells on the inner transwell membrane were removed carefully, and the membranes were stained with 1 mL of 0.5% crystal violet. The stained cells were solubilized with 10% acetic acid, imaged, and analysed using ImageJ v1.8.0.

Abbreviations: LA, local anaesthetic; ncRNA, non-coding RNA; miRNA, microRNA; PCR, polymerase chain reaction; sncRNA, small ncRNA; lncRNA, long ncRNA; EMT, epithelial-mesenchymal transition; ceRNA, competing endogenous RNA; MRE, miRNA response element; RISC, RNA-induced silencing complex; MYB, MYB proto-oncogene, transcription factor; *DANCR*, Differentiation Antagonising Non-Protein Coding RNA; qRT-PCR, quantitative real time polymerase chain reaction; PCA, principal component analysis.

Western Blotting

The proteins were extracted from the cells lysed with radioimmunoprecipitation assay buffer (RIPA) Lysis Buffer (EMD Millipore, Billerica, MA, USA) and the concentration was determined using Coomassie Protein Assay Reagent (Thermo Fisher, Waltham, MA, USA). The extracted protein samples (20 μg) were separated using 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (GE healthcare, Chicago, IL, USA). The membranes were incubated with primary rabbit polyclonal antibodies against c-MYB (Proteintech, Rosemont, IL, USA) and β-actin (GeneTex, Irvine, CA, USA), at 4°C overnight; the samples were hybridised with horseradish peroxidase (HRP) conjugated anti-rabbit IgG (GeneTex) at 25°C for 1 h. The blotted proteins were detected using an enhanced chemiluminescence (ECL) system (Millipore, Billerica, MA, USA) equipped with a BioSpectrum Imaging System (UVP, Upland, CA, USA). The images were analysed using ImageJ v1.8.0.

RNA Extraction, Reverse Transcription, and Quantitative Real Time Polymerase Chain Reaction

The RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). The RNA quality was detected by a spectrophotometer (NanoDrop; Thermo Scientific) with the A260/A280 ratio between 1.8∼2.0. Samples were kept in RNase free water buffer at neutral pH. All RNA samples were stored at −80°C. One μg of total RNA was reverse-transcribed by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). SuperScript IV Reverse Transcriptase (Invitrogen) was used for reverse transcription of the miRNAs in accordance with the manufacturer's instructions. Two point five percent of each reaction was used as template for quantitative PCR with OmicsGreen qPCR MasterMix (OmicsBio, New Taipei City, Taiwan) and the reactions were performed on StepOnePlus Real-Time PCR System (Thermo Fisher, Waltham, MA, USA). The primers were checked for only generating a single melting curve peak and detailed information are provided in **Supplementary Table 1**. The relative expression was normalised to that of 18S rRNA or U6 snRNA, using the $2^{-\Delta\Delta Ct}$ method.

Library Preparation and Sequencing

The total RNA was extracted using TRIZOL reagent (Invitrogen). The small RNA library was constructed from total RNA (2 μg) using the TruSeq Small RNA Library Prep Kit (Illumina Inc., San Diego, CA, USA), according to the manufacturer's instructions. A total of 75 single-read nucleotides were obtained from each miRNA library using NextSeq500 (Illumina Inc., CIC bioGUNE, Bilbao, Spain). The sequencing data were submitted to the Gene Expression Omnibus (GEO) (accession number: GSE171282).

Sequencing Data Analyses

The raw Illumina FASTQ sequencing reads were mapped to the miRBase mature miRNA reference (http://www.mirbase.org/) using Partek Flow™ v5.0 (Partek Inc., St. Louis, MO, USA) with Bowtie alignment algorithm. The Bowtie alignment method was generally thought to be optimal for sequences less than 50 bp. The adapter sequences were first removed from each FASTQ reads, and the remaining bases were trimmed from the 3′ end with a minimum Phred quality score of 20. A minimum read-length philtre of 15-bases in length was used in this work. A minimum seed length of 10 that was consistent with the standard setting provided by the Partek Flow pipeline was chosen for the Bowtie aligner. The remaining reads were aligned to the human genome reference (RefSeq Hg19). Raw miRNA expression reads were normalised by scale normalisation and then processed by $\log_2$ transformation. Then, miRNAs with extremely low expression ($\log_2$ <5 in all samples) were removed from further analysis. Then, differentially expressed miRNA profiles between groups were identified by $\log_2$ fold-change value ≧2X or <-1X and P-value < 0.05 using a two-sided Student's t-test. Principal component analysis using the expression values of total miRNAs was used to visualise the similarity of different groups.

Transfection

MCF-7 cells were cultured in an antibiotic-free medium to 70–80% confluence in 6-well plates (2.5×10^5 cells/well) and transfected with the 0.025 nmol of *miR-187-5p* mimic (GenePharma, Shanghai, China) or negative control (GenePharma), using Lipofectamine 2000 as the transfection reagent (Invitrogen), following the manufacturer's instructions.

The MCF-7 cells were transfected with 0.5 nmol of pcDNA-*DANCR* or the empty vector (Dharmacon, New Taipei city, Taiwan) using jetPRIME (Polyplus-transfection, New York, NY, USA) reagent, following the manufacturer's instructions. After 4 h, the medium was replaced with fresh medium containing serum. The cellular RNA expression after 48 h was determined using qRT-PCR.

Plasmid Construct and Site-Directed Mutagenesis

To determine promoter activity by luciferase assay, the luciferase expression plasmid pMIR-REPORT-*c-MYB 3′UTR* and pMIR-REPORT-*DANCR* was purchased from the BioMed Resource Core of the 1st Core Facility Lab, National Taiwan University College of Medicine (Taipei, Taiwan). Briefly, *c-MYB 3′UTR* region encompassing 2,493–3,684 bp relative to the transcription start site was amplified from the human genomic DNA by PCR. The *c-MYB 3′UTR* was subcloned into pMIR-REPORT vector, and the final construct was called pMIR-REPORT-*c-MYB 3′UTR*. *DANCR* encompassing 1–915 bp relative to the transcription start site was amplified from the human genomic DNA by PCR and the final construct was called pMIR-REPORT-*DANCR*.

miR-187-5p binding sites of *c-MYB 3′UTR* were located at 2,710–2,734 and 3,634–3,656 bp. Besides, *miR-187-5p* binding sites of *DANCR* were located at 134–157 and 380–408 bp. To determine binding activity of *miR-187-5p* by luciferase assay, the luciferase expression plasmid pMIR-REPORT-*c-MYB 3′UTR* S1, pMIR-REPORT-*c-MYB 3′UTR* S2, pMIR-REPORT-c-MYB 3′UTR S12, pMIR-REPORT-*DANCR* S1, pMIR-REPORT-*DANCR* S2, and pMIR-REPORT-DANCR S12 was purchased from the BioMed Resource Core of the 1st Core Facility Lab,

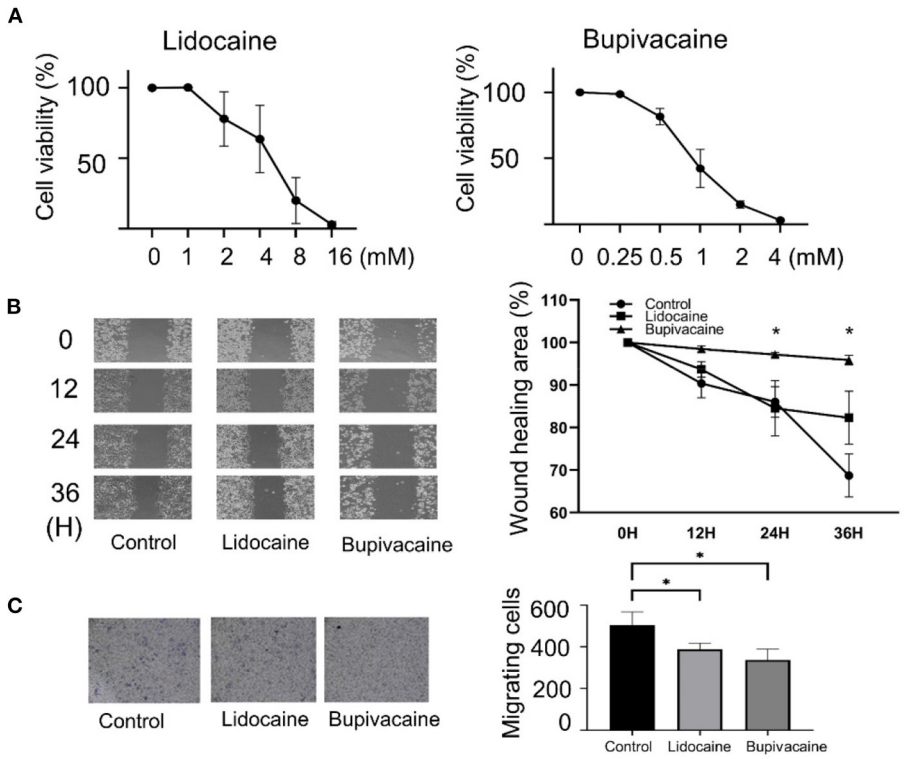

FIGURE 1 | Lidocaine and bupivacaine inhibited the proliferation and migration of MCF-7 cells. **(A)** Cell proliferation determined using the MTT assay. The growth of MCF-7 cells measured after 6 h of treatment with serially diluted concentrations of lidocaine or bupivacaine. The proliferation rate was normalised to that at 0 h. **(B)** Wound healing assay. Left: Images after 0, 12, 24, and 36 h of treatment with lidocaine (4 mM) or bupivacaine (1 mM). Right: The migratory ability quantified using the reduction in wound size over time by ImageJ software v1.8.0. **(C)** Transwell migration assay. Right: The cells were seeded after 24 h of treatment with lidocaine (4 mM) or bupivacaine (1 mM). Left: Cell migration measured after 36 h of seeding. All data are presented as mean ± SD ($n = 3$). *$p < 0.05$.

NTU-CM (Taipei, Taiwan). In addition, the mutated sequences were validated by sequencing.

Luciferase Reporter Assay

Luciferase assays were performed with HEK-293T cells using the Dual-Glo® luciferase reporter assay system (Promega, Fitchburg, WI, USA). Cells (5×10^4/well) were transfected with 100 ng reporter constructs containing the wild-type or mutant-type of *MYB*, the wild-type or mutant-type of *DANCR* and 2 ng Ranilla luciferase plasmid (BioMed Resource Core of the 1st Core Facility Lab, National Taiwan University College of Medicine, Taiwan), using the jetPRIME (Polyplus-transfection) reagent. The cells were additionally transfected with 0.025 nmol of *miR-187-5p* (GenePharma). After 24 h, the cells were lysed and the Renilla luciferase signals were used for normalisation, according to manufacturer's protocol.

Statistical Analyses

The experimental data are presented as the mean ± standard deviation (SD) of at least three independent experiments. The genes with statistically significant expression patterns in the different samples were determined by a two-tailed Student's *t*-test using GraphPad Prism v5 (GraphPad Software, Inc., La Jolla, CA, USA). *p*-values <0.05 were considered statistically significant.

RESULTS

LAs Significantly Suppressed Cell Viability and Migration

To determine the effects of lidocaine and bupivacaine on MCF-7 cells, the cell viability following treatment with different concentrations of lidocaine or bupivacaine for 6 h was determined. Lidocaine and bupivacaine inhibited the growth of MCF-7 cells in a dose-dependent manner (**Figure 1A**). The median lethal dose (LD_{50}) of lidocaine (4 mM) and bupivacaine (1 mM) were used as the concentrations for the following experiments, respectively. Wound healing and transwell invasion assays were performed using MCF-7 cells treated with lidocaine or bupivacaine for 12, 24, and 36 h. Both the LAs significantly reduced the migration and invasive ability of MCF-7 cells (**Figures 1B,C**), suggesting suppression of malignancy in the MCF-7 cells.

Differential miRNA Expression Profiles Following LA Treatment

To identify the miRNAs that mediated the effects of the LAs on MCF-7 cells, next-generation sequencing was used for identifying the differentially expressed miRNAs, following lidocaine or bupivacaine treatment. Principal component analysis (PCA) was

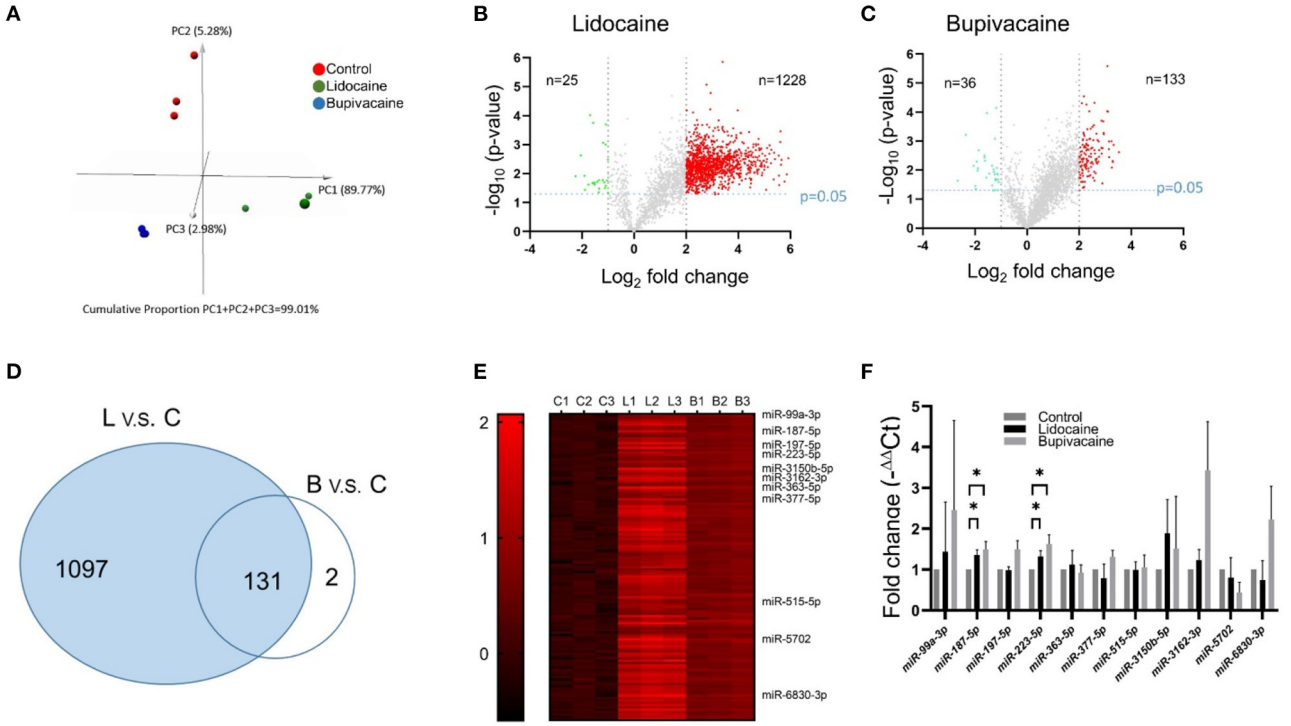

FIGURE 2 | Identification of miRNAs in MCF-7 cells that responded to treatment with lidocaine or bupivacaine. **(A)** PCA of differentially expressed miRNAs in response to treatment with lidocaine (4 mM) or bupivacaine (1 mM). **(B,C)** Volcano plot of the differentially expressed miRNAs in response to treatment with lidocaine **(B)** or bupivacaine **(C)** determined by next-generation sequencing. **(D)** Venn diagram of the differentially expressed miRNAs. The expression of 131 miRNAs was upregulated following treatment with lidocaine or bupivacaine. **(E)** Heat map of the upregulated miRNAs ($n = 131$) that responded to treatment with both lidocaine and bupivacaine. **(F)** PCR validation of the selective differentially expressed miRNAs in MCF-7 cells, following treatment with lidocaine and bupivacaine. The expression levels measured using qRT-PCR and normalised to that of U6 snRNA. L, lidocaine; B, bupivacaine; C, control. All data are presented as mean ± SD ($n = 3$). *$p < 0.05$.

performed for the samples (lidocaine, bupivacaine, and control groups, nine dots in total) using the data for 2,522 differentially expressed miRNAs in the three groups (**Figure 2A**). The miRNAs in the same group showed similar expression profiles, while the expression profiles in the lidocaine- and bupivacaine-treated groups were distinct from those of the control.

The miRNAs that were differentially expressed in the lidocaine- and bupivacaine-treated groups were filtered using the criteria: fold change ≥ 2.0X and p-value ≤0.05. The expression of 1,228 and 133 miRNAs was significantly upregulated in the lidocaine (**Figure 2B**) and bupivacaine groups (**Figure 2C**), respectively (red dots). The expression of 25 and 36 miRNAs was significantly downregulated (fold change <-1X and p-value ≤ 0.05) in the lidocaine (**Figure 2B**) and bupivacaine groups (**Figure 2C**), respectively (green dots). The genes that were not differentially expressed are depicted in grey. Among the upregulated miRNAs, 131 were common to both the lidocaine and bupivacaine groups (**Figure 2D**). The number of downregulated miRNAs common to both the groups was low; therefore, only the upregulated miRNAs were selected for subsequent studies. The expression of the selected miRNAs is depicted using a heatmap (**Figure 2E**).

The data from next-generation sequencing was validated by selecting the miRNAs whose expression was significantly higher and for which experimentally validated data were available. The expression of only *miR-187-5p* and *miR-223-5p* was significantly ($p < 0.05$) higher than that of the control. The expression of the other miRNAs examined using qRT-PCR was not statistically different between the lidocaine and bupivacaine groups (**Figure 2F**).

LAs Downregulated MYB Expression via *miR-187-5p*

To identify the genes regulated by *miR-187-5p* and ascertain their functional relevance, we used the TargetScan tool (http://www.targetscan.org/vert_72/) for identifying the downstream target genes. The *MYB* transcription factor, a proto-oncogene, was a predicted target gene of *miR-187-5p*. *In vitro* and *in vivo* studies indicated that *MYB* plays an important role in different cancers. *MYB* overexpression and dysregulation is observed in almost all breast tumours, and is associated with poor prognosis (20, 21). *MYB* also regulates cell proliferation, differentiation, and angiogenesis (22), and therefore, was selected for further studies.

qRT-PCR and western blotting were used to investigate the mechanism underlying the influence of *miR-187-5p* on cellular function. The mRNA and protein levels of MYB were significantly downregulated in the LA-treated groups (**Figures 3A,B**). The levels of *MYB* mRNA were significantly

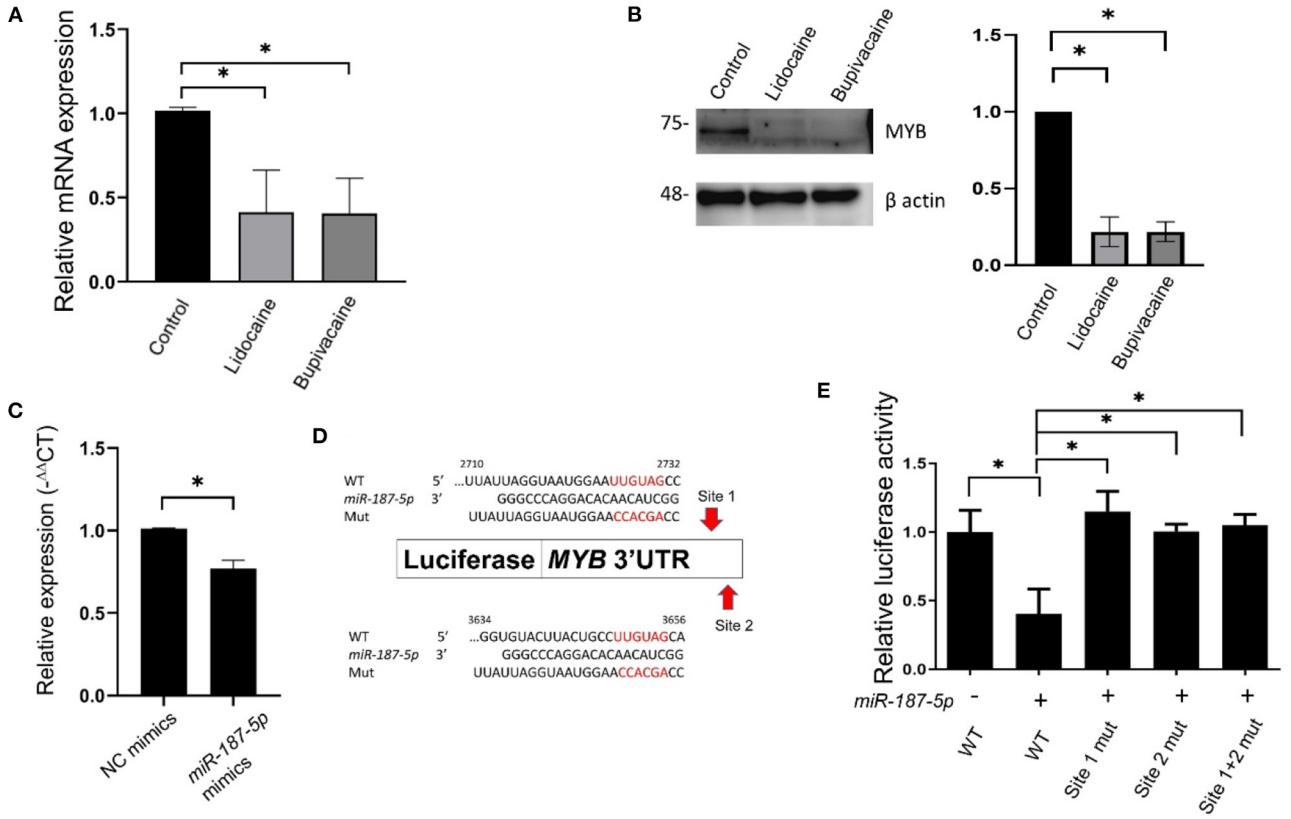

FIGURE 3 | Downregulation of *MYB* in MCF-7 cells following lidocaine and bupivacaine treatment. **(A)** The relative mRNA levels of *MYB* in MCF-7 cells following treatment with lidocaine (4 mM) or bupivacaine (1 mM). The expression levels of *MYB* were measured using qRT-PCR and normalised to that of 18S rRNA. **(B)** Western blots of MYB in MCF-7 cells following treatment with the LAs. Right: graphical representation of the results of western blotting. Left: quantification of the western blots. β actin was used as the loading control. **(C)** Relative expression levels of *MYB* in MCF-7 cells overexpressing *miR-187-5p*. The cells were transfected with 2 μg of *miR-187-5p* or control mimics. The expression levels were detected using qRT-PCR and normalised to that of 18S rRNA. **(D)** Schematic diagram of the putative binding site of *miR-187-5p* in the 3′-UTR region (2,493 ∼ 3,684 bp) of *MYB*. **(E)** Luciferase reporter assays of *MYB* 3′-UTR in HEK-293T cells overexpressing *miR-187-5p*. The HEK-293T cells were transfected with *miR-187-5p* mimics, firefly luciferase plasmids, and Renilla luciferase vectors. The relative activity of firefly luciferase was measured and normalised to that of Renilla luciferase. The data are presented as mean ± SD ($n = 3$). *$p < 0.05$.

reduced when *miR-187-5p* was overexpressed (**Figure 3C**). Using bioinformatics analysis, we validated *MYB* as a target gene of *miR-187-5p*; two binding sites (2,710–2,732 nt and 3,634–3,656 nt) were predicted in the 3′-UTR of *MYB*, which were complementary to the seed sequence of *miR-187-5p* (**Figure 3D**). The direct interactions between *miR-187-5p* and *MYB* were examined using luciferase reporter plasmids containing the wild-type *MYB* (*MYB*-wt) and binding site mutants (*MYB*-site1-mut, *MYB*-site2-mut, and *MYB*-site1+2-mut). The luciferase activity of *MYB*-wt decreased significantly, with the transfection of *miR-187-5p* mimics; however, they had no influence on *MYB*-site1, *MYB*-site2, and *MYB*-site1+2 (**Figure 3E**), indicating that *MYB* is a direct target gene of *miR-187-5p*. The LAs, lidocaine and bupivacaine, upregulated the expression of *miR-187-5p*, directly downregulating the expression of *MYB*.

DANCR Decoys *miR-187-5p* by Reciprocal Suppression

LncRNAs act as ceRNAs and influence mRNA levels by sequestering miRNAs that target both lncRNA and mRNAs. To identify the ceRNAs of *miR-187-5p*, we used DIANA-LncBase v2 (23) for predicting the lncRNAs that bind to *miR-187-5p*. Five possible lncRNAs were predicted and validated using qRT-PCR (**Figure 4A**). The expression of *DANCR* was downregulated by the LAs, while that of the *HIF1A-AS2* and *SNHG1* lncRNAs was upregulated; however, these lncRNAs did not function as ceRNAs of *miR-187-5p*. In addition, the expression of *DANCR* significantly decreased when the expression of *miR-187-5p* increased (**Figure 4B**).

Luciferase reporter assays were performed for validating the association between *DANCR* and *miR-187-5p*. Prediction revealed two potential binding sites of *miR-187-5p* at 134–157 nt and 380–408 nt of *DANCR*, which were individually positioned upstream of the luciferase reporter genes (**Figure 4C**). The binding site mutants (sites 1-mut, 2-mut, and 1+2-mut) were constructed and transfected into HEK-293T cells. The luciferase activity of *DANCR*-wt was significantly reduced by the *miR-187-5p* mimics; however, they had no effect on sites 1-mut, 2-mut, and 1+2-mut (**Figure 4D**), indicating that the *DANCR* lncRNA sponges *miR-187-5p* by direct binding.

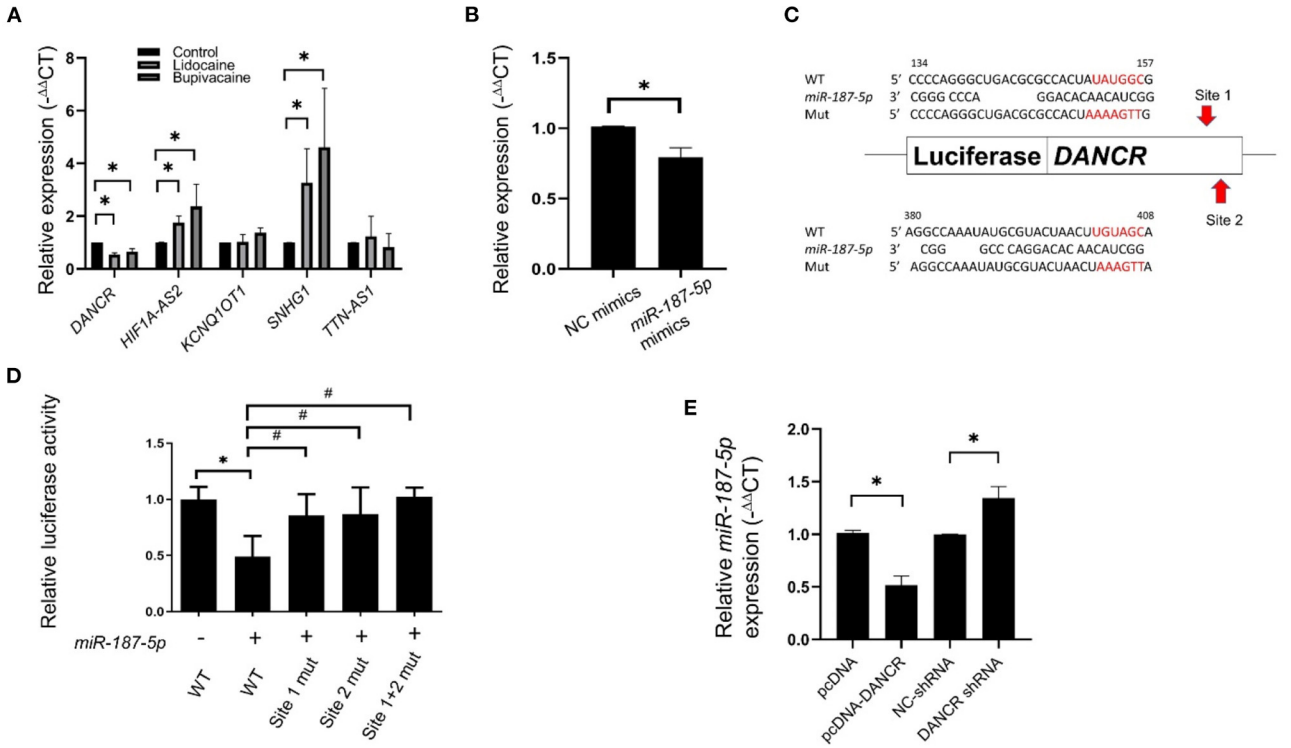

FIGURE 4 | Expression of *DANCR* lncRNA was downregulated in MCF-7 cells following treatment with lidocaine or bupivacaine. **(A)** The relative expression levels of selective lncRNAs in MCF-7 cells following treatment with lidocaine (4 mM) or bupivacaine (1 mM). The expression levels were measured using qRT-PCR and normalised to that of 18S rRNA. **(B)** Relative expression levels of *DANCR* in MCF-7 cells overexpressing *miR-187-5p*. The cells were transfected with 2 μg of *miR-187-5p* or control mimics. The expression levels were detected using qRT-PCR and normalised to that of 18S rRNA. **(C)** Schematic diagram of the putative binding site of *miR-187-5p* in the binding region of *DANCR*. **(D)** Luciferase reporter assays of *DANCR* in HEK-293T cells overexpressing *miR-187-5p*. The HEK-293T cells were transfected with *miR-187-5p* mimics, firefly luciferase plasmids, and Renilla luciferase vectors. The relative activity of firefly luciferase was measured and normalised to that of Renilla luciferase. **(E)** The relative expression levels of *miR-187-5p* in MCF-7 cells overexpressing *DANCR* or shRNAs against *DANCR*. The expression levels were detected using qRT-PCR and normalised to that of U6 snRNA. The data are presented as mean ± SD ($n = 3$). *$p < 0.05$.

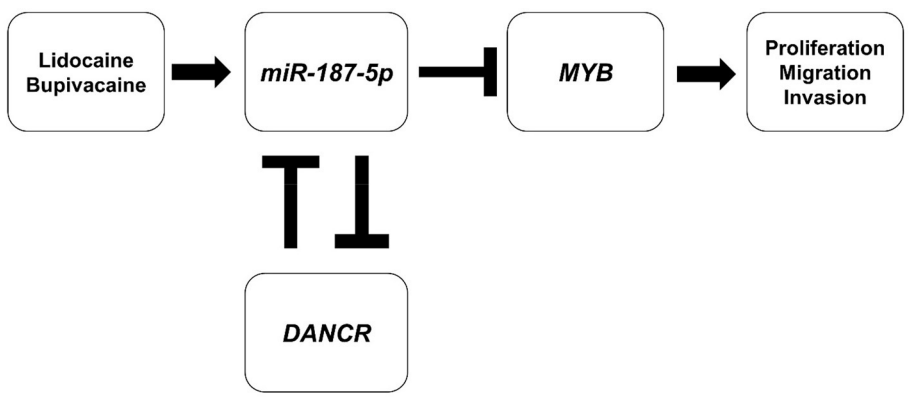

FIGURE 5 | Schematic diagram of the proposed mechanism underlying the inhibitory effect of LAs. Lidocaine and bupivacaine suppress the malignancy of MCF-7 breast cancer cells by upregulating *miR-187-5p*, which in turn downregulates the expression of MYB (MYB proto-oncogene, transcription factor) proteins and the DANCR (Differentiation Antagonising Non-Protein Coding RNA) lncRNA.

The overexpression of *DANCR* suppressed *miR-187-5p* expression, while the downregulation of *DANCR* increased *miR-187-5p* expression (**Figure 4E**). These results demonstrated a reciprocal inhibitory relationship between *DANCR* and *miR-187-5p*, and that *miR-187-5p* could mediate the inhibitory effect of the LAs on MCF-7 cell viability and migration.

DISCUSSION

The LAs, lidocaine and bupivacaine, inhibited MCF-7 cell proliferation and migration. Genomic studies and validation experiments revealed that the LAs promoted *miR-187-5p* expression, which downregulated *MYB* expression. Additionally, the LAs reduced the expression of *DANCR* lncRNA, which could be a ceRNA of *miR-187-5p*. Our study demonstrated a novel mechanism underlying the inhibitory effect of LAs on cancer cell proliferation, migration, and invasion, mediated via the *DANCR-miR-187-5p-MYB* regulatory axis.

LAs inhibit cancer cell proliferation, migration, and invasion by regulating miRNA expression (12, 13). Lidocaine inhibits proliferation and induces apoptosis in retinoblastoma cells by modulating the *miR-520a-3p*/EGFR axis (24). Bupivacaine inhibits gastric cancer progression by regulating the *circ_0000376*/*miR-145-5p* axis (25). Using NGS and qRT-PCR, we demonstrated that lidocaine and bupivacaine upregulated *miR-187-5p* expression. *MiR-187-5p* regulates cellular proliferation and apoptosis in lung cancer (26), hepatocellular carcinoma (27), bladder cancer (28), and acute lymphoblastic leukaemia (29), and is a possible indicator of drug sensitivity in breast cancer (30). This study is the first to identify that *miR-187-5p* regulates the inhibitory effect of LAs on the proliferation, migration, and invasion of MCF-7 cells.

Bioinformatics-based prediction revealed that *MYB* is a target gene of *miR-187-5p*, consistent with the reports that *MYB* functions as an oncogene in different tumours, including breast cancer (21, 31–33). *MYB* could be inhibited in MCF-7 cells through the upregulation of *miR-187-5p* by lidocaine or bupivacaine. These LAs reduced the mRNA and protein levels of MYB, and the *miR-187-5p* mimics significantly reduced *MYB* expression in MCF-7 cells. The luciferase reporter assay confirmed that *miR-187-5p* directly targets the 3′-UTR of *MYB*.

LncRNAs interact with various molecules and have key roles in regulating signalling processes at the epigenetic, transcriptional, and post-transcriptional levels (34, 35). A novel regulatory mechanism elucidates that all types of transcripts, including mRNAs, lncRNAs, and circular RNAs, can act as ceRNAs, forming a complex RNA interactome involving different RNA species, for regulating gene expression (35). The lncRNA-miRNA-mRNA crosstalk could have a prominent role in the anti-tumour effects of LAs. Among the lncRNAs, *DANCR*, encoded by a gene located in human chromosome 4q12, plays an important role in different cancers (36). The *DANCR*-SOCS3-EZH2 axis regulates the inflammatory phenotype and breast cancer cell metastasis (37). *DANCR* promotes progressive osteosarcoma by functioning as a ceRNA and sponging *miR-335-5p* and *miR-1972*, regulating ROCK1 expression (38). In this study, the expression of *DANCR* was inhibited by the LAs, and a reciprocal suppressive effect was observed between *DANCR* and *miR-187-5p*. The luciferase reporter assay confirmed that *DANCR* was the target of *miR-187-5p*. Therefore, the upregulation of *miR-187-5p* and downregulation of *DANCR* could mediate the inhibitory effect of LAs on the proliferation, migration, and invasion of MCF-7 cells. Together, we concluded that the DANCR-miR-187-5p-MYB axis is activated by lidocaine and bupivacaine; the proposed schematic diagram is presented in **Figure 5**.

This study has certain limitations. First, LAs are chemically categorised into amide-ester and amide-amide groups, and the new generation LAs, including ropivacaine and levobupivacaine, are used clinically. Only the older generation LAs, lidocaine and bupivacaine, were considered. LAs have variable anti-tumour effects and mechanisms of action in different cancers (39). Further studies are necessary for confirming whether the *DANCR-miR-187-5p-MYB* axis mediates the inhibitory effects of other LAs. Second, there are several breast cancer types; and therefore, further studies are necessary for confirming the effects of lidocaine and bupivacaine in other breast cancer cell lines and in an animal model, which may improve upon the present findings. Third, although luciferase studies proved the interaction between *DANCR-miR-187-5p-MYB* axis, whether the anti-tumour properties of LAs can be reversed requires more experiments to prove, such as using antagomir to inhibit *miR-187-5p* or overexpressing *MYB* in MCF-7 cells treated with LAs.

This study demonstrated that lidocaine and bupivacaine inhibited the growth and metastasis of breast cancer cells. The anti-tumour properties of these LAs were partially attributed to the upregulation of *miR-187-5p*, which inhibited *MYB* signalling by directly binding to *MYB*. Screening for the possible targets of *miR-187-5p* using a dual-luciferase reporter assay revealed that the *DANCR* lncRNA directly targets *miR-187-5p*, suggesting that *DANCR* could act as a *miR-187-5p* sponge. In summary, we report that the *DANCR-miR-187-5p-MYB* axis may be activated by lidocaine and bupivacaine.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found at: https://www.ncbi.nlm.nih.gov/geo/, GSE171282.

AUTHOR CONTRIBUTIONS

C-YL and L-CL: conception and design of experiments and manuscript preparation. C-YL and W-TT: performed experiments. C-YL and Y-YC: data analyses. L-CL, M-HT, and EC: contributed reagents, materials, and analytical tools. All the authors reviewed and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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Association Between Epidural Analgesia and Cancer Recurrence or Survival After Surgery for Renal Cell Carcinoma: A Propensity Weighted Analysis

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Whether epidural anesthesia and analgesia (EA) is beneficial for postoperative cancer outcomes remains controversial and we conducted this historical cohort study to evaluate the association between EA and long-term outcomes following surgery for renal cell carcinoma (RCC). We collected patients receiving RCC surgery from 2011 to 2017 and followed up them until February 2020. Patient attributes, surgical factors and pathological features were gathered through electronic medical chart review. The association between EA and recurrence-free and overall survival after surgery was evaluated using Cox regression models with inverse probability of treatment weighting (IPTW) to balance the observed covariates. The median follow-up time for the 725 included patients was 50 months (interquartile range: 25.3–66.5) and 145 of them (20%) received perioperative EA. We demonstrated EA use was associated with better recurrence-free survival [IPTW adjusted hazard ratio (HR): 0.64, 95% confidence interval (CI): 0.49–0.83, $p < 0.001$] and overall survival [IPTW adjusted HR: 0.66, 95% CI: 0.49–0.89, $p = 0.006$] in patients receiving surgical resection for RCC. More prospective studies are needed to verify this connection between EA and superior cancer outcomes after RCC surgery.

Keywords: epidural analgesia, inverse probability of treatment weighting, recurrence, renal cell carcinoma, survival

INTRODUCTION

Although life expectancy is increasing along with the progression and improvement of medical care, cancer remains one of the leading causes of death around the world and cancer treatment is still a great challenge in contemporary medicine (1). Surgical intervention is the mainstay treatment for the control and cure of most solid tumors but postoperative local or distant metastasis, which causes 90% of deaths, remains a common reason for morbidity and mortality in cancer patients (2, 3).

It should be noted that the perioperative period is associated with an increased formation of new metastatic foci and accelerated growth of micrometastatic disease (2). Surgical procedures

themselves also suppress the host's immunity which is inhibiting pre-existing micro-metastases, and manipulation during surgery can disseminate cancer cells which are shed from the primary tumor to the blood stream or lymphatic system intraoperatively (4). Moreover, surgery can directly activate the hypothalamic-pituitary-adrenal axis and sympathetic nerve system to increase the levels of catecholamine, prostaglandins and acute inflammatory cytokines (interleukin-6, interleukin-8) that further suppress the cytotoxic activity of macrophages and natural killer (NK) cells (5).

Also, accumulating evidence shows that anesthetic intervention and analgesia could affect the pathophysiological processes associated with long-term cancer outcomes (6). Since immunity plays a major role in cancer progression (7), perioperative pain management could be very important for preventing surgery-induced immunosuppression. Perioperative epidural anesthesia and analgesia (EA) effectively attenuate neuroendocrine stress responses related to surgery, they also reduce intraoperative volatile anesthetics and opioid consumption by blocking noxious afferent inputs transmitted to the central nervous system, and further preserve host immunity (4, 7, 8). Previous studies have demonstrated that patients with perioperative EA had better prostate, ovarian, colon, gastro-esophageal and breast cancer outcomes compared with those without (7, 9). Nevertheless, few studies have investigated the association between EA and postoperative outcomes after surgery for renal cell carcinoma (RCC) (8).

To fill this gap, we hypothesized that EA is beneficial to long-term outcomes after curative surgery for RCC and we conducted this retrospective study to evaluate the association between EA and cancer recurrence or overall survival. We used a novel propensity weighted analysis to promote analytical power and to reduce potential confounding effects by incorporating important prognostic factors in the analysis. Sensitivity analysis using the two other regression approaches was also employed to ensure the consistency of the estimated results.

METHODS

Setting and Patient Selection

The current study was approved by the Institutional Review Board (IRB) of the Taipei Veterans General Hospital, Taipei, Taiwan (IRB-TPEVGH no. 2018-06-009CC, Jul 2018), and written informed consent was waived by the IRB of the Taipei Veterans General Hospital. All methods were performed in accordance with the relevant guidelines and local regulations. Patients who underwent curative surgery for RCC between January 1st 2012 and December 31st 2017, as determined by reviewing the electronic medical records at our hospital, were included in the study. The exclusion criteria were: patients with benign pathological reports, non-RCC, reoperation for metastasis lesions, missing pathological data or perioperative pain management (**Figure 1**). All included patients were further classified into two groups based on whether they received perioperative EA or not. The reasons why patients did not receive EA included contraindications to EA, failed epidurals and the preference of the anesthesiologist, surgeon or patient, etc. For those who did not receive perioperative EA, intravenous patient-controlled analgesia with morphine was used to control postoperative pain.

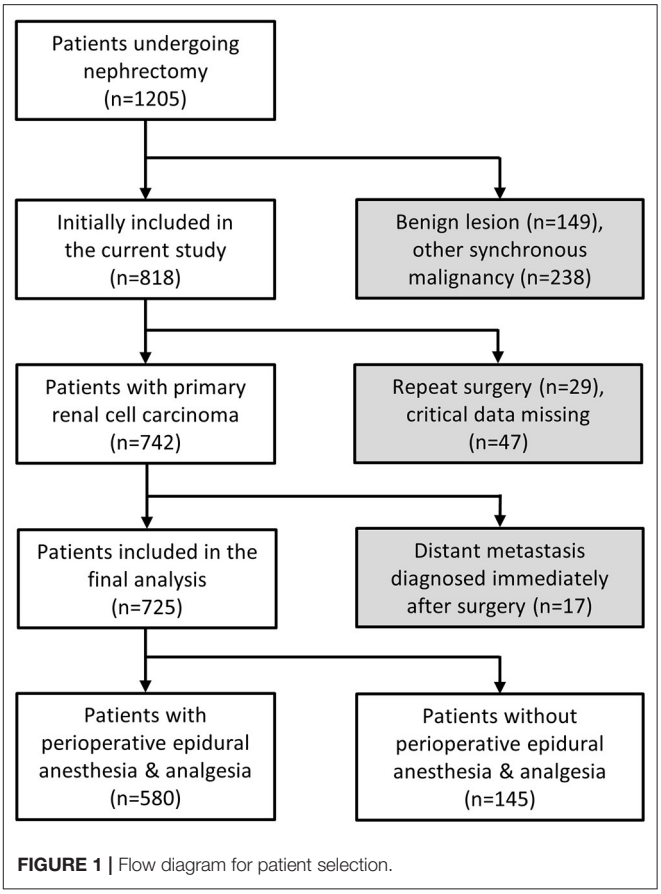

FIGURE 1 | Flow diagram for patient selection.

Anesthetic Management

During general anesthesia, the induction of anesthesia consisted in intravenous fentanyl 1–3 μg/kg, propofol 1–2.5 mg/kg and a neuromuscular blocking drug of either cisatracurium 0.15–0.2 mg/kg or rocuronium 0.6–1.2 mg/kg to facilitate tracheal intubation. Anesthesia was maintained with sevoflurane 2–3 vol% or desflurane 6–8 vol%. All epidural catheters were inserted at the lower thoracic or high lumbar region (T10–L2). Patients scheduled to receive EA had an epidural catheter implanted preoperatively which was tested using 2% lidocaine 2 ml to ensure it functioned properly. A loading dose of 1–1.5% lidocaine with or without fentanyl 50 μg was given before the surgical incision, and then bupivacaine 0.25% was continuous infused at a rate of 5–10 ml/h intraoperatively depending on the hemodynamic stability. EA was administered with bupivacaine 0.0625% for postoperative pain management and typically maintained for 48–72 h. Patients who did not receive EA had intravenous patient-controlled analgesia (PCA) *via* an ambulatory infusion pump (Gemstar Yellow, Hospira, IL, USA) to deliver morphine with an infusion rate of 0.5–1.0 mg/h and a bolus dose of 1 mg with a lockout time of 5–10 mins.

TABLE 1 | Patient demographics.

	Original data			After IPTW		
	Non-EA group (*n* = 580)	**EA group (*n* = 145)**	**SDD**	**Non-EA group (*n* = 725)**	**EA group (*n* = 622)**	**SDD**
Age	59 ± 14	56 ± 14	22.06	58 ± 14	58 ± 12	1.92
BMI	25.65 ± 4.10	25.39 ± 3.84	6.60	25.61 ± 4.10	25.68 ± 3.81	1.86
ASA physical status > 3	162 (27.9%)	38 (26.2%)	3.88	201 (27.7%)	168 (27.0%)	1.52
Charlson comorbidity index	4.08 ± 1.70	3.79 ± 1.76	16.93	4.03 ± 1.69	4.13 ± 1.78	5.84
Anesthesia time*	8.55 ± 0.36	8.57 ± 0.38	3.69	8.56 ± 0.36	8.58 ± 0.39	4.14
Intraoperative blood loss*	7.65 ± 1.89	8.03 ± 1.93	19.82	7.73 ± 1.90	7.65 ± 2.05	3.75
Sex, male	396 (68.3%)	100 (69.0%)	1.49	497 (68.6%)	450 (72.2%)	8.04
Smoking	134 (23.1%)	38 (26.2%)	7.21	172 (23.7%)	151 (24.2%)	1.13
Surgical year			42.61			9.87
< 2015	281 (48.4%)	100 (69.0%)		381 (52.6%)	358 (57.5%)	
Packed RBC transfusion	131 (22.6%)	41 (28.3%)	13.09	173 (23.8%)	148 (23.8%)	0.07
Laparoscopic or robotic surgery	321 (55.3%)	12 (8.3%)	117.12	333 (45.9%)	238 (38.2%)	15.73
Partial nephrectomy	351 (60.5%)	86 (59.3%)	2.46	437 (60.3%)	368 (59.1%)	2.34
Cancer subtype			12.87			7.46
Clear cell	441 (76.0%)	102 (70.3%)		543 (74.9%)	486 (78.1%)	
Others**	139 (24.0%)	43 (29.7%)		182 (25.1%)	136 (21.9%)	
Fuhrman grade > 2	215 (37.1%)	56 (38.6%)	3.20	271 (37.4%)	201 (32.3%)	10.70
Tumor necrosis	190 (32.8%)	54 (37.2%)	9.41	242 (33.4%)	179 (28.7%)	10.17
Capsular invasion	61 (10.5%)	10 (6.9%)	12.87	71 (9.8%)	40 (6.4%)	12.21
Hilar vein invasion	104 (17.9%)	27 (18.6%)	1.78	131 (18.1%)	138 (22.2%)	10.37
Renal sinus invasion	70 (12.1%)	22 (15.2%)	9.06	93 (12.8%)	92 (14.7%)	5.57
Cancer stage			7.18			2.76
I	369 (63.6%)	87 (60.0%)		454 (62.7%)	389 (62.5%)	
II	40 (6.9%)	14 (9.7%)		54 (7.5%)	39 (6.3%)	
III	141 (24.3%)	30 (20.7%)		171 (23.6%)	150 (24.2%)	
IV	30 (5.2%)	14 (9.7%)		45 (6.2%)	43 (7.0%)	

Values were mean ± SD or counts (percent), or median (interquartile range). Standardized difference (SDD) is the difference in mean or proportion divided by the pooled standard error, expressed as percentage; imbalance is defined as absolute value greater than 20 (small effect size).

**On base-2 logarithmic scale. **Other morphological types of RCC include chromophobe, papillary, Xp11.2 translocation, etc.*

BMI, body mass index; EA, epidural anesthesia and analgesia; IPTW, inverse probability treatment weighting; RBC, red blood cell; SDD, standardized difference.

Postoperative Cancer Control

RCC staging was defined according to the American Joint Committee on Cancer 2010 tumor-node-metastasis cancer classification system (10). After the primary tumor resection, additional surgeries for metastatic disease were performed depending on the lesion locations, including hepatectomy, colectomy, IVC thrombectomy, etc. Postoperative surveillance was performed regularly at an outpatient clinic and followed the National Comprehensive Cancer Network guidelines (11).

Data Collection

We reviewed the patient's electronic medical records and collected their demographic characteristics, including age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) physical status and Charlson comorbidity index (12). We also collected potential risk factors which might affect cancer prognosis, including cancer staging, pathological features (histologic tumor necrosis, capsular invasion, hilar vein invasion, renal sinus invasion), perioperative blood transfusion, minimal invasive or traditional open surgery, partial or radical nephrectomy and smoking. Current disease status and date of death were also obtained from the medical records. Local recurrence or distant metastasis was determined using imaging studies (computed tomography, magnetic resonance imaging, bone scan) or a tissue biopsy. The primary outcome was recurrence-free survival (RFS) which was defined as the time interval between the date of surgery and the discovery date of cancer recurrence or new metastatic foci. The secondary outcomes were overall survival (OS) and cancer-specific survival (CSS). All patients were followed until they were lost to follow-up, death or the 29th February 2020, whichever came first. For those without cancer progression, survival times were defined as the corresponding censored observation. Competing risk events were regarded as censoring in the analysis of cancer-specific survival.

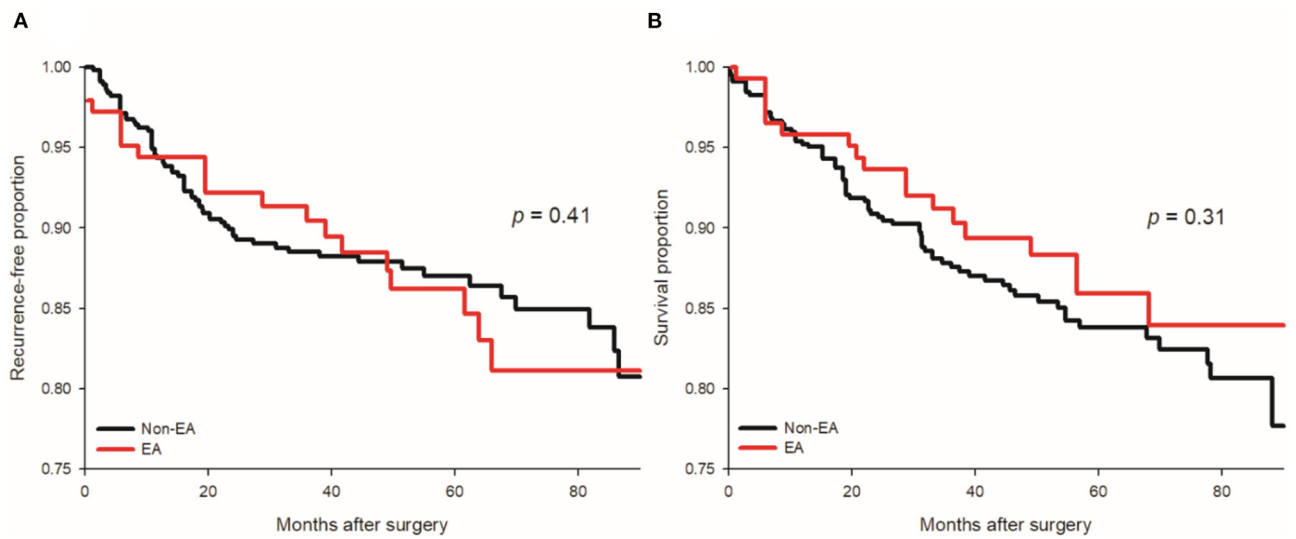

FIGURE 2 | Kaplan-Meier curves for cancer recurrence and all-cause mortality of EA (epidural anesthesia and analgesia) and non-EA groups. No significant difference in cancer recurrence **(A)** or all-cause mortality **(B)** was found after renal cell carcinoma resection between the EA with non-EA groups.

Statistical Analysis

All the patients were classified into the two groups depending on whether they received EA or not. Continuous and categorical variables are presented as the mean with standard deviation and count with the percentage, respectively. Logarithmic transformation was conducted to reduce the skewness of non-normal continuous variables. Standardized differences were used to evaluate balance in the collected variables between the two groups. The Kaplan-Meier method was used to compare the RFS and OS between the two groups. Univariate Cox regression analysis was also performed to assess the effects of EA and other covariates on RFS, OS and CSS. An inverse probability of treatment weighting (IPTW) method based on propensity scores was used to balance the distributions of the collected variables in the EA and non-EA groups and 1% of subjects at the end of weighting distribution were truncated to reduce the impact of large weights on the analytical results (13). Note that the propensity scores were generated from the logistic regression analysis (**Supplementary Table S1**) and reflected the probability of receiving EA given the collected variables. IPTW methodology weighted study subjects by the inverse probability of receiving EA or not to create a pseudo-population where the EA assignment is independent of the collected variables like randomization for unbiased estimation of average EA effects. Accordingly, weighted Cox regression analysis was applied to evaluate the association between EA and RFS, OS or CSS based on IPTW. For sensitivity analysis, all of the patients were further divided into the five equal groups using the quintiles of the generated propensity scores and a stratified Cox regression analysis was conducted to obtain a pooled hazard across the five strata to estimate the association of EA with RFS, OS and CSS. In addition, multivariable Cox regression analysis with a stepwise model selection strategy was used to identify independent predictors of RFS, OS and CSS, and to evaluate the effects of EA on these long-term outcomes. The significance level of all hypothesis testing was set at 0.05 and all the statistical analyses were conducted using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient Characteristics

A total of 725 patients were included in the study. They had a median follow-up interval of 50 with an interquartile range of 25.3 to 66.5 months and of these patients, 145 (20%) received EA. In the original sample, patients in the EA groups tended to be younger and have a higher chance of receiving open surgery (**Table 1**). Compared with the non-EA group, more cases in the EA group received surgery before 2015. However, after IPTW the imbalances in these covariate distributions between the two groups were removed (**Table 1**).

Recurrence-Free Survival

The 5-year RFS rates were 81.6% [95% confidence interval (CI): 74.5–88.7%] and 78.7% (95% CI: 74.8–82.6%) in the EA and non-EA groups, respectively. No significant difference in RFS distribution was noted between the EA and non-EA groups ($p = 0.408$ by log rank test, **Figure 2A**). The crude hazard ratio (HR) of the EA group was 0.84 with a 95% CI of 0.55–1.28. However, after IPTW, the weighted Cox regression analysis demonstrated a significant association between the EA and a better RFS (adjusted HR = 0.64, 95% CI: 0.49–0.83; $p < 0.001$). For sensitivity analysis, multivariable regression analysis identified six independent prognostic factors for cancer recurrence, including perioperative transfusion, anesthesia time, tumor necrosis, capsular invasion, cancer staging and EA (adjusted HR = 0.62, 95% CI: 0.40–0.96, **Table 2**). Notice that the association between EA and superior

TABLE 2 | Forward model selection for recurrence-free survival before weighting.

	HR	95% CI	*P*
Epidural analgesia	0.62	0.40–0.96	0.031
Anesthesia time*	2.12	1.29–3.47	0.003
Packed RBC transfusion	1.65	1.12–2.42	0.010
Tumor necrosis	2.40	1.63–3.54	<0.001
Capsular invasion	1.61	1.05–2.47	0.030
Cancer stage			<0.001
II vs. I	2.69	1.47–4.92	0.001
III vs. I	2.55	1.59–4.08	<0.001
IV vs. I	9.90	5.75–17.05	<0.001

On base-2 logarithmic scale. CI, confidence interval; HR, hazard ratio; RBC, red blood cell.

TABLE 3 | Forward model selection for overall survival before weighting.

	HR	95% CI	*p*
Epidural analgesia	0.63	0.37–1.07	0.086
BMI	0.93	0.88–0.99	0.025
Charlson comorbidity index	1.17	1.06–1.29	0.003
Anesthesia time*	3.15	1.84–5.39	<0.001
Tumor necrosis	2.83	1.75–4.60	<0.001
Capsular invasion	1.75	1.05–2.91	0.031
Cancer stage			<0.001
II vs. I	2.33	1.09–4.96	0.028
III vs. I	1.78	0.99–3.22	0.054
IV vs. I	11.44	6.26–20.94	0.000

On base-2 logarithmic scale. CI, confidence interval; HR, hazard ratio.

RFS was significant in the quintile-stratified analysis (pooled HR = 0.64, 95% CI: 0.40–1.00; $p = 0.05$).

Overall and Cancer-Specific Survivals

The 5-year OS rates were 86.8% (95% CI: 80.5–93.1%) and 83.8% (95% CI: 80.3–87.3%) in the EA and non-EA groups, respectively. There was no significant association between EA and better OS in the univariate analysis (p = 0.305 by log rank test, **Figure 2B**). The crude HR of EA was 0.76 with a 95% CI of 0.45–1.29. EA was significantly associated with superior OS after IPTW (adjusted HR = 0.66, 95% CI: 0.49–0.89; $p = 0.006$) and in the quintile-stratified analysis (HR = 0.54, 95% CI: 0.31–0.94; $p = 0.03$). The multivariable analysis identified six independent prognostic factors of OS, including BMI, Charlson comorbidity index, anesthesia time, tumor necrosis, capsular invasion and cancer stage (**Table 3**). The association between EA and better OS after RCC surgery was not significant after the adjustment for these significant predictors (HR = 0.63, 95% CI: 0.37–1.07, $p = 0.09$). With respect to the CSS, significant associations between EA and better CSS were noted after IPTW (adjusted HR = 0.68, 95% CI: 0.49–0.94; $p = 0.02$) and in the quintile-stratified analysis (HR = 0.52, 95% CI: 0.28–0.97; $p = 0.04$). Six predictors of CSS were identified after the model selection processes and a significant protective effect of EA on CSS was also noted (HR = 0.49, 95% CI: 0.27–0.89, $p = 0.02$, **Table 4**).

TABLE 4 | Forward model selection for cancer-specific survival before weighting.

	HR	95% CI	*P*
Epidural analgesia	0.45	0.27–0.89	0.011
Anesthesia time*	2.72	1.46–4.91	0.001
Packed RBC transfusion	1.86	1.12–3.05	0.016
Tumor necrosis	3.48	1.97–5.95	<0.001
Capsular invasion	1.91	1.15–3.32	0.017
Cancer stage			<0.001
II vs. I	2.41	0.87–5.27	0.060
III vs. I	2.56	1.17–4.37	0.007
IV vs. I	13.51	5.68–23.00	<0.001

On base-2 logarithmic scale. CI, confidence interval; HR, hazard ratio; RBC, red blood cell.

DISCUSSION

In recent decades, perioperative management has been identified as a factor that could impact cancer outcomes by altering the microenvironment and it has been receiving more clinical attention (14). All tissue trauma, including the sterile dissection carried out by surgeons, and inflammation have been associated with tumor progression (2). This study demonstrated the hypothetical benefits of perioperative EA for long-term cancer control and survival in patients following RCC resection. To the best of our knowledge, the current study is the largest comparative epidural study to date which has investigated the association between EA and long-term outcomes after RCC surgery. The current study had several strengths, including a relatively large sample size, and the fact that we took more prognostic and pathologic factors into account. We also used sound analytical approaches such as IPTW and other regression-based sensitivity analyses to ensure the consistency of the estimated results (9). Charlson comorbidity index was also used to control for the potential influence of comorbidity severity on the outcomes of interest in the analysis (15). All these efforts were used to try and provide more precise and reliable estimated results to determine the actual association between EA and RFS or OS after curative surgery for RCC.

Although opioids are widely used to control postoperative pain, they are believed to have negative effects on the immune system (8). The evidence from clinical observational studies indicates that opioids suppress cellular and humoral immunity, promote angiogenesis, and enhance progression of metastatic disease (2). Overexpression of μ-opioid receptors on cancer cells is observed and associated with angiogenesis and oncogenic signaling (4). Perioperative EA is administered near the nerve roots to block sensory and sympathetic nerves. It attenuates the neuroendocrine stress responses of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system activation (4) and minimizes volatile agent and opioid

consumption (7, 9). Therefore, it has been suggested that EA preserves immune function and prevents cancer recurrence after curative surgery but previous studies have reported inconsistent results (7, 14). Zimmitti et al. reported improved RFS but not OS in patients receiving general anesthesia with EA compared to those without after hepatic resection for colorectal liver metastases (16). However, the study did not take pre-existing medical conditions, perioperative blood transfusion or pathological features into account. Myles et al. (17) found no significant difference in RFS or OS between the EA and non-EA groups following abdominal cancer surgeries in a *post hoc* review of randomized control trials. A systemic review and meta-analysis revealed a lower risk of OS but not cancer recurrence among patients with perioperative regional anesthesia and analgesia (9). A recent study compared perioperative systemic analgesia (SA group) with perioperative EA in addition to systemic analgesia (EA group) following surgical resection of localized RCC, which suggested that EA usage was associated with a significantly improved OS but did not significantly impact cancer-specific survival (8). Accordingly, more prospective studies are needed to elucidate the associations between EA and long-term outcomes after curative cancer surgeries.

During cancer development, circulating tumor cells may leave the primary tumor and form clinically undetectable metastatic foci (2). Micrometastases remain in an immunologic equilibrium between tumor cell proliferation and host immunity (4). However, a cascade of local, systemic cellular and humoral inflammation events may reduce the ability of the host immune system to detect and eradicate cancer cells and could help to disseminated cancer cells which survive the host's defensive mechanisms (2, 14). Clinical evidence shows that tissue trauma caused during surgery can accelerate subsequent neoplastic disease (2, 6). Moreover, an experimental trial reported that the more extensive the surgery is the more potential there is for postoperative inflammation and complications, which further increase the recurrence rate (18). Some studies have suggested that open cancer surgery was associated with shorter disease-free survival compared with minimally invasive surgery, which limited surgical trauma (2, 19), however our investigation did not support the beneficial effects of minimally invasive surgery and partial nephrectomy compared with open surgery and radical nephrectomy, respectively. Notice that similar findings were also noted in another two studies which investigated oncological outcomes in patients undergoing minimally invasive surgery compared with open surgery for clinical T2 RCC and locally advanced RCC, respectively (20, 21). Similar findings were also noted in another study comparing partial or radical nephrectomy for clear cell RCC larger than 7 cm (22).

Interestingly, we also noted that a longer anesthesia time was associated with worse RFS and OS in the stepwise regression analysis. Singh et al. (23) had similar findings in an analysis of minimally-invasive surgeries for endometrial cancer. In their study, longer operative time was also associated with increased medical, surgical and overall complication rates. In fact, longer anesthesia time, as a surrogate for longer surgical time, may reflect the underlying aggressiveness of the disease or the complexity and difficulty of the surgery, or both. Since we have taken miscellaneous surgical and oncological factors into consideration to reduce confounding effects, anesthesia time is highly suspected as an independent risk factor of cancer recurrence and mortality in patients receiving RCC surgery.

Still, there are other factors which may have an effect on long-term cancer outcomes, including the use of steroids (14), non-steroidal anti-inflammatory drugs (4, 18, 19) and systemic lidocaine (4, 14), hypothermia (2, 14, 18), postoperative infections (2), blood transfusions (2, 14), etc. Red blood cells (RBC) are commonly given to cancer patients before, during or after major surgery for a number of different reasons. Although the value of blood transfusions for saving lives is indisputable (24), blood-component therapy can induce negative effects on the recipients' immune system (25), a condition called "transfusion-related immunomodulation" (25, 26). The detrimental effects of immunomodulation are thought to have an association with systemic inflammation (26, 27) and various immunologic changes, including inhibition of cytotoxic cell activity, and immunosuppressive prostaglandin release (26). In the sensitivity analysis, we observed that perioperative packed RBC transfusion was associated with a worse RFS in the multivariable regression analysis. Abu-Ghanem et al. (28) also reported that transfusion reduced RFS, CSS and OS in patients undergoing nephrectomy for RCC. Tsivian et al. (29) found that perioperative blood transfusion was independently associated with worse oncological outcomes for localized RCC after curative surgery and that the recipients were associated with roughly a two-fold increase in metastatic progression, all-cause and RCC-specific mortality. Moreover, negative clinical outcomes were also observed in colon (26, 27) and esophageal (30) cancer patients who received transfusion during curative-intent surgeries. Based on these findings, it has been suggested that blood transfusion can influence the different stages of tumor development including initiation, promotion, malignant conversion, invasion and metastasis (24). To reduce the potential confounding of perioperative blood transfusion on the outcomes of interest, the IPTW methodology was used to balance the exposure of transfusion in both the EA and non-EA groups.

The current study had several limitations. First, patients were not randomized to either group, they instead received EA depending on the preference of the patient, the surgeon or the anesthesiologist. Second, the influence of unmeasured covariates such as dose of opioid, local anesthetics or volatile agents and non-steroidal anti-inflammatory drugs use on cancer outcomes cannot be evaluated due to a lack of available data. Third, the clinical outcomes of loss to follow-up patients are unknown and the last observed censoring time was used in the analysis, which may have affected the results.

In conclusion, we demonstrated an association between perioperative EA use and better RFS and OS in patients undergoing curative surgery for RCC. Future prospective studies and randomized clinical trials with careful design are needed to confirm this relationship between EA and cancer outcomes after curative surgery for RCC and to elucidate the underlying mechanisms.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: the dataset is only available after the approval of the IRB of Taipei Veterans General Hospital. Requests to access these datasets should be directed to irbopinion@vghtpe.gov.tw.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of the Taipei Veterans General Hospital, Taipei, Taiwan (IRB-TPEVGH No. 2018-06-009CC, Jul 2018). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

F-YY: data collection and manuscript preparation. S-PL: data verification and interpretation. T-PL: data verification and manuscript revision. W-KC: statistical review and manuscript revision. K-YC: study design, data analysis, manuscript preparation, and critical content editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.782336/full#supplementary-material

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Evaluation of the Time Spent by Anesthetist on Clinical Tasks in the Operating Room

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Background: Changes in the health system in Western countries have increased the scope of the daily tasks assigned to physicians', anesthetists included. As already shown in other specialties, increased non-clinical burden reduces the clinical time spent with patients.

Methods: This was a multicenter, prospective, observational study conducted in 6 public and private hospitals in France. The primary endpoint was the evaluation by an external observer of the time spent per day (in minutes) by anesthetists on clinical tasks in the operating room. Secondary endpoints were the time spent per day (in minutes) on non-clinical organizational tasks and the number of task interruptions per hour of work.

Results: Between October 2017 and April 2018, 54 anesthetists from six hospitals (1 public university hospital, two public general hospitals and three private hospitals) were included. They were followed for 96 days corresponding to 550 hours of work. The proportion of overall clinical time was 62% (58% 95%CI [53; 63] for direct care. The proportion of organizational time was higher in public hospitals (11% in the university hospital ($p < 0.001$) and 4% in general hospitals ($p < 0.01$)) compared to private hospitals (1%). The number of task interruptions (1.5/h ± 1.4 in all hospitals) was 4 times higher in the university hospital (2.2/h ± 1.6) compared to private hospitals (0.5/h ± 0.3) ($p < 0.05$).

Conclusions: Most time in the operating room was spent on clinical care with a significant contrast between public and private hospitals for organizational time.

Keywords: clinical tasks, efficiency, anesthesia organization time, interruption task, anesthesia

HIGHLIGHTS

Most time in the operating room was spent on clinical care 62 % (58 % direct care and 4 % indirect care) with a significant contrast between public and private hospitals for organizational time (11% in the university hospital and 4% in general hospitals compared to 1% in private hospitals).

INTRODUCTION

Changes in the health system in Western countries have increased the scope of the tasks assigned to physicians in their daily lives. The burden of administrative tasks reduces the time spent with the patient. A recent work showed that in four different specialties (general medicine, internal medicine, cardiology and orthopedics), for every hour spent with a patient, a physician spent 2 h on tasks in the patient's absence (1). This result is consistent with that of the study of Wenger *et al.* which found a ratio of 1 to 3 for time spent with the patient vs. administrative tasks in a population of 36 internal medicine residents (2).

Anesthetists are confronted with the same reality as other specialties but no recent work has investigated this topic. A first study published in 1976 showed that anesthetists spent most of their time in contact with their patients: in direct observation (e.g. auscultation) or indirect observation (monitoring of constants) and adapting anesthesia drugs (3). A decade later, McDonald *et al.* reported a clinical time of 61% with 17% directly with the patient (4). In German multicentric study published in 2011, anesthesiologists spent 28.5% of each workday on indirect patient care, 14.7% on direct patient care and 18.8% on administrative work (5). Communication took up 19.9% of anesthesiologists' time, breaks and disruptions 15.2% and other job tasks 2.9%. The time spent on other non-clinical tasks (administrative, organization, etc) appears to be similar than specialties other than anesthesia (20% in the work of Sinsky *et al.*) (1).

A correlation has been shown between the importance of computer tasks in everyday routine and the occurrence of burnout (6). A reduction in clinical time with the patient could be a major source of dissatisfaction for physicians, which can lead to burnout. Anesthetists are more and more confronted with organizational tasks in the operating room that reduce their time spent with patients. No recent work has specifically evaluated the proportion of time spent by anesthetists on clinical and non-clinical tasks in the operating room. The primary objective of this social study was to evaluate the time spent per day by anesthetists on clinical tasks in the operating room. Secondary objectives were the time spent per day on non-clinical organizational tasks and the number of task interruptions per hour of work.

MATERIALS AND METHODS

Study Model

We conducted a multicenter, prospective and observational study in six hospitals in Normandy, France: one university hospital (Rouen University Hospital), two general hospitals (Dieppe General Hospital and Evreux General Hospital) and three private hospitals (Clinique du Cèdre, private hospital in Bois-Guillaume, Hôpital privé de l'estuaire, private hospital in Le Havre and Clinique Pasteur private hospital in Evreux). Fifty-four anesthetists were eligible and volunteered to participate in the study.

Study Protocol

We evaluated the time spent by anesthetists on clinical tasks during 1 day in the operating room. Each day between 8 a.m. and 3 p.m., an anesthetist, who had been selected the day before, was continuously monitored by an external observer, a student nurse anesthetist who was carrying out a research internship. There were 13 external observers, each of whom was followed up over the 3 weeks of the internship. All external observers had a 1-h training session by an anesthetist (TE) to explain the purpose of the study and the use of software for monitoring tasks. The training session consisted in a dedicated program on how to define and categorize clinical tasks and how to use recording devices. At the end of the training session, they were evaluated to assess their good comprehension of the different information. The information was also available on electronic devices. In case of difficulty, the two investigating anesthetists (TE and VC) were available at any time.

Organization of the Operating Rooms of Participating Hospitals

The three public hospitals (Rouen University Hospital, Dieppe General Hospital and Evreux General Hospital), operated with one nurse anesthetist in each operating room. An anesthetist was always in charge of two operating rooms. The organization was different according to the type of public hospital. The university hospital has specialty operating rooms (cardiac surgery, digestive and urological surgery, neurosurgery, pediatric surgery, orthopedic surgery and vascular and thoracic surgery) as well as shared operating rooms for emergency and ambulatory surgery, while the two general hospitals each have shared operating rooms integrating all specialties. The three private hospitals each have shared operating rooms. In the three private hospitals, an anesthetist was in charge of two operating rooms but there was only one nurse anesthetist for two operating rooms.

Data Collection

The data were anonymously collected using a digital tablet with atracker? software. This software was previously configured to monitor 15 items divided into five categories (**Table 1**): clinical time; non-clinical time; time spent on communication media to perform tasks; time spent in different locations; the number of task interruptions.

When the anesthetist started one of the tasks listed in the software, the external observer clicked on the corresponding task, triggering a stopwatch that stopped as soon as the observer clicked on it again. Several tasks could be followed in parallel. At the end of the day, data were automatically classified by the software and then sent in the form of a table on an Excel database.

For each anesthetist, observers recorded: age, the number of patients cared for during 1 day, the hospital, a satisfaction score of 0 to 10 filled by the anesthetist.

Study Outcomes

The primary endpoint was the evaluation by an external observer of the time spent per day (in minutes) by anesthetist on clinical tasks in the operating room.

TABLE 1 | Description of tasks.

Categories and daily tasks	Description
Clinical time	
Direct care (All tasks directly related to patient care)	- Direct communication with patient - Monitoring machines - Performing medical procedures
Indirect care (all tasks indirectly related to patient care)	- Searching for a patient's medical record - Preparing for a procedure
Pedagogical time	Time spent training residents
Non-clinical time	
Personal time	Breaks and lunch
Organizational time	Flow management in the operating room, staff meeting for patient programming
Administrative time	Meetings, scheduling, etc.
Other time	Any time not previously defined
Communication media	
Paper	Time spent writing on paper
Telephone	Time spent on the professional telephone
Computer	Time spent on the computer for professional tasks
Location	
Medical office	Time spent in the office
PACU	Time spent in the PACU
Operating room	Time spent in the operating room
Other	Time spent in the staff room, rest room etc.
Task interruption	
	Number of task interruptions

PACU, post anesthesia care unit.

Secondary endpoints were: the time spent per day (in minutes) on non-clinical organizational tasks; the proportion of time spent in different locations; the proportion of time spent on different communication media; the number of task interruptions per hour of work and stakeholders responsible for task interruptions; and anaesthetists' satisfaction of their working day. The definition used for task interruption was the unexpected cessation of human activity, temporary or permanent. The reason could be specific to the operator or, on the contrary, be external to him.

Ethical Considerations

The protocol was validated by the ethics committee for non-interventional research of Rouen University Hospital (E2017-27) and was registered in clinical trials (NCT03446482). The requirement for written informed consent was waived by the Committee.

TABLE 2 | Characteristics of anesthetists population.

	Anesthetists *n*	Age years	Treated patients *n*/day
Overall	54	39 (±10)	8 (±4.6)
General hospitals	13	44 (±11)$^{\#}$	8 (±3)
Private hospitals	14	43 (±8)	10 (±5.6)
University hospital	27	35 (±8.6)*	6.7 (±4.3)*

*Results are presented as means (± standard deviations) or absolute values. *$p < 0.05$ corresponding to a significant difference between the university Hospital and private hospitals. $^{\#}p < 0.05$ corresponding to a significant difference between the university hospital and general hospitals.*

Statistical Analysis

As described by Hauschild *et al.*, we wanted to include at least 500 h of anesthesiologist work in a minimum of five different hospitals (5). The values are presented as a mean (± standard deviation) for the characteristics of the study population. The results are presented in proportion, rounded to the nearest unit for the main results. The percentages (IC95%) expressed correspond to the time spent for each item per day and per anesthetist reported on the total observation time per physician. The number of task interruption is expressed as an average of the number of events per hour (± standard deviation). The different parameters were compared using the Kruskall Wallis test and Bonferroni correction. The correlation coefficients were calculated from the Pearson correlation test. We considered an alpha risk of 5%. The data were analyzed using Microsoft Excel ?, XLSTAT? and Prism ? software and the biostaTGV website.

RESULTS

Characteristics of Population

Between October 2017 and April 2018, 54 anesthetists from six hospitals were included. They were followed for 96 days corresponding to 550 h of assessed work hours. The characteristics of anesthetists according to hospitals are summarized in **Table 2**. The sex ratio was 2.6.

Evaluation of the Proportion of Time Spent on the Different Tasks

The proportion of time spent on clinical tasks was 58% 95%CI [53; 63]. The results are summarized in **Table 3**. The different stakeholders responsible for task interruptions are displayed in **Table 4**.

Correlation

There was no correlation between anaesthetists' job satisfaction and the number of patients cared for ($r = 0.19$, 95%CI[−0.03; 0.38], $p = 0.08$). Similarly, the correlation between the satisfaction score and the time spent in the patient's presence was not significant ($r = 0.18$, 95% CI[−0.03; 0.39], $p = 0.09$).

TABLE 3 | Time spent by anesthetists on clinical and non-clinical tasks in the operating room.

	Total	University hospital	General hospitals	Private hospitals
Anesthetists (n)	54	27	13	14
Task % [95%CI]				
Direct care	58% [53; 63]	53%*** [47; 59]	48% [40; 56]	78%+++ [71; 85]
Indirect care	4% [2.7; 5.4]	4% [2.2; 5.8]	2% [0.4; 3.6]	7%+ [3.3; 10.7]
Pedagogical time	4% [2.5; 6.2]	7%** [4.7; 9.3]	4% (2, 10)	<1%
Administrative time	4% [1.2; 5.9]	5% [0.5; 9.5]	1% (1, 3)	2% [0.3; 3.7]
Organizational time	7% [4.8; 9]	11%*** [7.6; 14.4]	4%## [0.3; 7.7]	1% [0; 2]
Personal time	14% [11.9; 5.3]	14% [11.3; 16.7]	15% [12.3; 17.7]	11% [7.5; 14.5]
Other time	9%	6%	26%	0
Communication media % [95%CI]				
Computer	7% [5.2; 9.7]	5%*** [3.2; 6.8]	1%## [0.2; 2.8]	17%+++ [12; 22]
Telephone	2% [1.7; 2.9]	3%* [1.4; 4.6]	1%## [0.4; 1.6]	2% [1.1; 2.9]
Paper	4% [2.7; 4.8]	5%*** [3.7; 6.3]	5% (2, 8)	<1%+
Location % [95%CI]				
Operating room	74% [69; 79]	77% [69; 85]	58%## [49; 57]	83%+++ [73; 93]
Medical office	6% [3.2; 8]	4% [1.2; 6.8]	13%## (6, 20)	2%++ [−0.1; 4.1]
PACU	13% [8.7; 15.6]	12% [4.4; 19.6]	16% [9; 23]	10% [2.7; 15.3]
Other	7% [3.7; 10.2]	7% [1.7; 12.3]	13% [5; 21]	5%+ [−0.1; 6.1]
Task interruption n/hour	1.5 (±1.4)	2.2 (±1.6)*	0.6 (±0.6)#	0.5(±0.3)
Satisfaction	7.2 (±1.6)	6.7(±1.7)**	7.4 (±1.8)	8 (±0.8)

Results are presented as percentages corresponding to the averages of proportions of anaesthetists' follow-up time for tasks, location and support and as an absolute value corresponding to the averages over the total observation time for interruptions and satisfaction.

**Corresponding to a difference between the university hospital and private hospitals.*
#Corresponding to a difference between the university hospital and general hospitals.
+Corresponding to a difference between the general hospitals and private hospitals.
, #, + $p < 0.05$, **, ##, ++ $p < 0.01$, *, ###, +++ $p < 0.001$.*

The correlation between the satisfaction score and the number of task interruptions during the day was significant and inversely proportional (r = −0.28, 95%CI[−0.46; −0.07], $p = 0.009$). The correlation between the satisfaction score and organizational time was significant and inversely proportional (r = −0.34, 95%CI[−0.52; −0.13], $p = 0.002$). The correlation between the satisfaction score and computer time was significant and positive (r = 0.26, 95%CI [0.05; 0.45], $p = 0.02$).

DISCUSSION

In this work based on an evaluation by external observers of the tasks in the operating room of 54 anesthetists corresponding to 550 h of follow-up in six centers, the overall clinical time of anesthetist was 62 % (58 % direct care and 4 % indirect care). This result is the same as that of an older work, published by Kennedy et al. in 1976, which focused on the proportion of time spent on the different tasks of the anesthetists during one day in the operating room. anesthetists spent most of their time in contact with their patients in the operating room to monitor and adapt anesthesia (>50%). These authors reported that anesthetists spent too much time (>30%) on tasks considered secondary (data recording, equipment preparation, etc.) because not related to direct care but which could be included in our definition of clinical time (3). About 10 years later, Mc Donald *et al.* evaluated the division of tasks of anesthetists in the operating room in order to analyze the impact of the modernization of monitoring on time spent in direct care. These authors found that 17% of anaesthetists' time was spent directly observing patients and about 40% on ancillary tasks (data recording, preparation, etc.) or observing the monitoring screens. However, if we add the clinical tasks related to anesthesia, the overall proportion of time spent on care was more than 70%, higher than our results (4). It is difficult to compare the results of our work with these two studies because of major changes in the practice of anesthesia over the past 30 years. Similarly, the definition of the tasks they used were not fully comparable with ours. The most recent wok shows that German anesthesiologists spent 28.5% of each workday on indirect patient care, 14.7% on direct patient care and 18.8% on administrative work (5). The type of hospital could change the ratio between different tasks. Indeed, Dexter *et al.* found 53.2% for direct clinical care, 11.5% for indirect clinical care, 10.3% for education and 12.9% for management (7).

Nevertheless, these results relative to ours suggest a continuous decrease in clinical time over the years. Our results are also consistent with those of different studies that focused on other specialties. In the study by Sinsky et al., the clinical time in the presence of patients was 33% in ambulatory practice (family medicine, internal medicine etc.) (1). Wenger et al. found similar results in a population of medical residents The authors attributed the decrease in clinical time to the increasing importance of computer tools (2). In an intensive care unit, the proportion of direct care ranged from 16 to 19% (8). Our results show that, on average, anesthetists spend 2–3 times longer on direct care than other medical specialties. One of the reasons is that in the French model, anesthetists are in charge of two operating rooms and are therefore responsible for two patients at the same time.

In our work, we found a significant difference in the time spent on direct care between public (48 and 53% for general hospitals and the university hospital, respectively) and private hospitals (78%). There are several explanations for this result. First, in the private hospitals observed, the anesthetist worked with only one nurse anesthetist for two rooms, which required the anesthetist to be present continuously in one of the two operating rooms and therefore the time in the presence of the

TABLE 4 | Stakeholders responsible for task interruptions.

Anesthetist	Nurse	Nurse anesthetist	Medical doctor	Resident	Surgeon	Surgical nurse	Unknown
3%	22%	15%	14%	4%	3%	7%	32%

patient was higher. In addition, in public hospitals, particularly in university hospitals, time is spent training residents (7%), which is not the case in private hospitals. Finally, this work highlights the higher organizational constraints in public hospitals (11% in the university hospital and 4% in general hospitals) compared to private hospitals (1%). This organizational time, which is at the expense of clinical time, is correlated with anaesthetists' satisfaction. This result suggests that in public hospitals, and more particularly in university hospitals, there is a difficulty in anticipating the organizational constraints of the operating room and that these latter regulate the work flow in real time. This organizational aspect, through a shift in tasks, is largely the responsibility of the anesthetist who may not be trained to assume this responsibility. Finally, organizational constraints could be a source of conflict between the many different medical professionals working alongside each other in the operating room (surgeons, surgical nurses, nurse anesthetists, nurse assistants).

The time spent on computer media was relatively low (7% of total observation time) compared to other specialties studied in the literature. Indeed, several studies showed that nearly a half of physicians' time was spent on computer tasks (1, 2). In our work, computer media were mainly used in private hospitals compared to public hospitals. In the private hospitals studied, intraoperative processes, including intraoperative monitoring, intra- and post-operative prescriptions and anesthesia consultation, were computerized, while in public hospitals, only post-operative prescriptions were computerized. Unlike other studies, we found a positive correlation between the use of computer media and anaesthetists' job satisfaction (6, 9). This result seems to be in discrepancy with that of another study which showed that the progressive use of electronic devices was rather a source of dissatisfaction for physicians in the U.S (10).

The number of task interruptions in our work was 1.5/h. Savoldelli et al. observed a frequency of distracting events of 5 per induction out of 37 inductions in the context of emergency surgery, the duration of which occupied 35% of the total induction time (11). In another work in the perioperative period, Campbell et al. followed 30 procedures during which they observed 13.8 distracting events per hour (17.4/h during induction, 9/h during maintenance of anesthesia and 30/h during the recovery phase) (12). Finally, out of 32 procedures, Jothiraj *et al.* observed 60 distractor events per hour of which 19.2 scored 2 on the Heavey scale (13). The difference observed between our study and these different works is the definition of task interruption. These other studies evaluated all the events likely to interfere with the physician's vigilance (distraction, disruption and interruption) whereas we only considered those that were responsible for cessation of activity, which reduced the number of our observations. In the context of critical care, several studies have focused on the quantification of interruption with a similar definition to ours. A work by Berg *et al.*, published in 2016, found a rate of 5/h in a population of physicians and nurses in an Emergency Department (14). A recent work by Li et al. evaluated task distribution and associated interruptions in an intensive care unit. The time spent in contact with the patient was only 16%, and 4.2/h of task interruptions were recorded In comparison, in a population of specialists not involved in critical care, Westbrook et al. found a 15% rate of patient contact time and a number of task interruptions of 2.9/h (8, 15). These task interruptions had consequences on the satisfaction of anesthetists since we found a correlation between their dissatisfaction and the number of task interruptions per day. Similarly, the work of Berg et al. found this result between dissatisfaction and task interruption (14).

Borrowed from aviation, some authors have put forward the concept of a sterile cockpit, particularly for the induction and recovery phases, to limit task interruptions by staying focused on clinical tasks (16). The International Civil Aviation Organization defines this concept as "the entire period during which the crew should not be disturbed except for matters essential to the safety of the aircraft" and in fact implies the restriction of crew members' activities to those that are operationally essential during particularly complex flight phases (take-off, landing,....). Apart from the exclusion of all non-management discussions, this concept also introduces the use of checklists and also a standardization of communication between the different professionals. This concept has not been specifically studied in anesthesia but other disciplines have shown interest in it. In a before/after study in cardiac surgery, the authors showed that formalizing the elements of communication made it possible to reduce the number of task interruptions (7.3 compared to 11.5 per case) (17). Without necessarily seeking total silence in the operating theater, simple measures such as banning unnecessary movement of people, reducing background noise, temporarily diverting telephone calls can create a more serene, professional and safe atmosphere. The physician (surgeon or anaesthesist) can also ask team members to refocus by warning them that a risky phase of a procedure is about to begin (18). Finally, training physicians to perform or manage multiple tasks simultaneously appears to be an interesting way (19, 20).

Our study presents a number of limitations that need to be included in discussion. We conducted a briefing session with external observers before their observation days to standardize the evaluation, but inter-observer variability between the 13 different external observers cannot be excluded. In addition, the choice of anesthetist to be followed during the day was defined by the study investigator a few days earlier without drawing lots from a population of volunteer physicians to be

followed. Since the evaluation took place over 7 consecutive hours and because of the multitude of tasks observed, the observers' attention may have fluctuated, the evaluation and the number of task interruptions were probably underestimated compared to reality especially since as described by Hauschild et al., we didn't perform a multitasking analysis (5). Although the anesthetists volunteered to participate in the study, there is a potential bias that there might not be happy with the working environment.

CONCLUSION

In this study, most time in the operating room was spent on clinical tasks with a significant contrast between public and private hospitals for organizational time. The enlargement of the scope of the anesthetists could in part lead to anaesthetists' dissatisfaction.

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

VC helped in the study conception and design, in resident recruitment, in study coordination, in interpretation of data, and in manuscript draft and revision. EB helped in the study conception and design, in acquisition of data, in analysis and interpretation of data and in manuscript draft. TC, NB, FL, FJ, NS, JH, and BD helped in interpretation of data and manuscript revision. TE helped in the study conception and design, in acquisition of data, in statistical analysis, in analysis and interpretation of data and in manuscript draft. All authors read and approved the final manuscript.

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Priorities Towards Fair Allocation of Ventilators During COVID-19 Pandemic: A Delphi Study

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Background: COVID-19 pandemic has resulted in severe shortage in vital resources, including invasive mechanical ventilators. The current imbalance between demand and supply of mechanical ventilators has called for investigations on the fair allocation of mechanical ventilators.

Objective: To determine the priorities of the medical experts towards the fair allocation of ventilators during the COVID-19 pandemic.

Methods: This study was conducted from May 28 to Aug 20, 2020. The questionnaire was sent to 50 medical specialists as the Delphi panel. Participants were asked to rate each prioritising factor: "−1" for low priority, "+1" for high priority, and "Zero" for equal priority.

Results: Among 38 experts who responded to the email, the responses of 35 were analysed. 31 (88.6%) participants recommended that pregnant women be considered high priority in allocating ventilators, 27 (77.1%) mothers of children<5 years, 26 (74.3%) patients under 80-years, and 23 (65.7%) front-line-healthcare-workers. In contrast, 28 (80.0) participants recommended that patients who are terminally ill should be considered as a low priority, 27 (77.1%) patients with active-malignancy, 25 (71.4%) neurodegenerative diseases, and 16 (45.7%) patients aged >80. The panel did not reach a consensus regarding the role of patients' laboratory profiles, underlying diseases, or drug abuse in the prioritisation of ventilators.

Conclusions: The panel considered pregnant mothers, mothers of children under 5 years, age groups younger than 80, and front-line healthcare workers to have high priority in allocating mechanical ventilators.

Keywords: coronavirus infections, health care rationing, ethics, health policy, resource allocation, SARS-CoV-2, mechanical ventilators

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is rapidly intensifying worldwide and continues to place an extraordinary burden on humankind (1). Since the early days of the pandemic, a severe shortage in vital resources, including invasive mechanical ventilators, has remained a significant concern of healthcare professionals (2, 3).

During the pandemic, of all patients diagnosed with COVID-19, 17–35% required hospitalisation at ICUs (4, 5) and 9–19% required invasive mechanical ventilation (4, 6). The estimated number of invasive mechanical ventilators in various countries would not be adequate to serve all clinically eligible patients during the current pandemic (3). Therefore, the current imbalance between demand and supply of mechanical ventilators has called for investigations on the fair allocation of mechanical ventilators. Although the research has been ongoing on the subject since the early days of the pandemic, significant concerns remain controversial (7–9).

Medical experts working at the COVID-19 care units interact with patients of different socioeconomic, clinical, paraclinical, and overall health statuses. Nevertheless, physicians should not be faced with situations where they would be obliged to decide which patient to treat due to the risk of human error and the life-long emotional toll (10). Therefore, prioritisation recommendations and guidelines are being developed in the hope of helping physicians, especially those less experienced, with the real-time decision-making process based on the resources and contexts (11, 12). Nevertheless, most studies on the subject have focused on experts' opinions from a single country or region, limiting their generalizability (7–9).

The objective of this study was to determine the priorities of the medical experts towards the fair allocation of ventilators during the COVID-19 pandemic via an international online Delphi survey.

MATERIALS AND METHODS

This online Delphi survey has been approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran, under the reference code IR.SBMU.RETECH.REC.1399.103. Participation was anonymous and upon the participant's own decision.

Review of Literature and Expert Selection

This study was conducted from May 28 to Aug 20, 2020. To design the Delphi questionnaire, an extensive literature review was conducted by the authors. The explored resources for data collection included the Centres for Disease Control (CDC), World Health Organisation (WHO), Infectious Diseases Society of America (IDSA), and European Centre for Disease Prevention and Control (ECDC). Electronic databases including PubMed, EMBASE, Medline, and Cochrane library were precisely investigated using the terms: COVID-19, SARS-CoV-2, mechanical ventilation, prioritisation, healthcare rationing, ethics, health policy, resource allocation, and invasive mechanical ventilators. After the initial preparation of the questionnaire, the Delphi survey was conducted in two phases.

Firstly, an expert panel of 10 individuals, two public health experts, two anesthesiologists, two emergency medicine specialists, two pulmonologists, and two infectious diseases specialists were asked to evaluate the questionnaire and provide other potential variables. The invitation link to participate was sent via email with a brief description of the aim of the study. Data were gathered through an online questionnaire via the Google Form platform.

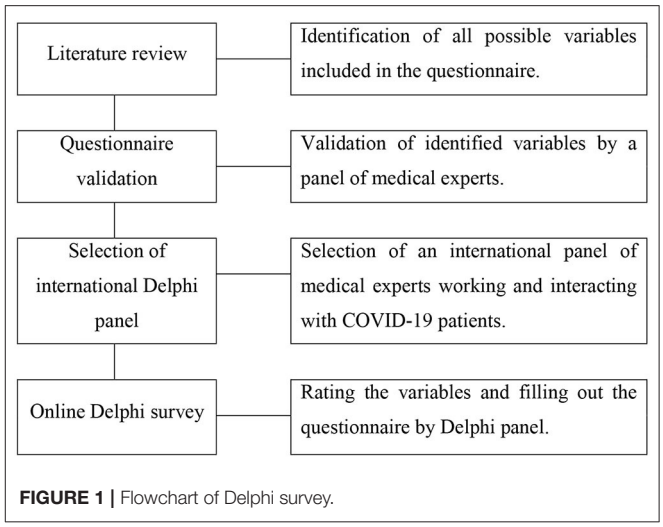

FIGURE 1 | Flowchart of Delphi survey.

Secondly, the revised version of the questionnaire based on the comments of the first-phase panellists was sent to a group of 50 medical specialists as our Delphi panel. All potential members were professionally involved in managing the patients with COVID-19 during the pandemic and were identified by investigating their professional academic curriculum vitae and the acquaintance of authors with them. Of them, 20 were intensive care experts, 10 were internal medicine specialists, 10 were emergency medicine specialists, 5 were forensic medicine experts, and 5 were infectious diseases specialists worldwide. Like the first phase, an email explaining the objective of the survey, their involvement in the study, how the Delphi study works, and the invitation link was sent to each potential participant. Among all participants who received the invitation email, 38 (76% response rate) agreed to participate in the study. Responses of three participants were incomplete, and therefore responses of 35 participants were analysed. Participants' responses in the second phase were considered as final responses. The authors of the article and the medical experts who contributed in the first phase were not included in the Delphi panel (**Figure 1**).

Variables and the Questionnaire

Variables included the personal information of the participants and the criteria for prioritising the ventilators during the pandemic. Personal information included participants' gender, age, area of study or speciality, affiliated institution, country, and the ventilator allocation experience in a setting of scarcity during the COVID-19 pandemic.

Among the criteria of prioritising the mechanical ventilators during the pandemic, all potential factors associated with the poor outcome of COVID-19 and the social responsibility of the patient were included and being divided into four sections: (1) non-medical determinants, (2) the underlying health conditions, (3) clinical, and (4) paraclinical presentation of COVID-19. It is worth mentioning that gender differences, religious beliefs,

various nationalities, chronic disabilities, and being refugees or immigrants were not included among the criteria of prioritising the mechanical ventilators due to ethical considerations (12).

Among non-medical determinants, various age groups of the patients, being healthcare professionals, smoking status, drug abuse, and being the mother of a child under 5 years were included. Giving the significance of various age groups in COVID-19 prognosis, age groups of patients were considered in eight different groups including below 19, 20 to 49 years, 50–59 years, 60–69 years, 70–74 years, 75–79 years, 80–84 years and above 85 years (13).

The underlying health conditions section included 12 health conditions, which would exacerbate the COVID-19 status of the patient. Obesity, pregnancy, uncontrolled hypertension, ischemic heart disease, poorly controlled diabetes mellitus, chronic kidney disease, neurodegenerative diseases, chronic respiratory failure, organ transplantation, hepatic failure, active malignancy, receiving immunosuppressive therapy, and being infected by Human Immunodeficiency Virus (HIV) were significant factors.

In the third and fourth sections, all clinical and paraclinical presentations of COVID-19 associated with the poor prognosis of the COVID-19 were included. Clinical presentations included clinicians' overall assessment of the COVID-19 prognosis based on the frailty scale (14), hypoxia based on disparate levels of oxygen saturation (SpO_2), hypotension and organ failure based on mean arterial pressure (MAP), the dosage of vasoactive agents required, having disseminated intravascular coagulation (DIC) and cardiac arrest. Paraclinical presentation of patients was included leukopenia, lymphopenia, low platelet counts, high bilirubin, creatinine, lactate-dehydrogenase (LDH), troponin, erythrocyte sedimentation rate (ESR), C-reactive protein (CPR), ferritin, and D-dimer.

Data Analysis

To quantify the opinions of the Delphi panel, we asked the participants to rate each prioritising factor based on three scores; "−1" was considered as low priority, "+1" high priority, and "Zero" which indicated that the factor should not be deciding at all and was considered "equal priority." The central tendency statistics, including mean, median, and mode, in addition to 95% confidence intervals (95% CI), standard deviation (SD), interquartile ranges (IQR), and skewness, were reported. The Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance tests were applied to define the differences between the means of two groups and three groups or more, respectively. Statistical analyses were performed using IBM SPSS Statistics 21. A probability level of <0.05 was considered significant.

RESULTS

Of 38 participants, responses of 35 (92.1%) participants were analysed. The mean (SD) age of participants was 50.1 (9.0), range 39–78, being 51.2 (9.8) among men and 47.2 (5.4) among women. Among participants, 22 (64.7%) declared that they had not encountered the situation of deciding to allocate invasive mechanical ventilators in the setting of scarcity during the COVID-19 pandemic. Other sociodemographic characteristics of participants are presented in **Table 1**.

TABLE 1 | Sociodemographic characteristics of participants.

Variable	*n* (%)
Sex	
Female	9 (25.7)
Male	26 (74.3)
Country	
Iran	14 (40.0)
Belgium	3 (8.6)
United States of America	3 (8.6)
United Kingdom	2 (5.7)
India	2 (5.7)
Norway	2 (5.7)
Other	9 (25.7)
Specialty	
Intensive care medicine	15 (42.9)
Internal medicine	7 (20.0)
Emergency medicine	7 (20.0)
Infectious diseases	4 (11.4)
Forensic medicine	2 (5.7)

Among various non-medical prioritising determinants, most participants believed that younger ages, healthcare workers, and the mothers of children under 5 years should be considered a high priority in allocating mechanical ventilators. As many as 26 (74.3%) participants reported high priority for patients under 80 years of age; however, 16 (45.7%) participants said they would give lower priority to patients aged 80 or more. There was no consensus regarding the prioritisation of ventilator allocation for patients' smoking and drug abuse status (**Table 2**).

When the Delphi panel was asked to prioritise patients regarding their underlying health condition, 31 (88.6%) participants recommended that pregnant women take high priority. Among underlying diseases, 27 (77.1%) and 25 (71.4%) participants reported low priority for active malignancy and neurodegenerative diseases, respectively. Although patients with BMI>40, diabetes mellitus, chronic kidney disease, and chronic respiratory failure received low priority, the number of participants reporting high or equal priority for the diseases was high as well. The participants' responses on prioritising ventilator allocation regarding underlying health conditions are presented in **Table 3**.

While the Delphi panel assigned a high priority for hypotensive and hypoxic patients, the end stages of hypotension, including DIC and cardiac arrest, received lower priority. Considering the clinician's judgment about the prognosis of COVID-19 based on the frailty scale, severely frail and terminally ill patients were given lower priority than very fit, well, and managing well patients (**Table 4**).

Considering the importance of paraclinical factors in anticipating the prognosis of COVID-19 among patients, we asked the panellists to rate every paraclinical factor, including blood cell counts, liver and kidney function tests, inflammatory

TABLE 2 | Responses on prioritising ventilator allocation regarding non-medical characteristics of patients.

Factor	Low priority (%)	Equal priority (%)	High priority (%)	Mean (SD)	95% CI	Median (IQR)	Skewness
Age							
<19 years	2 (5.7)	7 (20.0)	26 (74.3)	0.7 (0.6)	0.5, 0.9	1 (0, 1)	−1.7
20–49	1 (2.9)	6 (17.1)	28 (80.0)	0.8 (0.5)	0.6, 0.9	1 (1, 1)	−2.0
50–59	0 (0.0)	5 (14.3)	30 (85.7)	0.8 (0.4)	0.7, 1.0	1 (1, 1)	−2.0
60–69	0 (0.0)	6 (17.1)	29 (82.9)	0.8 (0.4)	0.6, 0.9	1 (1, 1)	−1.5
70–74	2 (5.7)	10 (28.7)	23 (65.6)	0.6 (0.6)	0.3, 0.7	1 (0, 1)	−1.2
75–79	8 (22.8)	10 (28.6)	17 (48.6)	0.3 (0.8)	0.0, 0.5	1 (0, 1)	−0.5
80–84	14 (40.0)	6 (17.1)	15 (42.9)	0.0 (0.9)	−0.4, 0.3	1 (−1, 1)	−0.1
≥85 years	17 (48.6)	5 (14.3)	13 (37.1)	−0.1 (0.9)	−0.4, 0.2	−1 (−1, 1)	0.2
Occupation							
Frontline HCW*	1 (2.9)	11 (31.4)	23 (65.7)	0.6 (0.5)	0.4, 0.8	1 (0, 1)	−1.0
Non-frontline HCW	1 (2.9)	14 (40.0)	20 (57.1)	0.5 (0.6)	0.3, 0.7	1 (0, 1)	−0.6
Smoking	10 (28.6)	15 (42.8)	10 (28.6)	0.3 (0.6)	−0.3, 0.3	0 (−1, 1)	−0.3
Drug abuse	14 (40.0)	16 (45.7)	5 (14.3)	−0.3 (0.7)	−0.5, 0.0	0 (−1, 0)	0.4
Mother of child under 5 years	1 (2.9)	7 (20.0)	27 (77.1)	0.6 (0.6)	0.5, 0.9	1 (0, 1)	−1.6

*Healthcare worker.

TABLE 3 | Responses on prioritising ventilator allocation regarding the underlying health condition of patients.

Factor	Low priority (%)	Equal priority (%)	High priority (%)	Mean (SD)	95% CI	Median (IQR)	Skewness
Obesity							
30< BMI ≤35	2 (5.7)	19 (54.3)	14 (40.0)	0.3 (0.6)	0.1, 0.6	0 (0, 1)	−0.3
35< BMI ≤40	8 (22.9)	18 (51.4)	9 (25.7)	0.0 (0.7)	−0.2, 0.3	0 (0, 1)	0.0
BMI >40	18 (51.4)	10 (28.6)	7 (20.0)	−0.3 (0.8)	−0.6, 0.0	−1 (−1, 0)	0.6
Pregnancy	0 (0.0)	4 (11.4)	31 (88.6)	0.9 (0.3)	0.7, 1.0	1 (1, 1)	−2.3
Uncontrolled hypertension	8 (22.9)	16 (45.7)	11 (31.4)	0.1 (0.7)	−0.3, 0.4	0 (−1, 1)	−0.1
Ischemic heart disease	10 (28.6)	15 (42.8)	10 (28.6)	0.0 (0.8)	−0.3, 0.3	0 (−1, 1)	0.0
Diabetes mellitus	13 (37.1)	9 (25.8)	13 (37.1)	0.0 (0.9)	−0.3, 0.3	0 (−1, 1)	0.0
Chronic kidney disease	13 (37.1)	12 (34.3)	10 (28.6)	−0.1 (0.8)	−0.4, 0.2	0 (−1, 1)	0.2
Neurodegenerative diseases	25 (71.4)	5 (14.3)	5 (14.3)	−0.5 (0.8)	−0.8, −0.3	−1 (−1, 0)	1.1
Chronic respiratory failure	20 (57.2)	4 (11.4)	11 (31.4)	−0.3 (0.9)	−0.6, 0.1	−1 (−1, 1)	0.6
Organ transplantation	6 (17.1)	14 (40.0)	15 (42.9)	0.3 (0.7)	0.0, 0.5	0 (0, 1)	−0.5
Immunosuppressive use	7 (20.0)	15 (42.9)	13 (37.1)	0.2 (0.8)	−0.1, 0.4	0 (0, 1)	−0.3
HIV/AIDS	10 (28.6)	19 (54.3)	6 (17.1)	−0.1 (0.7)	−0.4, 0.1	0 (−1, 0)	0.1
Hepatic failure	20 (57.2)	6 (17.1)	9 (25.7)	−0.3 (0.9)	−0.6, 0.0	−1 (−1, 1)	0.7
Active malignancy	27 (77.1)	3 (8.6)	5 (14.3)	−0.6 (0.7)	−0.9, −0.4	−1 (−1, −1)	1.7

factors, troponin, and D-dimer tests. The majority of paraclinical factors associated with the severity of COVID-19 were considered unimportant in resource allocation by panellists (**Table 5**).

No correlations were observed with participants' responses on resources allocation and their age, religion, country of residence, the field of study, and the dilemma of allocating invasive mechanical ventilators in the setting of scarcity during the COVID-19 pandemic.

DISCUSSION

The study showed that the panel considered younger age groups, healthcare workers, and mothers of children under 5 years for prioritising mechanical ventilators. While most participants reported high priority for patients under 80 years of age, almost half of participants said they would give lower priority to patients aged 80 or more. There was no consensus regarding the prioritisation of ventilator allocation for patients' smoking and drug abuse status.

Some 89% of participants recommended that pregnant women must take high priority. Many guidelines also prioritised pregnant women, younger age groups, and healthcare professionals to allocate ventilators (15). There is evidence that the mortality due to COVID-19 is lower among younger age groups. Nevertheless, young hospitalised patients with COVID-19 regularly require ventilators for extended periods (16). Thus, cohort and investigational studies could help healthcare professionals and experts.

TABLE 4 | Responses on prioritising ventilator allocation regarding clinical presentation of COVID-19.

Factor	Low priority (%)	Equal priority (%)	High priority (%)	Mean (SD)	95% CI	Median (IQR)	Skewness
Frailty scale							
Very fit	2 (5.7)	8 (22.9)	25 (71.4)	0.7 (0.6)	0.5, 0.9	1 (0, 1)	−1.6
Well	3 (8.6)	7 (20.0)	25 (71.4)	0.6 (0.6)	0.4, 0.9	1 (0, 1)	−1.6
Managing well	1 (2.9)	11 (31.4)	23 (65.7)	0.6 (0.5)	0.4, 0.8	1 (0, 1)	−1.1
Vulnerable	2 (5.7)	12 (34.3)	21 (60.0)	0.5 (0.6)	0.3, 0.8	1 (0, 1)	−1.0
Mildly frail	6 (17.1)	10 (28.6)	19 (54.3)	0.4 (0.8)	0.1, 0.6	1 (0, 1)	−0.8
Moderately frail	11 (31.4)	12 (34.3)	12 (34.3)	0.0 (0.8)	−0.3, 0.3	0 (−1, 1)	−0.1
Severely frail	22 (62.8)	3 (8.6)	10 (28.6)	−0.3 (0.9)	−0.7, 0.0	−1 (−1, 1)	0.8
Very severely frail	23 (65.7)	3 (8.6)	9 (25.7)	−0.4 (0.9)	−0.7, −0.1	−1 (−1, 1)	0.9
Terminally Ill	28 (80.0)	2 (5.7)	5 (14.3)	−0.7 (0.7)	−0.9, −0.4	−1 (−1, −1)	1.8
Hypoxia							
88<SpO2≤93%	4 (11.4)	9 (25.8)	22 (62.8)	0.5 (0.7)	0.3, 0.8	1 (0, 1)	−1.0
SpO2<88%	3 (8.6)	6 (17.1)	26 (74.3)	0.7 (0.6)	0.4, 0.9	1 (0, 1)	−1.7
Hypotension and organ failure							
MAP* <70 mmHg	2 (5.7)	12 (34.3)	21 (60.0)	0.5 (0.6)	0.3, 0.8	1 (0, 1)	−1.0
Dopamine ≤5 or Dobutamine (any dose)	2 (5.7)	13 (37.1)	20 (57.2)	0.5 (0.6)	0.3, 0.7	1 (0, 1)	−0.9
Dopamine >5, Epinephrine ≤0.1, or norepinephrine ≤0.1	4 (11.4)	12 (34.3)	19 (54.3)	0.4 (0.7)	0.2, 0.7	1 (0, 1)	−0.8
Dopamine >15, Epinephrine >0.1, or norepinephrine >0.1	7 (20.0)	11 (31.4)	17 (48.6)	0.3 (0.8)	0.0, 0.6	0 (0, 1)	−0.6
DIC**	13 (37.2)	11 (31.4)	11 (31.4)	0.0 (0.9)	−0.3, 0.3	0 (−1, 1)	0.7
Cardiac arrest	28 (80.0)	2 (5.7)	5 (14.3)	−0.7 (0.7)	−1, −0.4	−1 (−1, −1)	0.5

* *Mean Arterial Pressure.*

** *Disseminated intravascular coagulation.*

Although patients with BMI>40, diabetes mellitus, chronic kidney disease, and chronic respiratory failure received low priority, the number of participants reporting high or equal priority for the diseases was high as well. While the panel considered a high priority for hypotensive and hypoxic patients, the end stages of hypotension, including DIC and cardiac arrest, received lower priority.

More than two-thirds of participants considered patients with active malignancy and neurodegenerative diseases to have low priority. COVID-19 pandemic has disrupted the conventional care delivery for both malignancies and neurodegenerative diseases (17, 18). Patients with neurodegenerative diseases, which is more common among advanced age groups (19), often live in residential homes, which puts them at greater risk of COVID-19 transmission (20). Similar to considering these groups as low-priority in our study, many resource allocation guidelines have excluded these patients (21), which could put them at risk of systemic discrimination in the near future (22).

Among underlying diseases, COPD is reported to be an independent risk factor for all-cause mortality among patients with COVID-19 (23). Hypertension and uncontrolled hypertension were the most common comorbidity among hospitalised patients with COVID-19 infection (24).

The majority of paraclinical factors associated with the severity of COVID-19 were considered unimportant in resource allocation. There is evidence that LDH and CRP independently predicted ventilation requirements among COVID-19 patients (16).

Considering the clinician's judgment about the prognosis of COVID-19 based on the frailty scale, severely frail and terminally ill patients were given lower priority than very fit, well, and managing well patients. Evidence shows that the frailty scale is linearly associated with increased mortality due to COVID-19 (25). Although some studies proposed using Sequential Organ Failure Assessment (SOFA) score for prioritising ventilator allocation in the early days of the pandemic (26), the SOFA score has been shown to have inadequate accuracy for ventilator triage of patients with COVID-19 (27). The combination of the frailty scale with the SOFA score did not improve the performance of the SOFA score either (28). Thus, better alternatives are needed for prognostic prediction of patients with COVID-19 pneumonia requiring mechanical ventilation.

Unresolved ethical dilemmas regarding the fair allocation of ventilators threaten the success of the response to a public health emergency. Nevertheless, not all healthcare systems have developed allocation guidelines (26, 29, 30). Some studies challenge the "save the most lives" strategy. A study proposes that the following considerations be taken into account, when necessary while allocating scarce resources: maximising survival to hospital discharge, maximising the number of life-years saved,

TABLE 5 | Responses on prioritising ventilator allocation regarding laboratory presentation of COVID-19.

Factor	Low priority (%)	Equal priority (%)	High priority (%)	Mean (SD)	95% CI	Median (IQR)	Skewness
Leukopenia	5 (14.2)	20 (57.2)	10 (28.6)	0.2 (0.7)	0.0, 0.5	0 (0, 1)	−0.3
Lymphopenia	4 (11.4)	16 (45.7)	15 (42.9)	0.3 (0.7)	0.1, 0.6	0 (0, 1)	−0.5
Low platelet count							
100< PLT ≤149	1 (2.9)	27 (77.1)	7 (20.0)	0.2 (0.5)	0.1, 0.4	0 (0, 1)	0.6
50< PLT ≤99	2 (5.7)	20 (57.2)	13 (37.1)	0.3 (0.6)	0.1, 0.5	0 (0, 1)	−0.2
20< PLT ≤49	9 (25.7)	15 (42.9)	11 (31.4)	0.1 (0.8)	−0.2, 0.3	0 (−1, 1)	−0.1
PLT <20	11 (31.4)	15 (42.9)	9 (25.7)	−0.1 (0.8)	−0.3, 0.2	0 (−1, 1)	0.1
High LDH	3 (8.6)	21 (60.0)	11 (31.4)	0.3 (0.6)	0.1, 0.5	0 (0, 1)	−0.2
High troponin	6 (17.1)	16 (45.7)	13 (37.2)	0.2 (0.7)	0.0, 0.5	0 (0, 1)	−0.4
High bilirubin							
1.2–1.9 mg/dL	4 (11.4)	25 (71.5)	6 (17.1)	0.1 (0.6)	0.0, 0.3	0 (0, 0)	0.0
2.0–5.9 mg/dL	4 (11.4)	22 (62.9)	9 (25.7)	0.1 (0.6)	−0.1, 0.4	0 (0, 1)	−0.1
6.0–11.9 mg/dL	14 (40.0)	13 (37.1)	8 (22.9)	−0.2 (0.8)	−0.5, 0.1	0 (−1, 0)	0.3
≥12.0 mg/dL	16 (45.7)	10 (28.6)	9 (25.7)	−0.2 (0.8)	−0.5, 0.1	0 (−1, 1)	0.4
High creatinine							
1.2–1.9 mg/dL	0 (0.0)	24 (68.6)	11 (31.4)	0.3 (0.5)	0.2, 0.5	0 (0, 1)	0.6
2.0–3.4 mg/dL	3 (8.6)	21 (60.0)	11 (31.4)	0.2 (0.6)	0.0, 0.4	0 (0, 1)	−0.1
3.5–4.9 mg/dL	7 (20.0)	18 (51.4)	10 (28.6)	0.1 (0.7)	−0.2, 0.3	0 (0, 1)	−0.1
≥5.0 mg/dL	10 (28.6)	14 (40.0)	11 (31.4)	0.0 (0.8)	−0.2, 0.3	0 (−1, 1)	−0.1
High ESR	2 (5.7)	24 (68.6)	9 (25.7)	0.2 (0.5)	0.0, 0.4	0 (0, 1)	0.2
High CRP	2 (5.7)	20 (57.2)	13 (37.1)	0.3 (0.6)	0.1, 0.5	0 (0, 1)	−1.5
High D-dimer	1 (2.9)	21 (60.0)	13 (37.1)	0.3 (0.5)	0.2, 0.5	0 (0, 1)	0.1
High ferritin	2 (5.7)	25 (71.4)	8 (22.9)	0.2 (0.5)	0.0, 0.4	0 (0, 0)	0.3

maximising individuals' chances to live through each of life's stages, the severity of impairment, and patients' instrumental value into prioritisation considerations. In this sense, the public also needs to participate in choosing among ethically permissible allocation strategies (7, 31). Some studies have investigated people's opinions on the fair allocation of ventilators (32). A community-based survey reported that people considered age, expected ventilation effectiveness, smoking status, having dependents, being a healthcare worker, and having disabilities to be of importance in resource allocation (33).

STRENGTHS AND LIMITATIONS

This is among the few studies investigating the experts' opinions on priorities towards fair allocation of mechanical ventilators during the COVID-19 pandemic. Findings could empower public health authorities better to understand experts' opinions to be considered in future guidelines. It is worth mentioning that the COVID-19 pandemic disrupted the supply chain of medical resources, which was only successful when demand was predictable (34). Focusing on the fair allocation of ventilators during this crisis should not distract the authorities from optimising the supply chain.

We realise the limitations of the study. The number of participants was limited; however, the pandemic disrupted people's daily schedule worldwide, and experts were no exception (35). Nevertheless, given the response rate, the generalisation of results could be limited. Considering that the panel was approached based on the acquaintance of authors, our sample was over-representative of colleagues in the authors' network. Using the authors' network would increase the chance of the panel's participation in the study, given that experts would be too busy during the pandemic and would probably ignore emails from unfamiliar senders. While the study had a poor representation of some regions, especially considering different social, religious and healthcare systems approaches, the findings could be used as a basis for a broader represented experts' panel.

CONCLUSION

The panel considered younger age groups, healthcare workers, pregnant mothers, and mothers of children under 5 years for prioritising mechanical ventilators. There was no general consensus regarding the prioritisation of ventilator allocation based on the patient's laboratory profile, underlying diseases, or drug abuse. It could be suggested that more research is essential to develop comprehensive resource allocation strategies which are easy to apply, objective, accurate, reproducible, and would not discriminate against vulnerable populations.

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 Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran under the reference code IR.SBMU.RETECH.REC.1399.103. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

A-AK, NZ, HH-M, S-HG, and MA-K: study conception and design. A-AK, S-HG, and MA-K: acquisition, analysis, or interpretation of data. S-HG and MA-K: drafting of the manuscript. A-AK, HH-M, and NZ: critical revision of the manuscript for important intellectual content. A-AK: study supervision. The corresponding authors attests that all listed authors meet the authorship criteria and that no others meeting the criteria have been omitted. All authors contributed to the article and approved the submitted version.

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REVIEW
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Insights Into Thiamine Supplementation in Patients With Septic Shock

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Septic shock is associated with unacceptably high mortality rates, mainly in developing countries. New adjunctive therapies have been explored to reduce global mortality related to sepsis. Considering that metabolic changes, mitochondrial dysfunction and increased oxidative stress are specific disorders within the path of septic shock, several micronutrients that could act in cellular homeostasis have been studied in recent decades. Thiamine, also known as vitamin B1, plays critical roles in several biological processes, including the metabolism of glucose, synthesis of nucleic acids and reduction of oxidative stress. Thiamine deficiency could affect up to 70% of critically ill patients, and thiamine supplementation appears to increase lactate clearance and decrease the vasopressor dose. However, there is no evident improvement in the survival of septic patients. Other micronutrients such as vitamin C and D, selenium and zinc have been tested in the same context but have not been shown to improve the outcomes of these patients. Some problems related to the neutrality of these clinical trials are the study design, doses, route, timing, length of intervention and the choice of endpoints. Recently, the concept that multi-micronutrient administration may be better than single-micronutrient administration has gained strength. In general, clinical trials consider the administration of a single micronutrient as a drug. However, the antioxidant defense is a complex system of endogenous agents in which micronutrients act as cofactors, and the physiological interactions between micronutrients are little discussed. In this context, the association of thiamine, vitamin C and corticoids was tested as an adjunctive therapy in septic shock resulting in a significant decrease in mortality. However, after these initial results, no other study conducted with this combination could reproduce those benefits. In addition, the use of low-dose corticosteroids is recommended in patients with septic shock who do not respond to vasopressors, which can affect the action of thiamine. Therefore, given the excellent safety profile, good biologic rationale and promising clinical studies, this review aims to discuss the mechanisms behind and the evidence for single or combined thiamine supplementation improving the prognosis of patients with septic shock.

Keywords: thiamine supplementation, thiamine deficiency, septic shock, vitamin B1, mitochondrial dysfunction

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INTRODUCTION

Septic shock is a subset of sepsis characterized by profound hemodynamic alterations associated with organ dysfunction and is one of the most common causes of admission to intensive care units (ICUs) (1). Despite advances in management, rates of sepsis are still rising worldwide, and it is associated with high morbidity, disability and mortality (2).

In critical illness, and most notably in sepsis, the metabolic response to trauma, although necessary, can usually overwhelm the body's metabolism, leading to a wide range of clinical consequences. This response implies significant changes in intermediary metabolism, including increased glycogenolysis, inhibition of glycogenesis and increased lipolysis, producing glucose via gluconeogenesis of lactate, glycerol and amino acids (3). In this scenario, some vitamins and minerals are essential for energy metabolism and mitochondrial function; among these, thiamine deserves to be highlighted (4, 5). Currently, the role of thiamine in sepsis treatment has become of particular interest. Thiamine deficiency might be involved in the pathophysiology of septic shock because high serum lactate concentrations, metabolic acidosis and hypotension can occur in both conditions (6) (**Figure 1**).

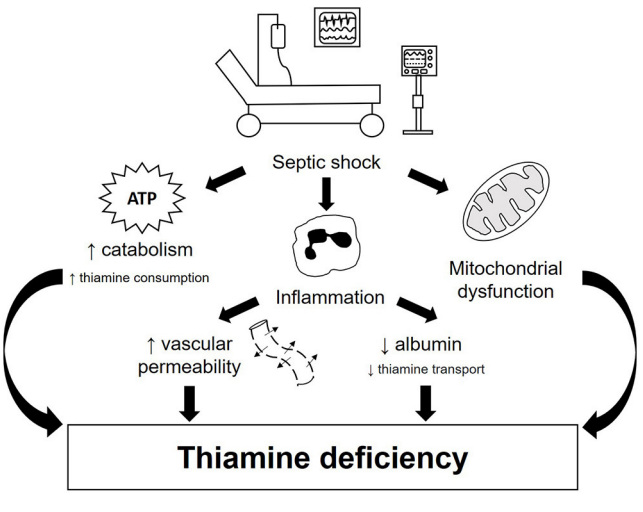

FIGURE 1 | Pathophysiology of thiamine deficiency in septic shock patients. Thiamine deficiency in septic shock occurs both due to increased need and impaired transport. This condition can be triggered by three main mechanisms: hypercatabolism, exacerbated pro-inflammatory response and mitochondrial dysfunction**.** During septic shock, there is an increase in the metabolic demand for energy, resulting in increased glycogenolysis, inhibition of glycogenesis and increased production of glucose via gluconeogenesis, considerably increasing the need for thiamine for glucose metabolism. Inflammation, on the other hand, promotes increased vascular permeability and reduced albumin production, impairing thiamine transport. In addition, tissue hypoxia present in septic shock is one of the main triggers of mitochondrial dysfunction, which contributes to an imbalance in glucose homeostasis, including lower availability of ATP and increased serum lactate concentration. All these mechanisms together contribute to the development and/or worsening of thiamine deficiency in patients with septic shock.

The evidence of thiamine deficiency in critically ill patients was discovered in the 1980s in patients who developed classical clinical manifestations, such as cardiac disorders and neuropsychiatric syndromes, after ICU admission (7–9). Afterwards, thiamine body status levels were first assessed in critically ill patients by Cruickshank et al. (10), who reported deficiency in 20% of adult patients upon admission to the ICU. Also, higher thiamine body status levels were associated with lower mortality. Despite important limitations, this previous investigation brought to light a considerable concern that thiamine deficiency might be related to poorer outcomes or even could be a potentially life-threatening condition in critically ill patients.

Indeed, thiamine deficiency is relatively prevalent in septic shock patients, with rates as high as 71.3% (11). In addition, it is essential to note that in sepsis, there are some severe metabolic derangements, so improvement in organ failure is essential. In this way, antioxidant–enforcement and mitochondrial stress attenuation are specific targets for the rationale of thiamine treatment during septic shock states (12). However, the results of studies assessing thiamine supplementation effects on outcomes remain inconsistent (11, 13–15).

Thus, understanding thiamine's multiple functions in several biological processes by examining past and ongoing studies will lead to the further definition of potential targets for septic shock treatment. This review aims to discuss the mechanisms and the evidence for single or combined thiamine supplementation on the prognosis of patients with septic shock.

THIAMINE: FUNCTIONS AND MECHANISMS OF ACTION

Thiamine or vitamin B1 is a water-soluble and thermosensitive vitamin that is not produced by our body and is indispensable in the human diet. It is considered an essential component of cell metabolism and is mainly involved in glucose metabolism (16).

In 9th-century appeared in Japan first descriptions of states associated with thiamine deficiency in the form of beriberi (17). Since then, knowledge about thiamine has increased along with the gradual recognition of diseases associated with its deficiency, which are divided into two classical clinical forms: encephalopathy (peripheral neuropathy and Wernicke-Korsakoff) and beriberi, which can be classified into dry beriberi (muscle weakness and anorexia), wet beriberi (high-output heart failure) and Shoshin beriberi (beriberi associated with shock) (18–20).

Thiamine is naturally present in several foods, such as meat (especially lean cuts of pork), cereals, yeasts, grains, fruits and other products of plant origin (21). Its biochemical structure consists of a thiazole ring and a pyrimidine group that together make up the sulfur-containing structure of two rings joined by a methylene group (16). Both fractions, pyrimidine and thiazole rings are necessary for thiamine biological activity (22). Its molecule can suffer the action of thiaminases, which are enzymes that cleave the thiamine in the methylene bridge, inactivating it. Thiaminases are produced by bacteria present in the small bowel

and colon and can also be found in some raw foods such as fish and shellfish (22). Thus, frequent intake of these foods heightens the risk of thiamine deficiency.

Six thiamine compounds are known in metabolism: free thiamine; thiamine monophosphate (TMP); thiamine diphosphate, also referred to as thiamine pyrophosphate (TPP); adenosine thiamine diphosphate (ATDP); thiamine triphosphate and adenosine thiamine triphosphate (17). The most important biological active form is TPP, and it accounts for 80–90% of total body thiamine content (17, 23). Approximately 90% of the total thiamine in the blood is found in erythrocytes (75%) and leukocytes (15%) in the TPP form (24). About 5–15% of the total vitamin B1 is in the form of free thiamine, which is bound to albumin in the blood or as TMP form. The remaining forms of vitamin B1 account for only 1% of the total thiamine in humans (17, 24).

There are no significant stores of thiamine in any human tissue (21), and its concentrations are highest in the skeletal muscles, heart, kidney, liver and brain respectively (24, 25). Due to its short half-life of 9.5–18.5 days (24), necessary role in multiple metabolic processes and increased requirements in some pathological states, appropriate dietary intake is crucial for avoiding deficiency states (26, 27).

The Recommended Daily Allowance (RDA) of thiamine for healthy adults is 1.1–1.2 mg/day (21). However, in individuals at risk or with established thiamine deficiency (TD) there is no consensus on the appropriate dose, frequency, or duration of supplementation. In critically ill patients, prophylaxis or treatment for TD typically consists of parenteral administration of thiamine (25, 26). The intravenous route is most frequently used due to rapidly achieving high plasma concentrations, flexible rate of dosing, and better site tolerance as compared to intramuscular injection (28). Also, thiamine replacement by oral or enteral route is possible in situations of non-emergent deficiency, though it is important to note that the gastrointestinal microenvironment is often perturbed during sepsis, resulting in gut dysfunction and nutrient malabsorption (24, 29).

Although thiamine is considered to have a very good safety profile, the tolerable upper intake level (UL) is not established (30). Studies have shown that parenteral doses >500 mg have occasionally led to anaphylaxis and minimal adverse effects such as nausea, anorexia, lethargy, mild ataxia, and a diminution of gut tone (26, 28, 31).

Considering the availability and half-life, the most recommended methods to detect thiamine deficiency are the assessment of transketolase activity (which uses TPP as a cofactor) and the measurement of ATDP, both of which conducted in erythrocytes (24, 32). However, the lack of availability and high costs of these methods limit their use in clinical practice (33).

Although vitamin B1 is involved in several related and simultaneous biological processes, we can divide the functions of thiamine into metabolic or enzymatic and structural or nonenzymatic roles (34, 35). Concerning metabolic functions, thiamine plays an essential role in energy transformation because TPP is a cofactor of two enzymes related to the extraction of energy from carbohydrate sources (16, 24, 34). These mitochondrial enzymes are involved in decarboxylation reactions and dehydrogenation reactions (24). The first enzyme is the multienzyme complex pyruvate dehydrogenase (PDH), which is made up of TPP-dependent pyruvate decarboxylase, a lipoic acid-dependent dihydrolipoyl transacetylase and a flavin adenine dinucleotide (riboflavin)-dependent dihydrolipoyl dehydrogenase (24, 36). In the related PDH reactions, TPP receives electrons in the redox processes (16), resulting in the conversion of pyruvate to acetyl-CoA, which then takes place in the Krebs cycle (37). At this point, we can infer that in states of thiamine deficiency, pyruvate access to the mitochondria is impaired with cytosolic conversion to lactate via lactate dehydrogenase and further lactic acidosis (**Figure 2**).

TPP is also a cofactor for α-ketoglutarate dehydrogenase, a mitochondrial enzyme responsible for converting α-ketoglutarate to succinyl-CoA in the Krebs cycle (24) through a decarboxylation reaction (16). This function highlights crucial redox mechanisms taking place inside the mitochondria, which is responsible for over 95% of adenosine 5-triphosphate generation and, consequently, oxygen reactive species production (12, 16). Mitochondrial dysfunction is well known in sepsis (12), and potential targets for thiamine supplementation might be located inside this organelle.

In turn, TPP is also useful in non-oxidative carbohydrate metabolism. The cytosolic enzyme transketolase needs TPP as a cofactor (24). This reaction is found within the pentose phosphate pathway, where sugars are interconverted and are essential for pentoses generation to nucleic acids synthesis and nicotinamide adenine dinucleotide phosphate (NADPH) for the production of fatty acids, maintenance of myelin sheaths, nerve membrane function and signal transmission (16, 24). At this point, both functions of thiamine are interconnected. NADPH, in turn, is involved in glutathione cycling, an important antioxidant pathway and potential target of thiamine as a metabolic resuscitator in patients with septic shock (38). It is also important to emphasize that thiamine requires magnesium as a cofactor for conversion into its TPP active form, playing a key role in aerobic metabolism as well. Magnesium absence, therefore, may result in altered metabolism of glucose and increased lactate production (39). Hence, in clinical practice, the correction of concomitant magnesium deficiency is essential to thiamine utilization, although it is often overlooked (40, 41).

Regarding the nonenzymatic functions of vitamin B1, we can highlight its relevance in the nervous system and its interface with the immune system. In the nervous system, thiamine is related to the transmission of nerve impulses. Playing important roles in sodium permeability, TPP is also involved in maintaining negative charge on the inner surface of the cell membranes and facilitates neurotransmission by acting on the release of some neurotransmitters, notably acetylcholine (24). It was demonstrated that thiamine deficiency provoked a significant decrease in the voltage-dependent K+ membrane conductance of

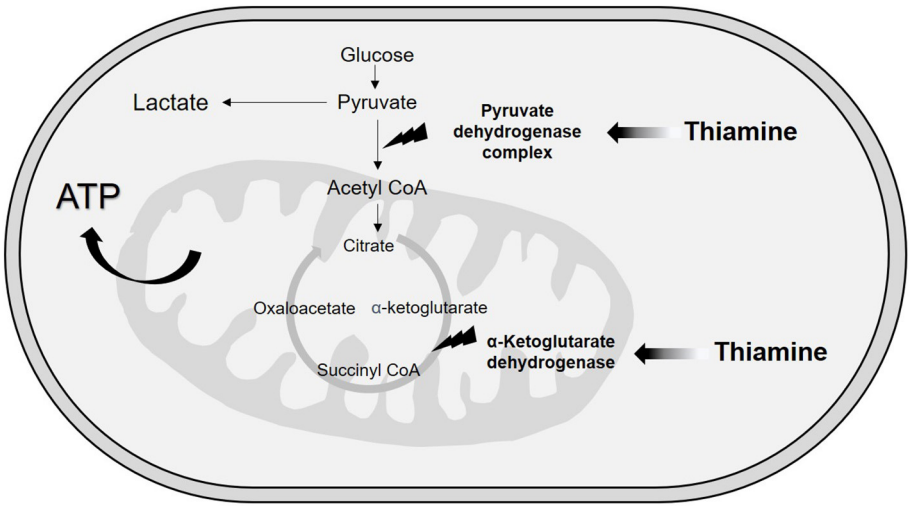

FIGURE 2 | Metabolic function of thiamine.

cerebellar neurons, mainly by suppressing an A-type K+ channel, which leads to important neuronal alterations (34) and a further reduction of nervous conduction velocity. In addition, TPP's role in fatty acid and NADPH synthesis is related to adequate neurologic functions (24).

In the immune system, thiamine has several functions in the regulation and activation of immune cells and proteins (16). The balance between glycolysis and Krebs cycle activities, where vitamin B1 is an important cofactor, is considered a determinant in controlling immune cell function, a concept referred to as immunometabolism (42). T-regulatory cells, resting macrophages and naïve T-cells generate energy mostly through the Krebs cycle, whereas activated macrophages, B-cells, Th1, Th2 and Th17 cells shift the balance toward aerobic glycolysis to complement energy from Krebs cycle (43).

Thiamine is also involved with hemin-dependent oxygenase, whose action affects the release of the specific members of the intracellular adhesion molecule (ICAM) proteins. ICAMs bind integrins during immunological reactions, affecting T-cell activity and other immune system cells (16). Vitamin B1 is important in immune system reactivity as well because it plays a pivotal part in the expression of immunoglobulins and, due to its antioxidative effects on neutrophils, by protecting the sulfhydryl groups on the cell surfaces from oxidation (16, 42). On macrophages, thiamine suppresses the oxidative stress-induced activation of the necrosis factor NF-kB, which induces the release of a variety of inflammatory markers such as cytokines, growth factors and immune-responsive proteins (44).

Given the crucial role of thiamine in the maintenance of metabolic functions and its supposed effect on the pathophysiology of mitochondrial dysfunction and microcirculatory changes that are characteristic of critical illness, it is essential to better understand the effectiveness of thiamine supplementation in patients with septic shock.

SINGLE THIAMINE SUPPLEMENTATION IN SEPTIC SHOCK

Several observational studies have explored the relationship between thiamine therapy and mortality rate in septic shock patients, with conflicting results (11, 14, 45–47). In a large retrospective matched cohort study, Woolum et al. (14) demonstrated that early intravenous (IV) thiamine supplementation (any dose between 100 and 500 mg) was associated with reduced 28-day mortality and improved lactate clearance. Additionally, lower mortality was also observed in septic shock patients with alcohol-use disorders who received thiamine within 48 h of sepsis onset (48).

In addition, a nationwide observational cohort study assessed the effect of thiamine administration on 28-day mortality in Japanese patients with septic shock over 7 years (46). The study included 68,571 patients, of which about 27% received 100 or 200 mg of IV thiamine within 2 days of admission. The findings of this large study did not support an association between early thiamine supplementation and a decrease in mortality. However, it is important to highlight the retrospective design of the study and the dose of thiamine used, which may be too low compared to the doses used in other studies (ranging from 200 to 500 mg daily).

To date, regardless of its relevance, only two randomized clinical trials have been published with IV thiamine supplementation as a single nutrient (13, 49). Donnino et al. (13) conducted the first randomized, double-blind, placebo-controlled trial. Patients with septic shock received 200 mg of IV thiamine twice daily or a matching placebo for 7 days or until hospital discharge. Contrary to expectations, supplementation was not effective in reducing serum lactate after the first 24 h of intervention. The prevalence of vitamin B1 deficiency among individuals was 35%, and supplementation was not associated with shock reversal time, disease severity or mortality. However,

in the subset of patients with thiamine deficiency, lactate levels were lower after the first dose of supplementation (2.1 [1.4–2.5] mmol/L vs. 3.1 [1.9–8.3] mmol/L, $p = 0.03$), and a decrease in mortality was observed (2 [13%] vs. 6 [46%)], $p = 0.047$).

Despite this being a pioneering study, the results should be taken with caution. First, the effect of thiamine supplementation on lactate reduction and mortality was found in a small sample size ($n = 28$). Second, shock resuscitation treatment by itself would be able to reduce serum lactate. Third, some confounding variables such as illness severity and organ dysfunction scores among non-surviving patients who had baseline disabilities and did not receive supplementation are not known. Finally, lactate levels were assigned based on a predefined plan of patients who died before the 24-h period. Thus, an increase of 20%, 15% and 10% from baseline was imputed for patients who died before the 6-h, 6–12-h and 12–24-h point, respectively (6, 13).

Afterward, a secondary analysis of this trial was performed by Moskowitz et al. (50). The authors found significantly lower serum creatinine levels and a lower need for renal replacement therapy in the thiamine-treated group compared to the placebo group. As in the original trial (13) overall mortality was not different but raises the hypothesis of thiamine's role as a renal protective in septic shock.

More recently, another randomized clinical trial assessed the vasopressor-free days over 7 days in septic shock patients who received 200 mg of IV thiamine or placebo every 12 h for 7 days or until hospital discharge (49). On the one hand, thiamine supplementation was not associated with a reduction in the need for vasoactive drugs in the first week of ICU admission or with 28-day mortality. On the other hand, the study had interesting findings such as a reduction in vasopressor dependency index and in the serum lactate concentration 24 h after the initial supplementation. However, it is important to note that these findings are questionable due to the small sample size ($n = 50$) and the early stopping point, limiting the validity of the results. **Table 1** describes the main studies that used single thiamine supplementation in patients with septic shock. Only two studies evaluated the concentration of vitamin B1 prior to its supplementation in patients with septic shock (13, 48). In addition, the studies involved patients with differences in sepsis severity, cause of infection, presence of risk factors and, when used, varied methods for assessing the vitamin concentration (11, 13–15, 46, 48–50). It is noteworthy that so far there are no reference values for detecting thiamine deficiency among septic patients. Therefore, it is essential to develop new studies that more assertively assess deficiency and establish specific reference values for septic patients to identify subgroups that can benefit more effectively from the treatment.

Studies with thiamine supplementation are still incipient, and therefore, it is essential to develop further clinical trials to conclusively determine the true role of thiamine in septic shock. Some problems related to the neutrality of these clinical trials are the study design, doses, route, timing, length of intervention and the choice of endpoints.

Recently, the concept that multi-micronutrient administration may be better than single-micronutrient administration has gained traction (12). In general, clinical trials consider the administration of a single micronutrient as a drug. However, the antioxidant defense is a complex system of endogenous agents in which micronutrients act as cofactors, and the physiological interactions between micronutrients are little discussed. Thus, current evidence does not support pharmacological use of single thiamine supplementation in septic shock patients, and future trials will probably focus on an early multi-micronutrient approach (12).

COMBINED THIAMINE SUPPLEMENTATION ON THE PROGNOSIS OF PATIENTS WITH SEPTIC SHOCK

The combined supplementation of thiamine with ascorbic acid and corticosteroids was tested in a retrospective before–after clinical study developed by Marik et al. (51). Patients with severe sepsis or septic shock were treated with the administration of the combination of vitamin C, (1.5 g every 6 h), hydrocortisone (50 mg every 6 h), and thiamine (200 mg every 12 h) for 4 days. During the control period, patients with sepsis did not receive intravenous vitamin C or thiamine. Surprisingly, the treated group had a significantly lower mortality rate than the control group (8.5 vs. 40.4%, $p < 0.001$). Despite this tremendous potential as a treatment for sepsis, this study warrants caution in extrapolating its results because notable limitations were found. In sum, the study had a small sample size ($n = 47$ patients), had one single-center and lacked blinding and randomization.

The use of low-dose corticosteroids is recommended in patients with septic shock who do not respond to vasopressors. Its main benefit is its immune-stimulating effects, which may limit the anti-inflammatory immunosuppressive state (52). Although mortality reduction is seen mainly in patients receiving higher vasopressor doses, its supplementation improves other secondary outcomes, such as shock recovery and ICU length of stay (53, 54). In addition, glucocorticoids and vitamin C appear to act synergistically in protecting or reversing endothelial dysfunction (55, 56) and have become an extremely interesting target in this population. Vitamin C deficiency is highly prevalent among critically ill patients and is related to increased need for vasopressors, larger organ dysfunction, kidney injury and shorter survival (54, 57). Despite its potent antioxidant action, the IV administration of vitamin C in high doses and for a long period requires caution due to the risk of hyperoxaluria and pro-oxidant action (55, 58). Additionally, the correction of thiamine deficiency acts as a cofactor in the oxidation of glyoxylate by the enzyme glyoxylate aminotransferase (56, 59) and may help to attenuate oxidative stress and inflammation in animal models of sepsis (55, 60). Consequently, a deficiency of both vitamins concomitantly can aggravate the oxidative mitochondrial injury and bioenergetic failure present in septic shock (54).

Despite the rationale for combining these vitamins with corticosteroids, the improvement in mortality was not reproducible in recent trials (55, 61–63). Subsequently, in search of more promising outcomes, studies were carried out with the supplementation of thiamine in higher doses associated

TABLE 1 | Clinical studies evaluating single thiamine supplementation in septic shock.

Authors	Diagnosis, number of patients and design	Dose and time	Results
Donnino et al. (13). *Crit Care Med*	Septic shock n = 88 Randomized, double-blind clinical trial Primary outcome: lactate levels 24 hours after the first dose Secondary outcomes: time to shock reversal, severity of illness and mortality	Thiamine 200 mg IV or placebo twice daily for 7 days or until hospital discharge	35% of the patients were thiamine deficient There was no difference in lactate levels at 24 hours (median: 2.5 mmol/L [1.5 - 3.4] vs. 2.6 mmol/L [1.6 - 5.1], p = 0.40) Overall mortality was 43% There was no difference in the proportion of patients with shock reversal between the thiamine and placebo groups (74% vs. 71%, p = 0.81) and mortality was also similar in both groups (42% vs 44%, p=1.00) There was no difference in APACHE II score (p = 0.15) and SOFA score (p= 0.41) between the groups Among thiamine deficient patients, those in the thiamine group had statistically significantly lower lactate levels at 24 hours (median 2.1 mmol/L [1.4 - 2.5] vs. 3.1 [1.9 - 8.3], p = 0.03)
Moskowitz et al. (50). *Ann Am Thorac Soc*	Septic shock n = 70 Secondary analysis of a randomized, double-blind trial Primary outcome: requirement for renal replacement therapy	Thiamine 200 mg IV or placebo twice daily for 7 days or until hospital discharge	32.8% of patients were deficient in thiamine Mortality of 37.1% (32.2% in the thiamine group and 41% in the placebo group; p=0.45) Lower serum creatinine levels (p=0.05) and a lower need for renal replacement therapy in the thiamine-treated group compared to placebo (3% vs. 21%, p=0.04) No differences regarding APACHE II, SOFA score, time of MV and other clinicals and demographics variables between the groups.
Holmberg et al. (48). *J Crit Care*	Septic shock with alcohol use disorders n = 53 Retrospective Primary outcomes: Mortality and practice patterns relating to thiamine administration in patients with alcohol use disorders	Low-dose (100 mg) was the most frequently ordered dose. Median time to administration was 9 (4–18) h	Thiamine deficiency was not evaluated 64% of the patients received thiamine at hospital admission Thiamine administration was associated with decreased mortality (44% vs. 79%, p = 0.02) No differences regarding SOFA score, hospital, and ICU-free days and other clinicals and demographics variables between the groups
Woolum et al. (14). *Crit Care Med*	Septic shock n = 1,049 Retrospective Primary outcomes: lactate clearance Secondary outcomes: 28-days mortality, change in SOFA score, AKI or RRT within the ICU, vasopressor-free, ventilator-free, and ICU-free days within the 28 days following ICU admission	High-dose thiamine (500 mg) was the most frequently ordered dose. Thiamine was administered for a median of 3 days	Thiamine deficiency not evaluated The median time from hospital admission to thiamine administration was 6.4 hours Thiamine administration was associated with improved lactate clearance (hazard ratio, 1.307; 95% CI, 1.002–1.704) and a reduction in 28-day mortality (hazard ratio, 0.666; 95% CI, 0.490–0.905) There were no differences in any other secondary outcomes
Harun et al. (15). *Crit Care and Shock*	Septic shock n = 72 Randomized clinical trial Primary outcome: lactate clearance	Thiamine 200 mg IV or placebo twice daily for 3 days	Thiamine deficiency not evaluated Supplementation was not associated with relative lactate changes (p= 0.091) No differences regarding SOFA score, ICU LOS and ICU mortality
Miyamoto et al. (46). *Crit Care Med*	Septic shock n = 68,571 Retrospective Primary outcome: 28-day mortality	Low-dose (100 mg and 200 mg) were the most frequently ordered doses within 2 days of admission	Thiamine deficiency not evaluated No significant differences between the 100-mg thiamine group and the control group (risk difference, 0.6%; 95% CI, −0.3% to 1.4%) and the 200-mg thiamine group and the control group (risk difference, −0.3%; 95% CI, −1.3% to 0.8%) regarding mortality
Petsakul et al. (49). *BMC Anesthesiology*	Septic shock n = 50 Randomized clinical trial Primary outcome: decrease in vasopressor requirement within 7 days	Thiamine 200 mg IV or placebo twice daily for 7 days or until hospital discharge.	Thiamine deficiency was not evaluated No difference in vasopressor-free days between the thiamine and placebo groups (p = 0.197) There was a reduction in the dependence index on vasopressors (0.14 $mmHg^{-1}$ vs.0.03 $mmHg^{-1}$, p = 0.02) and in the serum lactate concentration at 24 h (1.0 mmol/L vs. 0.5 mmol/L, p = 0.024) after initial supplementation No difference was observed in SOFA score within 7 days, vasopressor dependency index within 4 days and 7 days, or 28-day mortality

AKI, Acute kidney injury; APACHE II, Acute Physiology And Chronic Health Evaluation; IV, intravenous; ICU, intensive care unit; LOS, length of stay; MV, Mechanical ventilation; RRT, Renal replacement treatment; SOFA, sequential organ failure assessment.

TABLE 2 | Randomized clinical trials evaluating thiamine supplementation as adjunctive therapy in septic shock.

Authors	Diagnosis, number of patients and design	Nutrients. dose and time	Results	Strong points/limitations
VITAMINS Trial. Fugii et al. (61). *JAMA*	Septic shock n = 216 Multicentre, open-label, randomized clinical trial Primary outcomes: duration of time alive and free of vasopressor administration up to day 7 Secondary outcomes: 28-day, 90-day ICU, and hospital mortality, 28-day cumulative vasopressor-free days, 28-day cumulative mechanical ventilation-free days, 28-day renal replacement therapy–free days, change in SOFA score at day 3, 28-day ICU free-days, and hospital LOS	Intervention group: IV vitamin C (1.5 g every 6 h), hydrocortisone (50 mg every 6 h) and thiamine (200 mg every 12 h) Control group: IV hydrocortisone (50 mg every 6 h) Until shock resolution or up to 10 days	There was no significant difference in time alive and free of vasopressors up to day 7 (−0.6 h [95% CI, −8.3 to 7.2 h; p = 0.83]). There was no statistically significant difference in secondary outcomes.	Patients with septic shock within 24 h of diagnosis to maximize the possible effects of the intervention No serious adverse events were reported
HYVCTTSSS study. Chang et al. (62). *CHEST*	Sepsis and septic shock n = 80 Single-blind, randomized controlled trial Primary outcome: 28-day mortality. Secondary outcomes: duration of vasopressor use, ICU LOS, change in SOFA score within 72 h after experimental intervention, and PCT clearance rate within 72 h after experimental intervention	Intervention group: IV vitamin C (1.5 g every 6 h), hydrocortisone (50 mg every 6 h) and thiamine (200 mg every 12 h) Control group: placebo Hydrocortisone for 7 days and vitamin C and B1 for 4 days	There was no difference in mortality between the treatment and control groups (relative risk [RR],0.79; 95% CI, 0.41–1.52; p = 0.47) Thiamine treatment was associated with a significant improvement of 72-h change in ΔSOFA score (3.5 ± 3.3 vs. 1.8 ± 3.0; p = 0.02) and exhibited more incidents of hypernatremia (13 vs. 3; p = 0.005) In a subgroup diagnosed with sepsis within 48 h at ICU admission, an improvement in mortality in the treatment group was observed (13.6% vs 47.6%; RR, 0.29; 95% CI,0.09-0.90; p = 0.02)	Small sample size, single-blind and terminated early Did not include corticosteroids in the control group
ORANGES trial Iglesias et al. (55). *CHEST*	Sepsis and septic shock n = 137 Double-blind, randomized clinical trial Primary outcomes: resolution of shock and change in SOFA score Secondary outcomes: ICU mortality, hospital mortality, procalcitonin clearance (PCT-c), LOS, ICU LOS, and ventilator-free day	Intervention group: IV vitamin C (1.5 g every 6 h), hydrocortisone (50 mg every 6 h) and thiamine (200 mg every 12 h) Control group: placebo Maximum of 4 days	No statistically significant change in SOFA score was found between groups (p = 0.17) Intervention group showed quicker reversal of shock (27 ± 22 h vs 53 ± 38 h; p <0.001) No significant differences were found between study mortality, length of stay and ventilator-free days	Baseline ascorbic acid and thiamine levels were evaluated Homogenous (primarily white) cohort size, limiting the ability to detect differences in hospital mortality and length of stay Did not include corticosteroids in the control group

(Continued)

TABLE 2 | Continued

Authors	Diagnosis, number of patients and design	Nutrients. dose and time	Results	Strong points/limitations
Wani et al. (63). Infectious Disease	Sepsis and septic shock n = 100 Open-label, randomized controlled trial Primary outcomes: hospital mortality Secondary outcomes: 30-day mortality, duration of hospital stay, duration of vasopressor therapy, lactate clearance, change in serum lactate and the SOFA score over the first 4 days	Intervention group: IV vitamin C (1.5 g every 6 h), hydrocortisone (50 mg every 6 h) and thiamine (200 mg every 12 h) Control group: placebo Vitamin C and B1 for 4 days or until discharge from hospital; hydrocortisone for 7 days or until discharge from hospital	There was no difference between groups regarding hospital mortality (p = 0.82) and 30-day mortality (p = 1.00) Intervention group had shorter vasopressor use (96.13 ± 40.50 h vs. 75.72 ± 30.29 h; p = 0.010) and greater lactate clearance compared to control (41.8% vs. 56.8%; p = 0.031) No difference in mortality, length of stay and SOFA score	Geographical area (India) with high prevalence of antimicrobial resistance and mortality from sepsis Open-label and small sample size Did not include corticosteroids in the control group
ACTS trial Moskowitz et al. (38). *JAMA*	Septic shock n = 200 Multicentre, randomized, blinded clinical trial Primary outcome: change in the SOFA score between enrolment and 72-hour follow-up Secondary outcomes: kidney failure, 30-day mortality, ventilator-free days, and shock-free days during the first 7 days, days free of ICU stay, all-cause mortality to ICU and hospital discharge, post hospitalization disposition in survivors to hospital discharge, 72-hour change in individual SOFA score components, and delirium on day 3	Intervention group: IV vitamin C (1.5 g every 6 h), hydrocortisone (50 mg every 6 h) and thiamine (100 mg every 6 h) Control group: placebo For 4 days or until discharge from ICU	There was no statistically significant difference in SOFA score between groups (p = 0.12) The median number of shock-free days was higher in the intervention group compared with the placebo group (5 [IQR, 3–5] days vs 4 [IQR, 1–5] days; median difference, 1.0 days; 95% CI, 0.2-1.8 days; p < 0.01) There was no statistically significant difference in any other secondary outcomes	Conducted at 14 centres Adverse events were hyperglycaemia, hypernatremia and new hospital-acquired infection Large number of patients were screened (n = 4,569) but not randomized Did not include corticosteroids in the control group
ATESS trial. Hwang et al. (68). *Intensive Care Medicine*	Septic shock n = 111 Multicentre, double-blind, randomized clinical trial Primary outcomes: ΔSOFA score Secondary outcomes: 7-day, 28-day, 90-day, in-hospital and ICU mortality, shock reversal, vasopressor free days, vasopressor dose, duration of mechanical ventilation, ventilator-free days, AKI, RRT, RRT-free days, LOS ICU, ICU-free days, hospital LOS, reduction of C-reactive protein (CRP) and procalcitonin for 72 h	Intervention group: IV vitamin C (50 mg/kg, every 12 h, maximum daily dose 6 g) and thiamine (200 mg every 12 h) Control group: placebo For 48 h	There was no significant difference in ΔSOFA scores between the treatment group and the placebo group (3, interquartile range IQR – 1 to 5 vs. 3, IQR 0–4, respectively, p=0.96]) There was no significant difference in any secondary outcomes.	Glucocorticoid was administered to over half of the patients Interval for vitamin administration was longer (12 h vs. 6 h), while the duration of treatment was shorter (48 h vs. 96 h or more) compared to previous studies Intra-abdominal infection, either solid cancer or hematologic malignancy, accounted for almost half of the cases of septic shock
VICTAS Randomized Clinical Trial. Sevransky et al. (69). *JAMA*	Sepsis n = 501 Multicentre, double-blind, randomized clinical trial Primary outcomes: ventilator- and vasopressor-free days in the first 30 days Secondary outcomes: 30-day mortality	Intervention group: IV vitamin C (1.5 g), thiamine (100 mg), and hydrocortisone (50 mg) every 6 h Control group: hydrocortisone (of at least 200 mg) or matching placebo equivalent For 96 h or until discharge or death	There was no statistically significant difference between the intervention and control groups regarding ventilator- and vasopressor-free days (median difference of −1 day [95% CI, −4 to 2 days; p = 0.85]) There was no difference between groups in 30-day mortality (intervention = 22% vs placebo = 24%, p = 0.619)	Trial was terminated early for administrative reasons and may have been underpowered to detect a clinically important difference

AKI, Acute kidney injury; CI, confidence interval; CRP, C-reactive protein; IV, intravenous; ICU, intensive care unit; LOS, length of stay; PCT-c, Procalcitonin clrearence; RR, razard ratio; IQR, interquartile range; RRT, Renal replacement treatment; SOFA, sequential organ failure assessment.

with ascorbic acid and hydrocortisone. **Table 2** summarizes the main prospective and randomized studies published to date on the effect of combination therapy with ascorbic acid, hydrocortisone and thiamine in patients with septic shock. These combined therapy studies were also discussed in some systematic reviews (64–67).

To date, fewer than 10 randomized clinical studies have been published with combined thiamine supplementation in patients with sepsis and/or septic shock (38, 55, 61–63, 68, 69). The main outcomes involved organ dysfunction, time of need for vasopressor, ventilator-free days, development of acute kidney injury, lactate clearance, length of stay in the ICU and hospital mortality. Most studies did not observe any statistically significant difference regarding outcomes between treated individuals and the control group. Only in the study of Wani et al. (63) did the intervention group have shorter vasopressor use (96.13 ± 40.50 h vs. 75.72 ± 30.29 h, $p = 0.010$) and greater lactate clearance (41.81 vs. 56.83%, $p = 0.031$) compared to the control. It is noteworthy that the study in question has limitations, including being open-label, having a small sample size and having a high prevalence of antimicrobial resistance and mortality.

If the lack of beneficial effects of supplementation on the outcomes is evident, the assertiveness in the design of the work brings some questions. Between studies, doses are generally similar (vitamin C: 1,500 mg; hydrocortisone: 50 mg; thiamine: 100 mg, every 6 h); however, the administration time is highly variable (48 h, 4–10 days or until discharged from the ICU). Even with a more diverse design, the ATESS trial (68) did not include the administration of hydrocortisone in the treated group, and the interval for vitamin administration was longer (12 vs. 6 h), while the duration of treatment was shorter (48 vs. 96 h or more) compared to previous studies.

It is important to note that hydrocortisone monotherapy in patients with septic shock is associated with faster resolution of shock (70) and lower mortality (71) when compared with placebo groups. In this sense, the VITAMINS trial (61) compared IV thiamine combined with vitamin C and hydrocortisone with hydrocortisone administration alone and found no improvement on mortality or time free from vasopressors up to day 7, suggesting no synergic effect between them as previously postulated.

Still, for clinical practice, some questions still need to be clarified about the combined therapy for adjuvant management of septic shock. The current literature cannot sufficiently support use of single thiamine or combined administration outside of randomized controlled trials because most of the studies were interrupted early (69), a large number of patients were screened but not randomized (38), abdominal infections and tumors were highly prevalent (68) and the studies had mixed designs.

However, these efforts have unquestionably advanced care of septic shock patients. At the moment, it has been established that single thiamine administration or thiamine administration combined with vitamin C and hydrocortisone has a good safety profile with no adverse events, even at high doses. Consequently, studies are needed to fill the knowledge gaps regarding vitamin B1 supplementation as adjuvant therapy in septic shock.

CONCLUSION

Studies with thiamine supplementation in septic shock have notable differences in design, including dosage and time of supplementation; small sample sizes; and different septic phenotypes. Despite the excellent safety profile, good biologic rationale and promising clinical studies, no robust results support routine thiamine supplementation to improve outcomes. However, future trials should focus on combined multi-micronutrient therapy, higher doses, and early administration, which might be the key to improving mitochondrial function and reducing oxidative stress during hemodynamic resuscitation in patients with septic shock.

AUTHOR CONTRIBUTIONS

NC, AP, and LG: conceptualization, methodology, and writing—original draft. CS and NV: revision of the original draft. PA, SP, and LZ: writing—review and editing. BP and MM: writing—original draft and writing—review and editing and supervision. All authors revised the article critically for important intellectual content and approved the final version of the manuscript.

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Comparison of Deep and Moderate Neuromuscular Blockade on Intestinal Mucosal Barrier in Laparoscopic Gastrectomy: A Prospective, Randomized, Double-Blind Clinical Trial

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Deep neuromuscular blockade (NMB) improves the surgical conditions and is benefit for the postoperative recovery after laparoscopic surgery. However, the mechanisms of deep NMB in promoting the recovery of intestinal function have not been completely investigated. The objective of our study was to determine the impact of the deep NMB and moderate NMB strategy on the intestinal barrier function after laparoscopic gastrectomy. We collected patients undergoing elective laparoscopic gastrectomy. Patients were randomized to deep NMB (post-tetanic count 1–2) vs. moderate NMB (train-of-four count 1–2) during the surgery. Primary outcomes were time to flatus, serum diamine oxidase (DAO) and D-lactate, and gut microbiota. Other outcomes were surgical condition scores, postoperative visual analog pain scores, and length of hospital stay. Ninety patients in deep NMB group and sixty patients in moderate NMB group completed the study. Main results showed that the time to flatus was decreased in deep NMB group (74 ± 32 h) than that in moderate NMB group (93 ± 52 h, $P = 0.006$). The level of serum D-lactate was statistically increased in the moderate NMB group than that in the deep NMB group (1,209 ± 224 vs. 1,164 ± 185 ng/ml, $p < 0.001$). But no significant differences could be detected in the level of DAO between the groups. Additionally, the 16s rRNA analysis indicated that gut microbiota were similar in Alpha diversity but distinct in Beta diversity. Furthermore, the beneficial bacteria, such as genus *Lactobacillus* and *Bifidobacterium*, were more abundant in the deep NMB group, while the potentially harmful bacteria were more abundant in the moderate NMB group. Our findings suggested that the intestinal mucosal barrier and gut microbiota were better preserved in deep NMB, which greatly improved the postoperative recovery of intestinal function after laparoscopic gastrectomy.

Keywords: gut microbiota, intestinal function, intestinal mucosal barrier, laparoscopic gastrectomy, neuromuscular blockade

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INTRODUCTION

The intestinal mucosal barrier, which is composed of mechanical, chemical, microbial, and immunologic barriers, is essential for the normal intestinal function. The integrated intestinal mucosal barrier obstructs the harmful substances and plays a key role in human health and diseases. However, it would be easily damaged in surgeries or trauma with physical, chemical, and biological injuries (1).

Diamine oxidase (DAO) and D-lactate are common biomarkers to identify the intestinal barrier dysfunction. DAO is an enzyme abundant in intestinal mucosa which is localized mainly in the small intestinal mucosa, predominantly in the tips of the villi (2). It is the essential substance for the cell proliferation function and is the oxidative deaminating of several polyamines (3). Once the epithelia of intestinal mucosa were damaged, DAO released from the cells would be absorbed into blood. Thus, the level of serum DAO is a sensitive biomarker for evaluating the integrity of intestinal mucosa (4). D-lactate is another serum biomarker produced by some kinds of gut microbiota, which cannot be metabolized in human. A large amount of D-lactate can enter the circulation through the damaged mucosa when the intestinal mucosal permeability increases (5). With the advent of the genome era, gene sequencing has been widely used to display the microbial diversity. Moreover, 16s rRNA sequencing is one of the methods for the detection of intestinal microbiota at the level of phylum, class, order, family, and genus. Increased bacterial abundance and diversity are the important indicators of intestinal health (6).

Owing to less postoperative complications and faster rehabilitation compared with the traditional open surgery, laparoscopic surgery has become the major surgical approach for elective surgeries (7, 8). However, several factors, such as the surgery methods or the analgesic management, are considered to retard the recovery of intestinal function after laparoscopic surgery. Though the modified approach of the surgery and the multimodal analgesia partly reduce the worries about the intestinal side effects, previous studies have reported that the delayed recovery of bowel movement was associated with the long-lasting laparoscopic process with the continuous insufflation of carbon dioxide (CO_2) (9, 10).

Deep neuromuscular blockade (NMB) with low intra-abdominal pressure, which is an important element of enhanced recovery after surgery (ERAS) in gastrointestinal surgeries, may be responsible for remitting the postoperative intestinal dysfunction. It has been demonstrated that deep NMB improves the surgical conditions in laparoscopic surgery with a lower demand for CO_2 insufflation (11). Thus, deep NMB is recommended especially in the laparoscopic surgeries performed in proximity to the diaphragm, such as laparoscopic cholecystectomy or gastrectomy (12, 13).

Given the extensive use of deep NMB in laparoscopic surgeries, it is of great importance to investigate the deep NMB and moderate NMB strategy on the intestinal barrier function and prognosis. We therefore performed this prospective study to compare the effect of different depth of NMB on the intestinal mucosal barrier after laparoscopic gastrectomy surgery with a comparison of the biomarkers assessing the intestinal barrier dysfunction, 16s rRNA sequencing of gut microbiota, and other perioperative parameters.

METHODS

Study Design and Population

This prospective, randomized, and double-blind study was carried out from January 2019 to March 2021 at the First Affiliated Hospital of Nanjing Medical University. Informed written consent was obtained from each participant before enrolment. The protocol of this study was approved by the Hospital Research Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, Nanjing, China (No: 2018-SR-336). This clinical trial was registered at https://www.clinicaltrials.gov (NCT03782233).

Eligible patients with early- or intermediate-stage gastric cancer met the inclusion criteria: age 40–80 years.; body mass index (BMI) < 30 kg/m^2; American Society of Anesthesiologists (ASA) classification I–III; and scheduled for laparoscopic gastrectomy. Exclusion criteria included: inflammatory bowel diseases or intestinal obstruction; severe heart or lung diseases; severe renal insufficiency or liver diseases; long-term antibiotic therapy before surgery; history of abdominal surgery; neuromuscular diseases (e.g., gravis myasthenia); and allergy to the study medication.

All patients were randomized to either deep NMB group or moderate NMB group at a ratio of 1.5:1. The randomization sequence was produced with a random number generator and sealed with numbered envelopes providing randomized group allocation.

Study Intervention

The NMB monitor using an acceleromyograph (Mindray BeneView T9, Shenzhen, China) was set at the ulnar nerve for acquiring the train-of-four (TOF) responses or post-tetanic twitches. The deep NMB was maintained with a continuous infusion of rocuronium 0.5–0.6 mg/(kg·h) to a target post-tetanic count (PTC) of 1–2. A continuous infusion of rocuronium 0.2–0.3 mg/(kg·h) was titrated in the moderate NMB group to a target TOF count of 1–2. At the end of surgery, sugammadex 2 mg/kg was administrated intravenously at a TOF count of 2 for NMB reversal.

All surgeries were done by the same surgeon and assistants. Two anesthetists were responsible for anesthesia management. The depth of neuromuscular blockade was adjusted according to the group intervention by the chief anesthetist who was un-blinded to the group allocation, while the assistant, who was blinded to the group allocation, was responsible for recording perioperative outcomes. Both surgeon and patients were blinded to the group allocation.

Intraoperative Management

All patients were monitored with ECG, pulse oximetry, invasive arterial blood pressure, and bispectral index (BIS). Anesthesia was induced with etomidate 0.3 mg/kg, midazolam 0.02 mg/kg, and fentanyl 3–5 μg/kg. The acceleromyograph was standardized

when the patient was asleep. A single bolus of rocuronium 0.6 mg/kg was injected intravenously for intubation. Mechanical ventilation was performed in the volume control mode after intubation with tidal volume 6–8 ml/kg, respiration rate 12–16 times/min, positive end expiratory pressure (PEEP) 5 cmH_2O, and the air-oxygen mixture of 60% fraction of inspiration O_2 (F_iO_2). Anesthesia was maintained with propofol 1.5 mg/(kg·h), remifentanil 0.03–0.1 μg/(kg·min) and sevoflurane 0.6–2.3 age-adjusted minimal alveolar concentration (MAC) to a target BIS of 45–55. We standardized the perioperative pain management with low dosage of opioid anesthesia (fentanyl: 6–8 μg/kg), non-steroidal anti-inflammatory drugs (NSAIDs), and highly selective α2 adrenergic receptor agonists (Dexmedetomidine) in the present study.

There are four major factors affecting the splanchnic perfusion: (1) intra-abdominal pressure (IAP), (2) position of the patient, (3) carbon dioxide management, and (4) fluid management. In the present study, the intra-abdominal pressure with continuous CO_2 insufflation was controlled at 10–12 mmHg, the respiratory rate and tidal volume were adjusted to maintain the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) at 35–45 mmHg. In addition, anti-Trendelenburg position was used for better surgical condition occasionally, but the allowed level of tilt was controlled at 20 degrees, the effect of this position on the hemodynamic parameters was slight. Therefore, the sufficient fluid resuscitation should not be applied for keeping the stability of preload when using anti-Trendelenburg position. If anti-Trendelenburg position affected the hemodynamics, small dosage of phenylephrine hydrochloride (0.1–0.25 μg/kg·min) was used to keep the fluctuations of blood pressure and heart rate within 10% of base value. Furthermore, if the bad surgical conditions could not be improved by anti-Trendelenburg position or higher IAP level, the surgeons preferred to convert to open surgery and the case is to be excluded in this study.

Postoperative Management

After extubation, all patients were transferred to post-anesthesia care unit (PACU) for further observation. Patient-controlled intravenous analgesia (PCIA) with fentanyl 10 μg/kg, dexmedetomidine 2.5 μg/kg, and Granisetron 6 mg diluted with 0.9% normal saline to a total volume of 100 ml was administered for postoperative pain control. The background infusion was 2 ml/h and the bolus dose was 0.5 ml.

The standard enteral nutrition was started through the nasojejunal tube on postoperative day 1, with a protein:fat:glucose caloric ratio being approximate 20%:30%:50% of one's daily intake.

Laboratory Analysis

Furthermore, 3 ml of blood was drawn from the cubital vein 24 h before and after surgery and was centrifuged at 1,200 r/min at 4°C for 15 min and stored at −80°C. The level of serum DAO was detected with an enzymatic spectrophotometric assay and the level of serum D-lactate was detected with an enzyme-linked immunosorbent assay according to the instructions of manufacturer (Jiancheng Bioengineering Institute, Nanjing, China).

16s rRNA Sequencing of Feces

The postoperative fecal samples were collected in a test tube and immediately refrigerated at −80°C. The 16s rRNA sequencing of feces was entrusted to Suzhou Geneworks Technology Co., Ltd. (Suzhou, China). DNA was extracted using the Magen Hipure Soil DNA Kit (Magen, China) according to the protocol of manufacturer. DNA samples were quantified using a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Bacterial 16s rRNA genes of the V3–V4 region were amplified from extracted DNA using the barcoded primers: (5′-CCTACGGRRBGCASCAGKVRVGAAT-3′) and (5′-GGACTACNVGGGTWTCTAATCC-3′). The PCR products were checked for size and specificity by agarose gel electrophoresis and then purified. Finally, high-throughput sequencing was performed using the Illumina MiSeq platform (San Diego, CA, USA). The raw reads were filtered and clustered into operational taxonomic units (OTUs) at the level of 97% similarity using QIIME (Version 1.9.1) and the GreenGene database (Release 13_8_99).

Analysis for Diversity

Richness estimates and diversity indices (e.g., Chao1, Shannon index, and Simpson index) were calculated using QIIME (Version 1.9.1). A principal component analysis (PCoA) based on the weighted UniFrac distances was conducted to compare all samples by using the R language software (Version 2.15.3, vegan package).

Study Outcomes

The primary outcomes were the intestinal function recovery and intestinal mucosal barrier: (1) time to flatus and defection; (2) the levels of serum DAO and D-lactate; and (3) the analysis of gut microbiota. The secondary outcomes were: (1) duration of CO_2 insufflation and surgery; (2) surgical condition scores (five-point scale: 5 points, optimal; 4 points, good; 3 points, acceptable; 2 points, poor; 1 point, extremely poor); (3) time to discharge from the PACU; (4) postoperative pain at 12, 24, and 48 h after surgery using the visual analog pain scores (VAS) (0: no pain; 10: worst pain); (5) postoperative nausea and vomiting; and (6) length of postoperative hospital stay.

Sample Size Calculation

Sample size was estimated based on the levels of serum DAO and D-lactate. In the preliminary experiment, deep NMBs are more common and better accepted by the surgeons. The design therefore accords with the interest of patients from an ethical point of view. To this end, 1.5:1 randomization between deep and moderate NMB groups was used in this study. Type I error (α) was set at 0.05, and power was set at 80%. Considering a drop-out rate of 10%, a total sample size of 160 patients (96 patients in deep NMB group, 64 patients in moderate NMB group) was required.

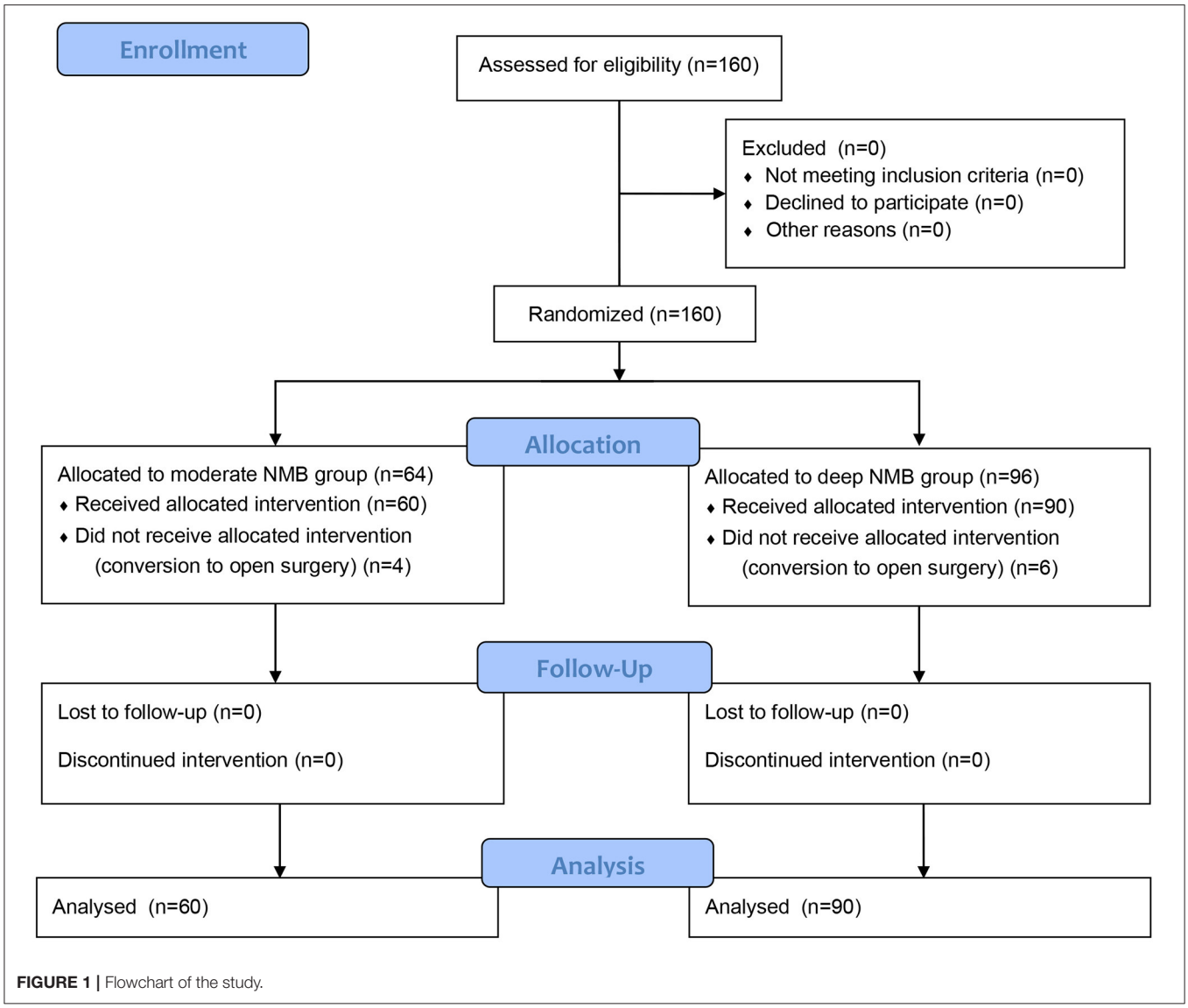

FIGURE 1 | Flowchart of the study.

Statistical Analysis

The analyses were conducted using IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables are reported as mean ± SD and were analyzed with two-sided Student's *t*-test. Skewed continuous variables are reported as median (range) and were analyzed with Mann–Whitney *U*-test. Categorical variables are reported as number (*n*) and percentage or rate (%), and χ^2 test, corrected χ^2 test, or Fisher's exact test was performed for the analysis. The value of $p < 0.05$ was considered statistically significant for all comparisons.

RESULTS

A total of 160 patients were enrolled in this study. Six patients in the deep NMB group and 4 patients in the moderate NMB group were excluded due to conversion to open surgery (**Figure 1**). The remaining 150 patients completed the study (90 patients in the deep NMB group and 60 patients in the moderate NMB group). The baseline characteristics were comparable between the two groups (**Table 1**).

As is shown in **Table 2**, ninety samples in the deep NMB group and sixty samples in the moderate NMB group were analyzed. The average time acquired to flatus after surgery in the deep NMB group was 74 ± 32 h while it took 93 ± 52 h the in moderate NMB group ($p = 0.006$). The serum concentration of D-lactate was statistically decreased compared with that of moderate NMB group (1,164 ± 185 vs. 1,209 ± 224 ng/ml, $p < 0.001$), suggesting that the damage of intestinal mucosa was more severe in the moderate NMB group compared with the deep NMB group. However, there was no significant difference in the level of serum DAO between the two groups (18 ± 4 vs. 23 ± 7 U/L, $p = 0.220$).

A total of 35 fecal samples, such as 12 samples in the moderate NMB group and 23 samples in the deep NMB group, were analyzed by 16s rRNA sequencing. The diversity of gut microbiota was more plentiful in the deep NMB group compared

TABLE 1 | Baseline characteristics and intraoperative parameters.

	mNMB ($n = 60$)	dNMB ($n = 90$)
Age (yr)	40–77	41–80
Sex, *n* (%)		
Male	41 (68.3)	59 (65.6)
Female	19 (31.7)	31 (34.4)
BMI kg · m^{-2}, mean (SD)	23.7 (2.7)	23.8 (2.7)
ASA classification		
I	2 (3.3)	5 (5.6)
II	45 (75.0)	68 (75.6)
III	13 (21.7)	17 (18.8)
Type of surgery, *n* (%)		
Distal gastrectomy	37 (61.7)	46 (51.1)
Proximal gastrectomy	2 (3.3)	2 (2.2)
Total gastrectomy	21 (35.0)	42 (46.7)

ASA, American Society of Anesthesiologists; BMI, body mass index; dNMB, deep neuromuscular blockade group; mNMB, moderate neuromuscular blockade group; SD, standard deviation.

TABLE 2 | Time to flatus and defecation, and the levels of serum diamine oxidase and D-Lactate.

	mNMB ($n = 60$)	dNMB ($n = 90$)	*P*-value
Time to flatus (h), mean (SD)	93 (52)	74 (32)	0.006
Time to defecation (h), mean (SD)	141 (59)	146 (44)	0.702
Diamine oxidase (U/L), mean (SD)			
Before surgery	16 (5)	16 (4)	0.931
24 h after surgery	23 (7)	18 (4)	0.220
D-Lactate (ng/ml), mean (SD)			
Before surgery	1,037 (186)	1,040 (192)	0.601
24 h after surgery	1,209 (224)	1,164 (185)	<0.001

dNMB, deep neuromuscular blockade group; mNMB, moderate neuromuscular blockade group; SD, standard deviation.

with the moderate NMB group (**Figure 2A**). The Alpha diversity, such as Chao 1, Shannon, and Simpson index, which refers to the diversity of microbiota within a habitat, was similar between the groups (**Figures 2B–D**). Beta diversity, such as PCoA analysis, indicates the variations of species between habitats. The PCoA analysis plots showed that the dots of deep NMB group were not close to the dots of moderate NMB group (**Figure 2E**). The heatmap at the genus level was displayed in **Figure 2F**. In detail, the beneficial bacteria (e.g., *Lactobacillus* and *Bifidobacterium*) were more abundant in the deep NMB group, while the potentially harmful bacteria (e.g., *Dialister*) was more abundant in the moderate NMB group.

The variations of gut microbiota between the groups were functionally relevant. The relative abundance of Family *Coriobacteriaceae*, Genus *Desulfovibrio*, and Genus *Collinsella* was significantly higher in the fecal samples of the deep NMB group (**Figures 3H,N,P**). In addition, the relative abundance of Order *SAR11 clade*, *MBMPE27*, and *Sneathiellales*; Family *Veillonellaceae*, *Clade I*, *Atopobiaceae*, and *Sneathiellaceae*; Genus *possible genus 04*, *Lachnoanaerobaculum*, *Dialister*, *Catonella*, *Veillonella*, and *Ferrovibrio* was significantly lower in the deep NMB group (**Figures 3A–G,I–M,O**).

The surgical condition scores were 3.4 ± 1.1 points in the moderate NMB group, and the scores were 4.8 ± 0.5 points in the deep NMB group ($p < 0.001$). The duration of the surgery in the deep NMB group (171.0 ± 30.8 min) was significantly reduced compared with the moderate NMB group (184.0 ± 33.7 min, $p = 0.017$). There were no statistical differences in the length of PACU stay and the postoperative hospital stay between the two groups. Meanwhile, postoperative VAS pain scores at 12, 24, and 48 h after surgery were similar in the two groups. Postoperative death was not observed in both groups before the patient was discharged from the hospital (**Table 3**).

DISCUSSION

This study demonstrated that deep NMB in laparoscopic gastrectomy could help to cut down the time to postoperative flatus and reduce the damage to intestinal mucosal barrier and gut microbiota. The beneficial bacteria were better preserved in the deep NMB group. In addition, deep NMB created better surgical conditions and shortened the average duration of the surgery.

Laparoscopic surgeries have been the main treatment option for abdominal surgeries and have been widely accepted with minimal incision compared with traditional open surgeries. But the indispensable and continuous insufflation of CO_2 during laparoscopic surgeries results in the artificial intra-abdominal hypertension, which would aggravate the perfusion of intra-abdominal organs (14). Studies reported a decrease of blood flow in both the superior mesenteric artery and hepatic portal vein under the CO_2 pneumoperitoneum of 14 mmHg (14, 15). The ischemic injury may cause deleterious effects to the intestinal mucosal barrier and postpone the postoperative recovery (16). In addition, studies have shown the benefits of deep NMB in optimizing surgical fields and reducing the morbidity of unexpected muscle retractions in major surgeries. Deep NMB is beneficial for the recovery of intestinal recovery and postoperative rehabilitations (10). But to date, the mechanisms of deep NMB on enhancing the recovery of postoperative intestinal function after laparoscopic gastrectomy are not clear.

In this study, time to flatus after surgery was significantly reduced in the deep NMB group (**Table 2**). However, mainly owning to the insufficient food intake, time to defection was similar between the groups. To evaluate the influence of different depth of NMB on the integrity of intestinal mucosal barrier, we tested the serum levels of DAO and D-lactate. A previous study showed that a receiver operating characteristic (ROC) analysis revealed that the sensitivity of D-lactate was 0.91, but the specificity was 0.70. The accuracy of D-lactate was very high, and that the areas under the curve (AUC) of the biomarker was

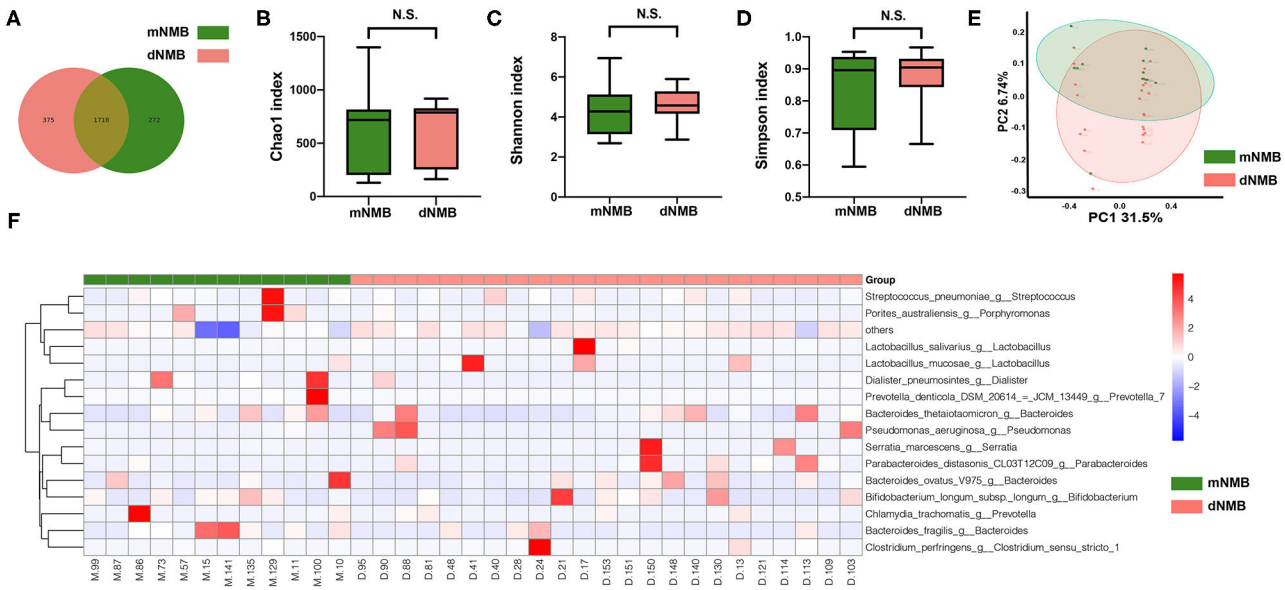

FIGURE 2 | Alpha diversity, Beta diversity, and heatmap of gut microbiota between the deep NMB and moderate NMB group. **(A)** Venn plot; **(B)** Chao 1 index; **(C)** Shannon index; **(D)** Simpson index; **(E)** PCoA of gut microbiota; **(F)** the heatmap at the genus level. dNMB, deep neuromuscular blockade; mNMB, moderate neuromuscular blockade; N.S., not significant; PCoA, principal coordinate analysis.

0.84. The sensitivity of DAO was 0.25, and its specificity was 0.92 (2). The present study showed that the increase of serum D-lactate was significantly lower in deep NMB group compared with in moderate NMB group, though the two biomarkers were increased after surgery among all the patients, suggesting that deep NMB was beneficial to protect the integrity of intestinal mucosal barrier. The relatively high levels of serum DAO and D-lactate observed in the moderate NMB group were consistent with the prolonged time to flatus after surgery. Therefore, we consider that deep NMB helps to enhance the postoperative the recovery of bowel movement by causing less damage to the intestinal mucosa in laparoscopic gastrectomy.

The important role of gut microbiota in human health and diseases, such as inflammatory or immune disorders, has been gradually recognized (17). Given the fact that alterations of the peritoneal fluid and the peritoneal microcirculation caused by the insufflation of CO_2 during the surgery, gut microbiota would transform to a more virulent phenotype (18–20). The alterations of normal gut microbiota could aggravate intestinal inflammation and prolong postoperative recovery (21, 22). Alpha and Beta diversity are effective indicators to reflect the within-habitat and between-habitat diversity of gut microbiota. This study showed that the Alpha diversity exhibited few differences (**Figure 2**). Interestingly, the Beta diversity demonstrated that the dots of deep NMB group were separated from those of moderate NMB group (**Figure 2**). The heatmap indicated that the relative abundance of several bacteria at the genus level was significantly different between the groups. The *Lactobacillus* and *Bifidobacterium* were more abundant in the deep NMB group, whereas the relative abundance of genera *Dialister* were much higher in the moderate NMB group. The *Lactobacillus* and *Bifidobacterium* are generally beneficial bacteria and are essential to enhance the intestinal microecology (23). However, the increased relative abundance of genera *Dialister* was observed among patients suffering from constipation (24, 25). The 16s rRNA sequencing demonstrated that the levels of Genus *Desulfovibrio* were significantly higher in the deep NMB group than those in the moderate NMB group. It has been reported that the levels of Genus *Desulfovibrio* were negatively correlated with intestinal inflammation (26). This may be a key bacterium to relieve the intestinal inflammation in moderate NMB group.

It has been proved that deep NMB could provide better surgical conditions with the same intra-abdominal pressure and reduce the incidence of muscular contractions (27). It is essential to be fully paralyzed, especially in laparoscopy, as the sudden body movement would result in hemorrhage or severe organ damages if it was in critical steps (28, 29). In this study, the incidence of muscle contractions was less in the deep NMB group than in the moderate NMB group. The surgeons were much more satisfied with the surgical conditions with deep NMB, which was consistent with previous studies (30). The relatively roomy surgical field could provide a better pre-judgment to probable situations and would improve the surgical process (31).

Postoperative pain and the opioid treatment are controversial factors prolonging the recovery of intestinal function. The side effect of opioid is constipation. So, we standardized the perioperative pain management with low dosage of opioid. A previous study found that the patients in the deep NMB group suffered from a lower intensity of postoperative abdominal pain after laparoscopic colorectal resection within the following 48 h after the surgery (10). However, low insufflation pressure with

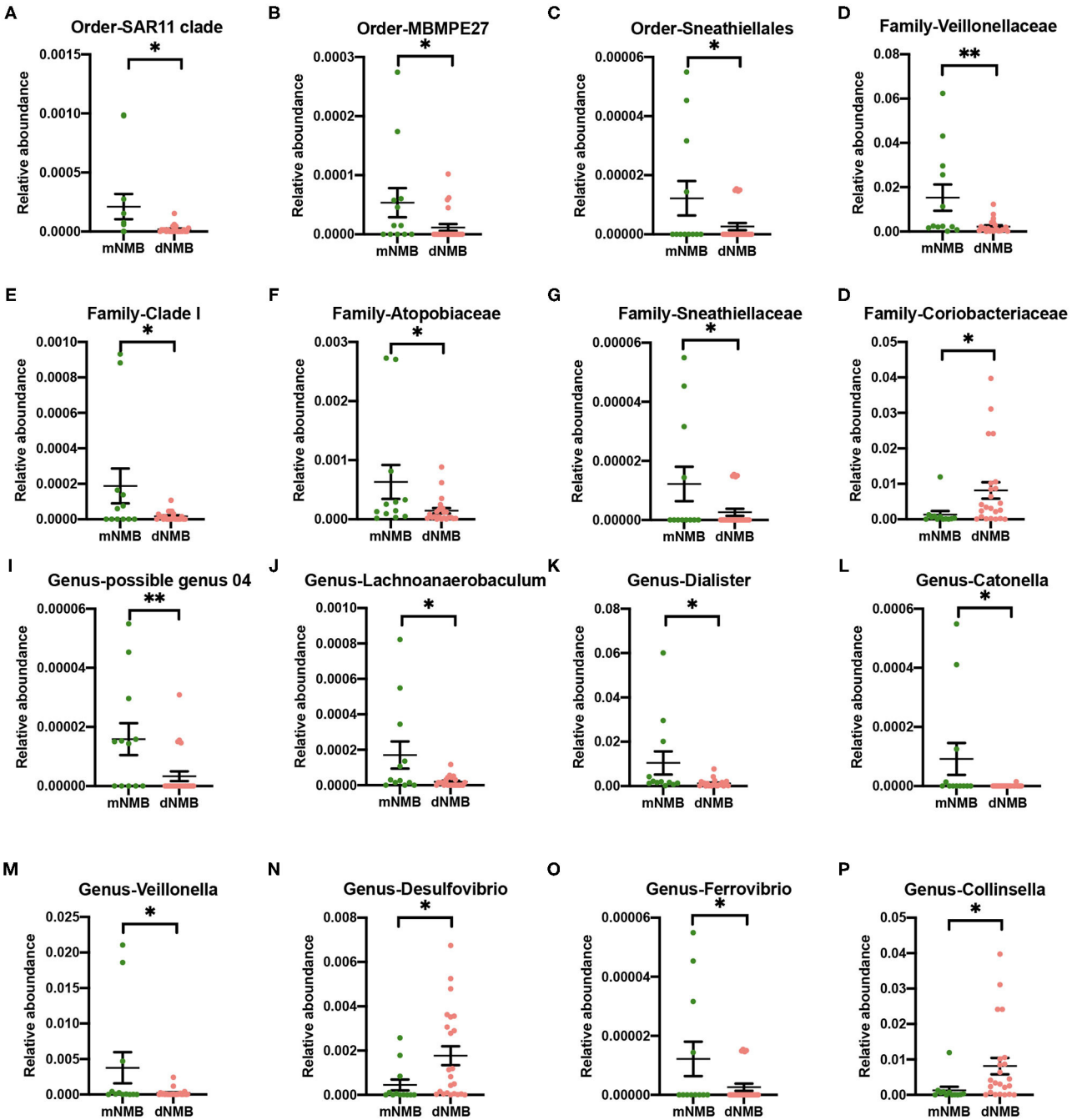

FIGURE 3 | Alterations in the levels of gut microbiota between the deep NMB and moderate NMB group. **(A)** Order *SAR11 clade*; **(B)** Order *MBMPE27*; **(C)** Order *Sneathiellales*; **(D)** Family *Veillonellaceae*; **(E)** Family *Clade I*; **(F)** Family *Atopobiaceae*; **(G)** Family *Sneathiellaceae*; **(H)** Family *Coriobacteriaceae*; **(I)** Genus *possible genus 04*; **(J)** Genus *Lachnoanaerobaculum*; **(K)** Genus *Dialister*; **(L)** Genus *Catonella*; **(M)** Genus *Veillonella*; **(N)** Genus *Desulfovibrio*; **(O)** Genus *Ferrovibrio*; **(P)** Genus *Collinsella*. $^{*}p < 0.05$, $^{**}p < 0.01$. dNMB, deep neuromuscular blockade; mNMB, moderate neuromuscular blockade.

deep NMB did not result in reducing the pain in laparoscopic cholecystectomy (32). In this study, there was no significant difference in postoperative pain between the groups owing mainly to the relatively minimally invasive surgical incision and complete postoperative analgesia in this study.

There are some improvements that could be applied in the following investigations. To detect a convincing correlation between intra-abdominal pressure and histologic lesions, a biopsy of intestinal tissue was more persuasive. But it was unethical in humans to get extra intestinal tissue. In addition,

TABLE 3 | Intra- and postoperative parameters.

	mNMB ($n = 60$)	dNMB ($n = 90$)	*P*-value
Intraoperative			
Surgical condition scores, mean (SD)	3.4 (1.1)	4.8 (0.5)	<0.001
5, *n* (%)	10 (16.7)	77 (85.6)	
4, *n* (%)	20 (33.3)	9 (10.0)	
3, *n* (%)	23 (38.3)	4 (4.4)	
2, *n* (%)	6 (10.0)	0 (0.0)	
1, *n* (%)	1 (1.7)	0 (0.0)	
Total time of surgery (min), mean (SD)	184 (33.7)	171 (30.8)	0.017
Total time of CO_2 insufflation (min), mean (SD)	155 (31.7)	141 (34.4)	0.010
Postoperative			
Duration of PACU stay (min), mean (SD)	10.06 (7.8)	8.9 (5.5)	0.349
VAS scores, mean (SD)			
12 h after surgery	4.0 (1.4)	3.8 (1.5)	0.551
24 h after surgery	3.2 (1.3)	2.9 (1.2)	0.225
48 h after surgery	1.7 (1.0)	1.6 (1.1)	0.748
Hospital stays after surgery (day), mean (SD)	10.1 (7.8)	8.9 (5.4)	0.349
PONV, *n* (%) ONV	7 (11.7)	8 (8.9)	0.30
Death, *n* (%)	0 (0.0)	0 (0.0)	0.99

CO_2, carbon dioxide; dNMB, deep neuromuscular blockade group; mNMB, moderate neuromuscular blockade group; PACU, post-anesthesia care unit; PONV, postoperative nausea and vomitting; VAS, visual analog scale; SD, standard deviation.

splanchnic perfusion is very important for laparoscopic surgery, the present study ensured the splanchnic perfusion by controlling the IAP at 10–12 mmHg and $PetCO_2$ at 35–45 mmHg, but we did not collect the data on hemodynamics and monitor the splanchnic perfusion. More studies should be carried out and better methods should be applied to monitor the parameters, such as intraoperative ultrasound in further studies. Moreover, the gut varies even in the same surgical procedure, and this could affect the findings. In the study, methods to evaluate the surgical difficulties were insufficient. Meanwhile, the mechanisms of the gut microbiota on health and diseases remain to be determined and the effect of perioperative probiotics remains to be identified in the future.

In summary, deep NMB, compared with moderate NMB, helps to preserve the intestinal function after laparoscopic gastrectomy with less damage to intestinal mucosa and gut microbiota. Therefore, deep NMB is worth taking into consideration for patients undergoing laparoscopic gastrectomy in terms of the effect of intestinal protection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA788087.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by First Affiliated Hospital of Nanjing Medical University, Nanjing, China (No: 2018-SR-336). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HH, CL, and CY: conceived and designed the experiments. HH, LZ, YY, and HX: performed the experiments. SL and ZX: data analysis and interpretation. HH, LZ, CL, and CY: manuscript preparation. All authors contributed to the article and approved the submitted version.

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frontiers in Medicine

REVIEW
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Clinical Importance of Potential Genetic Determinants Affecting Propofol Pharmacokinetics and Pharmacodynamics

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Interindividual variability in response to drugs used in anesthesia has long been considered the rule, not the exception. It is important to mention that in anesthesiology, the variability in response to drugs is multifactorial, i.e., genetic and environmental factors interact with each other and thus affect the metabolism, efficacy, and side effects of drugs. Propofol (2,6-diisopropylphenol) is the most common intravenous anesthetic used in modern medicine. Individual differences in genetic factors [single nucleotide polymorphisms (SNPs)] in the genes encoding metabolic enzymes, molecular transporters, and molecular binding sites of propofol can be responsible for susceptibility to propofol effects. The objective of this review (through the analysis of published research) was to systematize the influence of gene polymorphisms on the pharmacokinetics and pharmacodynamics of propofol, to explain whether and to what extent the gene profile has an impact on variations observed in the clinical response to propofol, and to estimate the benefit of genotyping in anesthesiology. Despite the fact that there has been a considerable advance in this type of research in recent years, which has been largely limited to one or a group of genes, interindividual differences in propofol pharmacokinetics and pharmacodynamics may be best explained by the contribution of multiple pathways and need to be further investigated.

Keywords: propofol, pharmacokinetics, pharmacodynamics, gene, polymorphism

INTRODUCTION

One of the most challenging areas of research in clinical pharmacology, pharmacy, pharmacoepidemiology, and especially pharmacogenetics is the attempt to understand why individuals respond differently to drug therapy. Problems with drug therapy can be divided into two main categories. The first problem is that the drugs are not equally effective in all patients. If it were possible to predict the efficacy of the drug in advance, interindividual variation of the drug would be avoided in patients in whom the drug does not work enough, and at the same time the costs would

be reduced. Another major therapeutic problem is the occurrence of adverse drug events (ADEs), which is especially important in the fields of medicine where drugs of small therapeutic range are used, among which are anesthesiology and intensive care.

Several evident examples of unfavorable outcomes from perioperative drugs are well-known (e.g., malignant hyperthermia, prolonged apnea, respiratory depression, and insufficient analgesia), leading to a better knowledge of the genetic susceptibilities behind these problems. Despite this, systematic genetic screening prior to surgery to determine drug risk is not currently common practice (1).

Adverse drug events or overdose are responsible for nearly half of anesthesia-related deaths (2), and one out of every 20 perioperative medication doses results in an unanticipated ADE or a medication error (3). Medications such as sedative-hypnotics, inhalation and intravenous anesthetics, analgesics, and cardiovascular drugs, among others, are frequently used in perioperative treatment. It should be noted that patients have generally never received these drugs before. For this reason, pharmacogenomics may play a part in the anesthesiologist's preoperative evaluation since it allows for individualized anesthetic plans.

In recent years, rapid breakthroughs in molecular biology and the Human Genome Project have resulted in the discovery of millions of new polymorphisms (4). The Clinical Pharmacogenetics Implementation Consortium (CPIC) was established in 2009 to provide a framework for understanding the levels of evidence required for pharmacogenetics to be incorporated into clinical practice, as well as to address the need to provide very specific guidance to clinicians and laboratories in order to ensure that pharmacogenetic tests are used wisely (5, 6).

To explain heterogeneity in drug therapy responses, anesthesiologists and other clinicians have focused on genetic variability that affects drug metabolizing enzymes. Many other essential proteins, including as transporter proteins and receptors, are now known to be affected by genetic variability (7).

Propofol is a short-acting intravenous anesthetic that is commonly used to induce and maintain general anesthesia as well as procedural sedation. Polymorphisms in cytochrome P450 (CYP) isoforms and UDP-glucuronosyltransferase (UGT), as well as drugs administered concurrently, could cause unpredictable interindividual variability of propofol pharmacokinetics and pharmacodynamics with forensic and clinically relevant adverse outcomes e.g., respiratory and cardiac depression, "propofol-related infusion syndrome – PRIS" (8).

METHODS

The electronic search for this narrative review included three databases, PubMed, EMBASE, and Google Scholar, and used search terms: "propofol," "pharmacokinetics," "pharmacodynamics," "gene," and "polymorphisms." The inclusion criteria were: articles for which full text was available, studies conducted in adults and children. The exclusion criteria were: articles that were not in English, or were gray literature. From the articles retrieved in the first round of search, additional references were identified by a manual search among the cited references. The search was limited to papers published between 2000 and 2021, and 66 papers were found to be eligible for study.

PROPOFOL – STRUCTURE AND PHYSICAL PROPERTIES

Propofol is by structure isopropyl phenol which is insoluble in water. Chemically it represents 2,6-diisopropylphenol. In commercial preparations, it is packaged in the form of an emulsion containing soybean oil, glycerol and egg lecithin. Propofol is a characteristic viscous, milky-white emulsion, called "milk of anesthesia." The lipoid emulsion of propofol possesses antioxidant properties when observed *in vivo* and *in vitro*. This property of propofol originates from its chemical nature because it has a structure similar to phenolic antioxidants, such as endogenous alpha-tocopherol (vitamin E) (9). Hence, it is particularly effective in preventing damage caused by ischemia and reperfusion (10, 11). Propofol increases expression of antioxidants, decreases production of reactive oxygen species (ROS), and thus alleviates DNA damage and cell death (12).

PHARMACOKINETICS OF PROPOFOL

Propofol's pharmacokinetics (PK) have been thoroughly investigated (13, 14). Propofol PK is commonly described using a three-compartmental model: a large central compartment, a peripheral compartment with lower perfusion (lean tissues), and a deep compartment with low perfusion (fat). Rapid start of action at the brain is ensured by high lipophilicity, and rapid redistribution from the central to peripheral compartment promotes rapid anesthetic action offset (15). Fat compartments at the periphery act as reservoirs, and redistribution from these compartments to the central compartment might take a long time, especially in obese and severely ill patients (16, 17).

Propofol should only be used intravenously. Because of its bitter taste and low oral bioavailability caused by a high first-pass effect and a high hepatic extraction rate (90 percent), it is not suited for enteral or other routes of administration. Propofol is significantly bound to plasma proteins (mostly albumin) and erythrocytes after intravenous administration.

Propofol crosses the blood–brain barrier (BBB) quickly and causes unconsciousness (sometimes in less than the time it takes for a drug to pass through the circulation once). Because of the rapid initial distribution, the period to offset clinical effects after a single bolus or brief infusion is short. Because redistribution of drug from the slow compartment is slower compared to the rates of metabolism and excretion, the offset of clinical effects is nevertheless relatively fast compared to other intravenous hypnotics even after prolonged treatment (18).

Propofol is metabolized in the liver to a multitude of metabolites, most of which are excreted in the urine. The biotransformation can occur in a variety of ways (**Figure 1**). The small intestines are also metabolically active, with an extraction ratio of 24% (19). The role of the lungs is still being discussed;

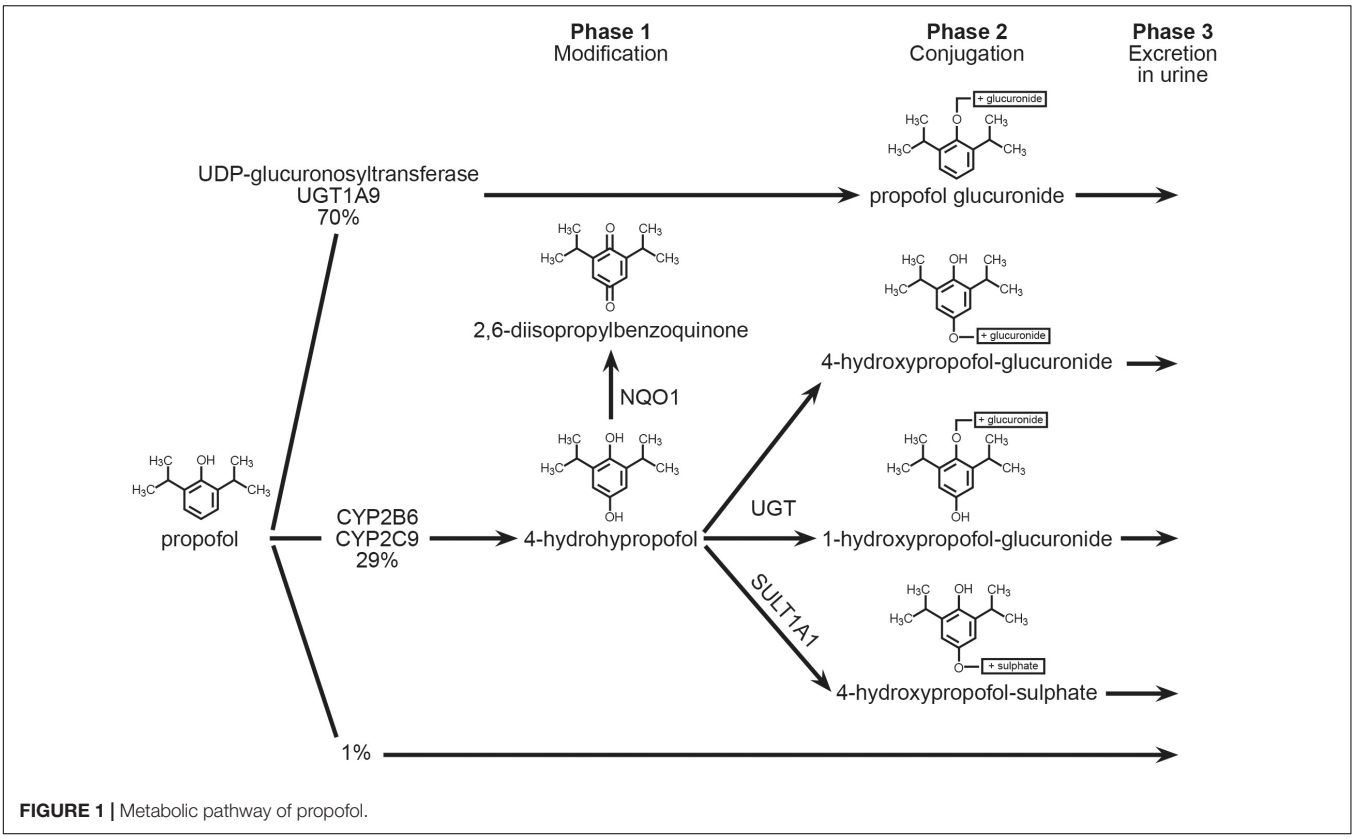

FIGURE 1 | Metabolic pathway of propofol.

some studies imply that the lungs play an active role (20), while others do not (21), or that the lungs are only a temporary propofol reservoir that later releases propofol from binding sites back into circulation (22). Furthermore, the kidneys likely account for about one-third of total body propofol clearance in patients undergoing cardiac surgery (21). Only about 0.3 percent of propofol administered is excreted unchanged. Propofol can also be exhaled.

The UDP-glucuronosyltransferase gene encoded by UGT1A9 (UDP glucuronosyltransferase 1 family, polypeptide A9, MIM 606434) is responsible for the majority of propofol's metabolism (about 70%) into propofol glucuronide (PG). The enzymes coded by the CYP2B6 (MIM 123930) and CYP2C9 (MIM 601130) genes, as well as the SULT1A (MIM 171150) and NQO1 (MIM 125860) genes, execute an alternate pathway of propofol biotransformation (about 29 percent) (23, 24). The cytochrome P450 enzymes (CYP2B6 and CYP2C9) are responsible for the formation of a hydroxyl derivative propofol-4-hydroxypropophol, which can further be transformed into 4-hydroxypropophol-1-ObD-glucuronide (Q1G) and 4-hydroxypropophol-4-ObD-glucuronide (Q4G). About 70–90% of propofol is eliminated by urine in the form of the glucuronide metabolite. It is possible that single nucleotide polymorphisms in the genes encoding these enzymes are responsible for the formation of individual variables of propofol metabolic products, resulting in unpredictable effects of standard anesthetic doses as well as prolonged waking time (recovery) from anesthesia (25).

CYP2C9 Gene

The enzyme CYP2C9 is a hemoprotein that participates in the first phase of biotransformation of xenobiotics and endogenous molecules and belongs to the large family of CYP2C genes. It makes up 15% of the total metabolism of the first phase of biotransformation. The gene for CYP2C9 is located on chromosome 10q24 and consists of 9 exons. More than 60 alleles of the gene for this enzyme are known. The most common polymorphic allele is CYP2C9*2 (rs1799853, 430C > T). This protein is characterized by weak metabolic activity. The gene encoding this enzyme is polymorphic, which is important for clinical practice because the enzyme CYP2C9 participates in the metabolism of several important drugs (phenytoin, tolbutamide, ibuprofen, and warfarin) (26) including anesthetic propofol.

CYP2B6 Gene

The gene for CYP2B6, encodes a number of cytochrome P450 enzyme superfamilies (CYP2A, CYP2B, and CYP2F). These enzymes belong to monooxygenases, catalyze reactions in the synthesis of cholesterol, steroids, and other lipids, as well as biotransformation reactions of many drugs, including propofol. Enzymes are localized in the endoplasmic reticulum. Phenobarbital is known to strongly induce the synthesis of these enzymes, while clopidogrel inhibits it (27). Families of this enzyme participate in the metabolism of xenobiotics, such as chemotherapeutics (cyclophosphamide and ifosphamide), anti-inflammatory drugs, anesthetics and benzodiazepines. The gene

for CYP2B6 is located on chromosome 19 (19q13.2), contains 9 exons, encodes a 48-kDa protein composed of 491 amino acids. The CYP2B6 gene contains over 28 alleles and over 100 SNPs and is considered a highly polymorphic P450 gene. A special variant of the CYP2B6*18 gene [I328T], predominantly present in Africans (4–12%) does not express proteins (enzymes). The CYP2B6 gene polymorphism is of particular importance in the treatment of HIV patients treated with a reverse transcriptase inhibitor (efavirenz), for whose metabolism this enzyme is responsible (28). The c.516G > T SNP allele variant in exon 4 (rs3745274) is responsible for reducing the amount of functional transcript for a given enzyme (29). Age, sex, nutritional status, disease state, drugs, and a patient's heredity all influence the expression of CYP enzymes.

UGT1A9 Gene

The UGT1A9 gene belongs to the family of genes responsible for the synthesis of the enzyme UDP-glucuronyl-sulfo-transferase. These enzymes participate in the glucuronidation reaction, in which glucuronic acid is conjugated to one of many different substances. In addition to the liver, enzymes are also present in the kidney, colon, ovary, testis and skin. The gene for UGT1A9 is located on chromosome 2 (q37.1). The gene locus comprises 13 unique alternative exons, of which the first 4 are considered pseudogenic, show significant variability, and encode a site in the substrate binding enzyme. UGT1A9 and UGT2B7 gene products participate in the second phase of biotransformation of endogenous and exogenous substrates. Variants of the UGT1A9 allele (c.98T > C as well as 766G > A) are the most common polymorphic forms that occur predominantly in the inhabitants of Asia and America. The gene for UGT1A9 is highly polymorphic (30), so carriers of some allelic variants can potentially face the described side effects, which leads to a significant reduction in the expression of the gene itself and, as a consequence, a decrease in glucuronidation of metabolites.

Effects of CYP2C9, CYP2B6, and UGT1A9 Genotypes on Propofol Pharmacokinetics

Eugene was the first to propose genotype-based dose modifications for patients who were administered propofol (31). The final PK (parametric pharmacokinetics) analysis covered 51 participants in total. The propofol concentration-time data was characterized using a two-compartment gamma multiplicative error model. The UGT1A9 and CYP2B6 G516T gene variants did not result in statistically significant differences in PK parameters, while the CYP2B6 A785G gene variants did result in statistically significant differences in elimination rate, especially in older patients. If no dosage adjustment is done, the CYP2B6 AA and AG patients will be exposed to roughly 250 percent greater blood propofol levels in a brief 1-h infusion, according to modeling and simulation. Because the maintenance infusion dose is proportional to the clearance rate, precision guided dose adjustments for the CYP2B6 AA and AG genotypes necessitate a 50% reduction in infusion dose to 25mg/kg/min, as indicated and demonstrated in the results (31).

Loryan et al. (32) evaluated common CYP2B6 and UGT1A9 SNPs in propofol patients, but no significant genotype-based findings were discovered. Similarly, Choong et al. (33) observed that women metabolize propofol faster than males despite no significant differences in CYP2B6 or UGT1A9 SNPs on propofol metabolism. However, through a functional estrogen response element in the upstream regulatory CYP2B6 sequences, estrogen receptors have been demonstrated to boost CYP2B6 gene expression (34). As a result, it's possible that sex hormone levels are a possible cause of the observed sexual dimorphism in propofol metabolite formation (33).

Several investigations have sought to see if the polymorphism CYP2B6 gene causes any substantial changes in propofol clearance and awareness after bolus doses and infusions, but none have resulted in gene-guided propofol dosage adjustments (35–38). Fujita et al. (39) investigated whether sex and cytochrome P450 (CYP) 2B6 and UDP glucuronosyltransferase (UGT) 1A9 polymorphisms influenced the discrepancy between predicted and measured plasma propofol levels during 4 h of target-controlled infusion (TCI). According to the authors, the propofol TCI system is more accurate in women than in men. Also, women's plasma concentrations of propofol decline more quickly than men's, and women recover from propofol anesthesia faster than males (39). This sex difference could be related to the fact that women's livers have 1.9-fold higher CYP2B6 protein levels than men's (32). On the other hand, Fujita et al. (39) concluded that the discrepancy between the predicted and actual plasma propofol concentrations in the perioperative period with continuous propofol infusion for 4 h was unaffected by CYP2B6 and UGT1A9 polymorphisms. Using stepwise multiple linear regression analysis to detect important factors of propofol pharmacokinetics in 94 patients (51 males, 43 females) who underwent lung surgery with total intravenous anesthesia (TIVA), Kobayashi et al. (37) concluded that sex differences, CYP2B6 polymorphisms, but not UGT1A, influenced propofol pharmacokinetics.

Kanaya et al. (40) found that body mass index (BMI) had an effect on propofol pharmacokinetics after a single intravenous dosage, whereas UGT1A9 and CYP2B6 SNPs, other clinical parameters, and hemodynamic variables had no effect. These findings suggested that BMI is a separate factor that influences propofol pharmacokinetics (40).

Mikstacki et al. (41) wanted to verify if the genetic mutations c.516G > T in the CYP2B6, c.98T > C in the UGT1A9, and c.1075A > C in the CYP2C9 genes had any effect on the individual propofol pharmacokinetic profile in Polish individuals having general anesthesia. A total of 85 patients were enrolled in the research. Rapid metabolizers were statistically more likely to be homozygotes c.516 T/T in the CYP2B6 gene. The pharmacokinetic profile of propofol was not affected by SNPs c.98T > C in the UGT1A9 and c.1075A > C in the CYP2C9 genes. The mean propofol retention time (MRT) was shown to be linked with the patient's BMI. According to Mikstacki et al. (41) only the polymorphism c.516G > T in the CYP2B6 gene and BMI have an effect on propofol metabolism and may have a role in propofol anesthesia optimization.

A total of 138 propofol-treated patients were enrolled, and environmental, clinical, and surgical data were gathered by Mourão et al. (38). The length of the surgery and the weight of the patient raised the propofol dose, whereas age and the presence of the T allele reduced the total dose of the medicine required. The total propofol dosages were 151.5 64.2 mg and 129.3 44.6 mg, respectively, based on the GG or GT/TT genotypes ($p = 0.043$). According to their findings, these factors account for 34% of the variation in the needed propofol dose, and the CYP2B6 c.516G > T polymorphism, which slows drug metabolism, contributes for about 7% of the drug dosage. Mastrogianni et al. (36) showed a substantial correlation between the CYP2B6 G516T variant and high blood propofol concentrations after a single bolus dosage.

The goal of Pavlovic et al. (25) study was to determine how UGT1A9 98T > C, CYP2B6 516G > T, and CYP2C9 430C > T genetic polymorphisms affected propofol pharmacokinetics in children of different sexes and ages who underwent total intravenous anesthesia (TIVA) and deep sedation during diagnostic and therapeutic procedures. This prospective study included 94 children aged 1–17 years old with an ASA I-II status who underwent a conventional anesthetic regimen for TIVA, which included continuous propofol administration. The results indicated that UGT1A9 genotype is an independent predictor of propofol concentration in children 10 min after the end of the continuous infusion. The propofol distribution constant was greater in carriers of the polymorphic UGT1A9 C allele. The polymorphic CYP2B6 T allele carriers received a considerably lower overall and first propofol dose. Unlike the UGT1A9 gene polymorphism, the investigated CYP2C9 and CYP2B6 gene polymorphisms are not independent predictors of propofol pharmacokinetics.

Khan et al. (42) concluded that patients with UGT1A9–331C/T had a greater propofol clearance and required a higher propofol induction dose. Patients with UGT1A9–1818T/C took longer to lose consciousness, while those with CYP2C9*2/*2 had higher propofol plasma concentrations than the others (42). Takahashi et al. (43) reported that the D256N polymorphism in UGT1A9 lowers enzyme activity in an *in vitro* study, suggesting that carriers of D256N may be at risk of propofol side effects.

ABCB1 Gene

The ABCB1 (MDR1, P-gp) gene was the first ABC transporter to be discovered and studied. This gene produces a transmembrane protein that facilitates ATP-dependent molecular transport (44). The P-gp protein is expressed on the luminal surface of blood-brain barrier (BBB) capillary endothelial cells and is known as the "guardian" of the brain. The P-gp transporter at the blood–brain barrier prevents active efflux of drugs into the CNS. It also allows harmful substances to be transported out of the brain (45). The absorption, distribution, and bioavailability of anesthetic drugs might be affected by variations in genes encoding P-gp protein. The MDR1 or ABCB1 gene, which encodes this transporter protein, has multiple functional polymorphisms, including 1236C > T, 2677G > T/A, and 3435C > T, which have been linked to anesthetic drug response variability (46). The c.3435C > T variant in exon 26 is one of over 100 polymorphic variants of this gene that have been found so far. This polymorphism has been linked to changes in P-gp expression and medication response in a variety of clinical settings (47).

According to the findings of Ivanov et al. (48) the ABCB1 (c.3435C > T) variation has no effect on clinical parameters in propofol patients. Although there is limited information on the impact of this variant on propofol therapy, their findings are consistent with those of Zakerska-Banaszak et al. (49), who found no statistically significant differences between propofol therapeutic effects and ABCB1 gene variants. Wolking et al. (50) pointed out that the genetic impact of ABCB1 polymorphisms on P-gp transporter expression and/or function is unclear and for that reason. for patients with the ABCB1 gene variation, no changes in drug dose or therapeutic substitution have been indicated (50). On the other hand, the mutation in ABCB1, SNP c.1236 C > T (rs1045642), was partly the reason for differences in the anesthetic effects when propofol was combined with remifentanil for pediatric tonsillectomy as reported by Zhang et al. (51). Liew et al. (52) used the databases PubMed, Medline, and Ovid to conduct a systematic search of the literature. The search was restricted to publications published between 2006 and 2020. In order to extract relevant papers from the databases, search phrases such as gene polymorphism, MDR1, ABCB1, opioid, propofol, children, pain, anesthetic, anesthesia, analgesic, analgesia, odds ratio, and surgery were used. For the analysis and summaries, a total of 2,554 patients from 17 papers were considered. The papers selected focused on the impact of SNPs in the ABCB1 gene (1236C > T, 2677G > T/A, and 3435C > T) on anesthetic and analgesic effects. Based on the evidence, genetic polymorphism in the ABCB1 gene had a substantial impact on anesthetic effects (mutational homozygous TT genotype in both ABCB1 1236C > T and 3435C > T was linked with a reduced anesthetic effect) but no apparent impacts on analgesia (51, 53).

PHARMACODYNAMICS OF PROPOFOL – MECHANISM OF ACTION

Propofol, like other intravenous anesthetics like benzodiazepines and barbiturates, acts by activating the central inhibitory neurotransmitter gamma-aminobutyric acid (GABA) to produce hypnosis (54). Gamma-aminobutyric acid type A (GABAA) receptors are ligand ion channels composed of different subunits (α, β, γ, δ, ε, θ, ρ, π) that form a pentameric structure containing a central chloride channel. Binding of propofol molecules to the receptor leads to increased influx of chloride ions and hyperpolarization of neurons, which leads to non-response to external stimuli. Propofol appears to be less effective at receptors containing β 1 than at those containing β 2 or β 3 subunits (55). Propofol also affects the presynaptic mechanisms of GABA transmission, such as GABA uptake and release (56). Its site of action appears to be different from that of barbiturates and benzodiazepines. The effect of propofol on other receptors has not been established with certainty. It has a very solid antiemetic effect (possible anti-serotonergic effect). It probably activates inhibitory glycine receptors at the spinal cord level, and inhibits

nicotinic acetylcholine receptors, as well as excitatory glutamate NMDA and AMPA receptors.

The inter-patient variability in the propofol dose necessary to achieve BIS < 70, as well as the estimated apparent systemic clearance and "time to eye opening" following TIVA, was studied by Iohom et al. (57). Although it appeared that genetic variants in the CYP2B6 and GABAA(ε) genes might explain for some of this variation *in vivo*, the genetic variants studied did not account for the majority of it. Ivanov et al. (48) found differences between the given propofol doses in patients with different genotype for the polymorphism studied i.e., in *GABRA1* (c.1059 + 15G > A) carriers the initial, additional, and total dose of propofol decreased with age ($p > 0.05$). GABRA2 rs35496835, GABRB1 rs1372496, GABRG2 rs11135176, GABRG2 rs209358, GAD1 rs3791878, SLC1A3 rs1049522, and gender were all revealed to be significant predictors of loss of consciousness latency following propofol administration (58). Blood pressure decrease during anesthesia induction was highly linked with GABRA2 rs11503014. Because there was no direct evidence of a link between hypotension and GABA, Zhang et al. (58) pointed out that future research should focus on the mechanisms underlying the effects of GABAAR gene polymorphisms on blood pressure during TIVA with propofol.

CLINICAL UTILITY OF PHARMACOGENOMIC TESTING IN ANESTHESIOLOGY

The patient's most vulnerable period is during the perioperative period, and personalized anesthetic approach will be the future standard. However, there is currently no clear clinical data addressing the efficacy and cost–effectiveness of pharmacogenomic testing for the majority of drugs (59, 60).

Over the past few years, the link between pharmacogenetics and anesthesiology has become even stronger due to the emergence of new data on the effects of genetic variations on the pharmacokinetics and pharmacodynamics of used drugs. Frequent conflicting results between studies make it difficult to include pharmacogenetics in anesthesiology (61). There are currently no clinically relevant guidelines in the field of anesthesiology for the individualization of the use of general anesthetics based on conducted pharmacogenetic analyzes of biological material in patients. From a scientific and professional point of view, this is especially important in anesthesiology because anesthesiologists could be considered as practicing pharmacologists and the use of total intravenous anesthesia (TIVA) using propofol is just a good example of "pharmacology in action" (62).

Since the Enhanced Recovery After Surgery (ERAS) practices emphasize a multidisciplinary comprehensive approach to the care of surgical patient (63) the most recent elements of ERAS involve drug administration and the feasibility of introducing pharmacogenomics testing in ERAS to guide drug administration (64).

Overall, there are two types of obstacles to clinical pharmacogenomic testing implementation: first, determining whether the testing should be done at all, based on the availability of evidence and cost-effectiveness, and second, overcoming challenges associated with integration into the clinical system and work flow (such as clinical labs' struggle to comply with regulatory frameworks designed for non-genetic or single-gene tests). Additionally, initial pharmacogenetic studies have concentrated on one gene and one drug, as is usual in genetic research. However, extrapolating data from one gene to a single drug is difficult due to the fact that drugs are rarely used alone and that the drug response pathway normally involves more than one gene (61). Furthermore, due to statistical power, tests are frequently repeated, and patients with lower frequency allele subgroups are constantly under-represented, resulting in potentially incomplete results. Besides all that, despite historically being the physician champions for pharmacogenetic testing to guide perioperative care, anesthesiologists sometimes had to rely on the other specialties to order the test (65). Nevertheless, further research is needed to define and describe polymorphic enzymes in order to better understand interindividual variations in the glucuronidation metabolic pathway, as well as their pharmacological and toxicological side effects.

Although positive pharmacogenetic polymorphic associations with clinical significance have been discovered, there is a lack of reproducibility because most studies focus on single variant associations, whereas interindividual differences in propofol metabolism may be best explained by the contribution of multiple pathways. Indeed, the perioperative phase has a high risk of serious adverse reactions due to the narrow therapeutic index and great variability in patient responses to anesthesia and surgery. Additional metabolites must be identified in order to validate xenobiotic exposure in a larger detection window, particularly in different samples. Besides, regardless of the fact that there are sex and racial/ethnic differences in propofol response, there is no strong evidence linking genetic variation to such findings, possibly due to the additional influence of weight, height, and lean body mass, environmental factors, and severe hepatic or renal impairment on propofol pharmacokinetics (17, 66).

Finally, propofol metabolomics has not yet been thoroughly investigated (8), and more research is needed to determine whether the various metabolomic patterns are clinically significant, taking into account sex, age, genetic polymorphisms, and other factors such as comorbidities.

CONCLUSION

There are many unresolved questions regarding the importance of pharmacogenetic studies in anesthesiology. In recent years, there has been a significant breakthrough in this type of research, which has been largely limited to one or a group of genes. In addition, the role of a number of well-known factors such as age, gender, associated diseases, BMI, type of surgery is unambiguous, so that our obtained results of pharmacogenomic studies can often confuse or lead to wrong conclusions. The value of pharmacogenomics in anesthesia has been firmly demonstrated throughout history, but whether it has a place in everyday clinical practice at this moment needs to be determined. What is certain

is that we will have to wait for more solid evidences from future studies and projects.

AUTHOR CONTRIBUTIONS

IB wrote and revised the manuscript. TJ contributed to the idea generation and edited the final manuscript. DP, VM, and ID searched the literature and revised final version. MS and DS reviewed and revised final version of the manuscript. All authors have read and approved the manuscript.

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A Positive Fluid Balance in the First Week Was Associated With Increased Long-Term Mortality in Critically Ill Patients: A Retrospective Cohort Study

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Introduction: Early fluid balance has been found to affect short-term mortality in critically ill patients; however, there is little knowledge regarding the association between early cumulative fluid balance (CFB) and long-term mortality. This study aims to determine the distinct association between CFB day 1–3 (CFB 1–3) and day 4–7 (CFB 4–7) and long-term mortality in critically ill patients.

Patients and Methods: This study was conducted at Taichung Veterans General Hospital, a tertiary care referral center in central Taiwan, by linking the hospital critical care data warehouse 2015–2019 and death registry data of the Taiwanese National Health Research Database. The patients followed up until deceased or the end of the study on 31 December 2019. We use the log-rank test to examine the association between CFB 1–3 and CFB 4–7 with long-term mortality and multivariable Cox regression to identify independent predictors during index admission for long-term mortality in critically ill patients.

Results: A total of 4,610 patients were evaluated. The mean age was 66.4 ± 16.4 years, where 63.8% were men. In patients without shock, a positive CFB 4–7, but not CFB 1–3, was associated with 1-year mortality, while a positive CFB 1–3 and CFB 4–7 had a consistent and excess hazard of 1-year mortality among critically ill patients with shock. The multivariate Cox proportional hazard regression model identified that CFB 1–3 and CFB 4–7 (with per 1-liter increment, HR: 1.047 and 1.094; 95% CI 1.037–1.058 and 1.080–1.108, respectively) were independently associated with high long-term mortality in critically ill patients after adjustment of relevant covariates, including disease severity and the presence of shock.

Conclusions: We found that the fluid balance in the first week, especially on days 4–7, appears to be an early predictor for long-term mortality in critically ill patients. More studies are needed to validate our findings and elucidate underlying mechanisms.

Keywords: acute kidney injury, critical care, cumulative fluid balance, fluid balance, long-term outcome, mortality, shock

INTRODUCTION

Fluid homeostasis is frequently altered in critical illness, and fluid balance has been attributed as one of the fundamental managements in patients admitted to the intensive care unit (ICU) (1). Fluid resuscitation is necessary to maintain tissue perfusion and improve cardiac output (2); however, excessive fluid accumulation may lead to detrimental effects, including prolonged tissue edema, impaired oxygen transport, reduced metabolite diffusion, and damaged cell–cell interactions (3). Increasing evidence has suggested that a positive fluid balance in the early stage of ICU admission might deteriorate outcomes in critically ill patients (4–7). One recent meta-analysis found that a positive cumulative fluid balance (CFB) in the first 3 days of ICU stay was associated with high hospital mortality [Relative Risk 2.15 (95% CI, 1.51–3.07)] (8). However, the optimal management of post-resuscitation fluid management, such as the day 4–7 fluid balance, remains unclear. Moreover, few studies have explored the association between early fluid balance and long-term survival (9).

Despite steady improvements in short-term mortality among critically ill patients, post-discharge mortality remains high (10). Among the ICU survivors, 15%−21% may die within the following year (11, 12), with ∼6–8% mortality per year in the subsequent 5 years (12). Therefore, long-term outcomes in critical medical illnesses, have become more important as more patients survive acute illness. Thus, the research priorities of critical care medicine have expanded to not only save lives while patients are in the ICU but also toward a goal of understanding and improving long-term outcomes. Nevertheless, outcome ascertainment of CFB in the meta-analysis was somehow restricted to short-term outcomes consisting of ICU mortality, hospital mortality, and 3-month mortality (8); data regarding the long-term effects of fluid balance on survival in medical ICU was sparse.

We linked data from the National Health Insurance Research Database (NHIRD) in Taiwan and Electronic Medical Records at Taichung Veterans General Hospital (TCVGH) to establish a critical care database. This database enabled us to test the hypothesis that a higher CFB at day 1–3 and day 4–7 might be associated with an increased risk of long-term mortality in patients admitted to the ICU.

Abbreviations: AKI, Acute kidney injury; APACHE, Acute Physiology and Chronic Health Evaluation; CCI, Charlson Comorbidity Index; CFB, Cumulative fluid balance; CFB 1-3, Cumulative fluid balance Day 1-3; CFB 4-7, Cumulative fluid balance Day 4-7; CI, Confident interval; HR, Hazard ratio; ICU, Intensive care unit; KDIGO, Kidney Disease: Improving Global Outcomes; NHIRD, National Health Insurance Research Database; RRT, Renal replacement therapy; TCVGH, Taichung Veterans General Hospital.

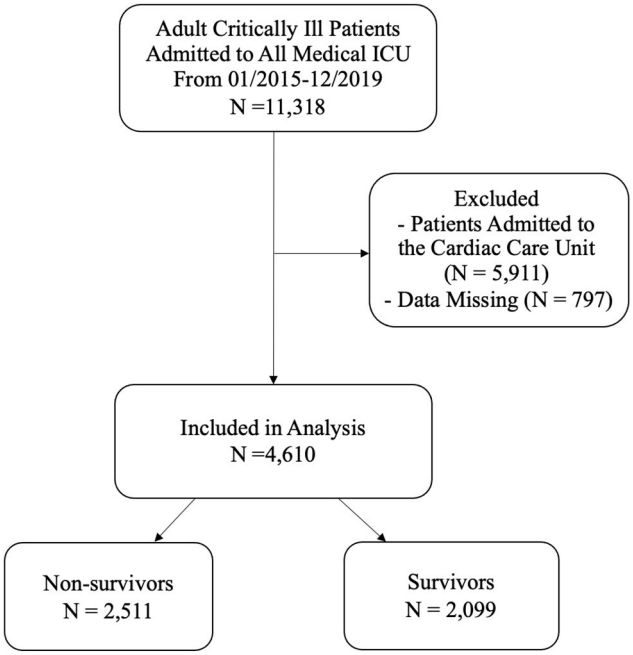

FIGURE 1 | Flowchart of enrollment of subjects eligible for analyses. ICU, intensive care unit.

MATERIALS AND METHODS

Study Population and Ethics Approval

This retrospective cohort study was conducted at TCVGH, a tertiary care teaching hospital in central Taiwan with 1,500 beds. The TCVGH Institutional Review Board approved this study with a waiver of informed consent since this was a retrospective analysis of anonymous data (number: CE20249B). All adult patients admitted to the medical ICU from January 2015 to December 2019 were included. We used the first ICU admission as the index admission. Of the available 11,318 patients admitted to medical ICU, we excluded patients in the Cardiac Care Unit ($N = 5,911$) and patients with missing data ($N = 797$); a total of 4,610 critically ill patients were eligible for analyses (**Figure 1**).

Data Source

We used two databases for this study: the clinical data warehouse at TCVGH and the cause-of-death data of the NHIRD in Taiwan. Demographic characteristics, comorbidities including Charlson Comorbidity Index (CCI), ICU admission, discharge diagnoses, daily fluid input and output, Acute Physiology and Chronic

Health Evaluation (APACHE) II score, mechanical ventilation usage, renal replacement therapy (RRT) commencement, use of vasopressors, serum creatinine, and hospital length of stay were obtained from the TCVGH clinical data warehouse. The presence of shock was defined as the requirement of vasopressors for more than 1 day. Acute kidney injury (AKI) was diagnosis, and the stage was determined according to the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines for AKI (13). A patient was diagnosed with AKI if they met the criteria for AKI stage 1 or higher within the 1 week of ICU admission. The encrypted TCVGH patient identification numbers were then linked to the cause-of-death data of the NHIRD to determine their date of death up to 2019.

Fluid Status and Study Outcome

The main exposure of interest in this study was daily fluid input, output, and balance. An intravenous or enteral fluid of any type was considered input. Output included urine, ultrafiltrate from RRT, all body fluid from drains, stool, and emesis. Fluid status was represented daily throughout the first week of ICU stay. We calculated the fluid balance for each patient as total fluid input minus total fluid output in each period. CFB of the first week was divided into two parts, the first 3 days from ICU admission and from admission day 4 to day 7 presented as CFB 1–3 and CFB 4–7, respectively. The primary outcome was the long-term all-cause mortality from the index ICU admission. The patients followed up until the ending date of NHIRD coverage or the end of the study on 31 December 2019, whichever came first. Given that National Health Insurance is a single-payer and mandatory program with a 99.9% coverage of Taiwanese population in 2019, the date of death and overall mortality in the present study should be accurate.

Statistical Analyses

Data for categorical variables are presented as numbers and percentages; data for continuous variables are shown as means ± standard deviation. We compared the baseline characteristics of the survivors vs. non-survivors by either Student's *t*-test or the chi-squared test. Kaplan–Meier analysis was used to analyze the association between long-term mortality and the day 1–3 or 4–7 CFB status, the Kaplan–Meier curve were presented up to 1-year. A Cox proportional hazards regression model was performed to identify independent variables that predicted long-term mortality. The adjusted hazard ratio (HR) and the corresponding 95% confidence interval (CI) for each variable were presented. We used the Wald test to determine the significance of modification effect by covariates, including age, sex, shock, and presence of sepsis. Additionally, we conducted a sensitivity analysis using a subset of our cohort consisting of 3,065 patients whose AKI stage could be determined. Moreover, we investigated the interaction of shock or AKI with CFB and long-term mortality by Kaplan–Meier analysis. All reported *p*-values were two-sided and considered significant if they are <0.05. Data cleaning and analysis were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline Characteristics of the Participants

Table 1 summarizes the baseline characteristics and clinical parameters of the participants. The mean age was 66.4 ± 16.4 years, and 2,943 patients (63.8%) were men. The reasons for ICU admission were sepsis ($n = 2,235$, 48.5%) followed by acute neurological disorder ($n = 444$, 9.6%) and respiratory disorder ($n = 420$, 9.1%). Among the total 4,610 patients, 1,291 (28%) passed away during the index admission, 1,741 (37.8%) died within 90 days, and 2,151 (46.7%) expired within 1 year after ICU admission. The patients were stratified into two groups, namely, survivors and non-survivors. The mean duration of follow-up among survivors was 2.1 ± 1.3 years. Compared with survivors, non-survivors were more likely to be older, male, with active cancer, and admitted due to sepsis or a respiratory disorder. Non-survivors compared with survivors had a significantly lower body mass index, a higher CCI, a higher APACHE II score, more mechanical ventilation, and a higher percent of shock. Besides, survivors had significantly less RRT initiated during admission compared with non-survivors. Collectively, there was high mortality among patients who survived after the index ICU admission.

Daily and CFB in the First Week

Table 2 presents the breakdown of fluid balance throughout the first week of ICU stay, categorized by survival status. The fluid balance was positive on day 1 in both survivors (1473.7 ± 2985.6 ml) and non-survivors (395.7 ± 2090.1 ml). The positive to negative fluid balance transition was earlier in survivors than non-survivors, which took place on day 2 and day 3, respectively. After day 3 from admission, daily fluid balance in either group was negative. Cumulative fluid positivity was common in our cohort, with a proportion of 49.6 and 23.9% positivity in CFB 1–3 and CFB 4–7, respectively. We further evaluated the CFB 1–3 and CFB 4–7 of the survivors and non-survivors. Notably, the survivors had a negative CFB 1–3, while the non-survivors had a positive CFB 1–3. Compared with the non-survivor group, a more negative CFB 4–7 was found in the survivor group (−580 ± 2995.6 vs. −910.7 ± 2664.5, $p < 0.01$). Together, **Table 2** shows that patients who survived presented a significantly lower daily fluid balance, CFB 1–3, and CFB 4–7, compared with those who died during long-term follow-up.

Both Day 1–3 and Day 4–7 Cumulative Fluid Positivity Are Correlated With Higher Long-Term Mortality

We further used CFB 1–3 and CFB 4–7 as a continuum to predict mortality. On univariate analysis, older age, male, lower BMI, CCI, APACHE II, shock, use of mechanical ventilation, active cancer, RRT initiated during ICU admission, CFB 1–3, and CFB 4–7 were significantly associated with an increased mortality risk. A multivariable model was done after adjusting for all these significant preexisting conditions and illness severity in the univariate model. Both CFB 1–3 and CFB 4–7 were independently associated with mortality (with per one liter

TABLE 1 | Patient characteristics by overall mortality ($N = 4,610$).

	All ($N = 4,610$)	Non-survivor ($N = 2,511$)	Survivor ($N = 2,099$)	*p*-value
Basic characteristics				
Age, years	66.4 ± 16.4	69.7 ± 15.5	62.5 ± 16.5	<0.01
Follow-up duration, years	1.2 ± 1.3	0.4 ± 0.7	2.1 ± 1.3	<0.01
Male	2,943 (63.8%)	1,658 (66.0%)	1,285 (61.2%)	<0.01
BMI	24.4 ± 4.7	24.0 ± 4.7	24.9 ± 4.7	<0.01
Charlson comorbidity index	2.4 ± 1.6	2.7 ± 1.6	1.9 ± 1.5	<0.01
Active cancer	627 (13.6%)	523 (20.8%)	104 (5.0%)	<0.01
Severity and managements				
APACHE II score	25.1 ± 7.5	27.8 ± 7.1	21.9 ± 6.7	<0.01
Shock	2,134 (46.3%)	1,500 (59.7%)	634 (30.2%)	<0.01
Ventilator	3,391 (73.6%)	2,074 (82.6%)	1,317 (62.7%)	<0.01
Renal replacement therapy (RRT)				
RRT initiated during ICU admission	738 (16%)	546 (21.7%)	192 (9.2%)	<0.01
RRT for end-stage renal disease	130 (2.8%)	69 (2.8%)	61 (2.9%)	0.75
Reasons for ICU admission				
Acute cardiac disorder	221 (4.8%)	42 (2.6%)	156 (7.4%)	<0.01
Acute gastrointestinal disorder	287 (6.2%)	146 (5.8%)	141 (6.7%)	0.22
Acute neurological disorder	444 (9.6%)	171 (6.8%)	273 (13.0%)	<0.01
Acute renal disorder	124 (2.7%)	57 (2.3%)	67 (3.2%)	0.06
Respiratory disorder	420 (9.1%)	289 (11.5%)	131 (6.2%)	<0.01
Sepsis	2,235 (48.5%)	1,402 (55.8%)	833 (39.7%)	<0.01
Others	879 (19.1%)	381 (15.2%)	498 (23.7%)	<0.01
Outcomes				
ICU-stay, days	9.9 ± 8.4	11.3 ± 8.8	8.3 ± 7.6	<0.01
Hospital-stay, days	24.2 ± 19.0	26.3 ± 19.7	21.8 ± 17.9	<0.01
Ventilator-day, days	9.7 ± 9.1	10.8 ± 9.6	8.1 ± 8.0	<0.01
In-hospital mortality	1,291 (28%)	1,291 (28%)	NA	
90-day mortality	1,741 (37.8%)	1,741 (37.8%)	NA	
1-year mortality	2,151 (46.7%)	2,151 (46.7%)	NA	

BMI, body mass index; APACHE II, acute physiology and chronic health evaluation; RRT, renal replacement therapy; ICU, intensive care unit; NA, not applicable.

increment, HR: 1.047 and 1.094; 95% CI 1.037–1.058 and 1.080–1.108, respectively) on multivariable analysis. Based on our results, the HR of mortality was even higher in per one liter increment of CFB 4–7 than CFB 1–3 (9.4 vs. 4.7%) in patients admitted to the medical ICU.

A Kaplan–Meier analysis for 1-year survival was completed using CFB positivity or negativity as a categorical variable. We disclosed that both CFB 1–3 and CFB 4–7 positivity were associated with a higher mortality risk (**Figure 2**). Considering that shock status may be an important confounder of survival, we constructed Kaplan–Meier survival curves for negative and positive CFB stratified by the presence of shock (**Figure 3**). Shock patients with a positive CFB 1–3 were more likely to die during long-term follow-up than patients with a negative CFB 1–3. However, CFB 1–3 was not a significant predictor of mortality in non-shock patients. Compared with CFB 1–3, CFB 4–7 positivity, an indicator of post-acute stage fluid balance, correlates with higher long-term mortality in either the shock or non-shock group.

Sensitivity Analysis

Sensitivity analysis limited the original cohort ($N = 4,610$) to a population without maintenance RRT for end-stage renal disease, stage 3 AKI at presentation in the medical ICU, or patients who stayed in the ICU <24 h, for a total analytic data set of 3,065 patients. The analysis produced robust results similar to those of the primary analysis (**Supplementary Table 1** and **Supplementary Figure 1** in the **Supplementary Material**).

Subgroup Analysis Stratified by AKI

The population in the subset was eligible for accessing in-hospital AKI status and stages. The multivariable model in **Table 3** reports that patients receiving new RRT during ICU admission were more likely to die during long-term follow-up than those without RRT (HR = 1.504; 95% CI 1.356–1.668, $p < 0.001$). Based on these results, we further explored the association of long-term outcomes and CFB in patients with different AKI status (**Supplementary Figure 2** in the **Supplementary Material**). Likewise, patients with a positive CFB 1–3 were more likely to die than those with a negative CFB 1–3,

TABLE 2 | Daily and cumulative fluid status in critically ill patients categorized by long-term mortality.

	All (*N* = 4,610)			Non-survivors (*N* = 2,511)			Survivors (*N* = 2,099)			*p*-value[a]
	Input (I)	Output (O)	IO balance	Input (I)	Output (O)	IO balance	Input (I)	Output (O)	IO balance	
Daily fluid status										
Day 1	3287.6 ± 2690.9	2196.9 ± 1715.5	982.9 ± 2670.4	3745.3 ± 3056.9	2036.2 ± 1763.3	1473.7 ± 2985.6	2782.8 ± 2106.9	2371.3 ± 1644.8	395.7 ± 2090.1	<0.01
Day 2	1710.7 ± 1221.1	1841.6 ± 1376.5	−61.4 ± 1388.4	1742.3 ± 1274.2	1708.4 ± 1269.5	65.5 ± 1417.5	1674.3 ± 1156.1	1993.8 ± 1475.1	−213.2 ± 1337.3	<0.01
Day 3	1641.5 ± 970.0	2013.4 ± 1384.2	−215.7 ± 1236.4	1634.4 ± 989.7	1903.6 ± 1369.1	−136.1 ± 1301.6	1650.2 ± 945.5	2145.7 ± 1391.2	−310.9 ± 1146.6	<0.01
Day 4	1632.2 ± 1035.7	2040.5 ± 1356.7	−228.0 ± 1172.4	1619.8 ± 1048.6	1951.4 ± 1337.0	−182.3 ± 1231.7	1648.1 ± 1019	2154.3 ± 1373.5	−282.7 ± 1094.9	<0.01
Day 5	1596.0 ± 935.7	2041.3 ± 1367.4	−216.6 ± 1070.5	1595.7 ± 966.5	1957.9 ± 1351.8	−177.6 ± 1130.5	1596.4 ± 892.3	2154.5 ± 1380.8	−263.3 ± 992.2	0.01
Day 6	1619.0 ± 911.7	1979.2 ± 1285.5	−159.5 ± 985.5	1606.3 ± 942.6	1926.0 ± 1246.2	−144.2 ± 1050.0	1637.4 ± 865.3	2054.6 ± 1336.1	−177.8 ± 902.3	0.25
Day 7	1610.5 ± 954	1939.9 ± 1271.4	−126.4 ± 897.9	1624.1 ± 1014.6	1842.2 ± 1280.1	−75.9 ± 969.1	1590.1 ± 855.0	2083.5 ± 1245.3	−186.9 ± 800.4	<0.01
Cumulative fluid status										
Day 1–3	6070.0 ± 3971.6	5328.4 ± 3484.3	705.8 ± 3710.0	6654.6 ± 4153.4	5075.6 ± 3409.5	1403.2 ± 4096	5427.2 ± 3656.2	5603.3 ± 3544.4	−128.4 ± 2981.3	<0.01
Day 4–7	4086.0 ± 3771.3	4993.3 ± 4711.1	−730.6 ± 2854.1	4523.5 ± 3761.8	5308.0 ± 4498.8	−580 ± 2995.6	3604.4 ± 3723.7	4650.3 ± 4910.4	−910.7 ± 2664.5	<0.01

[a] *Comparison of IO balance between the survivors and non-survivors. Data are presented as mean ± standard deviation.*

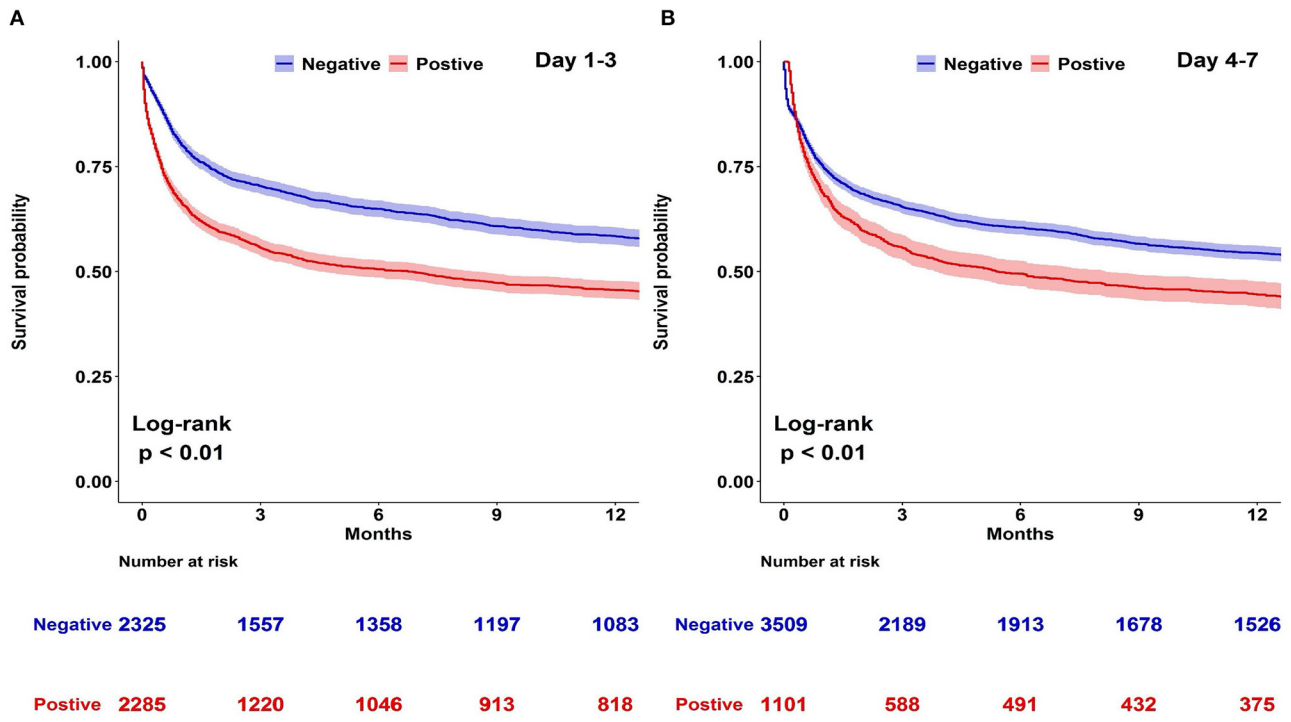

FIGURE 2 | Kaplan–Meier survival curves stratified by day 1–3 or day 4–7 cumulative fluid balance (CFB). Kaplan–Meier curves for long-term survival stratified by day 1–3 or day 4–7 CFB with log-rank test model among 4,610 patients admitted to medical intensive care units. **(A)** Day 1–3 CFB: negative vs. positive. **(B)** Day 4–7 CFB: negative vs. positive.

despite their AKI status. The analysis of CFB 4–7 and long-term mortality in patients with severe AKI (KDIGO stage 2 and 3) also produced similar results. Notably, in patients without AKI or mild AKI (KDIGO stage 1), those with a positive CFB 4–7 tended to have a higher likelihood of death. However, the difference was not significant. To conclude, CFB 1–3 and CFB 4–7 may have distinct impacts on long-term survival in patients with AKI. Despite these different risks, the results point in the risk of long-term survival of severe AKI patients in favor of the negative fluid balance group.

DISCUSSION

We addressed the association between early fluid balance and long-term mortality in critically ill patients and found that CFB 1–3 and CFB 4–7 positivity was independently associated with long-term mortality in patients with shock. Moreover, a positive CFB 4–7 was consistently associated with long-term mortality in critically ill patients without shock. These findings identify insights into the potential hazard of long-term mortality associated with positive fluid balance in critically ill patients and indicate future practical measures aiming a fluid status toward negative.

There is growing evidence that excess fluid administration may be detrimental to organ function (14). The FACCT trial addressed the 60-day mortality impact of liberal fluid management versus conservative therapy in patients with acute lung injury. They found no difference in mortality, but patients who received conservative fluid therapy had a shorter ventilator-day and ICU length of stay compared with those who underwent liberal fluid therapy (15). Messmer et al. performed a meta-analysis of 31 observational studies focusing on mortality association with fluid overload and found that a positive CFB 1–3 was associated with increased hospital mortality in critically ill patients (8). Several studies have further shown the clinical relevance of fluid balance in the first week among critically ill patients (5, 6, 16). Acheampong and Vincent (17) reported a positive association between the persistence of a positive fluid balance in the first week of ICU admission and high hospital mortality in 173 critically ill patients with sepsis. Similarly, Dhondup et al. (18), using a cohort comprised of 633 critically ill septic patients, reported that 61.1% of patients achieved a negative fluid balance during ICU admission, and a negative fluid balance tended to be associated with a lower 90-day mortality rate (36 vs. 44%; $p = 0.048$). In the present study, CFB 1–3 and CFB 4–7 positivity was common and highly associated with increased mortality. Collectively, this evidence indicates that a persistent positive fluid balance within the first week of ICU admission is relatively prevalent and associated with adverse outcomes among critically ill patients.

In this study, hospital mortality and 1-year mortality were 28.0% (1,291/4,610) and 46.7% (2,151/4,610), respectively. In other words, the post-acute 1-year mortality was 25.9%

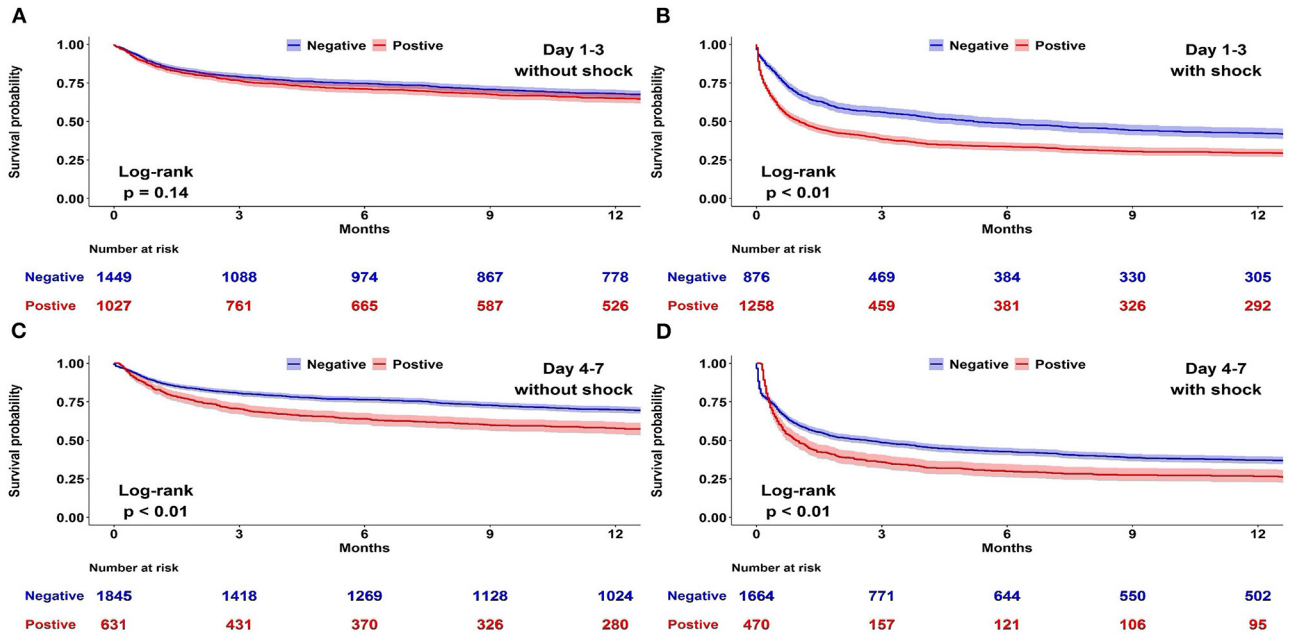

FIGURE 3 | Association between positive and negative cumulative fluid balance (CFB) and long-term survival stratified by shock status. Kaplan–Meier survival curves for negative and positive CFB among 4,610 patients admitted to medical intensive care units stratified by the presence of shock. **(A)** Day 1–3 CFB in patients without shock. **(B)** Day 1–3 CFB in patients with shock. **(C)** Day 4–7 CFB in patients without shock. **(D)** Day 4–7 CFB in patients with shock.

TABLE 3 | Cox proportional hazard regression analysis for long-term mortality.

Characteristics	Univariable		Multivariable**	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age, per year increment	1.017 (1.014–1.019)	<0.001	1.008 (1.006–1.011)	<0.001
Sex (male)	1.140 (1.049–1.238)	0.002	1.152 (1.060–1.252)	0.001
BMI, per 1 kg/m^2 increment	0.976 (0.967–0.984)	<0.001	0.963 (0.955–0.972)	<0.001
CCI, per 1 score increment	1.190 (1.164–1.217)	<0.001	1.093 (1.066–1.121)	<0.001
APACHE II score, per 1 increment	1.096 (1.089–1.102)	<0.001	1.063 (1.055–1.070)	<0.001
Presence of shock	2.564 (2.367–2.778)	<0.001	1.732 (1.582–1.897)	<0.001
Use of mechanical ventilation	2.096 (1.890–2.325)	<0.001	1.199 (1.072–1.340)	0.001
Active cancer	2.907 (2.637–3.206)	<0.001	2.633 (2.380–2.912)	<0.001
RRT initiated during ICU admission	2.305 (2.095–2.536)	<0.001	1.504 (1.356–1.668)	<0.001
RRT for end-stage renal disease	0.981 (0.772–1.246)	0.875	0.938 (0.735–1.197)	0.607
CFB of day 1–3*	1.096 (1.085–1.106)	<0.001	1.047 (1.037–1.058)	<0.001
CFB of day 4–7*	1.048 (1.033–1.064)	<0.001	1.094 (1.080–1.108)	<0.001

HR, hazard ratio; CI, confidence interval; BMI, body mass index; CCI, Charlson comorbidity index; APACHE II, acute physiology and chronic health evaluation II; RRT, renal replacement therapy; ICU, intensive care unit; CFB, cumulative fluid balance.

** Per 1 liter increment.*

*** Additionally adjusted for age, sex, BMI, CCI, APACHE II score, presence of shock, use of mechanical ventilation, active cancer, RRT initiated during ICU admission, CFB of day 1–3, and CFB of day 4–7.*

(860/3,319), and the prolonged mortality hazard tended to be prominent within the first 3 months. This finding was consistent with previous studies (10, 12, 19). For example, Mohr et al. recently investigated the short- and long-term outcomes of 830,721 patients with sepsis in Medicare claim database (10). They reported that hospital mortality and 90-day mortality were 20 and 48%, respectively. The long-term survival of our study coincides with prior research (**Figure 2**). Several studies have attempted to explore early determinants of long-term mortality in critically ill patients (12), but few studies have focused on fluid balance and long-term survival. Balakumar et al. conducted a retrospective single-center cohort study in which fluid balance during ICU stay was categorized as negative, even, or positive among critically ill patients (9). A positive

fluid balance was associated with a higher 1-year mortality than an even fluid balance. Although the aforementioned finding was consistent with our data; however, the population addressed in the study conducted by Balakumar et al. was different from our cohort in the medical ICUs, such as a high proportion of surgical admissions (59.2%) rather than medical admissions, fewer patients admitted due to sepsis than our study (15 vs. 48.5%), and a lower 1-year mortality (24.4%) than in our cohort (46.7%) (9). Therefore, fluid overload tends to be a crucial issue in patients admitted to either medical or surgical ICUs.

We recognized a positive fluid balance at different stages from the first week in ICU, including CFB 1–3 and CFB 4–7, as predictors of long-term mortality (**Figure 2**). Differences in survival between positive and negative fluid balance were most pronounced during the first 3 months. Similarly, 3-month is currently a critical time window to define the late recovery of organ failure after critical illness, such as AKI by the Acute Disease Quality Initiative (20). We hence postulate that the recovery of organ function in survivors may potentially mitigate the initial insults related to fluid balance. Aligned with our finding, van Mourik et al. found that a positive fluid balance after reversal of septic shock was associated with high long-term mortality (21). In detail, among the 636 patients with septic shock, a higher fluid balance in the ICU stay after reversal of shock correlated with an increased 30-day and 1-year mortality. Given that most patients achieved a reversal of shock within 2–3 days, the fluid balance in the study conducted by Mourik et al. appears to be comparable with the CFB 4–7 in our study. Indeed, the post-acute fluid balance might be overlooked after recovery from critical illness. Mitchell et al. explored fluid status on ICU-discharge in 247 patients who recovered from septic shock. They found that 35% (86/247) of patients had fluid overload, defined by an increase of body weight equal or higher than 10% of body weight on ICU admission (22). They reported that patients with a fluid overload on ICU discharge were less likely to ambulate on hospital discharge and tended to be discharged to a healthcare facility instead of home (22). Taken together, patient outcomes are not only dependent on early resuscitation but also potentially affected by the fluid status after resuscitation.

Indeed, fluid management may require different approaches depending on the time course of the disease (i.e., acute vs. post-acute period). We divided early fluid balance into CFB 1–3 and CFB 4–7 to address the different roles in distinct patient groups, such as patients without shock and with shock. In shock patients, the importance of early fluid balance and the achievement of a negative fluid balance in the de-escalation phase have been extensively studied (6, 23). However, evidence gaps exist regarding CFB and long-term mortality in non-shock patients. In patients without shock, accounting for 53.7% patients in our cohort, we found that CFB 4–7, instead of CFB 1–3, was associated with long-term survival. The long-term hazard of death of positive CFB 4–7 was not affected by age, sex, shock, or presence of sepsis (**Supplementary Table 2** in the **Supplementary Material**). Our finding highlights the importance of achieving a negative CFB in the post-acute phase even in patients without shock, which is potentially a modifiable target aimed at improved long-term outcomes.

We further point out the crucial role of fluid balance in patients with AKI. Owing to the fear that AKI might result from untreated hypovolemia, aggressive fluid administration is common in treating AKI patients. However, this practice is neither supported nor refuted by convincing clinical trials (1). The available knowledge about a higher CFB during AKI derives from observational studies focusing on short-term survival (24, 25). Thus, we specifically studied the role and the interaction of CFB with AKI and long-term survival. We found that negative CFB 1–3 had an inverse association with long-term mortality across all AKI stages (**Supplementary Figure 2**). In contrast, the significant survival importance of CFB 4–7 merely existed in AKI stage 2 and 3 patients, whose negative fluid balance was not easy to maintain without aggressive intervention. In a national sample of US veterans comprising 104,764 hospitalized patients (26), AKI developed in 16.3%. Most stage 1 AKI patients recovered within 2 days (71%), while slower AKI recovery was observed in patients with stage 2 and 3 AKI. The rapid restoration of renal function in less severe AKI echoes the lack of prognostic importance of CFB 4–7 in patients without AKI or mild AKI in our study. On the other hand, optimizing fluid balance in the post-acute phase among patients with severe and persistent AKI is of paramount importance and may be associated with long-term outcomes.

In the present study, the early fluid balance tended to be neutral in critically ill patients. For example, the mean CFB 1–3 was 1,403 ml and −128 ml among non-survivors and survivors, respectively. Therefore, we speculated less than half (48.5%) of enrolled patients had sepsis might account for the less requirement for aggressive fluid resuscitation. However, the enrollment of both septic and non-septic should reflect the nature of this real-world study. Moreover, the relative neutral fluid balance among patients enrolled during 2015–2019 aligns with a shift toward more restrictive fluid management in critically ill patients reported in recent trials (27).

A number of mechanistic studies have found prolonged fluid overload-associated deleterious effects, including microvascular abnormalities resulting from congestion within an encapsulated organ such as the kidney, impaired intestinal motility and nutrient absorption due to edema, dysregulated immunity possibly attributed to altered gut microbiota, and shedding of endothelial glycocalyx resulting in endothelial dysfunction (28–30). Furthermore, fluid overload may be alleviated by several measures, such as early vasopressor, higher dose of vasopressor, cautions for non-resuscitated fluid, fluid resuscitation guided by dynamic fluid responsiveness, early administration of diuretic, and protocolized diuresis (27, 31–34). Our data provides clinical evidence of a detrimental long-term impact of fluid overload and highlights that fluid balance during day 4–7 might potentially be an actionable target to alleviate fluid overload in critically ill patients.

There are limitations to be acknowledged. First, the observational design of the study does not allow the cause-and-effect relationship between CFB and outcomes to be inferred.

Second, this is a single-center study and the results may not be generalizable to other populations. Nevertheless, the main cause of ICU admission and the long-term mortality rate were consistent with previous studies (11, 35). Third, the confounders, such as diuretics prescription and the dose of vasopressors, cannot be assessed in this retrospective study although we have included variables such as RRT and the presence of shock in the regression model. Besides, we defined shock as a binomial variable although the presence of shock is not an on and off phenomenon. Fourth, we could not ascertain cause-of-death and data was presented as overall mortality. Fifth, we calculated CFB during ICU stay and did not include fluid management prior to ICU admission since fluid balance charting in the ward or emergency department might not be reliable (36). Given that bias could have resulted in underestimating the value of fluid overload, such a misclassification is more likely to influence the results toward the null hypothesis. Additionally, the individual physician made the decision of fluid therapy that could lead to a confounding effect. However, with dedicated intensivists and fluid management in accordance with the guidelines, it might partly mitigate the concern.

CONCLUSIONS

We linked two databases to address the long-term mortality association of fluid balance with the first week in critically ill patients. We found that patients with a positive CFB were independently associated with higher long-term mortality than those with a negative CFB. Patients with per one-liter increment of CFB 1–3 and CFB 4–7 significantly bore 4.7 and 9.4% risk odds of long-term mortality. Notably, we identified the role of a positive CFB 4–7 on long-term mortality in patients without shock or in those with severe AKI, indicating that CFB 4–7 might be a potentially modifiable factor to improve long-term outcomes. Further prospective studies focusing on both early fluid balance and post-acute fluid balance are warranted to validate our findings.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Taichung Veterans General Hospital (TCVGH: SE20249B). The Ethics Committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

T-JW, C-HC, C-LW, and W-CC: study concept and design. C-LW: study coordination. T-JW, C-LW, K-CP, L-TW, M-SW, and W-CC: acquisition of data. K-CP, L-TW, C-ML, and W-CC: statistical analysis. T-JW, C-TH, C-HC, C-LW, and W-CC: interpretation of data. T-JW and W-CC: drafting the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.727103/full#supplementary-material

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Global Research Status of Multiple Organ Dysfunction Syndrome During 2001–2021: A 20-Year Bibliometric Analysis

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Background: Multiple Organ Dysfunction Syndrome (MODS) is a major cause of high morbidity and mortality among patients in intensive care units (ICU). Although numerous basic and clinical researches on MODS have been conducted, there is still a long way to go to prevent patients from entering this stage. To our knowledge, no bibliometric analyses of MODS have been reported, this study, therefore, was conducted to reveal MODS research status and trends during 2001–2021.

Methods: All relevant literature covering MODS during 2001–2021 were extracted from Web of Science. An online analysis platform of literature metrology was used to analyze the publication trends. VOSviewer software was used to collect and analyze the keywords and research hotspots related to MODS.

Results: As of July 31, 2021, a total of 994 MODS-related articles from 2001 to 2021 were identified. The United States accounted for the largest number of publications (31.1%), followed by China and Germany, with 186 and 75 publications, respectively. Among all the institutions, the University of Pittsburgh published the most papers related to MODS (21). *Critical Care Medicine* published the most papers in this field (106). Professor Moore EE, who had the most citation frequency (1847), made great achievements in MODS research. Moreover, analysis of the keywords identified three MODS research hotspot clusters: "mechanism-related research," "clinical research," and "diagnostic research."

Conclusions: The United States maintained a top position worldwide and made the most outstanding contribution in the MODS field. In terms of publication, China was next only to the United States, but there was a disproportion between the quantity of

publications and citation frequency. The institution University of Pittsburgh and journal *Critical Care Medicine* represent the highest level of research in this field. During the 20 years from 2001 to 2021, basic MODS research has been in-depth yet progressed relatively slowly recently, but the outbreak of COVID-19 has to some extent set off an upsurge of clinical research in MODS field.

Keywords: MODS, bibliometric analysis, publication, keywords, research hotspots

INTRODUCTION

The high fatality rate of coronavirus disease (COVID-19) has posed a serious challenge to global health and epidemic prevention, and the latest research shows that this is closely related to the later progression of severe COVID-19 patients to Multiple Organ Dysfunction Syndrome (MODS) (1, 2). MODS is defined as the acute and potentially reversible dysfunction of two or more organs triggered by multiple clinical or non-clinical factors. The concept of MODS was first proposed in 1992, which was previously known as multiple organ failure (MOF) (3). Given that MOF could only be described statically, without showing a continuous process of multiple organ dysfunction, the concept of MODS came into being and gradually replaced MOF (4). The organ or system most easily affected by MODS successively include lung, cardiovascular system, liver, kidney, blood system, gastrointestinal tract, and central nervous system. The mortality in MODS patients increases with the number of organs involved. When only two organs become dysfunctional, the mortality is about 30%; when 3 or 4 organs are affected, the mortality will rise to 50–70% (5, 6).

Two primary causes of MODS are infectious and non-infectious factors, especially the former. Specifically, common causes, in addition to the most common cause of sepsis, include trauma, burn, surgery, shock, and so on (5, 7). Although an underlying pathophysiology for MODS remains elusive, global perfusion deficits (8), widespread endothelial damage (9), mitochondrial dysfunction/hibernation and associated energy deficit (10), intestinal bacterial product translocation (11), and apoptosis (12) have been implicated. These pathological mechanisms may aggravate the dysfunction of various organs (13–16). In recent years, mitochondrial dysfunction and abnormalities in the quality control methods of immune cells (the aggravation of apoptosis, the increase of pyrolysis, the dysfunction of autophagy and so on) have gradually attracted the attention of relevant researchers (17, 18). However, in the field of MODS, both basic research and clinical research are progressing slowly. It is no exaggeration to say that there has been no substantial breakthrough in the pathophysiology of MODS.

If we can summarize the current research status of MODS based on the available literature and analyze the current research difficulties and future research hotspots, it is expected to provide reference and develop new ideas for MODS related researchers. Bibliometrics is the tool to achieve the above goals. Bibliometrics refers to the cross-science of using mathematical and statistical methods to quantitatively and qualitatively analyze all knowledge carriers including books, periodicals and other publications (19). It is a comprehensive knowledge system that integrates mathematics, statistics, and philology, with a special focus on quantification. Bibliometric analysis can not only compare the contributions of different countries, institutions, journals, and scholars, but also describe a specific research field and predict the specific trends and future research hotspots, thus making important contributions to the prevention and treatment of diseases (20, 21). Although there are many bibliometric analyses on sepsis, the bibliometric research on MODS has not yet been reported, which means that there is a lack of comprehensive analysis of MODS and prediction of its research hotspots.

This research aims to analyze the content of MODS-related research literature based on Web of Science (WOS). We intend to use bibliometric methods to conduct a comprehensive analysis of the research status of MODS in the past 20 years, reveal research trends in this field and predict possible future research hotspots, in the hope of providing reference for the clinical treatment and scientific research of MODS.

MATERIALS AND METHODS

Data Sources and Search Strategies

The science citation index extension (SCIE) of Thomson Reuters Science Network is the most suitable database for bibliometric analysis. We used the WOS database to perform a comprehensive online search for literature published from 2001 to 2021. The article types were limited to original articles and reviews. The search strategy was as follows: TI = (Multiple Organ Dysfunction Syndrome) OR TI = (MODS) OR TI = (Organ Failure, Multiple) OR TI = (Multiple Organ Failures) OR TI = (Organ Dysfunction Syndrome, Multiple) AND Language = English. To avoid the prejudice caused by frequent updates of the database, all literature searches and data collection were completed within a single day on July 31, 2021. In addition, all data were obtained from public databases and did not involve any human subjects. For this reason, there was no need for informed consent. The

Abbreviations: MODS, Multiple Organ Dysfunction Syndrome; ICU, intensive care unit; PLA, People's Liberation Army; DC, dendritic cells; WOS, Web of Science; SCI-E, Science Citation Index-Expanded; TI, title; SIRS, systematic inflammatory response syndrome; AAY, average appearing year; IF, impact factor; JCR, Journal Citation Reports; JAMA, The Journal of the American Medical Association; SOFA, sequential organ failure assessment; APACHE II, acute physiology and chronic health evaluation II; RCTs, Randomized Clinical Trials.

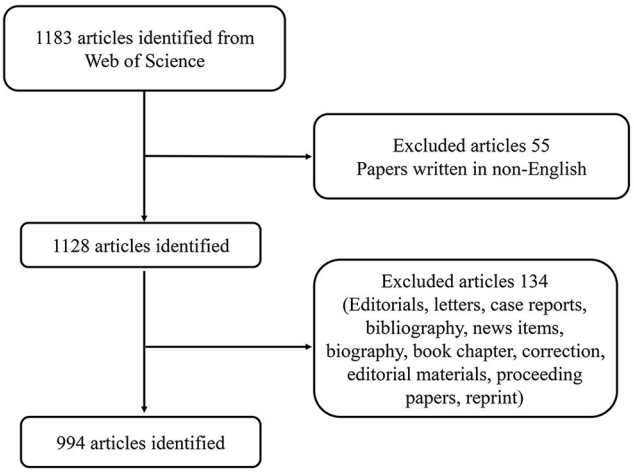

FIGURE 1 | Flow diagram of literature selection and screening in this study.

detailed process of literature selection and screening is shown in **Figure 1**.

Data Collection

Two reviewers (ZPY and XY) independently conducted primary search and extracted data from all qualified literature. The extracted data from WOS included titles, key words, publication dates, countries and regions, authors, institutions, journals, total number of citations, and H-index. Microsoft Excel 2016 (Redmond, Washington, USA), VOSviewer software (Leiden University, Leiden, Netherlands) and online platform of bibliometrics (http://bibliometric.com/) were used for qualitative and quantitative analyses.

Bibliometric Analysis

All publication characteristics of qualified documents in WOS, including country, institution, journal, author, H-index, etc., were recorded and described in detail. By examining the latest issue of JCR (Journal Citation Reports), we obtained the latest impact factor (IF) of the relevant journals, an important indicator to evaluate the academic influence of research (22). The H-index is an index to quantify an individual's scientific research output, which is defined as a scholar or a country has published h papers, and each paper have at least h citations (23). H-index can be obtained from WOS, reflecting the academic influence of scholars or countries/regions. In this study, we analyzed the number of publications and growth trends in different countries/regions annually using the online analysis platform of bibliometrics. VOSviewer software was applied to visualize keyword networks extracted from MODS research, thereby categorizing keywords into different clusters based on co-occurrence analysis. Moreover, it colored each keyword in line with their emerging time, for which we applied the definition of Average Year of Appearance (AAY) to quantify the relative novelty of keywords.

RESULTS

Global Growth Trends of Publications

A total of 994 articles published from 2001 to 2021 met our inclusion criteria (**Figure 1**). As shown in **Figure 2**, the global trend of published literature on MODS research was plotted. The years 2009 (63, 6.3%) and 2019 (57, 5.7%) were the 2 years with the highest volume of MODS-related publications. We also investigated the cumulative number of publications globally and found that the total publication of MODS-related literature has shown a trend of steady growth.

Meanwhile, we selected the top three countries with the highest volume of publications (the United States, China, and Germany), and compared their publication trends. The results showed that the trend of the United States was basically identical with that of the world, whereas publication in China displayed a relatively faster growth curve. Conversely, the Germany's growth trend showed a gradual downward curve (**Figure 3**).

Contribution of Countries to Global Publications

The United States ranked first regarding the number of publications (309, 31.1%), followed by China (186, 18.7%), Germany (75, 7.5%), England (71, 7.1%) and Japan (65, 6.5%). The detailed data of the top 10 countries/regions in terms of number of publications are presented in **Figure 4A**. The results of the cited frequency report from the WOS database showed that the 994 articles related to MODS were cited 21,363 times since 2001 (20,320 times without self-citation). The average citation frequency was 21.38 times per literature, and the H-index was 75. The citation frequency of the United States (9,773 times, 9,504 times without self-citation) accounted for 45.7% of the total. The average citations per literature was 31.63 times, with an H-index of 50. Publications by Germany were cited for 1,948 times (1,908 times without self-citation), with an H-index of 24, ranking second among all countries. China's publication number was second only to the United States in this field, but its citation frequency was merely 1,395 times with an H-index of 19, ranking 5th and 7th, respectively. What is worth mentioning is that the publications on MODS research conducted by Chinese scholars increased sharply since 2010. In 2012, China's annual publication volume surpassed the United States for the first time. Since then, the annual volume of MODS-related publications in China and the United States were comparable (**Figure 4B**).

To gain insight into the collaborative level between countries and regions worldwide, we mapped the cooperation of countries/regions using VOSviewer. Although the United States and China were the two leading countries with the greatest number of publications, the former had closer international cooperation compared to the latter, as evidenced by the central position of the U.S. among co-occurrence network (**Figure 5**).

Meanwhile, we conducted an analysis on publications in each country/region upon time course. The results showed that the papers in Europe, the United States, and other countries were predominantly published 10 years ago, while China published numerous MODS-related literature in the past 5 years (**Supplementary Figure 1**).

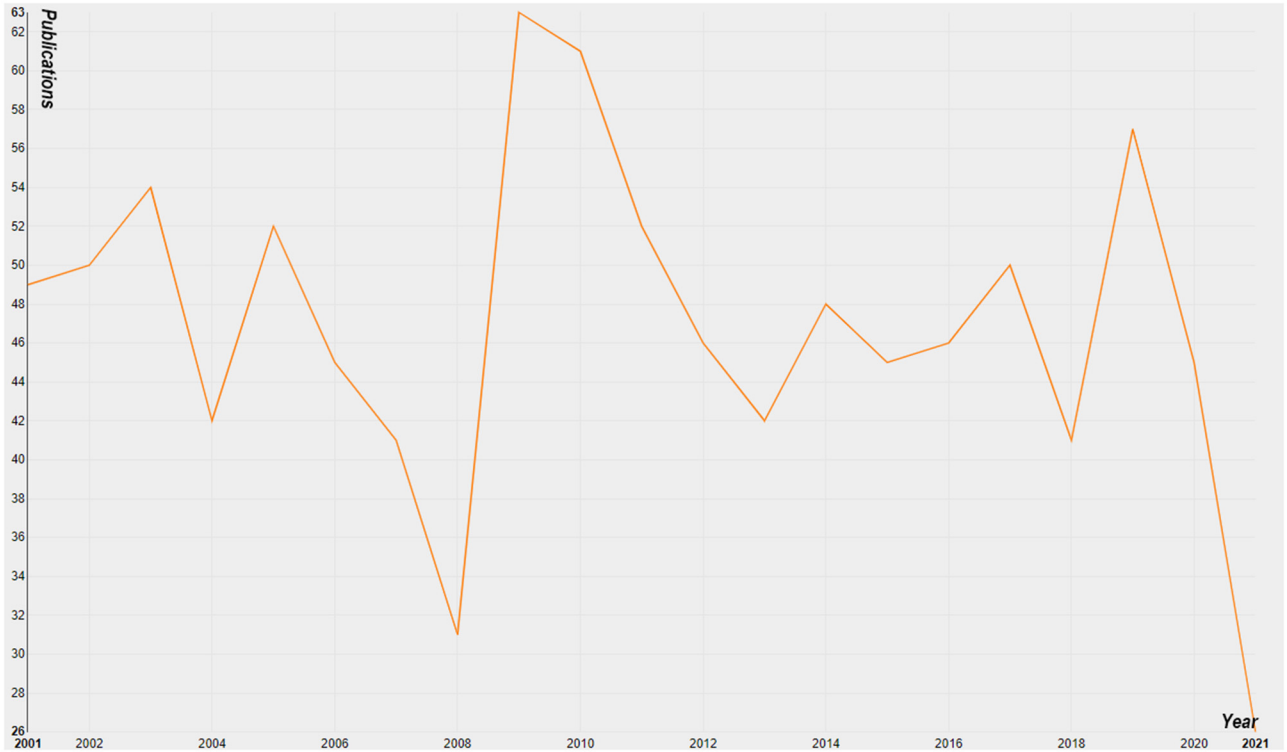

FIGURE 2 | The number of annual publications in MODS from 2001 to 2021.

Contribution of Institutions to Global Research on MODS

Globally, the University of Pittsburgh was the institution that published the highest number of MODS-related literature in the past 20 years (44, 4.4%). Among the top 10 institutions in this field, 7 of them were from the United States, and the other 3 were the University of Messina in Italy, the University of Halle-Wittenberg in Germany, and the University of Cayetano in Peru (**Figure 6A**). Furthermore, we analyzed the cooperative relationships of major institutions. The University of Pittsburgh and the University of Colorado in the United States located at the center of the map, indicating their close international cooperation. Besides, it was noteworthy to integrate and analyze the publications by major institutions over time. The results revealed that the top 10 institutions published MODS-related literature primarily 10 years ago, whereas the Third Military Medical University from China published several novel articles in recent 5 years (**Figures 6B,C**).

Journals and Authors Publishing Research on MODS

A total of 328 articles related to MODS were included in the top 10 journals with respect to the number of publications, accounting for approximately one-third (33%) of the overall publications. *Critical Care Medicine* (106), *Shock* (74) and *Intensive Care Medicine* (53) ranked the top three, with related literature accounting for 23.4% of all publications (**Figure 7**). Moreover, we listed the top 10 most influential MODS-related works based on the citation frequency, among which the most cited one entitled "Immunosuppression in Patients Who Die of Sepsis and Multiple Organ Failure" was conducted by Hotchkiss et al. and published on *Journal of the American Medical Association (JAMA)* in 2011 (24). The total citations and average annual citations of this paper were as high as 884 and 80.36, respectively. As to the top 10 papers, *Lancet* and *the Journal of Trauma* published two articles each, whereas the rest of articles were issued on other journals (**Table 1**).

There were 202 articles published by the top 10 authors, accounting for 20.3% of all MODS-related literature. Moore EE from the University of Pittsburgh published 30 MODS-related articles, which was cited 1,847 times, ranking first in both categories among global scholars. Italian professor Cuzzocrea S and American professor Carcillo JA published 27 and 24 articles on MODS research, ranking second and third, respectively. As shown in **Table 2**, among the top 10 most productive authors, four of them were from the United States, four came from Italy, and two were German authors. We further conducted the collaborative level analysis among the top-ranking authors using VOSviewer (**Supplementary Figure 2**).

Analysis of Keywords and Research Hotspots on MODS

We extracted keywords from the title and abstract of 994 eligible publications, and analyzed co-occurrence via VOSviewer. As

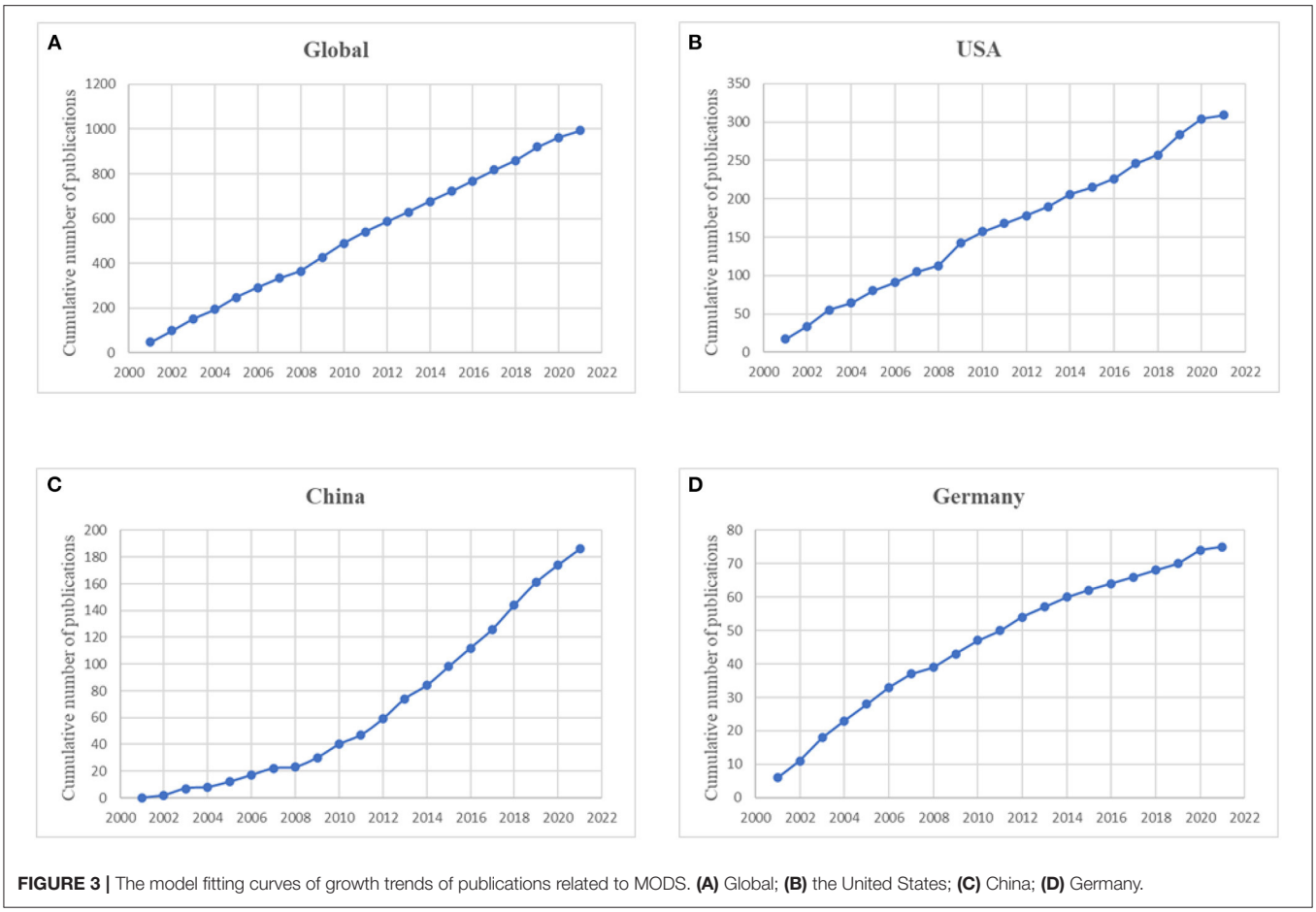

FIGURE 3 | The model fitting curves of growth trends of publications related to MODS. **(A)** Global; **(B)** the United States; **(C)** China; **(D)** Germany.

shown in **Figure 8A**, 128 keywords with more than 15 co-occurrences were subject to the mapping analysis, which yielded 3 distinct clusters: cluster 1 (mechanism-related research, red), cluster 2 (clinical research, green), and cluster 3 (diagnostic research, blue). The size of each keyword indicated its frequency of co-occurrence. In cluster 1, relevant keywords included effect (191 times), role (145 times), cell (97 times), expression (88 times) and increase (83 times). In cluster 2, frequently appearing keywords were score (171 times), intensive care unit year (135 times), age (93 times), year (93 times) and admission (90 times). In cluster 3, the main keywords were hospital (95 times), diagnosis (87 times), child (72 times), sample (66 times) and assay (51 times). **Supplemental Table 1** shows the detailed results of all 128 keywords.

In **Figure 8B**, we colored all keywords according to the time when the word appeared. The color of keywords represented their appearing time, with the color blue indicating early appearance and the color yellow recent emergence. As for 2001–2011, zymosan (cluster 1, keyword AAY 2007.5) peritonitis (cluster 1, keyword AAY 2007.5) and animal (cluster 1, keyword AAY 2007.9) were the main research topics. In addition, analysis of the hot vocabulary of cluster 1 revealed "oxidative stress" (cluster 1, AAY 2013.4), blood urea nitrogen (AAY 2012.7) and pathway (AAY 2012.2) might be the research hotspots. In cluster 2 (clinical research), the latest hot word was "area" (AAY 2014.3), appearing 47 times. In cluster 3 (diagnostic Science Research), "detection" (AAY 2013.7) and "performance" (AAY 2013.7) were considered as the novel keywords, which appeared 24 and 21 times, respectively.

DISCUSSION

Research Trends in MODS

It could be clearly seen from **Figure 2** that 2009 and 2019 were the 2 years with the highest volume of MODS-related publications. The search of MODS-related literature published in 2009 and 2019 revealed that many international conferences on critical illness, war trauma and microbiology were held in these 2 years. These conferences greatly stimulated the publication of MODS-related articles. Through further exploration, we found that the article "The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome" published in 2008 innovatively proposed that inflammatory factor storm was closely related to MODS, which might be one of its important pathogeneses (25). This conclusion undoubtedly provided a huge impetus for the related research of MODS. Of note, "The Third International Consensus

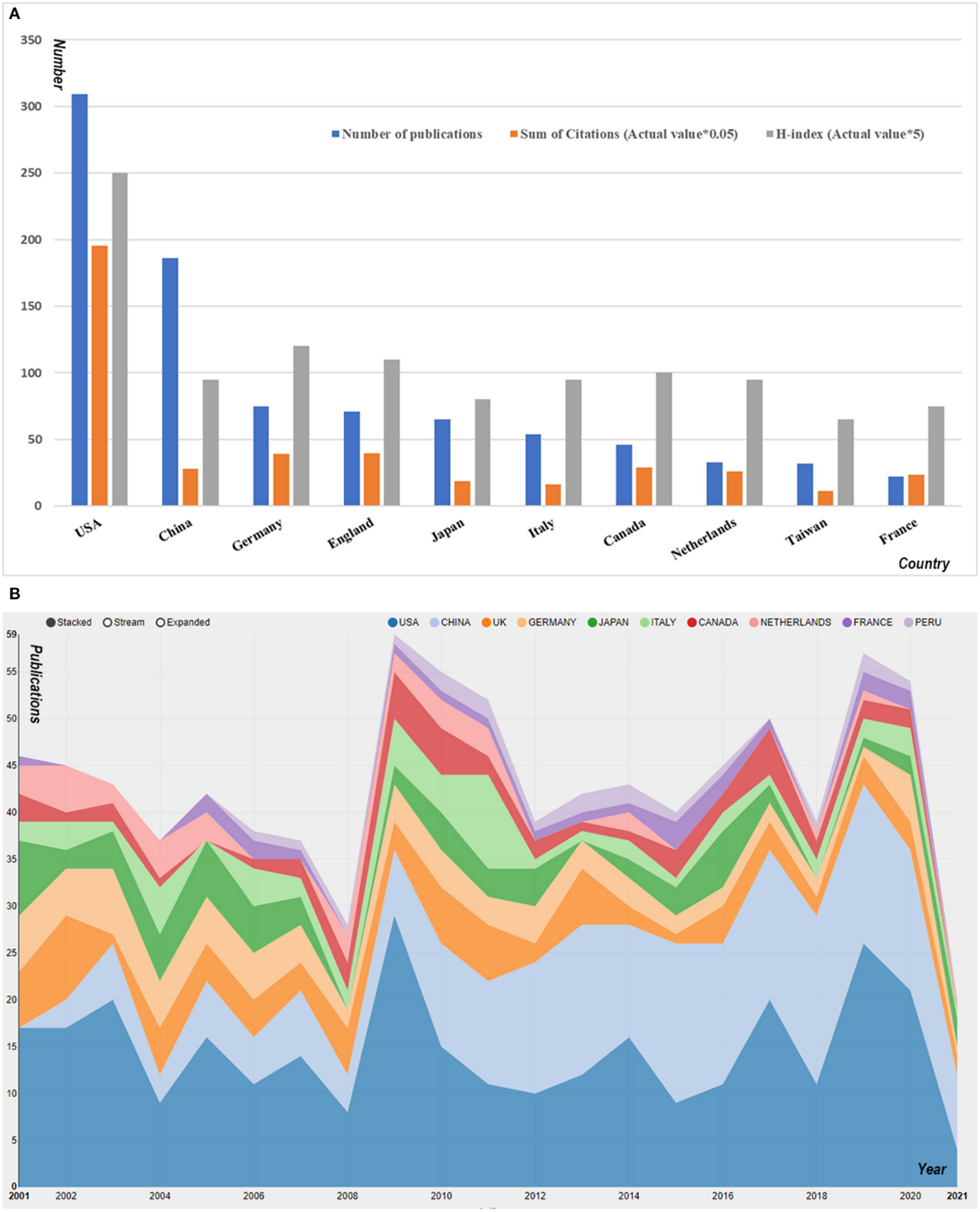

FIGURE 4 | The contributions of different countries/regions to the research field concerning MODS. **(A)** The number of publications, citation frequency (×0.05), and H-index (×5) in the top 10 countries or regions. **(B)** The growth trends of the top 10 countries/regions in MODS from 2001 to 2021.

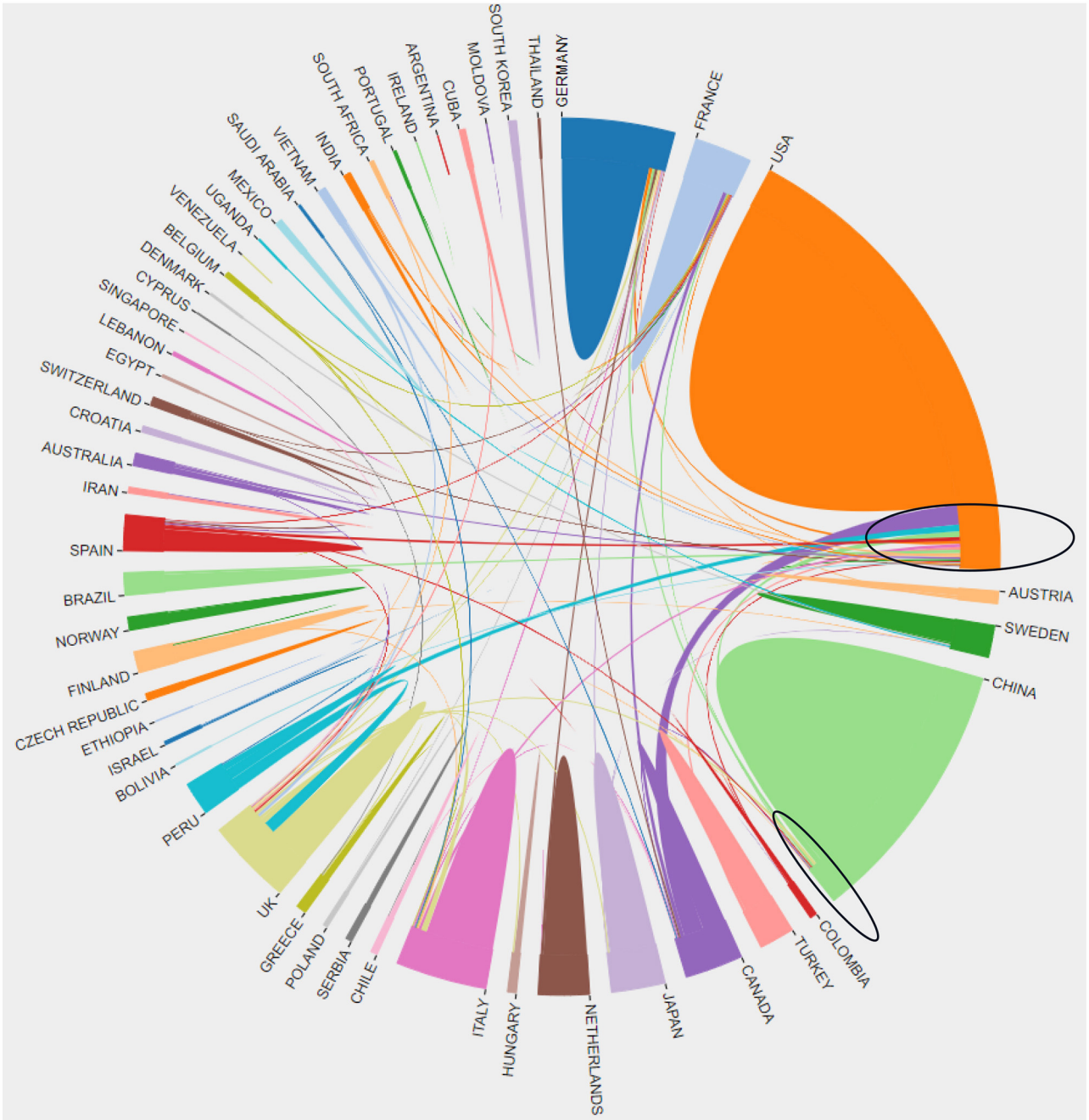

FIGURE 5 | The cooperation of countries/regions in MODS from 2001 to 2021.

on Sepsis and Septic Shock (Sepsis-3)" defined sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection, reflecting that MODS was the terminal stage of sepsis (26). This new definition of sepsis triggered a climax of MODS research. Given the periodicity of the publications, it was not difficult to understand the blowout of MODS-related articles in 2019.

The United States, Germany, and the United Kingdom ranked the top three in terms of total citations and H-index in the MODS field. The United States made the most outstanding contribution to the development of global MODS research, as evidenced by the number of published articles, the frequency of citations and the H-index. The concept of MODS was first proposed by American and European scholars, indicating that the United States paid attention to this issue earlier than most countries in the world. In addition, the conditions for basic medical research and clinical trials in the United States seemed to be superior, characterized by advanced equipment, professional researchers,

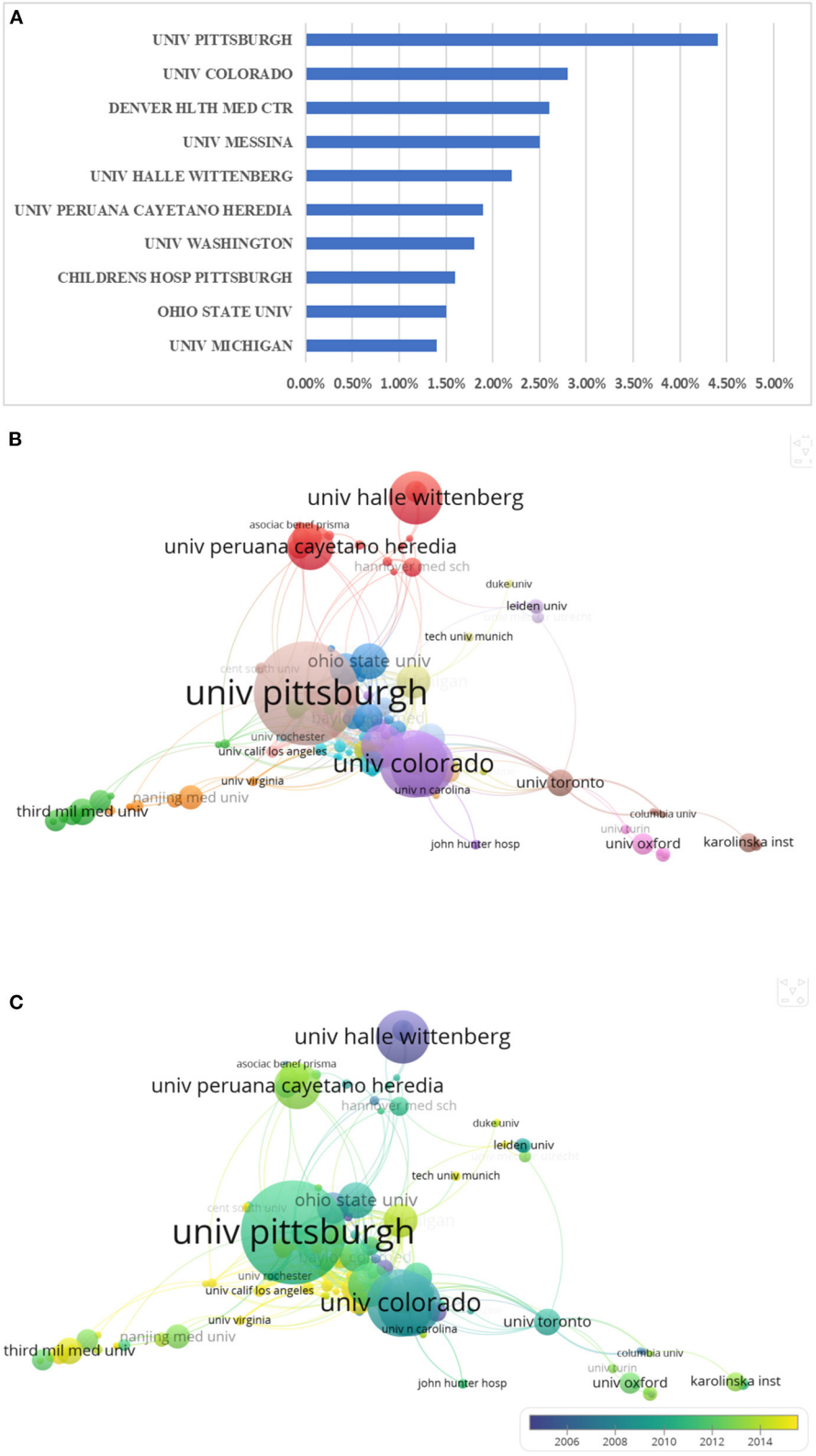

FIGURE 6 | The contributions of different institutions focusing on MODS. **(A)** Distribution of top 10 institutions focusing on MODS; **(B)** The cooperation of institutions in MODS, the circle with a large size represented the institution that published more articles; **(C)** Distribution of institutions was presented according to the appearance for the average time.

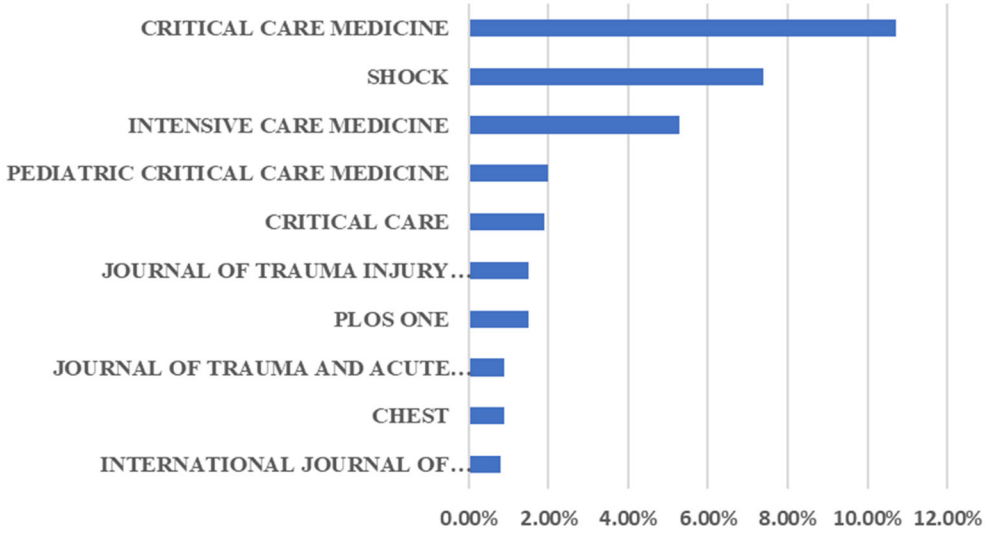

FIGURE 7 | The top 10 journals publishing research on MODS.

and sufficient funds. Moreover, the United States has some high-impact journals, excellent institutions and prolific authors related to MODS, and numerous high-level and influential international conferences were held in the United States. All of these advantages made the United States a leader in this field.

It was worth noting that although China ranked second in the total number of publications, it ranked only fifth and seventh in terms of citation frequency and H-index. There may be many reasons for the imbalance between the quantity and quality of Chinese publications. First of all, Chinese researchers first published an article in the related fields in 1992, but the number of annually-published articles was relatively small before 2009 (27). Therefore, it will take longer for China's citation frequency to catch up with that of other countries. Second, the diagnosis and treatment of MODS lacks standardization in China. In most Chinese hospitals, even in tertiary hospitals, medical staff do not regularly perform MODS-related scoring on critically ill patients, resulting in a high missed diagnosis rate for MODS patients. Third, China lacks high-quality multi-center randomized clinical trials (RCT) to provide reliable evidence for clinical practice. Additionally, compared with developed countries in Europe and the United States, the medical infrastructure and health system in China were still relatively backward, which to some extent limited the advance of basic and clinical MODS-related research in China. Finally, some high-quality literatures published in non-English journals may also be part of the reasons.

It can be seen from the time curve that since 2009, the number of articles published by China grew rapidly. Regrettably, although China has ranked second in the world in the total number of published articles in the past 20 years, it lags behind Germany, the United Kingdom and Canada in terms of the citation frequency and H-index. In addition, H-index of Italy and the Netherlands was comparable to that of China, although the number of publications by these two countries ranked only the 6th and 8th, respectively. Thus, it is urgent to improve the quality of scientific research papers in China.

Among the top 10 institutions in the MODS field, the United States boasted seven, demonstrating its absolute dominance in this field. The top three institutions that published the most articles in this field were the University of Pittsburgh, COLORADO University, and Denver HLTH MED CTR, which are all in the United States. These results indicate that the United States has the most elite institutions and also explain why this country maintains a leading position in the MODS field. There were three other universities on the top 10 list, namely the University of Messina in Italy, the University of Halle-Wittenberg in Germany, and the University of Cayetano in Peru. However, the imbalanced distribution of top institutions means we are still far from reaching the goal of "scientific research without borders," suggesting that more elite institutions outside the United States should involve in MODS-related research to provide more impetus for basic and clinical research.

As to the journals, *Critical Care Medicine* published 106 papers in the field of MODS, far ahead of other journals. *Shock* and *Intensive Care Medicine* were the other major journals publishing MODS-related articles. This indicates that the focus and hotspots of future advances in this field may appear in the above-mentioned journals.

In terms of authors, Moore EE and Carcillo JA from the United States and Cuzzocrea S from Italy published the most articles related to MODS. Moore EE and Carcillo JA mainly explored the dysfunction of regulatory T cells and macrophages in the pathogenesis of sepsis, while Cuzzocrea S focused on the potential role of neutrophils in sepsis and attempted to regulate the functions of neutrophils to reduce the pathological changes of sepsis. Although Cuzzocrea S from University of Messina ranked second in the total number of published papers, he ranked only seventh in terms of citation frequency, which may be

TABLE 1 | Top 10 high-cited papers related to MODS.

Title	Corresponding authors	Journal	IF	Publication year	Total citations	Average per year
Immunosuppression in Patients Who Die of Sepsis and Multiple Organ Failure	Hotchkiss, Richard S.	JAMA	56.272	2011	884	80.36
The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome	Aird, W. C.	BLOOD	22.113	2003	763	40.16
Neutrophils in development of multiple organ failure in sepsis	Treacher, D. F.	LANCET	79.320	2006	412	25.75
Post-injury multiple organ failure: The role of the gut	Moore, F. A.	SHOCK	3.450	2001	389	18.52
Continuous venovenous hemodiafiltration vs. intermittent hemodialysis for acute renal failure in patients with multiple-organ dysfunction syndrome: a multicenter randomized trial	Dhainaut, Jean-Francois	LANCET	79.320	2006	376	23.5
Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome	Marshall, J. C.	CRITICAL CARE MEDICINE	7.590	2001	284	13.52
Both primary and secondary abdominal compartment syndrome can be predicted early and are harbingers of multiple organ failure	Moore, F. A.	THE JOURNAL OF TRAUMA	NF	2003	271	14.26
Fresh Frozen Plasma Is Independently Associated With a Higher Risk of Multiple Organ Failure and Acute Respiratory Distress Syndrome	Peitzman, Andrew B.	THE JOURNAL OF TRAUMA	NF	2009	238	18.31
Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms	Sturk, A.	THROMBOSIS AND HAEMOSTASIS	5.243	2001	215	10.24
The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome	Ma, Sui	AMERICAN JOURNAL OF EMERGENCY MEDICINE	2.462	2008	213	15.21

IF, impact factor; NF, Not found.

TABLE 2 | Top 10 authors with most publications in research scope of MODS.

Author	Country	Affiliation	No. of publications	No. of citations	Average	H-Index
Moore EE.	USA	University of Pittsburgh	30	1,847	61.57	21
Cuzzocrea S.	Italy	University of Messina	27	458	16.96	14
Carcillo JA.	USA	University of Pittsburgh	24	1,032	43	14
Mazzon E.	Italy	University of Messina	21	335	15.95	11
Werdan K.	Germany	Martin Luther University	21	543	25.86	10
Di Paola R.	Italy	University of Messina	18	220	12.22	11
Hall MW.	USA	University of Pittsburgh	17	566	33.29	7
Sauaia A.	USA	Denver Health Medical Center	16	731	45.69	10
Schmidt H.	Germany	Martin Luther University	14	467	33.36	7
Thiemermann. C	Italy	University of Messina	14	204	14.57	6

related to the late publication of his papers. It was worth noting that the institutions of the top 10 highly productive authors were relatively concentrated. To be specific, the four professors in Italy were all from the University of Messina; the three professors in the United States except Sauaia A were all from the University of Pittsburgh; the two professors in Germany were both from Martin Luther University. In addition, cooperation between the authors is of great significance to the research of MODS. For example, Moore E. E. was listed as a co-author in many papers of the above-mentioned authors, indicating that he has close cooperative relations with authors from different institutions. We believe that these researchers may play a unique and indispensable role in this field. Their research not only have a wide-ranging impact on the past development of the field, but also point out the direction of the hotspots and future development in this field.

Research Focuses on MODS

The most cited papers have great academic influence in the field. The details of the top 10 cited in the MODS field are shown in **Table 1**. The paper "Immunosuppression in Patients Who Die of Sepsis and Multiple Organ Failure" has been cited 884 times since its publication and is the most cited paper in MODS fields. This research was published in *JAMA* in 2011, and the corresponding authors were Hotchkiss and Richard S. They put forward for the first time that the biochemical, flow cytometry, and immunohistochemical results of sepsis patients who died in the ICU were consistent with those of sepsis patients died from immunosuppression. This study was the first to confirm the existence of an immunosuppressive state at the onset of sepsis. They groundbreakingly proposed that targeted immune-enhancement therapy for sepsis patients may be an effective method (24). The second and third highly cited articles studied the important role of endothelial cells and neutrophils in the pathogenesis of MODS, which were published in *BLOOD* and *LANCET*, respectively (15, 28). Both articles focused on the pathogenesis of MODS, emphasizing that immunosuppression caused by cellular dysfunction was the main abnormality in MODS patients. Timely improvement of the endothelial cell and immune cell dysfunction and accurate immunotherapy would possibly show important clinical significance in the pathogenesis of MODS. In fact, most of the top 10 cited papers are directed at the pathogenesis of MODS. The relationship between inflammation, immunity and the pathogenesis of MODS has always been the focus of research (14, 25, 29). Early elucidations of these mechanisms will hopefully promote therapeutic advances and help to reduce the mortality of MODS patients, although current ongoing or completed clinical studies of MODS are mainly focused on immune-related drugs (NCT03769844, 01186783, 03119701, and 03518203).

The most recent hotspot was the "survival curve" (cluster 2, AAY is 2016.7). In fact, 2 of the 5 newly emerged words were from the "clinical research" group, namely "Area Under the Curve" and "Survival Curve." As shown in the **Figure 8**, the "clinical research" cluster received slightly less attention than the "mechanism-related research" cluster. However, the former contained some new words that appeared in recent years, indicating that the research focus of MODS has gradually shifted from mechanism research to clinical research. Hence how to improve the survival rate of MODS patients has become the focus of relevant scholars. According to the cluster relationship diagram formed by keywords, we can clearly see that clinical research cluster was closely related to "diagnostic research" cluster, whereas "clinical research" and "mechanism-related research" clusters were relatively less connected, indicating that the conversion speed of basic research and clinical research in the field of MODS needs to be accelerated. In terms of mechanism research, a relatively new keyword was "oxidative stress," which is a negative effect produced by free radicals in the body and is considered to be an important factor leading to aging and disease (30). The appearance of this term in "clinical research" cluster also shows that the relationship between oxidative stress and poor prognosis of MODS has become a relatively new hotspot (31). It is worth mentioning that "China" is a new keyword in the "diagnostics and laboratory research" cluster. On the one hand, the research of Chinese scholars in MODS-related fields has increased, with a growing number of publications in China in recent years. On the other hand, COVID-19 suddenly broke out in Wuhan, China at the end of 2019, and most of the patients with severe illness progressed to MODS in the later stage (32, 33). These are major reasons why "China" became a hot keyword.

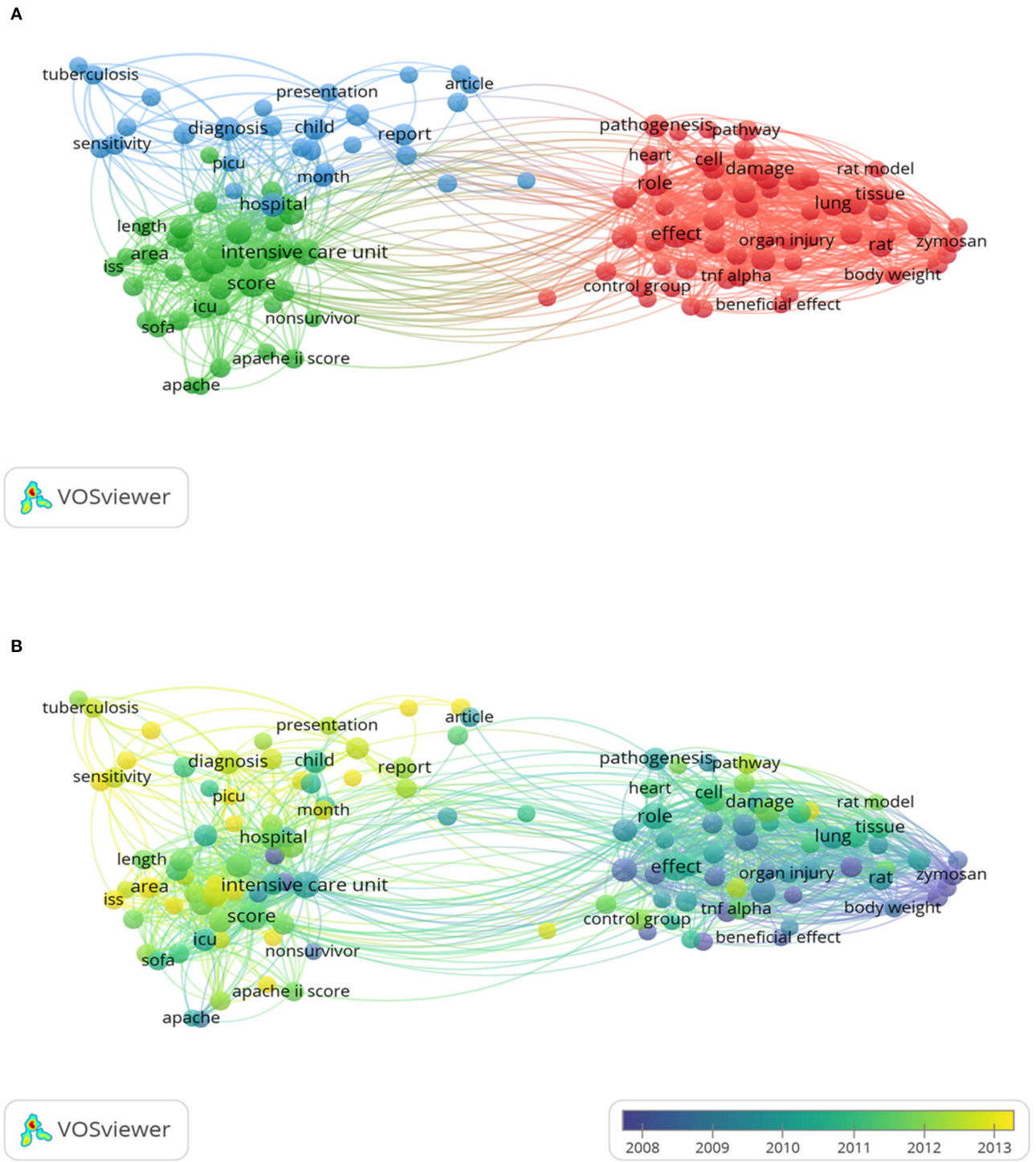

FIGURE 8 | The analysis of keywords in publications of MODS. **(A)** Mapping of the keywords in the area of MODS. The words were divided into 3 cluster in accordance with different colors generated by default: mechanism research (right in red), clinical research (left in green) and diagnostics and laboratory research (up in blue). The circle with a large size represented the keywords that appeared at a high frequency; **(B)** Distribution of keywords was presented according to the appearance for the average time. The blue color represented early appearance and yellow color recent appearance. Two keywords co-occurred if they both occurred on the same line in the corpus file. The smaller the distance between two keywords, the larger the number of co-occurrences of the keywords.

In general, through the analysis of the status quo and focus of MODS research, we hope to give some novel inspiration to relevant researchers. For example, in terms of basic research, the role of oxidative stress in the onset and deterioration of MODS is a current research hotspot. Mitochondrial metabolism and mitophagy are closely related to oxidative stress, thereby may mitochondrial metabolism and mitophagy be an innovative direction in the field of MODS? Currently, MODS-related clinical studies are mainly focused on survival and prognosis researches, the exploration of biomarkers that can predict poor outcomes in MODS patients and timely intervention are the priority of current and future research. In MODS diagnosis-related research, how to more accurately predict the occurrence of MODS in patients with COVID-19 is crucial to reducing the mortality rate of COVID-19 patients.

Strengths and Limitations

The MODS-related papers in this study were extracted from the core database of WOS, which is an extension of the scientific citation index. We comprehensively and objectively summarized the development status of MODS in the past 20 years from the perspective of bibliometrics and predicted and analyzed the research hotspots of MODS, which we believe will provide some reference for scholars in related fields to carry out researches. In addition, all searches were completed in 1 day, thus avoiding the deviation resulting from database updates. We assume that these latest publications may not be cited frequently in a short term, thus will not affect our conclusions. Nevertheless, some limitations are inevitable. First, due to our inclusion criteria, we only extracted English publications. Therefore, important researches in non-English language were ignored and excluded from the analysis, we tried to search these 55 non-English articles and found that these articles were mainly Russian or Latin ones, and conducting a separate analysis of these 55 non-English articles might be more reasonable. Second, we limited the types of articles to articles and reviews. Other influential work published in the form of conference abstracts or letters were also ignored. Third, we only included research papers published from 2001 to July 31, 2021, which means that no keywords before 2001 were included. Forth, in order to facilitate the analysis, we only searched the WOS core databases, and the MODS-related articles from the non-core and other database were ignored, which may also have a certain impact on our results. Finally, we mainly reflected the quality of publications from the perspective of citation frequency and H-index, and it is better to evaluate the quality of publications from a multi-dimensional perspective.

CONCLUSIONS

To sum up, this study summarizes global research status of MODS during 2001–2020. The United States made the greatest contribution in this important field. China, published the second largest number of papers, but the quality of these papers does need to be improved. The latest research and advances can be found in *Critical Care Medicine*, *Shock*, and *Intensive Care Medicine*. Moore EE, Cuzzocrea S, and Carcillo JA are academic leaders and have the most academic influence in this field. Basic research in MODS-related fields developed rapidly and received sufficient attention from researchers in the early stage. However, in recent years, the research hotspot of MODS has gradually transitioned from basic research to clinical research. The proportion of clinical research on MODS has gradually increased, but the transformation of basic mechanism research into clinical application still has a long way to go. We hope that this research could provide MODS-related researchers with a clear understanding of the current status and trends of MODS, and encourage more investigators worldwide to participate in this field for the sake of reducing the mortality rate of MODS patients.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

X-hD and R-qY conceptualized, supervised, and edited the manuscript. P-yZ, YX, and Z-bT extracted all data and performed the bibliometric analyses. ZM, S-yL, and X-pY undertook and refined the searches. P-yZ and YX co-drafted the paper. All authors contributed to and revised the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.814381/full#supplementary-material

Supplementary Figure 1 | The analysis of countries/regions in publications of MODS. **(A)** The cooperation of countries/regions in MODS, the circle with a large size represented the countries/regions that published more articles; **(B)** Distribution of countries/regions was presented according to the appearance for the average time.

Supplementary Figure 2 | The network map of productive authors engaging in MODS. **(A)** The circle with a large size represented the author that published more articles; **(B)** Distribution of authors was presented according to the appearance for the average time.

Supplementary Table 1 | The analytic consequence of 128 keywords with at least 15 occurrence times.

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ORIGINAL RESEARCH
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Effect of Serum Phosphate on the Prognosis of Septic Patients: A Retrospective Study Based on MIMIC-IV Database

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Objective: To assess the effect of serum inorganic phosphate (Pi) on the prognosis of patients with sepsis.

Methods: A retrospective analysis of patients with sepsis selected from the Medical Information Mart for Intensive Care (MIMIC)-IV database was performed. Sepsis was diagnosed according to the Third International Consensus Definition for sepsis and septic shock (Sepsis-3). The time-weighted values of the serum Pi measurements within the first 24 h of sepsis were analyzed. The association between serum Pi and in-hospital mortality was evaluated with a generalized linear model (log-binomial model).

Results: The analysis of 11,658 patients from six intensive care units (ICUs) showed a nearly linear correlation between serum Pi and in-hospital mortality in all patients with sepsis, especially in those with acute kidney injury (AKI). The increase of serum Pi was related to a higher risk of AKI, higher norepinephrine doses, ICU mortality, and in-hospital mortality. The generalized linear model showed that serum Pi was an independent predictor for in-hospital mortality in all patients with sepsis even within the normal range. The adjusted risk ratios (RRs) were also significant in subgroup analyses according to kidney function, gender, respiratory infection, vasopressor use, and Sequential Organ Failure Assessment (SOFA) score.

Conclusion: Higher levels of serum Pi, even within the normal range, were significantly associated with a higher risk of in-hospital mortality in patients with sepsis regardless of kidney function, gender, respiratory infection, vasopressor use, and SOFA score.

Keywords: sepsis-3, phosphate levels, prognosis, MIMIC-IV, septic shock

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INTRODUCTION

Sepsis is a complex condition that remains the major cause of morbidity and mortality worldwide, and the true global burdens of sepsis are likely much higher than reported (1, 2).

A better understanding of sepsis has been researched in the past three decades (3). In 2016, the Third International Consensus Definition for Sepsis and Septic Shock (Sepsis-3) defined sepsis as life-threatening organ dysfunction resulting from dysregulated host responses to infection, which offers greater consistency for epidemiologic studies and clinical trials (4). The hour-1-bundle

proposed by Surviving Sepsis Campaign in 2018 encourages clinicians to act as quickly as possible to make an accurate diagnosis and start appropriate treatment if clinically indicated (5).

Although great progress has been made into the pathobiology, management, and epidemiology of the disease, the high mortality is still unacceptable (2). The early identification of patients with sepsis at high risk of death allows clinicians to administer treatment in time. Inorganic phosphate (Pi) plays crucial roles in several aspects of physiological processes including energy metabolism, cellular signal transduction, and membrane transport (6, 7). Serum Pi disorders are common in critically ill patients, which can be attributed to several factors including gastrointestinal dysfunction, acute kidney dysfunction, and redistribution of Pi from the extracellular space into the cells (8, 9).

The predictive value of serum Pi has been studied in several specific patient populations. It was reported that even a minor increase in serum Pi was associated with a higher risk of several adverse outcomes including worsened heart failure and all-cause mortality in patients with heart failure (10–12). Numerous studies have confirmed the association between higher serum Pi and adverse outcomes in patients with chronic kidney disease (CKD) with or without kidney transplantation (13, 14). In the general population, it was also reported that a minor increase in phosphate at the base level was associated with a significant increase in all-cause mortality (15, 16).

Though serum Pi was considered to be a good predictor of adverse outcomes for several diseases, its prognostic value for patients with sepsis has not been well investigated yet, and the conclusions are mixed. For instance, in Shor et al's study, severe hypophosphatemia in sepsis increased the risk of death by nearly 8-fold (8). In contrast, in Miller et al.'s study, they concluded that patients with hyperphosphatemia had higher 28-day in-hospital mortality while those with hypophosphatemia did not (17).

Moreover, the heterogeneity of disease severity and classification are great in intensive care units (ICUs). Thus, it is of vital importance to re-evaluate the association between serum Pi and mortality in patients with sepsis and different septic subgroups. We aimed to assess the effect of serum Pi within the first day of sepsis on the prognosis of patients with sepsis. In particular, the subgroup analyses were performed according to the presence or absence of acute kidney injury (AKI) or CKD, gender, respiratory infection, vasopressor use, and Sequential Organ Failure Assessment (SOFA) score in this study.

MATERIALS AND METHODS

Study Design and Participants

The Medical Information Mart for Intensive Care (MIMIC)-III database provided critical care data for over 40,000 patients admitted to ICUs at the Beth Israel Deaconess Medical Center (BIDMC) from 2001 to 2012. Importantly, patient identifiers were removed according to the Health Insurance Portability and Accountability Act (HIPAA) Safe Harbor provision. MIMIC-IV, an update to MIMIC-III, incorporates contemporary data and improves on numerous aspects of MIMIC-III. The project was approved by the Institutional Review Boards of BIDMC (Boston, MA, United States) and the Massachusetts Institute of Technology (Cambridge, MA, United States). The latest version of MIMIC-IV was available on the PhysioNet, an online forum for the dissemination and exchange of recorded biomedical signals and open-source software for analyzing them (Johnson, Alistair, et al. "MIMIC-IV" (version 1.0), PhysioNet (2021), https://doi.org/10.13026/s6n6-xd98) (18).

The database is accessible to researchers who have completed a 'protecting human subjects' training. The data presented in this study were extracted by author Li, who completed the online training course of Data or Specimens Only Research (certification number: 38455531). Data extraction was performed using PostgreSQL tools V 12.4.

Patients meeting the diagnostic criteria of Sepsis-3 and older than 18 years old were enrolled in the study. For clinical operationalization, organ dysfunction associated with sepsis can be represented by an increase [Sepsis-related] in the SOFA score of 2 points or more, which is associated with an in-hospital mortality greater than 10%, and patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dl) in the absence of hypovolemia(4, 19).

Data Collection

The following information was extracted: age, gender, weight, pre-ICU comorbidities [hypertension, diabetes, CKD, coronary artery disease (CAD)], AKI, hospital and ICU length of stay (HLOS and ILOS), in-hospital mortality, ICU mortality, SOFA score, vasopressor use, white blood cell count, Serum Pi, serum creatinine, and blood lactate, infection sites. The definition of vasopressor use was any use of the following vasopressors, including norepinephrine, epinephrine, dopamine, and dobutamine, within the first 24 h of sepsis. The diagnosis and staging of AKI were according to the criteria of the Kidney Disease: Improving Global Outcomes (KDIGO) AKI Guideline Work Group (20).

The measurements of serum Pi and blood lactate within 24 h after the diagnosis of sepsis were extracted, and the time-weighted mean (TWM) values were calculated to represent the serum Pi and blood lactate level over the course by taking the area under the time-value curve divided by the time between the first and the last measurement assuming a linear trend between measurements (17). The serum levels of creatinine and the white blood cell count at the diagnosis of sepsis were extracted. The normal range of serum Pi is 2.7–4.5 mg/dl in MIMIC IV. Thus, crude outcomes were compared among the three groups based on the TWM values of the serum Pi measurements: hypophosphatemia (<2.7 mg/dl), normophosphatemia (2.7–4.5 mg/dl), and hyperphosphatemia (>4.5mg/dl).

Primary Exposure and the Primary Outcome

The primary exposure was serum Pi level and the primary endpoint was in-hospital mortality. The secondary endpoints

included ICU mortality, development of AKI, HLOS, ILOS, SOFA scores at diagnosis of sepsis, and norepinephrine doses.

Statistical Analysis

The data were analyzed using the software Stata V.12.1. Continuous variables are presented as mean ± SD or median with interquartile ranges (IQR) and compared by the Student's *t*-test and the Wilcoxon rank-sum test as appropriate. Categorical variables are reported as numbers and percentages and the comparisons were analyzed by Chi-squared and Fisher's exact tests. One-way ANOVA or the Kruskal–Wallis test was performed for comparisons of crude outcomes between the three groups. Locally weighted scatterplot smoothing (LOWESS) regression and logistic regression were used to explore the crude relationship between serum Pi and in-hospital mortality. It has been studied that the odd ratio always overstates the relative risk when the outcome incidence is common (>10%). A generalized linear model (log-binomial model) utilized to determine the adjusted RRs is statistically appropriate in this situation. This model is applied because the overall in-hospital mortality in this study was not rare (21.3%) (21).

The subgroup analyses were based on the diagnosis of CKD or AKI for the kidney is the main regulator of extracellular fluid Pi concentration by virtue of having a tubular maximum reabsorptive capacity for Pi (22). Also, subgroup analyses based on gender, respiratory infection, vasopressor use, and SOFA score were performed. In the subgroup analysis, according to SOFA score, the patients were divided into two groups according to the median of the SOFA score (3 in this study).

RESULTS

Demographic Data and General Clinical Characteristics

The MIMIC-IV contains records for 524,520 admissions, of which 69,619 were admitted to ICUs. Of these, 54,177 admissions without suspicious infections were excluded for no antibiotic

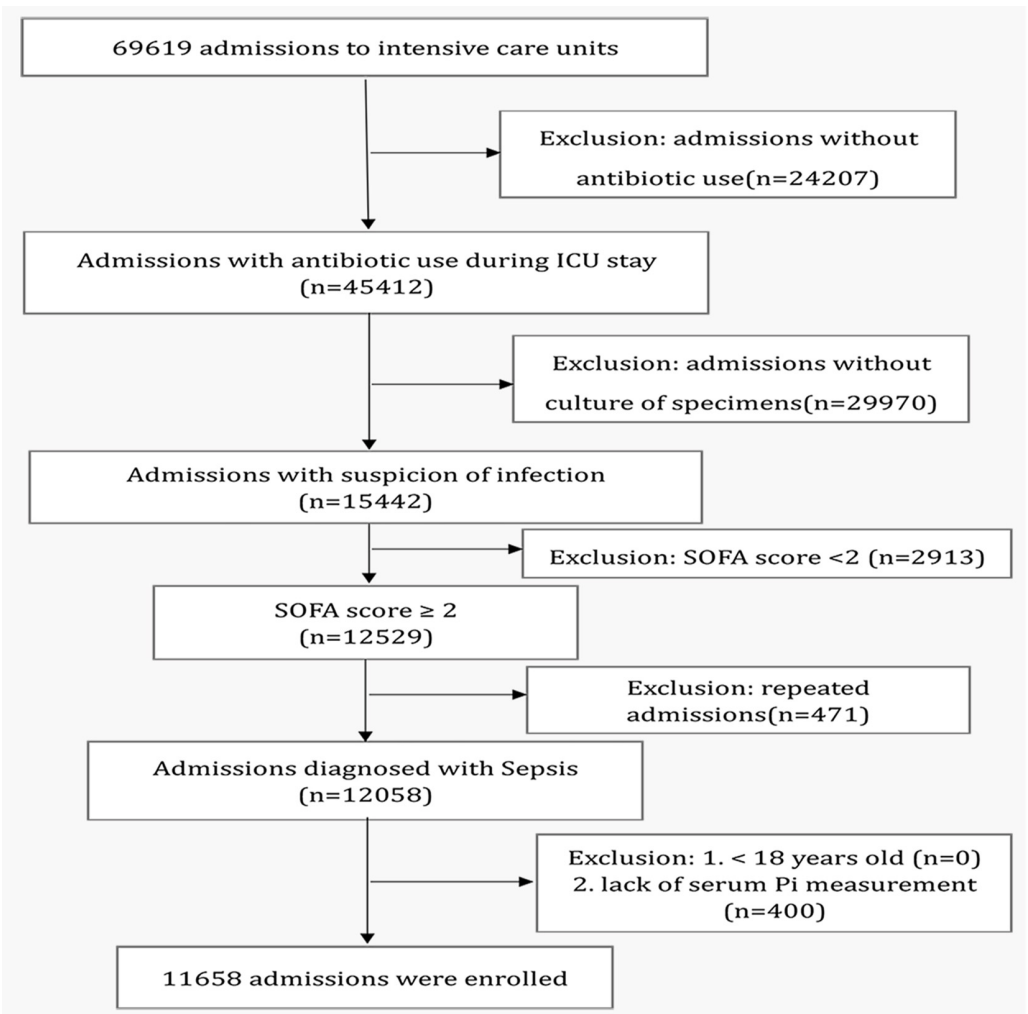

FIGURE 1 | Study flowchart. ICU, intensive care unit.

TABLE 1 | Comparisons of demographics between survivors and non-survivors.

	Survivors (*n* = 9179)	Non-survivors (*n* = 2479)	*P* value
Age (years)	66.7 ± 16.2	70.2 ± 14.9	< 0.01
Female(*n*%)	4427(48.2%)	1169(47.2%)	0.34
Weight (kilograms)	81.8 ± 26.9	80.2 ± 24.7	0.01
SOFA score	3(2-4)	4(3-6)	< 0.01
Vasopressor use[*n* (%)]	2130(32.2%)	1132(45.7%)	< 0.01
Hypertension [*n* (%)]	1962(21.4%)	479(19.3%)	0.03
Diabetes [*n* (%)]	2470(26.9%)	637(25.7%)	0.23
CKD [*n* (%)]	2251(24.5%)	694(28.0%)	< 0.01
CAD [*n* (%)]	1387(15.1%)	429(17.3%)	0.01
WBC (*10^9/l)	13.4 ± 10.9	15.3 ± 13.6	< 0.01
Creatinine (mg/dl)	1.77 ± 1.79	2.04 ± 1.66	< 0.01
Blood lactate (mmol/l)	1.92 ± 1.15	3.42 ± 3.06	< 0.01
Serum Pi (mg/dl)	3.96 ± 1.23	4.22 + 1.74	< 0.01
AKI [*n* (%)]	958(10.4%)	454(18.3%)	< 0.01
Infection site [*n* (%)]			
Respiratory system [*n* (%)]	2744(29.9%)	979(39.5%)	< 0.01
Digestive system [*n* (%)]	983(10.7%)	261(10.5%)	0.80
Urinary system [*n* (%)]	3057(33.3%)	621(25.1%)	< 0.01
Blood system [*n* (%)]	659(7.18%)	128(5.16%)	< 0.01
Other sites [*n* (%)]	3586(39.1%)	982(39.6%)	0.62

BMI, body mass index; SOFA score, the Sequential Organ Failure Assessment; CKD, chronic kidney disease; CAD, coronary artery disease; WBC, white blood cell; Pi, inorganic phosphate; AKI, acute kidney disease.

administration or culture of specimens during ICU stays. A total of 2,913 admissions were excluded for the SOFA scores were less than 2. A total of 12,529 admissions were considered to be patients with sepsis according to the definition of Sepsis-3. A total of 471 repeated admissions were excluded for we only take the patient's first admission to ICUs. In total, 400 admissions were excluded due to the lack of serum Pi measurement. Finally, 11,658 admissions (9,179 survivors and 2,479 non-survivors) from 6 ICUs, including trauma surgical ICU, neurosurgical ICU, medical ICU, postanesthesia care unit, coronary care unit, and cardiac vascular ICU were analyzed. All enrolled patients were older than 18 years old (**Figure 1**).

The baseline characteristics of the survivors and non-survivors are presented in **Table 1**. The overall in-hospital mortality was 21.3%. The non-survivors were older ($p < 0.01$) and had lower weight ($p = 0.01$), higher SOFA scores ($p < 0.01$), higher white blood cell count ($p < 0.01$), higher serum creatinine levels ($p < 0.01$), higher blood lactate levels ($p < 0.01$), and higher serum Pi levels ($p < 0.01$). A higher percentage of patients had hypertension ($p = 0.03$), CKD ($p < 0.01$), CAD ($p = 0.01$), AKI ($p < 0.01$), and vasopressor use ($p < 0.01$) in the non-survivor group than in the survivor group. Respiratory infection seemed to be associated with higher in-hospital mortality compared with urinary system infection and blood system infection.

Serum Pi and Outcome of Patients With or Without Kidney Dysfunction

The results for crude outcomes are listed in **Table 2**. These results revealed that hyperphosphatemia was associated with a longer duration of ICU stay ($p < 0.01$) and higher SOFA scores ($p < 0.01$). And the increase of serum Pi, even in the normal range, was related to higher risks of AKI ($p = 0.01$, $p < 0.01$), higher norepinephrine doses ($p < 0.01$, all), ICU death ($p < 0.01$, all), and hospital death ($p < 0.01$, all). **Figure 2** shows the unadjusted relationship between serum Pi and in-hospital mortality using LOWESS smoothing technique in different kidney function subgroups. A nearly linear relationship was found in all patients with sepsis, especially those with AKI (**Figures 2A,C**). This relationship was less clear for patients with sepsis with CKD and those without AKI and CKD (**Figures 2B,D**).

Serum Pi and In-Hospital Mortality of Patients With or Without Kidney Dysfunction

A logistic regression model with Pi < 1.5 mg/dl as the reference group was used to evaluate the unadjusted relationship between serum Pi and the risk of in-hospital mortality. The results were shown in **Figure 3**. It showed that the increase of Pi was related to higher odds ratios (ORs) of in-hospital mortality even in the normal range. But it was only significant for the extremely high values in the CKD and AKI subgroups (**Figures 3B,C**). Also, the OR of in-hospital mortality was higher among those with a serum Pi level less than 1.5 mg/dl compared with those with a serum Pi level between 1.5 and 2.5 mg/dl in the overall septic population and patients with sepsis without CKD and AKI though they are not statistically significant (**Figures 3A,D**).

Predictive Value of Serum Pi for In-Hospital Mortality

In order to eliminate the influences of possible confounding factors, an adjusted risk ratio was used to confirm the relationship between the Pi and in-hospital mortality. The results were shown in **Table 3**. Variables considered to be associated with in-hospital mortality and serum Pi homeostasis, i.e., variables with a *p*-value less than0.1 in **Table 1** and gender in this study, were included in the analysis. After adjusting for other confounders, including age, gender, SOFA scores, weight, vasopressor use, hypertension, CAD, CKD, AKI, white blood cell (WBC) count, serum creatinine level, blood lactate level, respiratory infection, urinary infection, and bloodstream infection, the log-binomial model indicated that Pi was an independent predictor of in-hospital mortality (RR 1.11, 95%CI 1.08–1.23, $p < 0.01$). It means that a 1 mg/dl increase in Pi was associated with an incremental increase of 11% in in-hospital mortality. Older age, higher SOFA scores, lower weight, more vasopressor use, more AKI, lower serum creatinine levels, and respiratory infection were also significantly associated with an increased risk of in-hospital mortality. The same model was built for the subgroup analyses. The results showed that Pi performed well in these subgroups too (RR 1.13, 95%CI 1.08–1.18, $p < 0.01$ for patients with sepsis with CKD; RR 1.08, 95%CI 1.03–1.13, $p < 0.01$ for patients with sepsis with AKI; RR 1.09, 95%CI 1.05–1.13, $p < 0.01$ for patients with sepsis without CKD and AKI; RR 1.09, 95%CI 1.05–1.13, $p < 0.01$ for male patients; RR 1.11, 95%CI 1.07–1.16, $p < 0.01$ for female patients; RR 1.13, 95%CI 1.08–1.18, $p < 0.01$ for patients with

TABLE 2 | Unadjusted relationships between serum inorganic phosphate (Pi) groups and crude outcomes.

Serum Pi [mg/dl, (*n*)]	<2.7(2749)	2.7-4.5(6486)	> 4.5(2423)	P_1P_2 value
ICU stay [day, median (IQR)]	4.58(1.96–12.1)	4.57(2.0–11.8)	5.16(2.19-12.7)	0.42 <0.01
Hospital stay [day, median (IQR)]	10.7(6.09–19.4)	10.8(6.06–19.8)	11.7(5.98-21.8)	0.78 0.14
SOFA score [median (IQR)]	3(2–4)	3(2–4)	4(3-6)	0.11 <0.01
Norepinephrine rate [mcg/kg/min, median (IQR)]	0.12(0.06–0.24)	0.15(0.08–0.29)	0.25(0.10–0.42)	<0.01 <0.01
AKI [*n* (%)]	235(8.55%)	674(10.4%)	502(20.7%)	0.01 <0.01
ICU mortality [*n* (%)]	312(11.3%)	931(14.4%)	713(29.4%)	<0.01 <0.01
In-hospital mortality [*n* (%)]	415(15.1%)	1221(18.8%)	843(34.8%)	<0.01 <0.01

P1 represents the p-value of comparisons between the hypophosphatemia group and the normophosphatemia group and p2 represents the p-value of comparisons between the normophosphatemia group and the hyperphosphatemia group; Pi, inorganic phosphate; SOFA score, the Sequential Organ Failure Assessment; AKI, acute kidney disease.

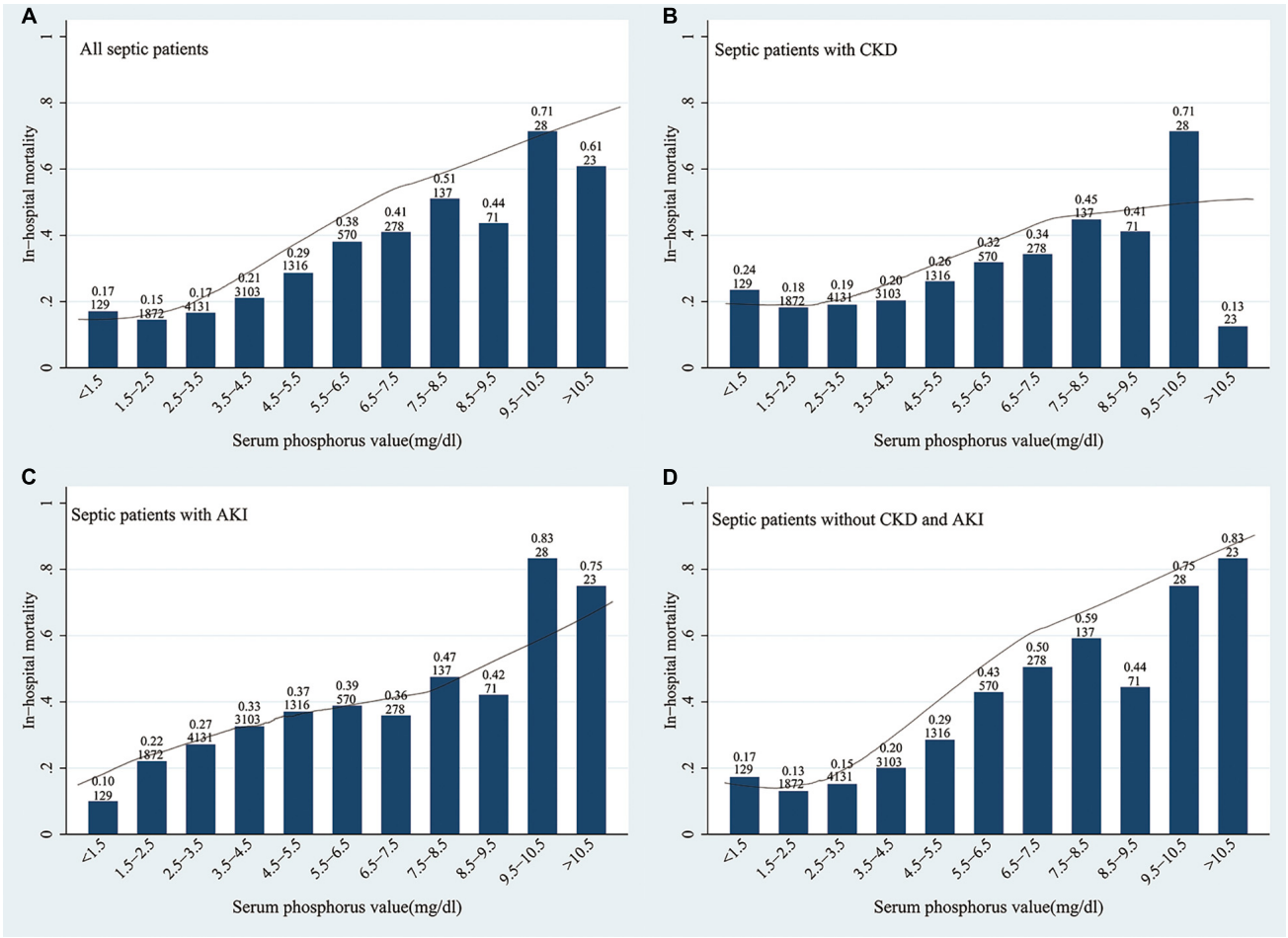

FIGURE 2 | Association between serum inorganic phosphate (Pi) and the in-hospital mortality of patients with sepsis with **(A–C)** or without **(D)** chronic kidney disease (CKD) and acute kidney injury (AKI). A nearly linear relationship was found in this figure, especially in the overall population **(A)** and the AKI subgroup **(C)**. Pi, inorganic phosphate; CKD, chronic kidney disease; AKI, acute kidney injury. The legends on the top of each bar mean the in-hospital mortality and number of admissions in each serum Pi group.

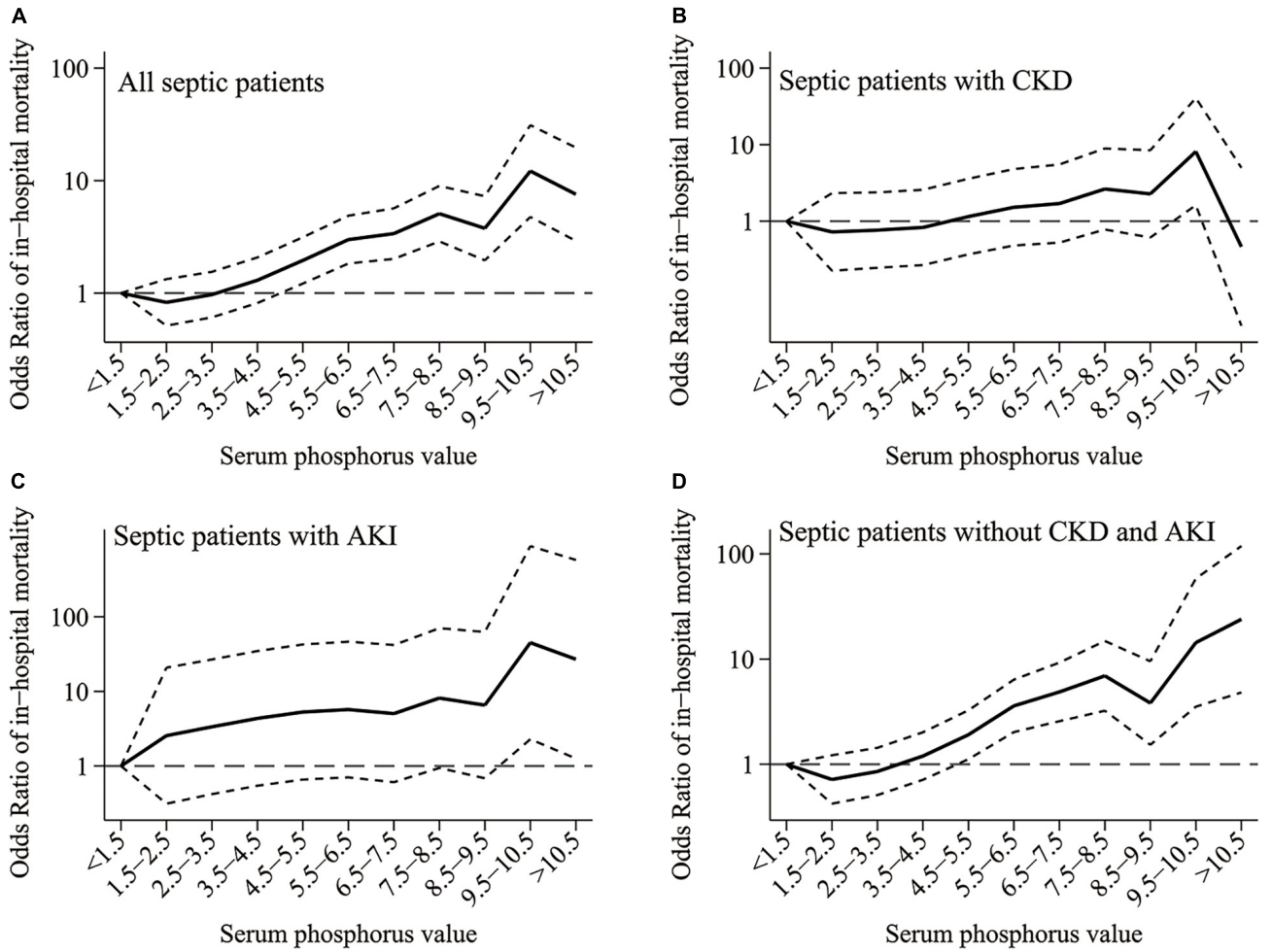

FIGURE 3 | (A–D) Unadjusted odds ratios (Ors) of in-hospital mortality with <1.5 mg/dl as the reference group in patients with sepsis with CKD and AKI or not. The figure shows higher Pi was related to a higher risk of in-hospital mortality even in the normal range though it was only significant for extremely high value in the CKD and AKI subgroups. CKD, chronic kidney disease; AKI, acute kidney injury.

respiratory infection; RR 1.10, 95%CI 1.06–1.13, $p < 0.01$ for patients without respiratory infection; RR 1.10, 95%CI 1.07–1.13, $p < 0.01$ for patients with vasopressor use; RR 1.09, 95%CI 1.05–1.14, $p < 0.01$ for patients without vasopressor use; RR 1.11, 95%CI 1.05–1.18, $p < 0.01$ for patients with SOFA score ≤ 3; RR 1.10 95%CI 1.07–1.13, $p < 0.01$ for patients with SOFA score >3. **Figure 4**).

DISCUSSION

In this study, we found that serum Pi was an independent predictor of in-hospital mortality in the overall septic population and subgroups categorized according to kidney function, gender, respiratory infection, vasopressor use, and SOFA score. Even in the normal range, a minor increase of Pi was also associated with a higher risk of in-hospital mortality. However, this study did not reveal hypophosphatemia as a risk factor for mortality. To our best knowledge, this is the first research studying the relationship between serum Pi and in-hospital mortality in patients with sepsis with a large sample size.

The prognostic values of serum Pi were well studied in many diseases, while published studies in critically ill patients mainly focused on hypophosphatemia (8, 23–25). Most of the studies either included too few cases or were single-centered. The conclusions about the relationships between serum Pi abnormalities and ICU outcomes were inconsistent. In Shor et al.'s study, they reported that severe hypophosphatemia increased the risk of death by nearly 8-fold (8). However, only 55 patients were included. The algorithm for selecting and calculating the serum Pi levels was not clear. A recently published study including patients with bloodstream infection (BSI) from different ICUs concluded that hypophosphatemia was independently associated with a two-fold increase in 90-day mortality in ICU patients with BSI (24). However, the serum Pi level was assessed by only a single measurement obtained during ICU admission. Similarly, the conclusion of a large, multicenter retrospective study that hypophosphatemia at admission was

TABLE 3 | Results of log-binomial model analysis.

Variables	B	SE	Z	Adjusted RRs	*P* value
Age (years)	0.02	0.00	10.36	1.02(1.01–1.02)	< 0.01
Gender (Female)	−0.00	0.04	−0.07	1.00(0.92–1.08)	0.92
SOFA score	0.07	0.01	10.11	1.07(1.06–1.09)	< 0.01
Weight (kilograms)	−0.00	0.00	−2.38	1.00(1.00–1.00)	0.02
Vasopressor use	0.41	0.07	9.45	1.51(1.38–1.64)	< 0.01
Hypertension	−0.08	0.05	−1.54	0.92(0.83–1.02)	0.12
CAD	−0.04	0.05	−0.67	0.97(0.87–1.07)	0.50
CKD	−0.01	0.05	−0.17	0.99(0.90–1.10)	0.86
AKI	0.23	0.07	4.48	1.26(1.14–1.40)	< 0.01
WBC(*10^9/l)	0.00	0.00	1.35	1.00(1.00–1.00)	0.18
Creatinine(mg/dl)	−0.05	0.01	−3.59	0.95(0.92–0.98)	< 0.01
Blood lactate(mmol/l)	0.09	0.01	13.16	1.10(1.08–1.11)	< 0.01
Respiratory infection	0.27	0.05	6.49	1.31(1.21–1.42)	< 0.01
Urinary infection	−0.27	0.03	−5.63	0.76(0.69–0.84)	< 0.01
Bloodstream infection	−0.18	0.07	−1.89	0.84(0.69–1.00)	0.06
Serum Pi(mg/dl)	0.10	0.01	7.68	1.11(1.08–1.23)	< 0.01

SOFA score, sequential organ failure assessment score; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; AKI, acute kidney injury; WBC, white blood cell; Pi, inorganic phosphate.

independently associated with increased risk of death was questionable for hypophosphatemia was defined as at least one measurement meeting the criteria of hypophosphatemia and no measurement of hyperphosphatemia (26). In contrast, using a similar definition of hypo- and hyperphosphatemia, Broman et al. did not find any difference between the hypophosphatemic group and the control group (27). In our study, the TWM values of serum Pi were calculated. We think this algorism could reflect the level of serum Pi more accurately. Similar to another study by Suzuki et al. (28), we found that the OR of in-hospital mortality of severe hypophosphatemia (<1.5mg/dl in this study) was higher but not statistically significant in the overall septic population and patients with sepsis without CKD and AKI. Although conclusions regarding the effect of hypophosphatemia on the prognosis of critically ill patients were different, these studies agreed that hyperphosphatemia was an independent predictor of poor prognosis (26, 27). Several other studies also confirmed the association between hyperphosphatemia and adverse outcomes in critically ill patients (17, 29, 30). In these studies, the higher risk of hyperphosphatemia for unfavorable outcomes was all compared to the normophosphatemia group. Many studies have shown that even a slight increase in serum Pi within the normal range was associated with a higher risk of adverse outcomes significantly in the non-critically ill population. However, this relationship in patients with sepsis has never been studied. In our study, the serum Pi was evaluated as a continuous variable. It showed that minor elevation was independently associated with a higher risk of in-hospital mortality whether in the normal range or not in septic patients.

The mechanism is still unclear. Possible explanations include low muscle strength, subclinical vascular disease, vascular calcification, cardiovascular disorders, etc. (12, 17, 31–34). Several studies have shown that an increase of serum phosphate within the normal range can independently predict a greater likelihood of vascular calcification or increased arterial stiffness in CKD and the general population (32). In Ginsberg et al.'s study, they reported that higher serum phosphate levels, even within the normal range, are associated with microvascular dysfunction in community-living individuals (33). In this study, we found that the higher serum Pi level was associated with a higher

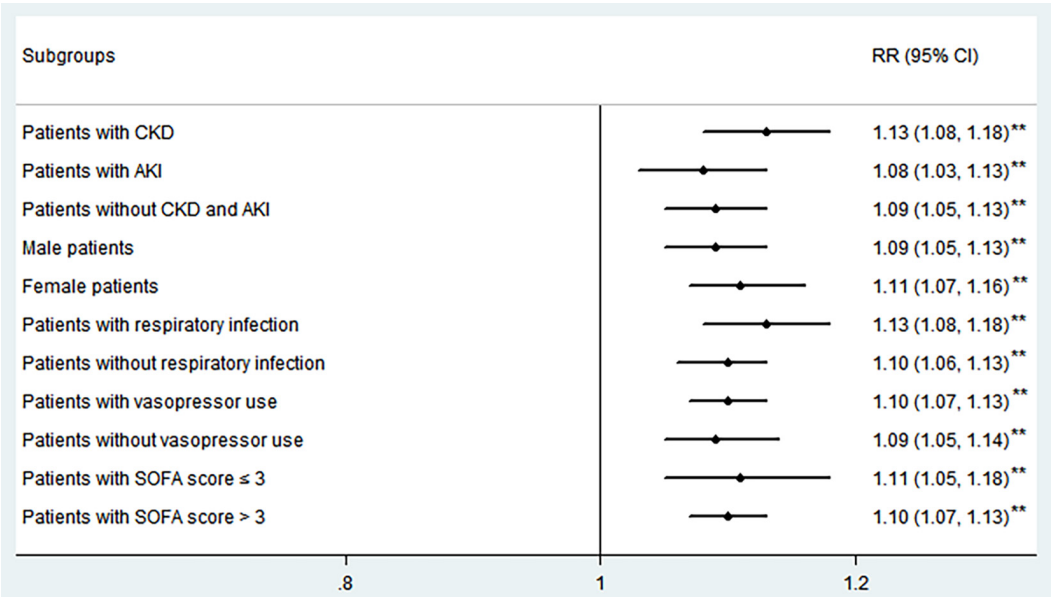

FIGURE 4 | Adjusted risk ratio (RR) of serum Pi for in-hospital mortality in different septic subgroups. $^{**}p < 0.01$; CKD, chronic kidney disease; AKI, acute kidney injury. SOFA score, sequential organ failure assessment score.

norepinephrine infusion rate. This may be attributed to vascular disorders associated with increased serum Pi as described above.

Also, higher quartiles of serum phosphate were found to have a significant association with lower muscle strength and a higher risk of dynapenia (31). This may explain the longer ICU stay associated with hyperphosphatemia in our study.

Whether higher Pi was a direct cause of increased mortality or a marker of disease severity is still unclear. More research studying the potential mechanisms and assessing the potential benefits of lowering serum Pi are needed.

The present investigation had several limitations. First, this study only used serum Pi measurements within the first 24 h of sepsis and did not study the effects of all measurements obtained during ICU stay and the changes in serum Pi on the prognosis of septic patients. Second, the baseline SOFA score was assumed to be zero, as we did not know if the patient has preexisting organ dysfunction before the onset of infection.

CONCLUSION

A minor increase of serum Pi, even in the normal range, could be closely associated with a higher risk of in-hospital mortality significantly in septic patients regardless of kidney function, gender, respiratory infection, vasopressor use, and SOFA score.

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://physionet.org/content/mimiciv/1.0.

AUTHOR CONTRIBUTIONS

ZL, TS, and YH conceived the idea. ZL completed the online training course of data or specimens only research and extracted the date. ZL and TS performed the analysis and drafted the manuscript. YH interpreted the results and helped revise the manuscript. All authors contributed to the article and approved the submitted version.

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Transcutaneous Electrical Acupoint Stimulation Decreases the Incidence of Postoperative Nausea and Vomiting After Laparoscopic Non-gastrointestinal Surgery: A Multi-Center Randomized Controlled Trial

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Importance: Postoperative nausea and vomiting (PONV) gives patients a bad experience and negates their good recovery from surgery.

Objective: This trial aims to assess the preventive effectiveness of transcutaneous electrical acupoint stimulation (TEAS) on the incidence of PONV in high-risk surgical patients.

Design: The large sample size, multicenter, evaluator-blinded, and randomized controlled study was conducted between September 3, 2019 to February 6, 2021.

Setting: The 12 hospitals were from different Chinese provinces.

Participants: After obtaining ethics approval and written informed consent, 1,655 patients with Apfel score $\geq$ 3 points were enrolled for selective laparoscopic non-gastrointestinal surgery under general anesthesia.

Interventions: Patients were randomly allocated into the TEAS and Sham group with a 1:1 ratio. The TEAS group was stimulated on bilateral Neiguan and Zusanli acupoints after recovery from anesthesia on the surgical day and the next morning for 30 min, while the Sham group received an identical setting as TEAS but

without currents delivered. Electronic patient self-reported scale was used to evaluate and record the occurrence of PONV.

Main Outcomes and Measures: Primary clinical end point is the incidence of PONV which was defined as at least one incidence of nausea, retching, or vomiting after operation within postoperative 24 h.

Results: Compared with the Sham treatment, the TEAS lowered the PONV incidence by 4.8% (29.4 vs. 34.2%, $P = 0.036$) and vomiting incidence by 7.4% (10.4 vs. 17.8%, $P < 0.001$). TEAS also lowered persistent nausea incidence and PONV scores and decreased PONV related complications and Quality of Recovery−40 scores ($P < 0.05$). TEAS lowered the 24 h PONV risk by 20% (OR, 0.80, 95% CI, 0.65 −0.98; $P = 0.032$), and lowered hazard ratio by 17% (HR, 0.83, 95% CI, 0.70–0.99; $P = 0.035$). Both TEAS and palonosetron were the independent PONV risk protective factors for 24 h PONV incidence and cumulative PONV incidence. The combination of TEAS and palonosetron was the most effective strategy to reduce the PONV incidence ($P < 0.001$).

Conclusions and Relevance: TEAS attenuated the PONV incidence and severity in high-risk surgical patients and may be applied clinically as a complement therapy to prevent PONV.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT04043247, identifier: NCT04043247.

Keywords: antiemetic drugs, laparoscopic non-gastrointestinal surgery, nausea, transcutaneous electrical stimulation, vomiting

KEY POINTS

- **Question:** Dose transcutaneous electrical stimulation prevent postoperative nausea and vomiting (PONV) in high-risk surgical patients?
- **Findings:** The electrical stimulation significantly reduced the PONV incidence and its severity. Both TEAS and palonosetron were independent PONV risk protective factors for 24 h PONV incidence and cumulative PONV incidence.
- **Meaning:** TEAS may enhance recovery of PONV high-risk surgical patients and should be routinely used clinically as a complementary strategy.

INTRODUCTION

Postoperative nausea and vomiting (PONV), characterized as nausea, retching, or vomiting or any these symptoms in combination after surgery, is one of the most common complaints after surgery with an overall incidence of 30%, but its incidence can be as high as to be 60–80% in high-risk patients who have three or four PONV risk factors (1). Nausea gives patients an extremely afflictive medical experience, induces stomach discomfort including the feeling of keck and poor appetite, and even causes dizziness and headache. Vomiting increases the potential risks of electrolyte imbalances, pain, incision dehiscence or hernia, dehydration, esophageal damage, or airway aspiration (2). PONV prevention has been included in enhanced recovery after surgery (ERAS) management strategy. Even though the incidence of PONV can be decreased by using less opioids and inhalational anesthetics and even using antiemetic drugs, its occurrence is still high, with up to 20% of high-risk patients treated with three antiemetic prophylaxis (1, 3).

Owning to multiple mechanisms triggering PONV, several antiemetic drugs are often used clinically in high-risk PONV patients (4), but their effectiveness is still limited and include unwanted side effects such as headaches, xerostomia, abnormal liver function, and extrapyramidal reactions (5–8). Compared to antiemetic drugs, non-pharmaceutical therapy including acupuncture has considerable advantages, for example, non-toxic effects, which has been widely used in postoperative pain analgesia and gastrointestinal function rehabilitation (9, 10). Indeed, acupuncture, a safe and effective non-pharmacotherapy, can directly or indirectly inhibit the emesis center or regulate gastrointestinal function through multiple regulatory mechanisms, such as modulating gastric motility through somatovisceral reflex and influencing the endogenous opioid system and serotonin transmission (11–14). Acupoint Neiguan (P6) stimulation was reported to be comparable to antiemetic drugs in reducing PONV incidence but its effectiveness in combination with antiemetic drugs is inconclusive (1). Transcutaneous electrical acupoint stimulation

Abbreviations: PONV, Postoperative nausea and vomiting; TEAS, transcutaneous electrical acupoint stimulation; QoR-40, the 40-item Quality of Recovery; P6, Neiguan; ST36, Zusanli; EX-LE5, Xiyan; 5-HT3, 5-hydroxytryptamine 3; PACU, postoperative recovery unit; VAS, visual analog scale score.

(TEAS) with transcutaneous electrical nerve stimulation applied to acupuncture points has been used to prevent PONV occurrence (15, 16). However, its effectiveness in preventing PONV has not been established yet due to small sampling size and being not well controlled in the previous studies (1). Therefore, the large sample size, multicenter, evaluator-blinded, and randomized controlled study was carried out to verify the effectiveness of TEAS on P6 and Zusanli (ST36) in reducing the incidence of PONV.

METHODS

Study Design and Participants

This multicenter randomized controlled trial was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTU1AF2019LSK-084) and then registered at ClinicalTrials.gov (NCT04043247). The detailed trial protocol is available online as **Supplementary Material 1**. The study was carried out in accordance with clinical research CONSORT and acupuncture clinical trial STRICTA to provide reliable concrete evidence for clinical application of TEAS in PONV prophylaxis (**Supplementary Material 2**). The following inclusion criteria were included: (1) selective laparoscopic non-gastrointestinal surgery under general anesthesia; (2) age 18–50 years, BMI 15–40 kg/m^2, ASA class I–III; (3) Apfel score $\geq$ 3 points; and (4) ability to understand, sign informed consent, and coordinate intervention and evaluation. The following exclusion criteria were included: (1) pregnant and breast-feeding patients; (2) TEAS contraindications such as skin allergy, breakage, infection or itching of test points, allergic to adhesive tape, pacemaker implanted; (3) history of alcohol, opioids, or other drug abuse; (4) likely admission to ICU after surgery; and (5) participation in other clinical studies within 3 months before admission to this study. All investigators were trained according to the standardized acupoints selection and TEAS manipulation by a senior licensed acupuncturist with 20 years of practicing experience.

Enrolment, Randomization, and Blinding

After written informed consent was obtained, patients scheduled for selective laparoscopic non-gastrointestinal surgery under general anesthesia were screened for potential enrolments from 12 hospitals (listed in the authorship) in China from September 3, 2019 to February 6, 2021. The eligible patients were randomly divided into the Sham and TEAS groups in a 1:1 ratio (with a block size of 4) by Crabyter scientific research system (Xinyu Information Technology Co., Ltd.). All patients, anesthesiologists, surgeons, and evaluators were blinded for group allocation, screening, intervention treatment, and statistical analysis. Designated evaluators who were totally blinded took charge for follow-up data collections.

Clinical End Points

Primary clinical end point is the incidence of PONV, which was defined as nausea, retching, and/or vomiting after operation within postoperative 24 h. Secondary clinical end points were the postoperative 24 h PONV severity and the 40-item Quality of Recovery (QoR-40) and PONV-related complications. The PONV severity was assessed with the time and visual analog scale (VAS) score of first PONV, the cumulative numbers, and VAS score in postoperative 24 h. The specific contents of QoR-40 score include five aspects: physical comfort (12 items, 60 scores), emotional state (9 items, 45 scores), physical independence (5 items, 25 scores), psychological support (7 items, 35 scores), and pain (7 items, 35 scores). PONV-related complications are dizziness and headache, electrolyte imbalances, and pain.

Anesthesia and Surgery

After receiving 0.5 mg pre-medication of anticholinergics drug penehyclidine, atropine, or scopolamine, patients were inducted with propofol (2 mg/kg), sufentanil (0.3 μg/kg), and cisatracurium (0.15 mg/kg), and maintained with remifentanil (0.1 μg/kg/h), cisatracurium (0.1 mg/kg/h), dexmedetomidine (initial dose 1 μg/kg for 10 min, maintenance dose 0.4 μg/kg/h), and sevoflurane (1% in 2 L/min enriched oxygen). Propofol (4–8 mg/kg/h) as necessary was used for anesthesia maintenance to keep BIS 40–60 throughout surgery. Patients were treated with 50 mg non-steroidal anti-inflammatory drug flurbiprofen axeril or 40 mg parecoxib at the end of surgery, and were intravenous injection pumped with sufentanil individualized postoperative analgesia pump to keep the perioperative pain numerical score at <4 points.

Interventions

Both groups received 5 mg dexamethasone (before induction) combined with 0.075 mg palonosetron (before induction) as the first choice. When palonosetron was not available at some hospitals, patients received 5 mg dexamethasone (before induction) combined with 2 mg tropisetron (at the end of surgery) instead. According to the criteria of WHO acupuncture points, P6 and ST36 were selected in this study (**Supplementary Figure 1**). These acupoints are far from the operation site. P6 is located at 2 inches above palm wrist transverse striation, between the palm long tendon and the temporal flexor tendon. ST36 is located at 3 inches below EX-LE5 (the lateral pit of the patella and patellar ligament), and one transverse finger outward lateral tibia. The true or sham TEAS was given to the TEAS or sham group patients, respectively, in the PACU when recovered from anesthesia (to be awake and calm, Richmond Agitation-Sedation Scale = 0) on the same surgical day and on the next morning of surgical ward for 30 min. The bilateral P6 and ST36 were pressed to achieve a tingling sensation called "de qi", and electrode slices were stuck and fixed on those acupoints. Interveners connected electrode slices to electrical stimulator (TEAS stimulator, SDZ-V; Hwato, China) and selected the distant-dense wave with the frequency of 2/10 Hz. After adjusting the intensity to the patient's maximum tolerance, the treatment lasted for 30 min. Patients in the sham group received identical settings and manipulation to the TEAS group, except that the wire between electrode pads and TEAS stimulator was cut so that no current could be delivered. The 5-HT$_3$ receptor

antagonists or propofol or haloperidol were used for PONV rescue when necessarily.

Data Collection

Electronic patient self-reported scale (Jiangsu Rehn Medtech Technology Co., Ltd.) was used to evaluate and record the occurrence of PONV, including time and corresponding severity. The severity was evaluated with VAS score which is a psychometric response scale to measure the intensity of subjective characteristics or attitudes and has been widely used for pain quantification. In our study, the severity of nausea and vomiting was rated on the VAS score from 0 to

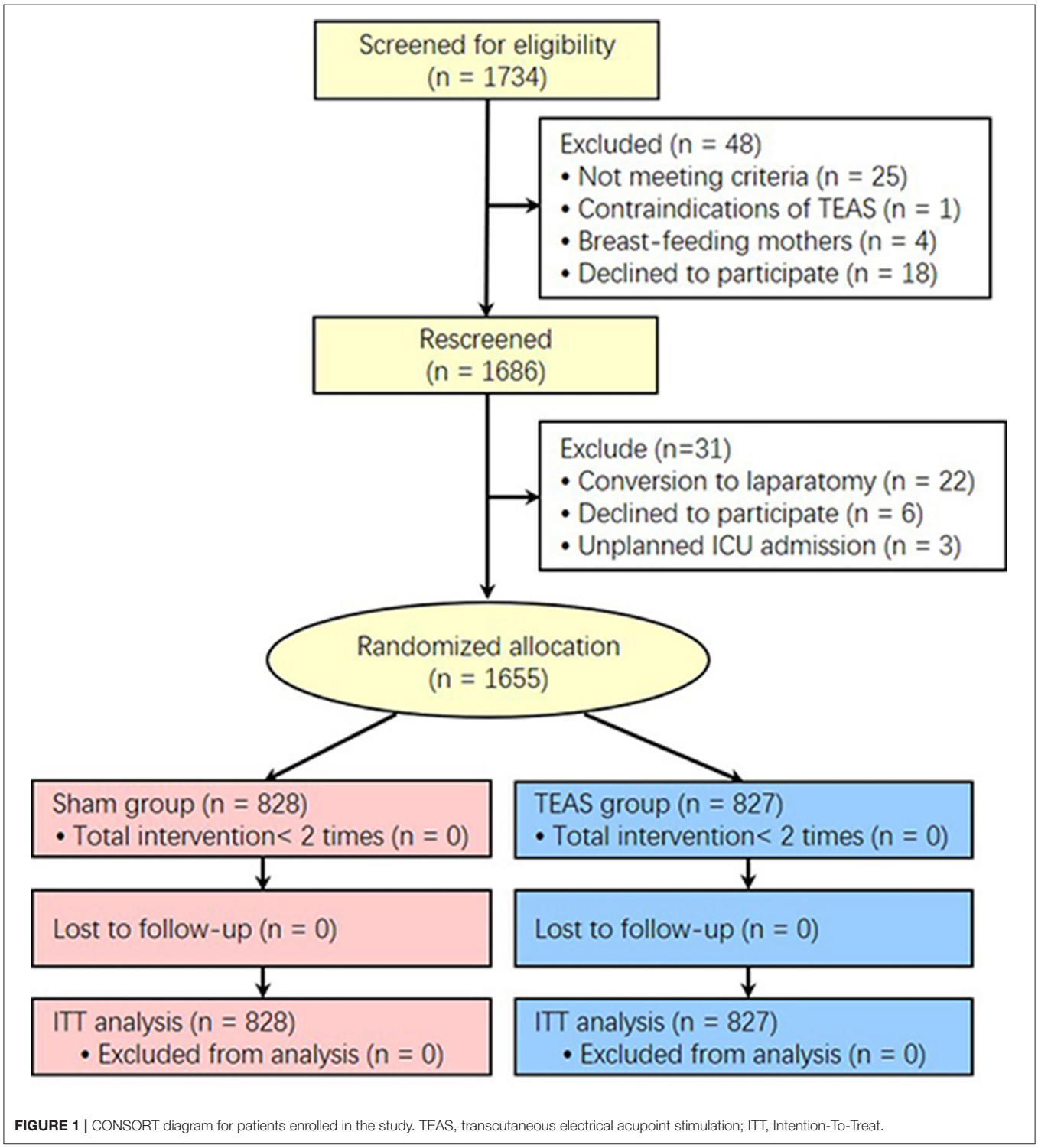

FIGURE 1 | CONSORT diagram for patients enrolled in the study. TEAS, transcutaneous electrical acupoint stimulation; ITT, Intention-To-Treat.

10 (0 = no nausea or vomiting, 10 = maximum severity). Once patients suffered PONV, patients or their caregivers pressed the buttons on an electronic scale and reported the related information (**Supplementary Figure 2**). Postoperative 24 h cumulative PONV incidence refers to the cumulative number of PONV episodes in the postoperative 24 h period. Persistent nausea was defined as continuous nausea lasting over 5 min. To avoid the leakage of allocation, evaluators went to the wards after 12 a.m. at the 1st and 2nd postoperative day to check the electronic patient self-reported system running condition and obtain the information of QoR-40 and complications. The postoperative 30-day complications and adverse events were obtained through telephone follow-ups and were stored in the electronic medical record system.

TABLE 1 | Baseline characteristics.

	TEAS (*n* = 827)	Sham (*n* = 828)	*P*-value
Age, yr	39.0 (31.0, 46.0)	39.0 (31.0, 46.0)	0.693
BMI, kg/m^2	23.1 (21.0, 25.4)	22.7 (20.6, 24.8)	0.001
Education level, *n* (%)			0.154
≤Primary education	223 (27.6)	209 (25.6)	
Secondary education	147 (18.2)	179 (22.0)	
Tertiary education	439 (54.3)	427 (52.4)	
Apfel's score, *n* (%)			
Female	803 (97.1)	803 (97.0)	0.888
Non-smoker	821 (99.3)	818 (98.8)	0.316
Use of postoperative opioids	734 (88.8)	737 (89.0)	0.869
History of PONV or motion sickness	477 (57.7)	485 (58.6)	0.712
4 scores	354 (42.8)	359 (43.4)	0.821
Comorbidities, *n* (%)			
Coronary heart disease	3 (0.4)	5 (0.6)	0.479
Stroke	2 (0.2)	1 (0.1)	0.563
Diabetes	10 (1.2)	5 (0.6)	0.194
Hypertension	40 (4.8)	56 (6.8)	0.094
SAS anxiety score	40.0 (32.0, 45.0)	40.0 (32.0, 45.0)	0.942
The operation types, *n* (%)			0.147
Gynecology	610 (73.8)	587 (70.9)	
Cholecystectomy	142 (17.2)	145 (17.5)	
Urinary	53 (6.4)	57 (6.9)	
Hernia repairment	14 (1.7)	28 (3.4)	
Liver surgery	8 (1.0)	11 (1.3)	
Operation time, hr	1.6 (1.1, 2.3)	1.6 (1.1, 2.3)	0.373
Anesthesia time, hr	2.0 (1.5, 2.8)	2.0 (1.4, 2.8)	0.503
RASS score after extubation	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.144
PACU electrolyte index			
Na^+, mmol/L	139.0 (137.0, 141.0)	140.0 (137.9, 141.0)	0.734
K^+, mmol/L	3.8 (3.5, 4.0)	3.8 (3.6, 4.1)	0.512
Cl^-, mmol/L	109.0 (107.0, 111.0)	109.0 (108.0, 111.0)	0.810
Glu, mmol/L	6.3 (5.4, 7.4)	6.1 (5.3, 7.2)	0.111

ASA, American society of anesthesiologists physical status classification system; RASS, Richmond agitation-sedation scale; PACU, post anesthesia care unit.
Data are presented as median (IQR) or number (%).

Statistical Analysis

Power Calculation

In previous studies, the PONV incidence was reported to be ∼40%, and the PONV incidence was reduced by 7% with the TEAS treatment (15). With significance set at 0.05 and power set at 80%, the sample size required to detect differences was 742 patients in each group calculated with the Pass 11 software (NCSS, LLC. Kaysville, Utah, USA). Taking into account a loss-to-follow-up rate of about 10%, 1,634

TABLE 2 | Anticholinergics drugs, antiemetics, and analgesic drug records.

	TEAS (*n* = 827)	Sham (*n* = 828)	*P*-value
Anticholinergics drugs, *n* (%)	461 (55.7)	438 (52.9)	0.245
Penehyclidine	393 (47.5)	383 (46.3)	0.606
Atropine	68 (8.8)	61 (7.6)	0.389
Scopolamine	4 (0.5)	3 (0.4)	0.669
Prophylaxis antiemetic, *n* (%)			
Dexamethasone	770 (93.1)	758 (91.5)	0.233
Palonosetron	431 (52.1)	438 (52.9)	0.750
Tropisetron	396 (47.9)	390 (47.1)	0.750
Dexamethasone+ Palonosetron	407 (49.2)	417 (50.4)	0.640
Dexamethasone+ Tropisetron	363 (43.9)	341 (41.2)	0.265
Rescue medication, *n* (%)	41 (5.0)	36 (4.3)	0.556
Nerve blocking, *n* (%)	81 (9.8)	70 (8.5)	0.344
NSAIDs or cyclooxygenase-2 inhibitors, *n* (%)	274 (33.1)	275 (33.2)	0.972
Flurbiprofen axetil	234 (30.2)	251 (31.2)	0.658
Parecoxib	40 (5.0)	24 (2.9)	0.034
Analgesic pump sufentanil, *n* (%)	597 (72.2)	588 (71.0)	0.596
24 h analgesic pump, μg	33.0 (0.0, 50.0)	30.0 (0.0, 60.0)	0.197
Intraoperative medication			
Sufentanil, μg	30 (30, 40)	30 (30, 40)	0.580
Remifentanil, μg	1,000 (600, 1,200)	1,000 (600, 1,255)	0.908
Propofol, mg	450 (300, 600)	450 (300, 600)	0.936
Sevoflurane, ml	20 (15, 30)	20 (15, 30)	0.798
Intraoperative fluid, ml			
Crystalloid solutions	1,000 (600, 1,500)	1,000 (600, 1,500)	0.149
Solute solutions	500 (500, 500)	500 (500, 500)	0.855

Data are presented as median (IQR) or number (%).

patients in total were enrolled to meet the minimum sample size requirement.

Intention-to-treat analysis was used for all enrolled patients. Continuous variables were presented as mean ± SD if data are normally distributed; otherwise, they were presented as median (interquartile range, IQR). Categorical values were presented as numbers (percentages). The differences between two groups were compared using the Mann-Whitney or Chi-square tests. The Kaplan-Meier log-rank test was used to illustrate the cumulative incidence of nausea and vomiting. Variables with significant differences ($P < 0.10$) in univariate analysis were included in multivariate analysis, which was used to identify the associated risk factors of the PONV occurrence. The comparison of independent risk factors related to PONV was further analyzed by chi-square tests or Kaplan-Meier log-rank test. A 2-sided *P*-value < 0.05 was considered to be of statistical significance. All these statistical analyses were performed using the SPSS software (version 25.0, IBM Corporation, NY, USA). All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Characteristics of Study Population

A total of 1,734 patients were scheduled for laparoscopic non-gastrointestinal surgery at 12 hospitals in China between September 3, 2019 and February 6, 2021. At two-step recruitment screenings, 48 patients were excluded at the first screening and of those, 31 patients were excluded at the second screening. A total of 1,655 patients were enrolled and randomly divided into two groups: 828 patients allocated to the Sham group and 827 patients to the TEAS group. All 1,655 patients were included in the intention-to-treat analysis (**Figure 1**). No TEAS-related adverse effects occurred in the study. The demographics, comorbidities, preoperative SAS anxiety score, surgical and anesthesia data, RASS scores after extubation, and PACU electrolyte index between the TEAS and Sham group were presented in **Table 1**. The selective laparoscopic non-gastrointestinal surgery under general anesthesia included gynecology (>70%), cholecystectomy, urinary, hernia repairment, and liver surgery. The anticholinergics, antiemetics, and multimode analgesics used during the perioperative period showed no significant differences between the two groups, except the parecoxib usage was more often higher in the TEAS group than in the Sham group (**Table 2**). The patients with or without parecoxib had no significant difference of PONV incidences.

Primary and Secondary Outcomes

Compared to the Sham group, patients in the TEAS group had significantly lower PONV incidence (29.4 vs. 34.2%, $P = 0.036$) and lower vomiting incidence (10.4 vs. 17.8%, $P < 0.001$). For nausea, the TEAS group showed lower persistent nausea incidence (2.2 vs. 5.1%, $P = 0.003$), lower first-time nausea score, and 24 h nausea highest scores ($P < 0.001$). Specifically, in 827 patients of the TEAS group, 29.4% (243) patients suffered nausea, including 0.7% (6) patients who suffered both persistent nausea and vomiting, 1.5% (12) patients who suffered persistent nausea without vomiting, 9.7% (80) patients who suffered vomiting without persistent nausea, and 17.5% (145) patients who suffered nausea without persistent nausea or vomiting. Correspondingly, in 828 patients of the Sham group, these incidences were, respectively, 2.1% (17), 3.0% (25), 15.7% (130), and 13.4% (111) (**Supplementary Figure 3**).

TABLE 3 | PONV outcomes and related complications.

Outcomes	TEAS ($n = 827$)	Sham ($n = 828$)	*P*-value
24 h PONV, *n* (%)	243 (29.4)	283 (34.2)	0.036
Vomiting, *n* (%)	86 (10.4)	147 (17.8)	<.001
Nausea			
Persistent nausea, *n* (%)			0.003
Persistent nausea	18 (2.2)	42 (5.1)	
Non-persistent nausea	225 (27.2)	241 (29.1)	
Non-PONV	584 (70.6)	545 (65.8)	
First-time nausea score	1.2 (2.3)	1.65 (2.8)	<.001
24 h nausea highest score	1.3 (2.4)	1.7 (2.9)	<.001
Vomiting			
First-time vomiting score	0.6 (1.8)	0.9 (2.0)	0.005
24 h vomiting highest score	0.6 (2.0)	0.9 (2.1)	0.008
24 h vomiting times	0.3 (0.9)	0.4 (1.2)	0.001
Related complications			
Dizzy, *n* (%)	321 (38.8)	363 (43.8)	0.038
Headache, *n* (%)	37 (4.5)	58 (7.0)	0.027
Electrolyte disturbance, *n* (%)	14 (1.7)	20 (2.4)	0.300
24 h pain VAS score	2.0 (1.7)	2.2 (1.8)	0.006

PONV, Postoperative nausea and vomiting; VAS, visual analog scale score. Data are presented as mean (SD) or patient's number (%).

In the vomiting patients, the TEAS group showed lower first-time vomiting score ($P = 0.005$) and lower highest vomiting scores ($P = 0.008$) together with vomiting times at the postoperative 24 h ($P = 0.001$). TEAS also significantly decreased the PONV-related postoperative 30 days complications including dizziness ($P = 0.038$), headache ($P = 0.027$), and postoperative 24 h Pain ($P = 0.006$) (**Table 3**). Compared to the non-PONV patients, the PONV patients had worse QoR-40 scores in all the five items (all $P < 0.001$). Compared to the 24 h QoR-40 scores in the Sham group, patients in the TEAS group had better physical comfort ($P = 0.003$), emotional state ($P = 0.001$), psychological support ($P = 0.002$), and pain ($P = 0.012$). Compared to the vomiting patients, the non-vomiting patients had significantly better scores in the same items (all $P < 0.001$) (**Supplementary Table 1**).

Multivariate Binary Logistic and COX Regression Analysis for PONV

Both TEAS and palonosetron were independently protective factors of PONV (**Table 4**). Compared with the Sham group, the TEAS group had 20%lower risk of PONV (OR, 0.80, 95% CI, 0.65–0.98; $P = 0.032$) and 48%lower risk of vomiting occurrence (OR, 0.52, 95% CI, 0.39–0.70; $P < 0.001$) by multivariate logistic

TABLE 4 | Multivariates binary logistic and cox regression associated with PONV.

Variable	Multivariate logistic		Cox regression	
	OR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
PONV				
TEAS	0.80 (0.65, 0.98)	0.032	0.83 (0.70, 0.99)	0.035
Palonosetron	0.62 (0.50, 0.77)	<0.001	0.68 (0.57, 0.81)	<0.001
Vomiting				
TEAS	0.52 (0.39, 0.70)	<0.001	0.57 (0.43, 0.74)	<0.001
Palonosetron	0.31 (0.23, 0.42)	<0.001	0.34 (0.26, 0.45)	<0.001

regression analysis; the TEAS group delayed PONV occurrence time by 17% (HR, 0.83, 95% CI, 0.70–0.99; $P = 0.035$) and delayed vomiting occurrence time by 43% (HR, 0.57, 95% CI, 0.43–0.74; $P < 0.001$) by COX regression analysis (**Supplementary Figure 4**). Compared with the tropisetron antiemetic, palonosetron reduced risk of PONV by 38% (OR, 0.62, 95% CI, 0.50–0.77; $P < 0.001$) by multivariate logistic regression analysis; the palonosetron delayed postoperative 24 h PONV occurrence time by 32% (OR, 0.68, 95% CI, 0.57–0.81; $P < 0.001$) by COX regression analysis.

The combined TEAS and palonosetron group had the lowest PONV incidence (25.1%), followed with combined Sham and palonosetron (28.8%) and combined TEAS and tropisetron (34.1%), and the combined Sham and tropisetron group had the highest PONV incidence (40.3%) ($P < 0.001$). The similar distribution of cumulative PONV incidences and vomiting among the four groups was noted (**Figure 2**).

DISCUSSION

Our study found that the TEAS significantly decreased the 24 h PONV incidence and also lowered persistent nausea incidence, PONV scores, and postoperative 24 h cumulative PONV incidences. Further, the TEAS also significantly decreased PONV-related complications, and improved postoperative 24 h physical comfort and emotional state, psychological support scores, and pain score in 24 h QoR-40 Scores. We also found that both TEAS and palonosetron were the independent PONV protective factors for 24 h PONV incidence and cumulative PONV incidence.

PONV is triggered by the nerve projected to the vomiting center, which is influenced by the cerebral cortex, vestibular and cerebellar nuclei, and chemoreceptor trigger band (4). The surface of the chemoreceptor trigger band covers various receptors, such as 5-HT_3, 5-HT_4, opioid, cholinergic, cannabis, and dopamine receptor (17). It has been reported that the incidence of PONV can be decreased by 26% for each additional type of antiemetic drug. The incidence of PONV was 52, 37, 28, and 22% in the absence or presence of 1, 2, and 3 types of antiemetic drugs, respectively (3). The Enhanced Recovery after Surgery Society recommended that more than two types of antiemetics should be used for patients undergoing gynecologic procedures (18), while three types of antiemetics did not show improved efficacy over the two agents (19). Thus, two types antiemetics in combination (dexamethasone with tropisetron/palonosetron) were used in our study. Compared to the first-generation 5-HT_3 receptor antagonists of tropisetron, the second-generation 5-HT_3 receptor antagonist palonosetron has a higher binding affinity and longer half-life and duration of action, and a single injection of a 0.075 mg dose of palonosetron for PONV prevention was used for up to 24 h after surgery (20).

However, the combination of two types of antiemetic drugs only inhibited part of the PONV-related receptors, and the prophylaxis effect reached the maximum effect and increasing their doses did not increase antiemetic effects due to receptor occupancy saturation. However, the side effects were increased (7). Correspondingly, the TEAS can modulate comprehensive PONV triggering receptors and reflex pathways to prevent PONV. For example, TEAS stimulates neuronal pathways from the soma splanchnic neurons to the paraventricular nucleus of the hypothalamus (21); TEAS also reduces secretion of 5-HT in the duodenum and suppresses the activation of the nucleus tractus solitarii in the brain-stem (22). TEAS inhibits sympathetic nerve and stimulates parasympathetic nerve and hence increases the activity of acetylcholinesterase (23); TEAS also stimulates receptors such as NO, CCK-A, cannabinoid, opioid receptor, and others and regulates neurotransmitters such as serotonin, GABA, and catecholamines (14). All these result in a decreased incidence of PONV.

Furthermore, the PONV prophylaxis outcome of TEAS might be due to stimulating acupoints of P6 and ST3. P6 is currently recognized as the standard acupoint for the prevention of PONV (24), and its effect was comparable to that of antiemetic drugs (25). Stimulation of P6 and ST36 in laparoscopic radical gastrectomy for gastric cancer significantly reduced the incidence of PONV, decreased early postoperative pain intensity and analgesic dosage, shortened the time of exhaust and excretion, promoted the recovery of gastrointestinal function, and improved patient satisfaction (26). Stimulation of P6 alone or combined with ST36 for 30 min before the lumbar anesthesia of cesarean section decreased PONV, and the mechanism is related to the reduction of plasma 5-HT5 concentration (27). Electroacupuncture at P6 and St36 together was reported to decrease period-dominant frequency in the electrogastrograph, and this effect was abolished by naloxone, indicating a central opioid pathway involvement (12).

It is worth noting that we enrolled the PONV high risk patients who had Apfel ≥ 3. The Apfel score includes female gender, non-smoker, history of motion sickness or PONV,

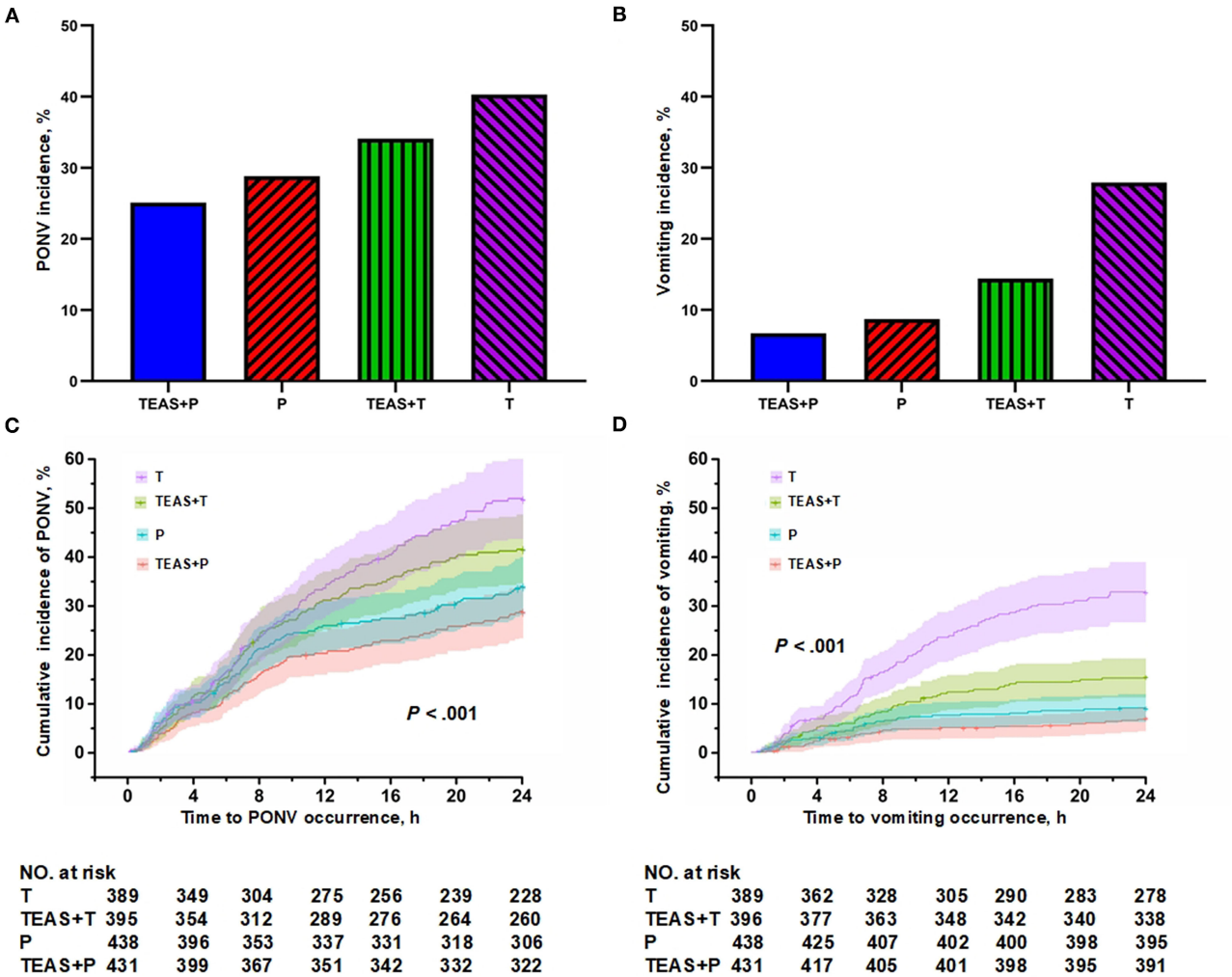

FIGURE 2 | The postoperative 24 h **(A)** PONV incidences, **(B)** vomiting incidences, postoperative 24 h cumulative **(C)** PONV and **(D)** vomiting incidences among combined TEAS and palonosetron (TEAS + P), combined Sham and palonosetron (Sham + P), combined TEAS and tropisetron (TEAS + T), and combined Sham and tropisetron (Sham + T).

and postoperative opioid use. In our study, 97.0% of patients were female. Besides accounting for a quarter of Apfel score, females were more likely to be non-smoking (28) and have motion sickness than males (29). This agreed with the previous epidemiological study, in which females accounted for 93.3% in Apfel score 3 population and 100% in Apfel score 4 population (30). Without any PONV prophylaxis, the estimated probability of PONV in Apfel $\geq$ 2 patients were between 39 and 78%, whereas if Apfel $\geq$ 3 is present, it may rise up to 78% (1). In addition, we enrolled high PONV risk surgical types. The laparoscopic gynecological operation and the laparoscopic cholecystectomy accounts for about 70 and 18% in our study, respectively (**Table 1**). Without any PONV prophylaxis, the PONV incidence of gynecological surgery and cholecystectomy surgery were 69 and 59.6% (31). When PONV prophylaxis with dexamethasone and tropisetron, the PONV incidence of laparoscopic gynecological surgery had been reported even up to 77.4% (32). In contrast, we found that the PONV incidence was much lower. What factors caused these discrepancies between previous studies and the current study remain unknown. However, the study heterogeneity was avoided in our study which may be one of reasons; for example, almost all patients were female and non-smokers and the SAS anxiety score were comparable among patients.

Limitations

The strengths of our trial are its large sample size, multiple centers, high PONV risk patients and surgeries, and the effectiveness of TEAS group in reducing PONV was clearly demonstrated. However, the unavoidable limitation in our study was that patients cannot be blinded to the intervention totally as they can sense the acupoint stimuli in the TEAS group whilst others in the sham group did not. As such, any "placebo" effects of TEAS are unknown. In addition, the

balance anesthesia as a potential factor to reduce PONV, the non-standardization of anesthesia management, and the use of different regimens to prevent PONV during surgery may cause potential bias on the results. Antiemetic prophylactic medication used during our study remained similar but not identical due to different medical supplies in various centers. We have narrowed 5-HT_3 receptor antagonists down to two specific drugs (palonosetron or tropisetron). Meanwhile, only 92.3% patients received dexamethasone, although there is no significant difference regarding dexamethasone usage between the TEAS group and Sham group (**Table 2**). Lastly, the sample size calculation was based on the previous publications without any antiemetic prophylaxis medication (15) which might induce sample size bias. However, our study is a large sample size and a multi-centers trial and, therefore, all those limitations were unlikely to have affected our conclusions.

CONCLUSIONS

This multi-center randomized controlled study concluded that the use of postoperative TEAS (at anesthesia recovery arrival and in the following morning) in conjunction with antiemetic prophylaxis may significantly reduce PONV incidence and its severity. Both TEAS and palonosetron were independent PONV risk protective factors for 24 h PONV incidence and cumulative PONV incidence. The combination of TEAS and palonosetron was the most effective strategy to reduce the PONV incidence. Our work may suggest that one should consider implementing TEAS clinically for patients' better and smoother postoperative recovery.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTU1AF2019LSK-084). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WG and QW: study conception, critically revised the manuscript for important intellectual content, and obtained funding. WG, LZ, XH, LW, JF, XZ, JZ, HW, QZ, CW, WC, XN, LY, RD, GW, and BL: acquisition or interpretation of data. WG and YL: statistical analyses. WG, LY, and QW: drafted the manuscript. WG, LZ, XH, LW, JF, XZ, JZ, HW, QZ, CW, WC, XN, LY, RD, GW, BL, and QW: provided administrative, technical, or material support.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.766244/full#supplementary-material

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ORIGINAL RESEARCH
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The Effect of Relaxation Therapy on Hypoxia During Intravenous Propofol Anesthesia in Patients With Pre-operative Anxiety: A Prospective Randomized Controlled Trial

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Background: This study aimed to investigate the effect of relaxation therapy on hypoxia during intravenous propofol anesthesia in patients with pre-operative anxiety.

Methods: Two-hundred and eighty patients were randomly categorized in the experimental group (relaxation therapy group) and control group. The Spielberger State-Trait Anxiety Inventory (S-STAI) was administered 30 to 60 min pre-operatively to assess the patient's current anxiety status and select appropriate patients. Patients in the experimental group received pre-surgical relaxation therapy. Decrease in oxygen saturation during the procedure was recorded for each patient group, and the relevant data were compared between the two groups.

Results: The basic S-STAI scores of the experimental and control groups were 56.88 ± 2.91 and 57.27 ± 3.56, respectively ($p = 0.331$). The difference was not statistically significant. The incidence of hypoxia in the experimental group during painless artificial abortion [routine blood oxygen saturation (SpO_2) <95%, duration >15 s] decreased from 30 to 12.3%.

Conclusion: Relaxation therapy may effectively reduce the incidence of hypoxia during painless artificial abortion by using less dose of propofol. It may help patients relieve their anxiety and improve perioperative safety.

Trial Registration: Chinese Clinical Trial Registry (ChiCTR2000032109).

Keywords: intraoperative hypoxia, pre-operative anxiety, propofol anesthesia, relaxation therapy, induced abortion

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INTRODUCTION

Pre-operative anxiety is a psychological reaction before surgery. It manifests as discomfort, apprehension, and fear (1). The three main reasons for pre-operative anxiety are unknown surgery, disease, and the possibility of death (2). It is understandable that patients have mild anxiety before and after surgery. However, severe anxiety often causes intraoperative hemodynamic problems (3) and affects the progress of rehabilitation (4–7). The incidence of pre-operative anxiety in adult patients can be as high as 80% (8). High pre-operative anxiety independently predicts increased post-operative mortality and the incidence of serious complications (9). Moreover, anxiety is an independent risk factor for intraoperative hypoxia (10). Individuals under the age of 30 years and women are more likely to have higher levels of anxiety (11–13). Hence, young women were asked to participate in this study. Patients who underwent painless artificial abortion in this study were at a high risk of pre-operative anxiety. Anti-anxiety medications are not recommended for temporary anxiety, such as that before an operation. Contrastingly, relaxation therapy is a simple, easy, and effective non-pharmacological treatment to relieve anxiety (14) and can be implemented with high compliance. Therefore, relaxation therapy can be used to alleviate anxiety and reduce the incidence of intraoperative hypoxia. This study aimed to evaluate whether relaxation therapy can reduce the incidence of intraoperative hypoxia among patients with pre-operative anxiety before undergoing painless artificial abortion. The results of this study can guide in the further improvements in perioperative safety and comfort.

METHODS

The study protocol was approved by the Ethics Committee of Shanghai Tenth People's Hospital (approval number: SHSY-IEC-4.1/20-47/01). The study was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR2000032109) on April 20, 2020 at https://www.chictr.org.cn/index.aspx. This randomized controlled trial was conducted between September 2020 and January 2021. The participants included 280 anxious female patients who underwent painless artificial abortion in the outpatient department of Shanghai Tenth People's Hospital (Shanghai, China). All patients signed an informed consent form before surgery. Inclusion criteria were as follows: (1) Spielberger State-Trait Anxiety Inventory (S-STAI) score >45.5, (2) age 18–45 years, (3) body mass index 18.5–25.0 kg/m^2, (4) 6–9 weeks of pregnancy, (5) American Society of Anaesthesiologists (ASA) physical status class I/II, (6) Mallampati airway classification class I/II, and (7) no coagulation dysfunction. Exclusion criteria were the following: (1) previous motion sickness, hypertension, heart disease, asthma, epilepsy, Parkinson's disease, depression, and other diseases, (2) history of drug allergy, and (3) routine blood oxygen saturation (SpO_2) ≤98%.

Data were collected 1 h before surgery through a questionnaire. The participants were asked about their general information, anxiety scores using the S-STAI, and informed consent. They were categorized into a control group and an experimental group through a random number generated by a computer. Patients in the experimental group received pre-operative relaxation therapy. The surgeon and the anaesthesiologist did not intervene in the assignment of numbers and were blinded to the groups.

Half an hour before the operation, patients in the experimental group received audio therapy and progressive relaxation training in a separate room in the presence of an observer. The patients were instructed to close their eyes in an open and quiet place, to keep quiet, and to perform 10 min of progressive muscle relaxation training according to the audio instructions (15). Voluntary and orderly muscle relaxation and contraction were required until all muscle groups were relaxed. Progressive muscle relaxation started with the hands, followed by the arms, shoulders, chest, legs, and feet to exercise all muscle groups. Six different types of music were played in a loop, such as the piano, harp, orchestra, jazz, Chinese orchestra, and synthesizer. The music rhythm ranged from 60 to 80 beats per min, the pitch was low, melody lines were smooth, and the volume and rhythm did not change significantly (16, 17).

The anaesthesiologist conducted routine monitoring by measuring the blood pressure, heart rate, SpO_2, and baseline bispectral index scores before the operation (T0) and 1 min (T1) and 5 min (T5) before the beginning of the operation. Oxygen at 6 L min^{-1} (37°C, oxygen concentration 100%) was administered to the patient through a face mask. The patient then received an intravenous bolus of 0.01 mg kg^{-1} butorphanol tartrate (Nuoyang) for analgesia and 2 mg kg^{-1} of propofol for anesthesia induction. The operation started after the disappearance of ciliary reflex. In case of intraoperative body movement, 0.5 mg kg^{-1} of propofol was administered. Simultaneously, we recorded the number of body movements and the number of times additional propofol was required. The aim was to maintain the bispectral index score between 50 and 70. Hypoxia events were recorded when the patients' SpO_2 was <95% and lasted more than 15 s (18). Sedation-related adverse events were defined as systolic blood pressure <90 mmHg, heart rate <50 beats min^{-1}, mild hypoxia (75% ≤ SpO_2 < 90%, with a duration <60 s), or severe hypoxia (SpO_2 < 75% or 75% ≤ SpO_2 < 90%, with a duration ≥60 s). In case of adverse effects, norepinephrine (20–40 μg) was administered to patients with hypotension, and atropine (0.25–0.50 mg) to patients with bradycardia. In patients with hypoxia and severe hypoxia, the jaw was lifted to open the airway and increase oxygen flow. Thereafter, mask ventilation was used to treat hypoxia. If hypoxia could not be treated by mask ventilation, endotracheal intubation was performed. Patients with sedation-related adverse events were excluded from the study. All sedation procedures in this study were performed by an anaesthesiologist.

Based on the results of our previous clinical trials, we determined that the cut-off S-STAI score should be >45.5 points. There is an increased risk of intraoperative hypoxia when the score is >45.5 points. The primary outcome indicator of this study was the incidence of hypoxia, and the secondary outcome indicators were the dosage of propofol, number of times additional propofol was required, incidence of body movement, and change in heart rate and mean arterial pressure.

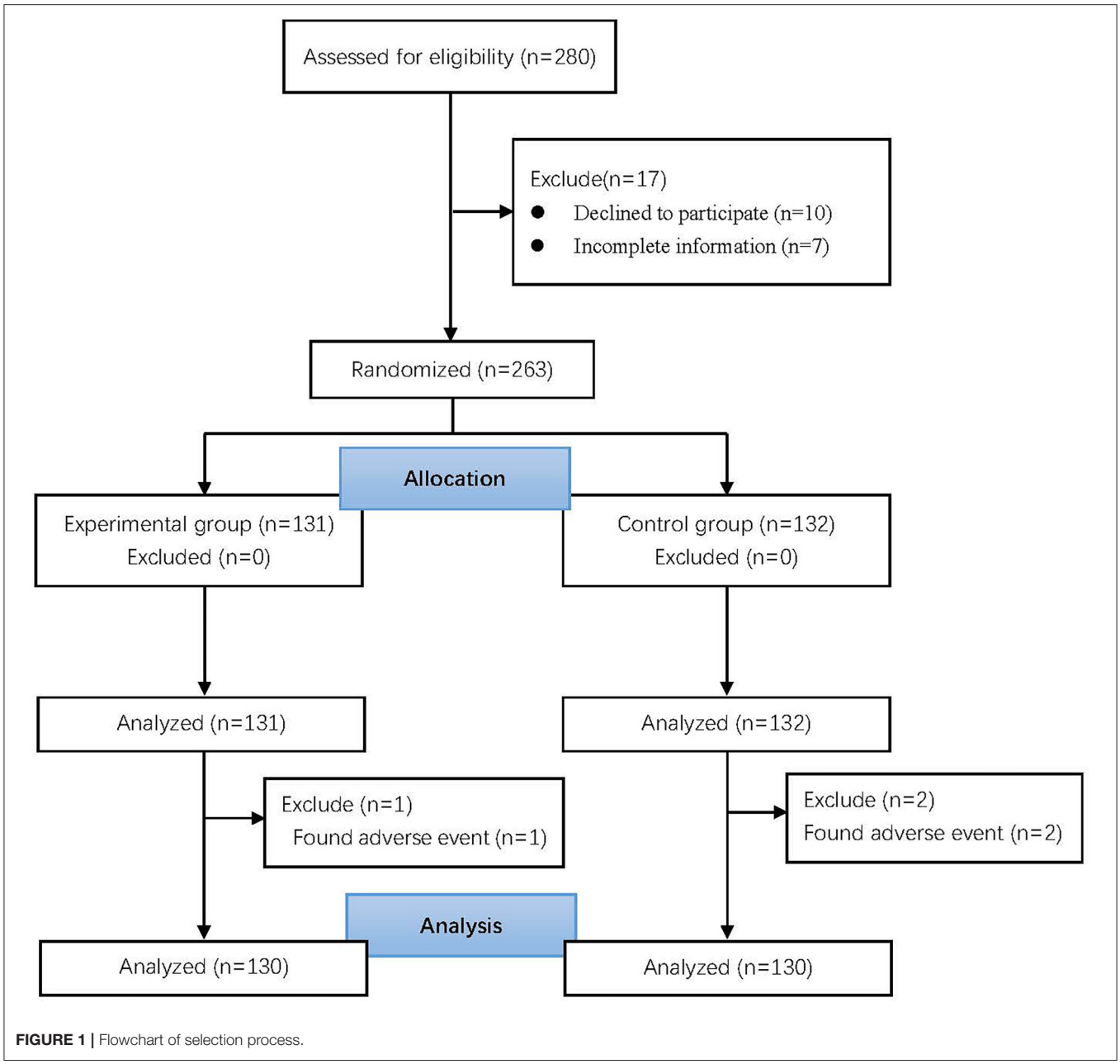

FIGURE 1 | Flowchart of selection process.

Sample Size Calculation

The calculation of the sample size was based on the pre-experimental prevalence rate of 23.3%. The sample size ratio of the control and experimental groups was 1:1; the number of cases and controls was calculated at 110 cases each. We selected 280 patients to account for the 20% dropout rate.

Statistical Analysis

SPSS version 20.0 software was used for data analysis. Continuous variables are represented as mean and standard deviation and categorical variables are represented as frequency and proportion. Continuous variables were analyzed by two-sample *t*-test and the categorical variables were analyzed by independent-sample *t*-test and Fisher test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Of 1,985 patients, 280 were recruited based on anxiety scores. Among them, 17 were excluded (10 patients refused to participate and 7 did not complete the pre-operative survey). The remaining 263 patients were randomly categorized into two groups. Three patients (2 in the control group and 1 in the experimental group) had adverse reactions and were eventually excluded. Finally, 260 patients were analyzed (**Figure 1**).

TABLE 1 | Patient characteristics.

Factors	Experimental group ($n = 130$)	Control group ($n = 130$)	*P*
Age (years)	30.96 ± 6.11	31.05 ± 5.98	0.902
Height (cm)	162.05 ± 4.31	161.68 ± 4.51	0.500
Body mass index (kg/m^2)	21.10 ± 2.66	21.49 ± 2.50	0.221
Pre-operative heart rate (times/min)	83.22 ± 14.88	87.51 ± 16.71	0.262
Pre-operative mean arterial pressure (mmHg)	88.63 ± 10.12	85.61 ± 10.20	0.575
Pre-operative oxygen saturation (%)	100	100	/
Menopausal days (days)	51.45 ± 5.70	50.45 ± 6.04	0.174
Smoking (%)			
Yes	13.1	8.5	
No	86.9	91.5	0.230
Drinking (%)			
Yes	4.6	5.4	
No	95.4	94.6	0.776
S-STAI score (points)	57.27 ± 3.56	56.88 ± 2.91	0.331

Values are presented as mean ± standard deviation or n (%).
S-STAI, Spielberger State-Trait Anxiety Inventory.

TABLE 2 | Perioperative indicators.

Factors	Experimental group ($n = 130$)	Control group ($n = 130$)	*P*
The incidence of hypoxia (%)	12.30%	30%	<0.001
Propofol dosage (mg)	13.91 ± 2.26	14.58 ± 3.07	0.046
Propofol bolus (times)	1.08 ± 0.88	1.26 ± 1.06	0.047
Incidence of body movement (%)			
Yes	50	53.8	0.535
No	50	46.2	

Values are presented as mean ± standard deviation or %.

There was no significant difference between the two groups in terms of age, height, body mass index, heart rate, mean arterial pressure, the number of days since last menstruation, smoking and drinking habits, and S-STAI scores (**Table 1**).

In the experimental group, the incidence of hypoxia dropped from 30 to 12.3%, which was a 59% reduction, after pre-operative muscle relaxation. Moreover, the consumption of propofol in the experimental group during induction of anesthesia was significantly less than that in the control group (13.91 ± 2.26 vs. 14.58 ± 3.07, $p = 0.046$). The number of times additional propofol was required in the experimental group was significantly less than that in the control group (1.08 ± 0.88 vs. 1.26 ± 1.06, $p = 0.047$; **Table 2**). The changes in heart rate and mean arterial pressure over time after anesthesia induction are shown in **Figure 2**, and the hemodynamic data are shown in **Tables 3**, **4**. Compared with baseline (T0), the heart rates of both the experimental and control groups were significantly reduced 5 min (T5) after the induction of anesthesia.

DISCUSSION

Pre-operative anxiety is a potential conscious response of the human brain. Anxiety is magnified when people feel threatened with externalities. Mild anxiety can improve a person's ability to handle stress, while excessive anxiety may lead to hemodynamic changes during the operation, and this can seriously affect the patient's perioperative safety.

People usually have a stress response due to pre-operative anxiety (19) that leads to increased sympathetic nerve activity and increased release of catecholamines from the adrenal medulla (20). This results in increased blood pressure and heart rate, accelerated breathing, other adrenergic responses, and pituitary–adrenal cortex reactions (21, 22). The administration of anti-anxiety drugs for pre-operative anxiety is not advisable because it may cause adverse reactions such as excessive sedation, hypotension, allergies, vomiting, and shock (23, 24). These complications increase costs and side effects. However, relaxation therapy provides a good anti-stress effect that can reduce sympathetic nerve activity. Consequently, there is a reduction in the respiratory rate, heart rate, and blood pressure, and an increase in the feeling of wellness. The relaxation therapy employed in this study included music therapy and progressive muscle training. Both relaxation methods can reduce pre-operative anxiety (25, 26) and have a good synergistic effect.

The main purpose of this study was to explore the effect of relaxation therapy on hypoxia in patients with anxiety before painless abortion. We defined hypoxia as SpO_2 <95% and lasting more than 15 s. The monitoring of SpO_2 was delayed because apnea and altered respiration frequently precede hypoxemia with a significant time lag of up to 2 min (27, 28). According to the obtained results, the incidence of hypoxia in anxious patients after relaxation therapy was reduced by 59%. Further, the number of times additional propofol was required and total consumption of propofol were significantly reduced. Considering the respiratory depression effect of propofol, we believe that the lower incidence of hypoxia in the experimental group may be the combined effect of using lesser amount of propofol during surgery.

In this experiment, six different types of music were played in a loop. Music can heal the body and mind (29). Although personal preferences for music vary, music can play a direct physiological role through the autonomic nervous system (30). Furthermore, unlike other relaxation methods such as massage and yoga, it is a low-cost intervention. Since the early 1990s, music therapy has been used to aid in sleeping and to reduce anxiety related to surgery (31). Now, music therapy is used in different fields. Listening to music can significantly reduce anxiety ($p = 0.003$) in cardiac catheterization. In a previous study, the music group exhibited decreased heart rate and increased skin temperature ($p < 0.001$) (16). Music therapy is effective in reducing anxiety in critically ill patients (32). Additionally, music therapy is a safe and low-cost intervention that can relieve pain and anxiety associated with surgery (33).

Progressive relaxation training helps patients relax all muscle groups. All relaxation processes ensured rhythmic breathing, muscle tension reduction, and increased patient awareness. The

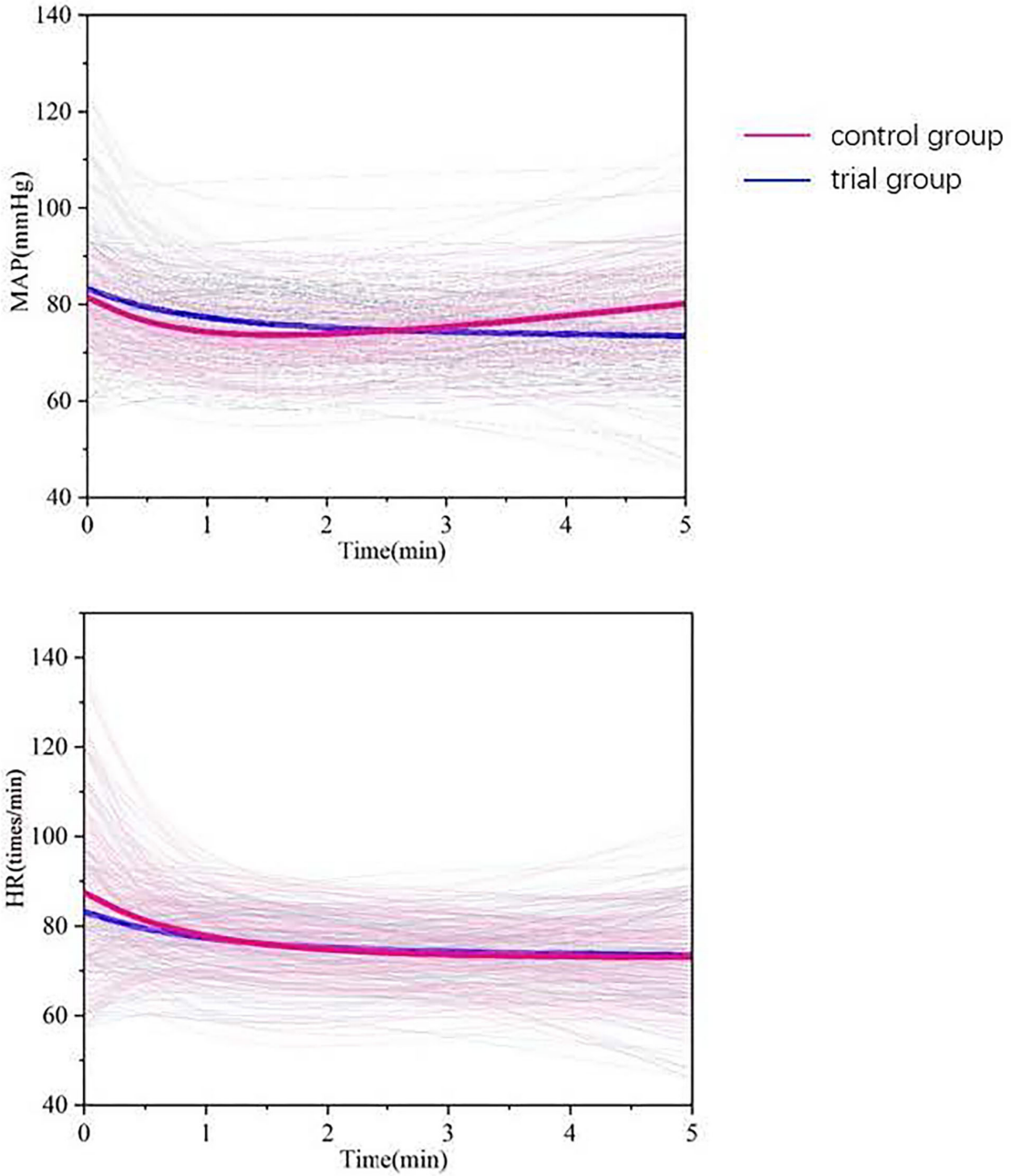

FIGURE 2 | Average values of the hemodynamic variables studied (thick line) and individual patient data (thin line) : the red line represents the control group, and the blue line represents the trial group.

method is simple and easy to implement, without external intervention. As an auxiliary method, progressive muscle relaxation can reduce patient anxiety. For example, it can help improve sleep quality in patients with coronavirus disease 2019 (34). In patients undergoing gastrointestinal surgery, progressive relaxation training can increase their pain threshold and reduce their stress and anxiety (35).

In our previous study, we found that anxiety is the main risk factor of hypoxia. We assumed that relaxation therapy would reduce the rate of hypoxia of anxious patients. The results of this study showed that the incidence of hypoxia in the experimental group was 12.3% and in the control group was 30% ($p < 0.001$).

The current study also showed that relaxation therapy can effectively reduce the consumption of propofol. The comparison of the average of the two groups showed that the dose of propofol in the control group was higher than that in the experimental group. Therefore, for painless abortion surgery, anxious patients who used relaxation therapy required less amount of propofol.

TABLE 3 | Intraoperative haemodynamic data.

Factors	Experimental group ($n = 130$)	Control group ($n = 130$)	*P*
Changes in heart rate (times/min; T5–T0)	−9.68 ± 11.46	−14.45 ± 14.19	<0.001
One min mean arterial pressure changes (mmHg; T1–T0)	−12.07 ± 11.19	−12.44 ± 9.45	0.745
Five min mean arterial pressure changes (mmHg; T5–T0)	−1.63 ± 12.04	−1.42 ± 10.82	0.867

Values are presented as mean ± standard deviation.

TABLE 4 | Intraoperative haemodynamic data.

		T0	T5	T5–T0
Heart rate (times/min)	Experimental group	83.22 ± 14.88	73.54 ± 10.46	−9.68 ± 11.46
	Control group	87.51 ± 16.71	73.05 ± 10.22	−14.45 ± 14.21
	P	0.262	0.662	<0.001
Mean arterial pressure (mmHg)	Experimental group	88.63 ± 10.12	86.99 ± 11.18	−1.63 ± 12.04
	Control group	85.61 ± 10.20	84.19 ± 11.01	−1.42 ± 10.84
	P	0.575	0.954	0.867

Values are presented as mean ± standard deviation.

Higher levels of anxiety among patients increased their needed amount of propofol intraoperatively. These results were similar to the results of Li Ruiyun (36). Music has a sedative effect on patients. It can change their physiological parameters and reduce pain and discomfort, thereby reducing the required analgesic dose (37). Our results show that relaxation therapy can reduce the dosage of intraoperative propofol by relieving pre-operative anxiety, thus reducing the incidence of intraoperative hypoxia.

Like most studies, this study has some limitations that may affect the experimental results. First, the sample size of the experiment is small. Not all anxious patients were included in the group due to the S-STAI score cut-off at 45.5 points. Second, this is a randomized single-blind experiment. Since the experimental group required relaxation therapy, a double-blind control could not be achieved. Third, there are differences in the surgical techniques of different gynecological surgeons, even if uniform training is carried out. Fourth, due to the time constraints, we did not record the S-STAI scores after relaxation therapy. Furthermore, participants were unable to individually choose their favorite music during the experiment. This may reduce the effect of music therapy.

CONCLUSION

This study focused on the effect of relaxation therapy on hypoxia caused by intravenous propofol anesthesia in patients with pre-operative anxiety. We found that relaxation therapy can reduce the occurrence of hypoxia in anxious patients, and it may be the combined effect caused by the use of less dosage of propofol during the operation.

IMPLICATION STATEMENT

This study focused on the effect of relaxation therapy on hypoxia caused by intravenous anesthesia with propofol in patients with pre-operative anxiety. We found that relaxation therapy can reduce the occurrence of hypoxia in anxious patients and improve perioperative safety.

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 Ethics Committee of Shanghai Tenth People's Hospital (approval number: SHSY-IEC-4.1/20-47/01). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YF and QJ: data collection. YF, QJ, and CL: data analysis. YF and CL: writing. QJ, SC, XS, HZ, LT, and CL: study design. YF: patient recruitment. YF and QJ: contributed equally to this work. All authors: revision. All authors contributed to the article and approved the submitted version.

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REVIEW
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Pathogenesis of Multiple Organ Failure: The Impact of Systemic Damage to Plasma Membranes

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Multiple organ failure (MOF) is the major cause of morbidity and mortality in intensive care patients, but the mechanisms causing this severe syndrome are still poorly understood. Inflammatory response, tissue hypoxia, immune and cellular metabolic dysregulations, and endothelial and microvascular dysfunction are the main features of MOF, but the exact mechanisms leading to MOF are still unclear. Recent progress in the membrane research suggests that cellular plasma membranes play an important role in key functions of diverse organs. Exploration of mechanisms contributing to plasma membrane damage and repair suggest that these processes can be the missing link in the development of MOF. Elevated levels of extracellular phospholipases, reactive oxygen and nitrogen species, pore-forming proteins (PFPs), and dysregulation of osmotic homeostasis occurring upon systemic inflammatory response are the major extracellular inducers of plasma membrane damage, which may simultaneously operate in different organs causing their profound dysfunction. Hypoxia activates similar processes, but they predominantly occur within the cells targeting intracellular membrane compartments and ultimately causing cell death. To combat the plasma membrane damage cells have developed several repair mechanisms, such as exocytosis, shedding, and protein-driven membrane remodeling. Analysis of knowledge on these mechanisms reveals that systemic damage to plasma membranes may be associated with potentially reversible MOF, which can be quickly recovered, if pathological stimuli are eliminated. Alternatively, it can be transformed in a non-resolving phase, if repair mechanisms are not sufficient to deal with a large damage or if the damage is extended to intracellular compartments essential for vital cellular functions.

Keywords: multiple organ failure, plasma membrane, reactive oxygen species, phospholipase A2, pore forming protein, systemic inflammation and sepsis, hypoxia

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INTRODUCTION

Considerable number of critically ill patients develop multiple organ failure (MOF) (also called multiple organ dysfunction syndrome (MODS), which is the leading cause of morbidity and mortality in intensive care patients (1–3). Despite major advances in intensive care medicine, we still know very little about this syndrome. It is commonly accepted that MOF has a multifactorial

character, based on two general pathological processes, namely, hypoxia and overwhelming inflammatory response (4, 5). Surprisingly, even with advanced organ dysfunction occurring in MOF, all the failed organs appear normal and manifest minimal signs of cell death (6, 7). Normal appearance of failed organs was observed even in patients who died of non-resolving MOF (4, 8, 9). Furthermore, it is striking that if critically ill patients recover from MOF, failing organs can recover relatively fast, even those organs that have poor regenerative capacity (10). There are some other unresolved questions about MOF highlighted elsewhere (9, 11, 12). The fact that the dysfunction occurs parallel in several organs suggests a similar pathologic mechanism(s) operating in quite different organs. The organ failure occurs despite the centralization of circulation, which increases blood flow in vital organs often occurring in critically ill patients. A decrease in oxygen consumption and ATP levels is observed in tissues, despite of adequate tissue oxygen supply. These unresolved questions suggest that the pathological changes causing MOF occur on molecular level and do not influence cellular morphology. Even electron microscopy did not reveal continuously reproducible data. In single studies, specific changes in the ultrastructure such as swollen mitochondria (13), delayed endoplasmic reticulum (ER) (6), and formation of autophagosomes (7) have been shown for specific, but not for all the cases and they were not reproducible in different studies/models. The manifestation of MOF described above is particularly characteristic for MOF mediated by severe inflammatory response, such as in septic patients. In contrast, hypoxia, accompanied by nearly complete inhibition of ATP synthesis, causes cell death and formation of necrotic areas in affected organs (14). The mechanism leading to necrosis upon hypoxia is much better understood than MOF mediated by systemic inflammatory response. According to our current knowledge, mechanisms causing MOF should be reversible at least at the beginning of the disease and they should induce cell dysfunction, but not cell death. They should undergo fast recovery pathway(s); the pathways of damage and recovery should operate in all the susceptible to failure organs and there should be a reasonable explanation, why increased blood flow through the organs (centralization of circulation) does not preserve organ function often even deteriorating the situation.

Strikingly, damage to plasma membranes impairs their barrier function as well as other important cellular functions. Impairment of these functions leads to serious and quickly developed cellular dysfunction (15, 16). Since integrity of plasma membranes is very important for all the cells, they have developed robust membrane repair mechanisms (15), which include exocytosis, endocytosis, ectosome shedding, and protein-driven membrane remodeling (15). The recovery of the phospholipid bilayer is the major aim of the cell membrane repair strategy. The damage to the plasma membrane impairs the entire cellular homeostasis, but if the mechanism of damage is terminated, then the membrane can quickly repair by the abovementioned efficient mechanisms (17). Over the last decade, the knowledge about the mechanisms of membrane damage and recovery has been strongly extended and describes in details a number of such mechanisms (18). It is striking that the majority of mechanisms damaging membranes obligatorily accompany MOF. Membrane disruptions can be caused by so-called pore-forming toxins (PFTs) [pore-forming proteins (PFPs)] secreted by most pathogenic bacteria (19). The pores formed by these proteins can be permeable not only for small molecules, but also for proteins (20). Hypoxia and reoxygenation can also induce membrane damage *via* changes in Ca^{2+} homeostasis and generation of intracellular reactive oxygen and nitrogen species (RONS), which cause widespread oxidation of both the proteins and lipids associated with membrane damage occurring predominantly in intracellular compartments (18, 21). It has been proposed that plasma neuronal membrane disruption is a major contributor to morbidity of neurological patients (22). The assumption that plasma membrane critically contributes to organ function satisfactorily explains MOF because mechanisms damaging biological membranes are universal in each organ. Below we consider the major mechanisms of membrane damage obligatorily accompanying MOF.

EXTRACELLULAR REACTIVE OXYGEN AND NITROGEN SPECIES

Upon systemic inflammation, RONS formed by immune cells is released into extracellular fluid to attack pathogens, but also host cells in case of overwhelmed activation of immune cells. Superoxide radicals ($O_2^{\bullet -}$) are the primary reactive species generated by immune cells. It can be converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) or *via* spontaneous dismutation. Both the $O_2^{\bullet -}$ and H_2O_2 are not very aggressive species and serve predominantly for signaling purposes (23). However, $O_2^{\bullet -}$ and H_2O_2 contribute to the generation of two chemically aggressive species, peroxynitrite (ONOO) and hypochlorite (HClO), respectively. ONOO and HClO are used by immune cells to kill bacteria, but they can also damage host cells in the place they are produced (23). They cannot move to another location due to their high reactivity and short half-life time. In contrast, H_2O_2 can diffuse at longer distances from the place it was formed. H_2O_2 can induce oxidative stress upon reaction with heme iron or ferrous ions. This, so-called Fenton reaction, yields hydroxyl radical ($HO^{\bullet}$), which is a very reactive species activating lipid peroxidation and damaging biological membranes. Free ferrous ions occur only within the cells, while heme iron is predominantly present in the blood in a form of hemoglobin (24). In the blood, hemoglobin bound to haptoglobin (cell-free hemoglobin) and is the major catalyzer of the Fenton reaction (25). It has been shown that elevated levels of cell-free hemoglobin are associated with an increased risk of death in septic patients (26). This effect is attributed to the induction of oxidative stress, which impairs endothelial permeability, in turn having particularly dramatic consequence in the lung (26). Moreover, H_2O_2 can also undergo a redox reaction with methemoglobin yielding ferryl species, which are extremely

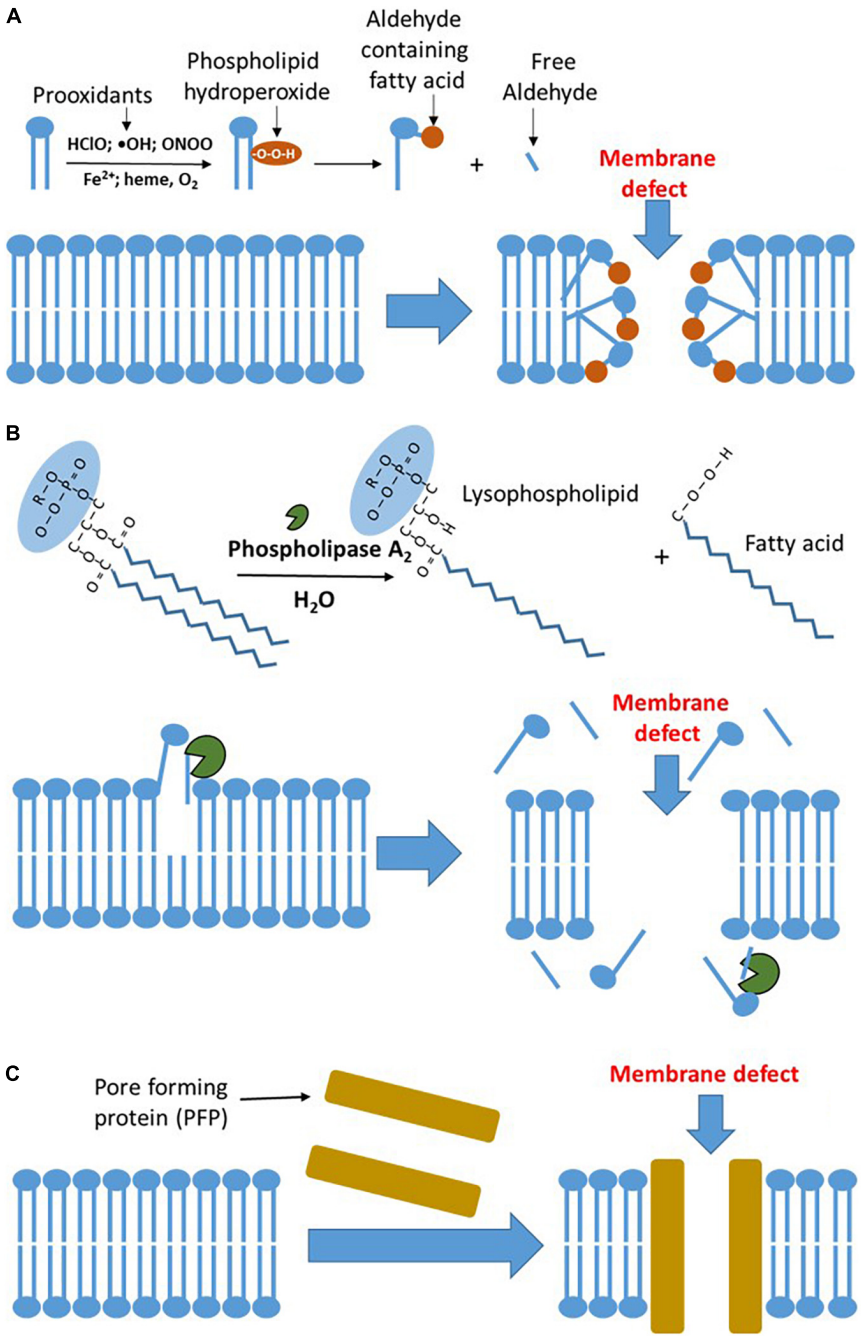

FIGURE 1 | The major mechanisms of plasma membrane damage. **(A)** Oxidative damage to the plasma membrane. Prooxidants formed by inflammatory sources circulating in the blood induce lipid peroxidation in plasma membranes. The latter results in the formation of polar, hydrophilic species, which form defects in the membrane and increase its permeability. **(B)** Damage to the plasma membrane mediated by phospholipases. Elevated concentration of phospholipase A_2 (PLA_2) in the circulating blood hydrolyzes phospholipids in the plasma membranes. This reaction results in the formation of lysophospholipids and free fatty acids, which are prone to leave the membrane and increase its permeability facilitating lipid peroxidation in plasma membranes. The latter results in the formation of polar, hydrophilic species, which form defects in the membrane and increase its permeability. **(C)** Pore-forming proteins (PFPs) mediated damage to plasma membrane. Upon binding to lipid membranes, they convert from the soluble form into an oligomeric state, undergo conformational change, and form transmembrane pores, which dramatically increase the membrane permeability. HClO, hypochlorite; ONOO, peroxynitrite; and OH, hydroxyl radical.

reactive pro-oxidants and they are prone to damage host cells (27). The major target of RONS in plasma membranes is the lipid bilayer. RONS induce lipid peroxidation in biological membranes resulting in the formation of polar oxidation products, which increase the permeability of biological membranes (**Figure 1A**).

INTRACELLULAR REACTIVE OXYGEN AND NITROGEN SPECIES

Reactive oxygen and nitrogen species are released not only in extracellular fluids, but also within the cells. Generation of intracellular RONS is predominantly associated with the mitochondrial electron transport chain generating $O_2^{\bullet-}$ and nitric oxide (NO) synthases (NOSs), a family of enzymes generating NO. Both the $O_2^{\bullet-}$ and NO serve as intracellular messengers, if produced in physiological amounts (23, 28). The levels of mitochondrial RONS are elevated upon the systemic inflammatory response *via* a mechanism called RONS-NOS cycle (29), which comprises several steps. First, inflammatory mediators upregulate inducible NOS (iNOS) causing drastic increase in the intracellular NO levels. NO reversibly binds to complex IV at the same site as the oxygen (30). This inhibits the electron flow through the mitochondrial electron transport chain to complex IV and its two electron reduction to water (31). Instead, the electrons leak to oxygen from complexes I and III being reduced by one electron to $O_2^{\bullet-}$ (29). The intracellular interaction between NO and $O_2^{\bullet-}$ induces mRONS-NOS vicious cycle mentioned above, which continuously elevates cytoplasmic levels of $O_2^{\bullet-}$ and NO until their levels are sufficient to form ONOO and damage cellular membranes causing the release of intracellular enzymes into extracellular fluid (29). This is accompanied by a drop in cellular ATP levels impairing the entire cellular metabolism. In specific cell types and under hypoxic conditions, xanthine oxireductase (32) and NADPH-oxidase also contribute to the intracellular RONS pool, predominantly by generation of H_2O_2 (33). NADPH oxidase also contributes to the crosstalk with mitochondria *via* RONS generated at both the sites (33, 34).

An additional mechanism activating oxidative stress upon hypoxia is the release of ferrous ions from the ferritin. Ferrous ions are extremely strong activators of oxidative stress, particularly of lipid peroxidation (LPO) (35). Iron-mediated LPO can irreversibly damage intracellular compartments such as mitochondria; this action of iron can be prevented by chelation of ferrous ions, for instance, by NO (36). Of note, NO can be deleterious in the presence of oxygen upon inflammation forming ONOO and beneficial under hypoxic conditions inactivating ferrous ions. There are clinical observation supporting the key role of RONS. It has been shown that common antioxidant therapies reduce MOF and inflammation (37). *In vivo*, it is difficult to dissect the effects of inflammation and hypoxia and they often appear together due to systemic interactions. Precisely cut liver slices, which maintain tissue structure, but are free of systemic influences, can be used to separate effects of inflammation and hypoxia in the tissue. In these slices, it has been shown that either treatment with a cocktail of inflammatory mediators or hypoxia induce the release of liver damage markers aspartate aminotransferase/alanine aminotransferase (AST/ALT), while mitochondrial dysfunction was observed only under hypoxia (38). This supports the assumption that inflammatory mediators predominantly impair plasma membrane, while hypoxia affects both the plasma membranes and intracellular compartments.

EXTRA- AND INTRACELLULAR PHOSPHOLIPASES

Phospholipases are a family of enzymes that cleave ester bonds within phospholipids. This hydrolytic reaction releases free fatty acids and lysophospholipids. These products control a number of cellular signaling pathways. Phospholipase A (PLA) occurring in all the human cells is particularly often involved in pathophysiological processes (39). PLA is a family of enzymes including several forms. The Ca^{2+}-dependent cytosolic and secreted PLA_2 as well as Ca^{2+}-independent PLA_2 are predominantly associated with physiological and pathological processes (40, 41). PLA_2 is also the major toxic component of snake venom (42). Extracellular (secreted) PLA_2 belongs to the acute phase proteins and releases in response to inflammatory stimuli. PLA_2 hydrolyzes glycerophospholipids in position two and destabilizes lipid part of the membranes increasing membrane permeability. This reaction, called phospholipolysis, is essential for bactericidal activity (43). The enzyme type IIA of the secreted phospholipase A2 (s PLA_2-IIA) is particularly important for the mammalian innate host defense against bacterial infection (44). The expression of PLA_2 in mammalian cells is regulated by proinflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) (45) *via* nuclear factor-kappa B (NF-κB) and PPARg pathways (46). PLA_2 is not specific for bacterial phospholipids and hydrolyzes phospholipids in all the membranes, including host cell membranes.

The levels of PLA_2 are drastically increased in septic patients (47) and the high levels of PLA_2 are associated with the increased mortality in these patients (48). This supports the assumption that systemic damage to plasma membranes is critical for outcome of septic patient. PLA_2 also plays a pivotal role in the development of acute respiratory distress syndrome (ARDS) (49). Activation of PLA_2 upon inflammation is very fast; for instance, IL-1 activates intracellular PLA_2 within several minutes (50). Activation of intracellular PLA_2 damages mitochondrial membranes (51). Mitochondrial dysfunction results in a decrease in ATP levels, subsequently increases cytosolic calcium levels, which further activates phospholipase. Accumulation of lipid breakdown products, unesterified free fatty acids, acyl carnitine, and lysophospholipids, which have a detergent effect, further aggravate membrane damage and induce the leak of intracellular content into extracellular fluids (52). Thus, the spectrum of phospholipase actions is ranging from contribution to regulatory pathways and defense against bacterial infection to the damage of host cell membranes and subcellular organelles. It has been shown that septic mice, pretreated with GW4869, an inhibitor of PLA_2, ameliorate the disease decreasing the levels of circulating cytokines and extracellular vesicles elevated in response to sepsis (53). An overview of PLA2-mediated membrane damage is given in **Figure 1B**.

DIRECT DAMAGE BY HOST AND PATHOGEN PROTEINS

The third mechanism of membrane damage accompanying the development of MOF is the interaction of membranes with so-called PFTs, also termed as PFPs. These proteins disturb biological membranes and increase permeability by forming large pores (18) (**Figure 1C**) due to cytolytic transmembrane assemblies; this not necessarily kills the cells, but substantially changes cellular functions (54). PFPs induce the influx of calcium and efflux of potassium, altering intracellular ion homeostasis. Particularly critical are elevated Ca^{2+} levels because PFPs strongly facilitate the influx of Ca^{2+} and its levels quickly become toxic (55, 56) causing desensitization of immune cells (57), destabilization of tissue barriers, and subsequently leading to organ failure (58). PFPs are best characterized in bacteria (18) and they are the largest group of bacterial toxins comprising about 30% of all the bacterial toxins (59). PFPs have been shown to play a critical role in the initial stages of neonatal sepsis and to be associated with poor clinical outcome (60). PFPs also occur in eukaryotic cells. The mammalian immune system has adopted PFPs to kill pathogens (61). PFP gasdermin D is active in neutrophils and plays a crucial role in the release of neutrophil extracellular traps (62). However, excessive formation of these traps aggravates organ failure in septic patients, while its inhibition attenuates MOF (62). Another important PFPs formed by the mammalian immune system are the complement components (C5, C6, C7, and C8), also named as membrane attack complex (MAC) (63). Excessive activation of the complement system has also been attributed to the worsening of MOF (64, 65).

OSMOTIC SHOCK AND MECHANICAL DAMAGE

Osmotic dysregulation causes severe cell damage resulting in cell swelling and ultimately cell lysis and necrotic cell death. Osmotic damage occurs due to disturbance of the electrolyte balance, particularly due to pathologic changes in potassium and sodium homeostasis as well as in extracellular fluid volumes. Water/electrolyte balance disorders are commonly found in hospitalized patients, most often in the elderly patients (66) and are associated with an increased risk of death (67). The kidneys play a particularly important role in the regulation of body water and osmotic balance and they are very susceptible to failure in patients presenting hypernatremia (68). Hyponatremia has also been associated with an increased mortality following bacterial infectious diseases (69). It has been shown that both the hypernatremia (70) and hyponatremia (71) increase the risk of death. Severe hyponatremia is directly associated with the development of MOF (72).

In addition to osmotic, also mechanical deformation of tissues may damage membranes. Mechanical deformation may appear due to external blast or lateral tension appearing upon trauma or excessive physical exercises. Muscle fibers are particularly prone to injury mediated by mechanical deformation (73). Muscle damage can also appear in patients suffering from muscular dystrophy, due to changes in cytoskeletal protein, dystrophin (74). Osmotic shock and mechanical deformation may aggravate organ dysfunction induced by hypoxia and inflammation. For instance, it has been shown that in patients with mechanical trauma, the overwhelmed activation of complement is the predictor of MOF (75).

Thus, there are numerous mechanisms potentially causing membrane damage, which are associated with MOF. The mechanisms connecting damage of plasma and intracellular membranes and organ function/dysfunction will be considered below.

CONSEQUENCES OF DAMAGE TO PLASMA MEMBRANES

Plasma membrane integrity is obligatory for physiological cellular homeostasis; the impairment of the plasma membrane causes dysregulation of ion homeostasis and the release of cellular content in extracellular fluids (76). Moderate damage to the plasma membrane does not lead to cell death, but affects cellular functions and communication between the cells (76–78), both are important for organ function and they are almost reversible. These reversible changes often occur in muscle tissues, which are particularly susceptible to damage during exercises and the body has well-developed mechanisms repairing this damage. However, in severe local trauma, such as traumatic brain injury, trauma exceeds the repair capacity of the cells and this results in membrane lesions and severe neuronal dysfunction (79). The damage to cell membranes caused by microbial pathogens and immune cells can also exceed repair capacity and can have deleterious consequences for cellular functions (58, 80).

It is still unclear how to determine critical size of the membrane damage and compare it with potential repair capacity as well as how different mechanisms of membrane repair cooperate in order to recover membrane integrity (76). The progress in this field is limited by a number of technical restrictions. The major technical problem is that visualizing and characterizing damages to plasma membrane are difficult due to low resolution of analytical methods and rapid speed of damage and repair processes (76). In the majority of the studies, the damage to plasma membrane is determined by three indirect means, including the entry of cell-impermeable molecules, calcium influx, and the release of intracellular contents into the extracellular fluid (81). However, already these approaches have delivered a number of examples showing that instability of plasma membranes is a prerequisite of MOF. Intestinal epithelial injury determined by entry in the tissue of 4 kDa fluorescein-dextran has been shown to predict subsequent development of MOF (82). Hyperpermeability of endothelium triggered by inflammation or ischemia promotes edema, exacerbating disease progression, and slowing down recovery (83). Increased permeability of endothelium allows the inflammatory mediators/acute phase proteins diffuse into tissue (84) extending the process of disintegration of plasma membrane to parenchymal cells in different organs. Increased permeability

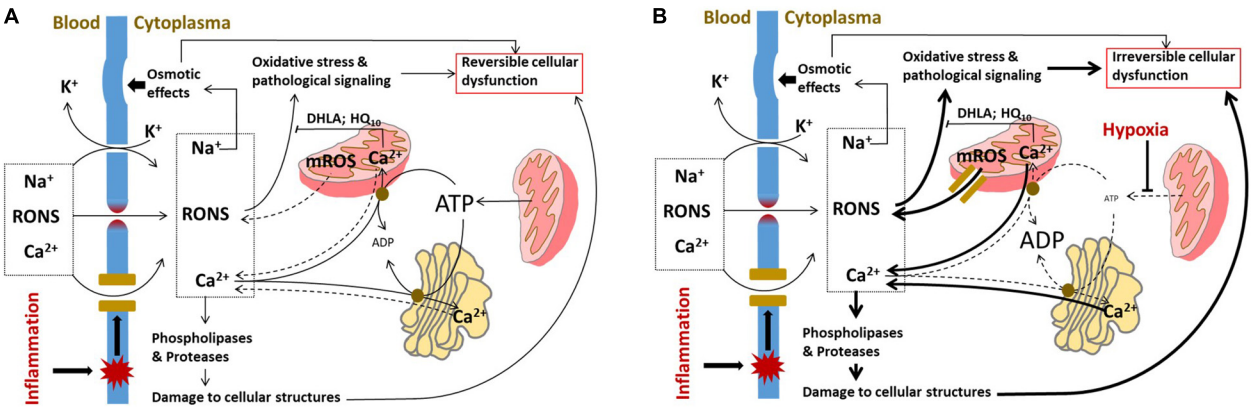

FIGURE 2 | Induction of cellular dysfunction occurring due to damage to cellular membranes. **(A)** Pathologic mechanisms operating in parenchymal cells upon damage to plasma membrane. The influx of RONS and Ca^{2+} through the defects in plasma membrane is partially compensated by intracellular organelles, which scavenge RONS and take up Ca^{2+} from the cytoplasm by ATP-dependent Ca^{2+} pumps. In addition, the influx of Na^+ and leak of K^+ are compensated by Na^+/K^+ pump, which uses ATP to pump the ions (not shown). At this phase of disease, the cells are exposed to oxidative stress, osmotic shock, and elevated activities of phospholipases and proteases; however, all these changes are still reversible because if sources of damaging substances in the blood are removed, the plasma membrane will be repaired and the functional intracellular organelles will restore the normal cellular metabolism. **(B)** Pathologic mechanisms operating in parenchymal cells upon damage to plasma membrane combined with damage to intracellular compartments induced by hypoxia. Under hypoxic conditions, the ATP levels in cells are drastically decreased and all the ATP-dependent processes are slowed down. The mitochondria instead of taking up Ca^{2+} and scavenging RONS will release Ca^{2+} and RONS; the latter leak through permeability transition pore, an intracellular PFP formed in mitochondria. The pathological changes in ion homeostasis are decompensated and Na^+/K^+ imbalance will be further aggravated. The cell comes in a kind of decompensation phase and the cellular dysfunction will become irreversible. RONS, reactive oxygen and nitrogen species; DHLA, dihydrolipoic acid; HQ_{10}, ubiquinone; and mROS, mitochondrial reactive oxygen species.

of plasma membranes for Ca^{2+} is particularly deleterious for severe patients. This is supported by the fact that infusion of Ca^{2+} exacerbates organ failure and mortality in septic patients (85). The leak of potassium from cells into extracellular fluids also has a critical pathological impact. Hyperkalemia is associated with poor outcomes of patients in different pathological settings, including the acutely ill patients (86); the primary mortality risk in these patients is cardiac electrophysiological disturbances (87).

The damage to the plasma membrane and influx of extracellular Ca^{2+} can be at least partially compensated by intracellular organelles. RONS can be scavenged by intracellular antioxidant systems and excess of Ca^{2+} can be removed from the cytoplasma by ATP-dependent Ca^{2+} pumps in the ER and the mitochondria (**Figure 2A**). After repair of the plasma membrane, cell functions can be recovered.

CONSEQUENCES OF DAMAGE TO INTRACELLULAR MEMBRANES

The integrity of intracellular compartments is critical for vital cellular functions. Impairment of both the outer and inner mitochondrial membranes compromises mitochondrial function. Damage to outer mitochondrial membrane causes the release of cytochrome c. This inhibits electron transport along mitochondrial electron transport chain to oxygen and consequently inhibits ATP synthesis. Released cytochrome c can also induce apoptosis. Increased permeability of the inner mitochondrial membrane does not affect the electron transport, but reduces mitochondrial membrane potential and uncouples oxidation from phosphorylation causing a drop in ATP synthesis. The formation of the mitochondrial permeability transition pore, a kind of intracellular PFP, is considered as the major mechanism of uncoupling (88). Iron-mediated lipid peroxidation is another mechanism impairing outer mitochondrial membrane leading to the release of cytochrome c under hypoxic conditions (36). Mitochondrial dysfunction has been suggested to cause MOF upon systemic inflammation (89, 90), although it may be the result of secondary hypoxia (38). Irrespective of the underlying mechanism, patients with sepsis have better prognosis, if they have higher levels of tissue ATP (91). Decreased levels of ATP cause the release of Ca^{2+} and activation of numerous intracellular phospholipases and proteases, which normally exert only a modest activity.

However, low levels of ATP can also be beneficial by protecting against apoptosis. Oxidative damage to ER often induces so-called ER stress. ER stress during critical illness has been less thoroughly studied than mitochondrial dysfunction, but it has been shown to play a critical role in a number of acute pathologies accompanied by MOF (92). In animal models, markers of increased ER stress have indeed been observed in heart and liver during sepsis (92) as well as in failing organs following hemorrhage, trauma, and ischemic injury (92–95). In animal models accompanied by MOF, ER stress markers were associated with organ failure (96, 97) suggesting that ER stress contributes to the development of MOF. The effect of ER stress on function of different organs during critical care diseases needs to be further investigated. A critical situation appears, if the plasma membrane damage occurs simultaneously with hypoxia. Hypoxia slows down the mechanisms compensating damage to the plasma membrane. Mitochondrial permeability transition pore formed under hypoxic conditions facilitates the

release of mitochondrial ROS and cytochrome c into cytoplasm. The decrease in ATP synthesis results in the release of Ca^{2+} from intracellular storage (mitochondria and ER) instead of taking Ca^{2+} up from cytoplasm. We assume that overwhelmed activation of intracellular Ca^{2+}-dependent phospholipases and proteases along with excessive RONS generation are the major mechanisms causing irreversible cell damage (**Figure 2B**).

MECHANISMS OF MEMBRANE REPAIR

Membrane defects can be efficiently repaired, if they do not exceed the critical size of the damage. Membrane fusion and replacement are two main strategies to repair the plasma membranes (15, 18). Membrane fusion is realized by exocytosis, while the removal of damaged membranes is executed by endocytosis or shedding. The closure of membrane defect can be achieved *via* protein-driven membrane remodeling and wound closure (16, 98, 99). Repair mechanisms can be activated in a very fast mode; in some cases, exocytosis can be activated for less than 1 min (100). The activation of exocytosis and shedding results in an increase in the quantity of so-called extravascular vesicles (EVs) into blood or other extracellular fluids (**Figure 3**). EVs consist of exosomes (∼40–200 nm), microvesicles (∼200–1,000 nm), and apoptotic bodies (500–3,000 nm), a nomenclature based on the biogenesis pathways (101).

Judging from patterns of EVs, one can distinguish between activation of repair (shift to small EVs) or death pathways (shift to large EVs). It has been shown that EV sizes in septic patients and cecal ligation and puncture (CLP) mice are remarkably smaller than in corresponding controls (102, 103), suggesting that in septic patients, the membrane repair process rather than apoptosis is initiated as a response to plasma membrane damage. Indeed, except lymphocytes, no other apoptotic cells have been reported in sepsis patients (7) and animals (6) even in those dying from sepsis. The role of EVs in sepsis is controversial; it has been associated with both the proinflammatory (102, 104) and anti-inflammatory (105) effects. The literature on metabolic effects of EVs in sepsis was recently reviewed in detail by Burgelman et al. (101). Intriguingly, in septic patients, significantly higher levels of endothelial-derived EVs were determined in the blood of survivors compared to non-survivors (106). This is in line with the assumption that endothelium membranes are the primary targets for membrane damage upon systemic inflammatory response and consequently repair mechanisms are first activated in those cells.

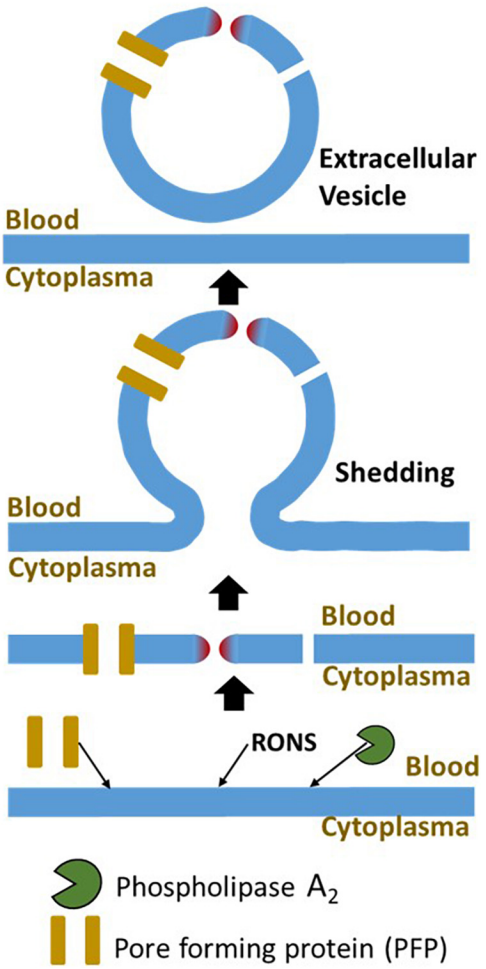

FIGURE 3 | The major mechanisms of damage to plasma membrane and its repair. The plasma membrane is damaged by three major mechanisms, interaction with PFP, lipid peroxidation, and elevated phospholipase activity. The major mechanism repairing of this damage is membrane shedding. The damaged part of the plasma membrane will be excluded from the cell in form of an extracellular vesicle. RONS, reactive oxygen and nitrogen species.

REGULATION OF MEMBRANE REPAIR

There are several mechanisms regulating cell repair. As mentioned above, inflammatory response is accompanied by the release of PLA_2, which on one hand damages biomembranes, but on the other hand activates exocytosis, a repair mechanism. The critical role in the repair activation plays soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. SNARE is responsible for the connection between vesicular and plasma membranes; this connection is essential for activation of exocytosis facilitating the fusion between vesicles and the plasma membrane (107). The fusion with the damaged plasma membrane is facilitated by polyunsaturated fatty acids (PUFAs) generated from PLA_2 activity. Thus, PLA_2 activity has a double function. On one hand, it damages the membranes, but simultaneously it can induce repair processes in a product-dependent manner. For instance, it has been shown that PUFAs have a protective effect recovering increased permeability of endothelium. Arachidonic acid and sphingosine are particularly strong enhancers of exocytosis (108, 109). More details about interaction between PUFAs and SNARE can be found in the review by Virginia Garcia-Martinez (110). The second class of molecules regulating membrane repair are RONS. Similarly to PLA_2, RONS are involved in both the membrane damage and regulation of membrane repair. RONS facilitate exocytosis by specific activation of lysosomal TRPML1 (the first member of the mammalian mucolipin TRP

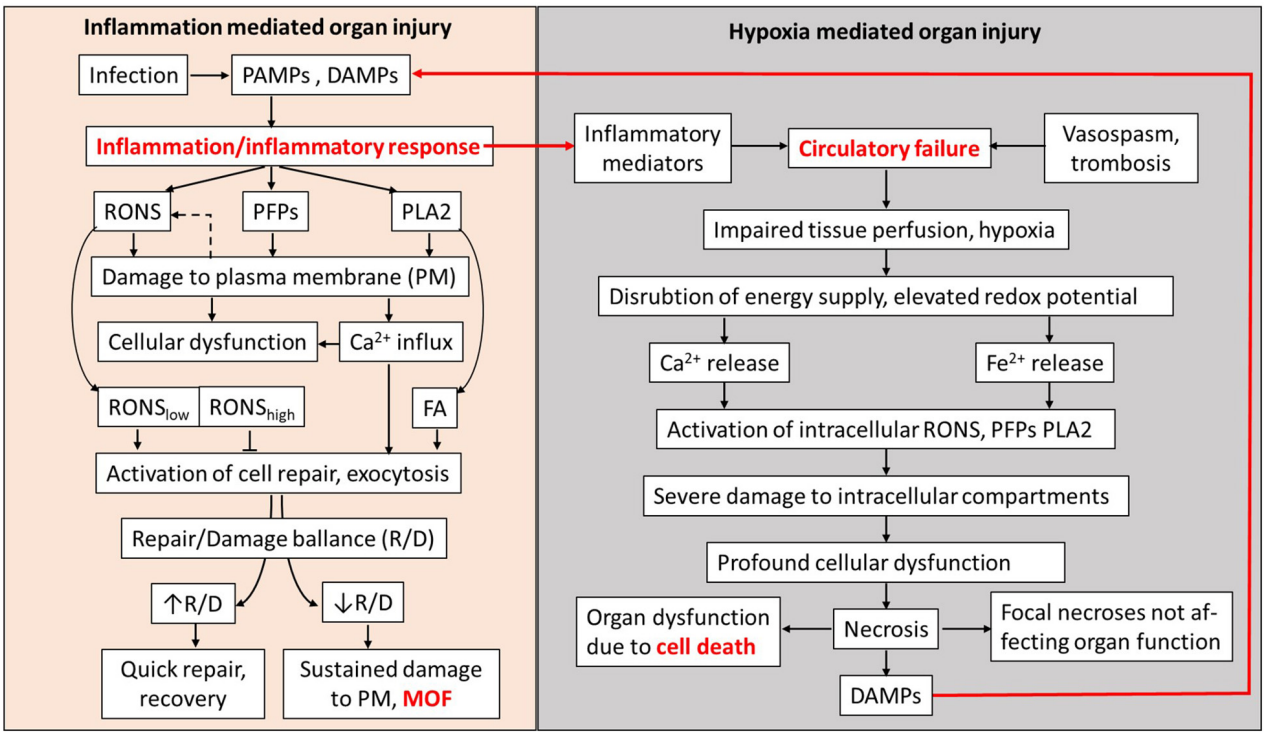

FIGURE 4 | Schematic presentation of MOF pathogenesis mediated by plasma membrane damage. RONS, PFPs, and PLA_2 released in blood and extracellular fluids upon inflammatory response cause damage to plasma membranes (PMs) of well-perfused organs. Influx of Ca^{2+}, RONS, and products of PLA_2 simultaneously activate PM repair mechanisms. RONS may also be released from cells (dashed arrow). Damage and the counteracting repair establish a repair/damage balance, which is critical to define whether involved organs will recover or undergo MOF. Hypoxia occurring concomitantly with inflammatory response causes the increase in RONS and Ca^{2+} levels and PLA_2 activity inside the affected cells, leading to severe damage of intracellular organelles causing cell death and the release of DAMPs. Inflammatory response and hypoxia interact *via* the release of inflammatory mediators and DAMPs. In well-perfused organs, the damage to PM and development of MOF have higher impact than hypoxia-mediated damage to intracellular compartments. PAMPs, pathogen-associated membrane patterns; DAMPs, damage-associated molecular patterns; RONS, reactive oxygen and nitrogen species; PFPs, pore forming proteins called also protein forming toxins (PFPs); PLA_2, phospholipase A_2; FA, fatty acids; R/D balance, repair/damage balance; PM, plasma membrane; and MOF, multiple organ failure called also multiple organ dysfunction syndrome (MODS).

channel subfamily) channels, inducing lysosomal Ca^{2+} release, which, in turn, stimulates lysosomal exocytosis *via* calcineurin, a Ca^{2+}-dependent phosphatase (111). Activation of repair mechanisms by RONS depends on their concentration. At low concentrations, RONS act as a second messenger stimulating lysosomal exocytosis and facilitating membrane repair. In contrast, high levels of RONS inhibit lysosomal exocytosis manifesting exclusively damaging potential (112). The process of repair is also facilitated by Ca^{2+} influx through injured plasma membrane areas. Influxed Ca^{2+} has two functions. First, it facilitates the fusion of preexisting intracellular vesicles to form so-called membrane "patches." Second, the location of Ca^{2+} influx indicates the place of the membrane damage and facilitates the fusion of membrane "patches" with the injured part of the plasma membrane using Ca^{2+} as an anchor (113).

DISCUSSION

In this review, we have considered two general pathological processes, systemic inflammatory response and hypoxia, which are associated with MOF and collected evidence that the release of substances causing damage to plasma membranes in diverse organs is a key process leading to MOF (**Figure 4**). Inflammatory response causes damage to plasma membranes mediated by elevated levels of PLA_2, RONS, and PFPs in extracellular fluids. The damage to plasma membranes is potentially reversible and the rate of recovery depends on the size of the damage and the repair capacity. Similar mechanisms mediated by PLA_2, RONS, and PFPs are induced by hypoxia. However, upon hypoxia, these mechanisms are activated within the cells and their primary targets are intracellular compartments, such as mitochondria and ER, which are essential for vital cellular functions. Damage to these organelles causes irreversible cellular dysfunction and ultimately leads to cell death. We assume that the focal necrosis sometimes observed in severe preclinical models of systemic inflammatory response or sepsis is due to secondary hypoxia rather than to the action of inflammatory mediators. Since repair of plasma membranes is a fast process, it can be achieved in a short time after elimination of pathologic stimuli.

Membranes have similar structure in all the organs; that is why, several organs can be affected simultaneously. The

circulatory centralization often occurring upon shock ameliorates oxygen supply to vital organs, but simultaneously delivers more inducers of plasma membrane damage (RONS, PLA_2, and PFPs); that is why, damage appears despite improved tissue perfusion. It looks like the body decides to induce reversible dysfunction caused by inflammation in several organs to avoid the "death" of single organs induced by hypoxia. This can be a good solution for short-term acute critical phases, but on the long run, this causes the death of the whole body due to systemic dysregulation induced by MOF, although each failed organ would have a chance to recover.

Although MOF is not characterized by noticeable death of parenchymal cells in failed organs, it is accompanied by elevated death of immune cells. The programmed death of immune cells was associated for a long time with apoptosis induced by the damage to mitochondrial membranes and the release of cytochrome c (114). More recently, the death of immune cells was attributed to necroptosis, which is activated by the inflammatory mediator, TNF-alpha. This pathway has already been attributed to critical care diseases and it is characterized by the loss of plasma membrane integrity (115); consequently, this supports the key role of plasma membrane damage in the development of MOF. As we mentioned above, the hypoxic conditions facilitate the release of ferrous ions from the ferritin and can potentially activate ferroptosis, a programmed cell death regulated by iron-containing compounds (116). Indeed, recently ferroptosis was associated with the vascular leakage upon septic conditions (117). Damage to intracellular compartments such as mitochondria stimulates autophagy and formation of autophagosomes. It has been shown that autophagy is activated in septic patient and animal models of sepsis (7). Moreover, autophagy has been shown to play a protective role against sepsis (118). We assume that the release of damaged membranes with exosomes formed in autophagosomes is the mechanism underlying beneficial effects of autophagy.

We are convinced that current knowledge on membrane damage upon MOF summarized here will attract attention of researchers and clinicians to this field stimulating basic and clinical research and creating the basis for novel powerful diagnostic and therapeutic tools improving clinical outcome of patients with MOF.

AUTHOR CONTRIBUTIONS

AK conceived the review, drafted the manuscript and the figure, and approved the final version of the manuscript. JG wrote the section on extravascular vesicles and approved the final version of the manuscript. Both authors contributed to the article and approved the submitted version.

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The Amplitude of Diaphragm Compound Muscle Action Potential Correlates With Diaphragmatic Excursion on Ultrasound and Pulmonary Function After Supraclavicular Brachial Plexus Block

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Objective: This prospective, double-blind, randomized study assessed (1) the associations between diaphragm compound muscle action potential (CMAP), hemidiaphragmatic excursion, and pulmonary function after supraclavicular brachial plexus block (SCBPB) and (2) diagnostic efficacy of pulmonary function for hemidiaphragmatic paralysis evidenced by diaphragm CMAP as an assessment of diaphragm strength was evaluated.

Methods: Eighty-six patients were scheduled for the removal of hardware after healing of a right upper limb fracture distal to the shoulder who were randomly assigned in a 1:1 ratio to two groups: Group A (diaphragmatic excursion), or Group B (pulmonary function). Phrenic nerve conduction studies (PNCSs), M-mode ultrasonography of the diaphragm, and pulmonary function tests (PFTs) were performed before and 30 min after SCBPB. PNCSs were used to determine the latency and amplitude of diaphragm CMAP. Ultrasonography of the diaphragm was performed with patients in a supine position using a low-frequency probe over the subcostal space at the midclavicular line. The diaphragmatic excursion was measured during quiet breathing and deep breathing. Pulmonary function, i.e., forced vital capacity (FVC), predicted value of FVC, and forced expiratory flow in the first second (FEV1), was measured with spirometry. Receiver Operating Characteristic (ROC) curve analysis was used to assess the diagnostic efficacy of pulmonary function for hemidiaphragmatic paralysis evidenced by diaphragm CMAP as an assessment of diaphragm strength.

Results: There were significant associations between the reduction in amplitude of diaphragm CMAP and reductions in diaphragmatic excursion during quiet breathing ($r = 0.70$, $p < 0.01$) and deep breathing ($r = 0.63$, $p < 0.01$) when expressed as a

percentage of baseline values. There were significant associations between the reduction in amplitude of diaphragm CMAP and reductions in FVC ($r = 0.67$, $p < 0.01$), FVC% ($r = 0.67$, $p < 0.01$), and FEV1 ($r = 0.62$, $p < 0.01$), when expressed as percentage of baseline values. The area under the ROC curve for FVC was 0.86. A decrease of >8.4% in FVC compared to pre-block predicted hemidiaphragmatic paralysis (determined by diaphragm CMAP) with sensitivity and specificity of 79.2 and 100%, respectively.

Conclusions: The relative reduction in diaphragm CMAP amplitude after SCBPB was correlated with relative reductions in diaphragmatic excursion and pulmonary function. FVC has potential as a useful diagnostic indicator of hemidiaphragmatic paralysis, evidenced by diaphragm CMAP, after SCBPB. These data establish diaphragm CMAP as a direct and objective index of diaphragmatic paralysis after SCBPB.

Keywords: diaphragm compound muscle action potential (CMAP), supraclavicular brachial plexus block, phrenic nerve conduction studies, hemidiaphragmatic excursion, pulmonary function

INTRODUCTION

Brachial plexus block is an effective method for providing anesthesia and post-operative analgesia in patients who underwent surgery of the upper limb. Compared to general anesthesia, brachial plexus block is associated with reduced opioid consumption and opioid-related side effects, decreased length of stay in the post-anesthesia care unit (PACU), and improved patient satisfaction (1). However, brachial plexus block may result in ipsilateral phrenic nerve block and hemidiaphragmatic paresis (2). The incidence of ipsilateral hemidiaphragmatic palsy in the interscalene brachial plexus block is almost 100% (3). However, the distance between the phrenic nerve and the brachial plexus increases caudally to the cricoid cartilage; therefore, the incidence of ipsilateral hemidiaphragmatic palsy in supraclavicular brachial plexus block (SCBPB) is 36–67% (2).

The diaphragm is a main inspiratory muscle, accounting for 75% of the total tidal volume in quiet breathing (4). Healthy individuals at rest are able to tolerate hemidiaphragmatic paresis, but they may experience exercise-associated dyspnea and reduced exercise tolerance (5–8). Patients with unilateral paralysis of the diaphragm usually have no symptoms, as the contralateral diaphragm can compensate. In some patients, clinical signs of respiratory failure after diaphragmatic paralysis cannot be resolved through compensation by the contralateral diaphragm. In one report, a patient with no obesity or history of respiratory disease experienced dyspnea in the post-operative recovery unit after total shoulder replacement (9). In patients with obesity and respiratory diseases, dyspnea and decreased blood oxygen saturation may be more serious.

Diagnosis of diaphragmatic paralysis is essential to avoid respiratory morbidity and can be achieved with a variety of tests. Spirometry techniques are commonly used to measure pulmonary function by assessing forced vital capacity (FVC), predicted value of FVC, and forced expiratory flow in the first second (FEV1). The quality of the spirometry test is influenced by the effort and cooperation of a patient. With the evolution of ultrasound technology, M-mode imaging has been used to identify abnormal diaphragm movement/position during quiet or deep breathing (4); however, ultrasound does not account for the influence of the contralateral diaphragm and accessory respiratory muscles. Phrenic nerve conduction can overcome these challenges. Phrenic nerve conduction is an emerging technique that can be used to evaluate diaphragmatic dysfunction (10, 11) by measuring the change of diaphragm compound muscle action potentials (CMAPs).

Physicians should be aware of diaphragmatic paralysis caused by brachial plexus block, diagnosis of diaphragmatic paralysis, and that early detection of diaphragmatic paralysis can prevent the onset of dyspnea (9). The objective of this prospective, double-blind, randomized study was to assess the associations between diaphragm CMAP, hemidiaphragmatic excursion, and pulmonary function after SCBPB. The diagnostic efficacy of pulmonary function for hemidiaphragmatic paralysis evidenced by diaphragm CMAP as an assessment of diaphragm strength was evaluated.

METHODS

Patients

Patients scheduled for the removal of hardware after healing of right upper limb fracture distal to the shoulder between September 1, 2019 and January 30, 2020 at the 98th Hospital of the People's Liberation Army (PLA) were eligible for this study. Inclusion criteria were as follows: (1) aged 18–60 years; (2) American Society of Anesthesiologists physical status class I or II; (3) body weight 45–85 kg; and (4) consented to SCBPB. Exclusion criteria were as follows: (1) history of the cardiopulmonary disease; (2) body mass index (BMI) >30 kg/m^2; (3) sensory or motor impairment; (4) coagulopathy or severe renal or hepatic failure; (4) known allergy to local anesthetics; (5) extremely atypical diaphragm CMAP; or (6) mild block.

Included patients were randomly assigned in a 1:1 ratio (using a computer-generated random number table and the sealed envelope system for allocation concealment) to two groups: Group A, in which diaphragm CMAP and diaphragmatic excursion were measured, or Group B, in which diaphragm

CMAP and pulmonary function were measured. Two groups of patients were required as it takes 2 min to measure diaphragmatic excursions and 3 min to measure pulmonary function. We could not adjust for the differences in the time it takes to obtain these measurements; therefore, both measurements could not be obtained from a single group of patients.

This prospective, double-blind, randomized study was approved by the Medical Ethics Committee of the 98th Hospital of the PLA. Written informed consent was obtained from all patients before enrollment. This study was an extension of a clinical trial registered on the Chinese Clinical Registry (http://www.chictr.org.cn/showproj.aspx?proj=16992; ChiCTR-IND-17012166, 2017/7/27).

Supraclavicular Brachial Plexus Block

Patients were routinely prepared for surgery. Intravenous access was established prior to the brachial plexus block. ECG, oximetry (SPO_2), and blood pressure (non-invasive) monitoring were performed while patients breathed room air. The patients, the anesthesiologist who managed the patients in the surgery preparation room, and the researchers who collected and analyzed data were blinded to the group allocation.

All the patients were placed in the supine position with the head rotated in the contralateral direction. After sterile skin preparation with 2% chiorhexidine, an ultrasound probe was placed in the supraclavicular fossa to view the brachial plexus and subclavian artery. The skin and subcutaneous tissues were infiltrated with 1 ml of 2% lidocaine. Using the in-plane approach, 0.375% ropivacaine was injected in a lateral-to-medial direction with a sterile 22-gauge, 50-mm insulated nerve stimulating needle. Initially, 15 ml of 0.375% ropivacaine was injected into the "corner pocket" at the junction of the subclavian artery and first rib or pleura (12). The remaining 15 ml of 0.375% ropivacaine was injected into the most superficial portion of the lateral aspect of the cluster formed by the brachial plexus trunks and divisions (13). Adverse events associated with performing the block, such as vascular puncture and dyspnea, were recorded.

The success of the block was assessed after 30 min by an anesthetist who was blinded to the group allocation. The anesthetic effect was categorized as excellent, moderate, or mild. Excellent was defined as no need for supplemental local anesthetics and/or opioids or conversion to general anesthesia. Moderate was defined as the patient can undergo surgery with the need for 1–2 μg/kg fentanyl (14). Mild was defined as the patient was unable to tolerate the pain caused by the skin incision at the initiation of surgery or fentanyl administration did not increase the pain. Patients were followed up for 24 h after completion of infiltration of local anesthetic. Adverse events, such as local anesthetic toxicity, lack of return of normal motor and sensory function, SpO_2 decline (accounting for a 5% decrease as the patient was breathing room air), difficulty in breathing, and hoarseness, were recorded.

Diaphragm CMAP With Phrenic Nerve Conduction

Phrenic nerve conduction studies (PNCSs) were performed by a single technician using Cascade software (Cadwell Laboratories, Kennewick, WA, USA) according to previously published protocols (15–17). The patient was placed in the supine position with the head slightly elevated and rotated 30° in the contralateral direction. A bipolar surface stimulating electrode was placed at the posterior border of the right sternomastoid muscle at the level of the cricoid cartilage. One needle was inserted into the diaphragm at the xiphoid process, 16 cm from a second needle that was placed at the ipsilateral costal margin. A ground disc electrode was placed between the stimulator and the recording electrode. The right phrenic nerve was stimulated at end-expiration using rectangular pulses of 0.2 ms duration. The intensity of the stimulus was increased until reproducible response and maximal diaphragm CMAP amplitude were obtained (maximal stimulation). The phrenic nerve was supramaximally stimulated to 10–20% above maximal stimulation. The latency of the diaphragm CMAP, expressed as phrenic nerve conduction time (PNCT), was measured from stimulus to CMAP onset in milliseconds (ms). The amplitude of the diaphragm CMAP was measured from the negative peak to the positive peak of the waveform in microvolts (μV). Mean latency and amplitude of the diaphragm CMAP measured on three tests before and 30 min after SCBPB were used in the analyses. Paralysis of the diaphragm was defined as >50% reduction in the amplitude of the diaphragm CMAP.

Diaphragm CMAP with phrenic nerve conduction was performed after an ultrasound of the diaphragm and spirometry.

Ultrasound of the Diaphragm

Measurements were performed with the patient in the supine position with the head of the bed at a 30° angle (18) using a SonoSite M-Turbo system with a 2–5 MHz convex transducer. The right hemidiaphragm was visualized by placing the probe over the right anterior subcostal region between the midclavicular and anterior axillary lines. The probe was directed cranially at 30° to the coronal plane. Initially, the right hemidiaphragm was visualized in 2-dimensional (2D) mode with the liver serving as an acoustic window to the right. Subsequently, the probe was raised to the apex of the diaphragmatic dome, and M-mode was used to measure the amplitude of the diaphragmatic excursion during quiet and deep breathing. The means of 3 consecutive measurements before and 30 min after SCBPB were used in the analyses. "Complete paralysis" was defined as >75% reduction in diaphragmatic movement. "Partial paralysis" was defined as a 25–75% reduction in diaphragmatic movement, and "no paralysis" was defined as a <25% reduction in diaphragmatic movement. The number of patients with "paralysis" (partial or complete paralysis) or "no paralysis" was recorded.

Spirometry

Spirometry (Multi-Functional Spirometer H1-701, Japan) was performed in the surgery preparation room according to the recommendations of the American Thoracic Society (19, 20). Patients were placed in the seated position with the head of the bed at a 90° angle and instructed how to perform the test. The FVC, predicted value for FVC, and FEV1 were measured three times at 2-min intervals before and 30 min after SCBPB. The

means of 3 acceptable measurements before and 30 min after SCBPB were used in the analyses.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, version 22. Variables are reported as mean ± SD or medians and interquartile range (IQR). Between-group comparisons of baseline demographic and clinical characteristics were conducted using the Student's *t*-test. Associations between diaphragm CMAP amplitude, diaphragmatic excursion, and pulmonary function were evaluated using Pearson correlation analysis. The diagnostic efficacy of pulmonary function for hemidiaphragmatic paralysis determined by diaphragm CMAP was evaluated with Receiver Operating Curve (ROC) analysis. $p < 0.05$ was considered statistically significant.

The sample size required to obtain minimum correlation coefficients between reduction in amplitude of diaphragm CMAP and diaphragmatic excursion during quiet breathing and deep breathing of 0.75 and 0.53, respectively, in Group A and minimum correlation coefficients between reduction in amplitude of diaphragm CMAP and FVC, FVC%, and FEV1 of 0.6, 0.6, and 0.7, respectively, in Group B was calculated using the following formula ($n = 4 \times \left[(u_\alpha + u_\beta)/\ln(\frac{1+r}{1-r})\right]^2 + 3$,

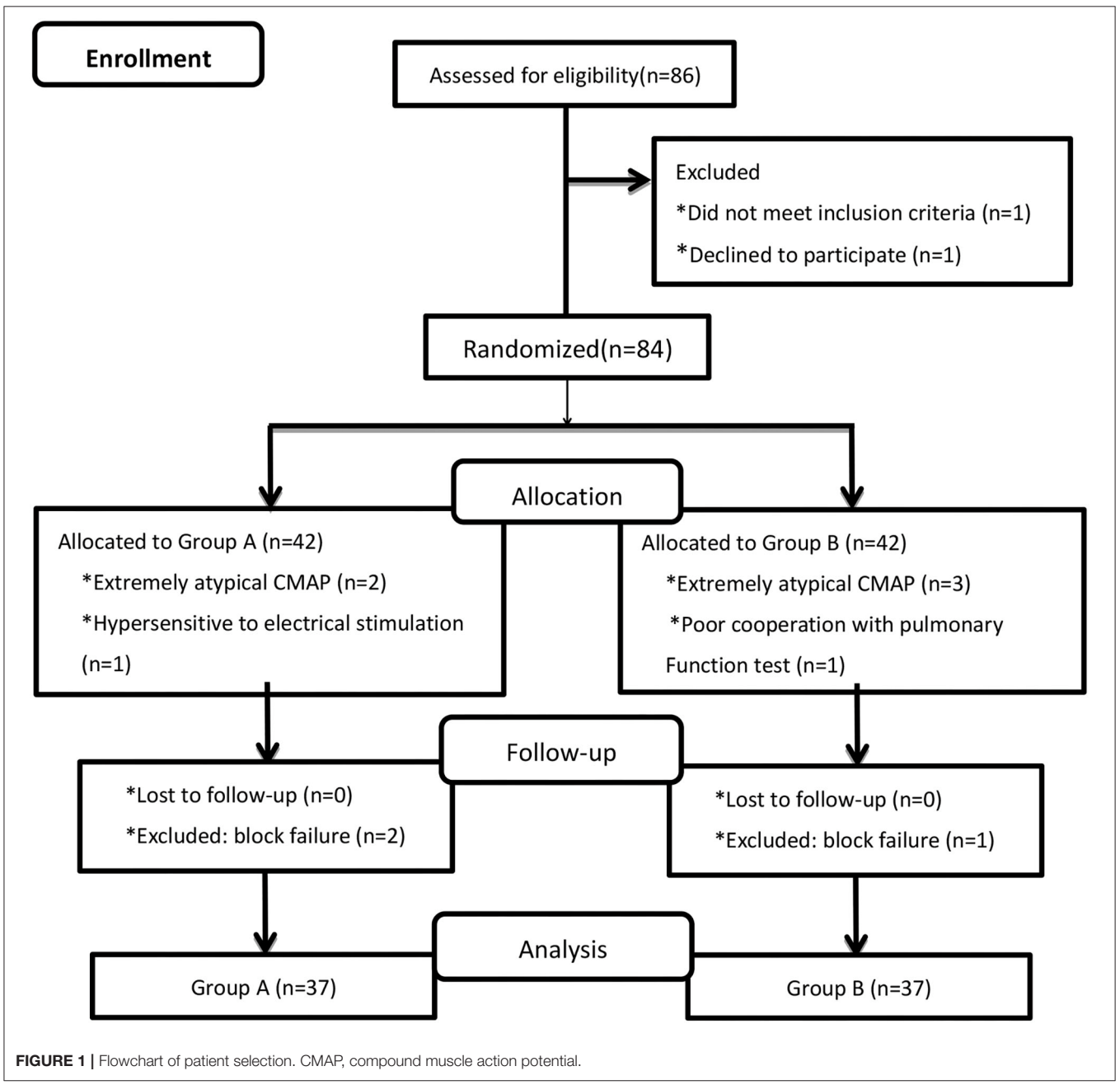

FIGURE 1 | Flowchart of patient selection. CMAP, compound muscle action potential.

$u_\alpha = 1.96, u_\beta = 1.282$). In total, 34 patients were required in each group. Assuming that 20% of study participants were lost to follow-up or dropped out, 86 patients were enrolled in the study.

The sample size for ROC curve analysis was determined using Power Analysis Software (PASS 11). Based on a preliminary study with 10 patients in each group, to obtain an area under the ROC curve of 0.75 with a statistical power of 80%, an alpha error of 0.05, and assuming that 20% of study participants were lost to follow-up or dropped out, 32 patients were required in Group B.

RESULTS

Patient Population

A total of 86 patients were randomized, and data for 74 patients were included in the analyses ($n = 37$ in each group; **Figure 1**). The demographic and clinical characteristics of the patients in Groups A and B were similar (**Table 1**).

Overall, 49 of the 74 (66.2%) patients experienced hemidiaphragmatic paralysis evidenced by diaphragm CMAP as the reference standard. Two patients in Group A and one patient in Group B were converted to general anesthesia because the anesthetic effect of the block was mild. The latency and amplitude of the diaphragm CMAP were extremely atypical in two patients in Group A and three patients in Group B, possibly due to stimulation of the brachial plexus. Among these, one patient in Group A was hypersensitive to the electrical stimulation as the amplitude of the diaphragm CMAP was >3,000 μV. This may be because the long thoracic, lateral pectorals, and/or medial pectoral nerves, which are derived from the brachial plexus, were stimulated.

Associations Between Diaphragm CMAP, Hemidiaphragmatic Excursion, and Pulmonary Function After SCBPB

Respiratory function, diaphragmatic excursion, and diaphragm CMAP are presented in **Table 1**. There were no differences in the effectiveness of SCBPB between Groups A and B. In

TABLE 1 | Baseline demographic and clinical characteristics of the study population.

	All patients ($N = 74$)	Group A ($n = 37$)	Group B ($n = 37$)	*P*-value (A vs. B)
Age (years)	41.95 ± 11.32	42.73 ± 11.86	41.16 ± 10.85	$P = 0.81$
Weight (kg)	62.81 ± 11.68	61.19 ± 11.41	64.43 ± 11.87	$P = 0.78$
Height (cm)	166.55 ± 7.66	165.57 ± 7.86	167.54 ± 7.43	$P = 0.31$
BMI(kg/m^2)	22.52 ± 3.13	22.22 ± 3.15	22.82 ± 3.12	$P = 0.68$
Sex (F/M)	17/57	10/27	7/30	$P = 0.41$
ASA physical status (I/II)	25/49	11/26	14/23	$P = 0.46$
Amplitude (μV)	1034.91 ± 497.96	1039.19 ± 511.06	1030.64 ± 491.52	$P = 0.61$
Amplitude (μV) of diaphragm CMAP after block	382.25 ± 303.77	385.10 ± 334.45	379.40 ± 274.31	$P = 0.48$
Latency (ms)	5.78 ± 1.65	5.69 ± 1.42	5.87 ± 1.86	$P = 0.15$
Quiet excursion (cm)		1.53 ± 0.29 ($P = 0.16$)		
Deep excursion (cm)		7.05 ± 1.03 ($P = 0.11$)		
FVC			3.44 ± 0.75 ($P = 0.2$)	
FVC%			84.48 ± 9.37 ($P = 0.2$)	
FEV1			2.98 ± 0.65 ($P = 0.17$)	

Values are mean ± SD or number. Group A, diaphragm excursion; Group B, pulmonary function; ASA, American Society of Anesthesiologists; FVC, forced vital capacity; FVC%, predicted value of FVC; FEV1, forced expiratory flow in the first second.

TABLE 2 | Diaphragm compound muscle action potential, diaphragm excursion during SCBPB, and respiratory function post-SCBPB.

	All patients ($N = 74$)	Group A	Group B	*P*-value
ΔCMAP pre-block to post-block	−73.6% (−86.1∼−30.4%)	−73.1% (−87.1∼−38.3%)	−74.6% (−84.5∼−27.2%)	0.95
ΔPNCT pre-block to post-block	25.1% (4.1∼67.6%)	24.2% (4.0∼60.6%)	27.7% (4.6∼70.3%)	0.67
Δquiet excursion pre-block to post-block		−40.0% (−100∼−6.5%)		
Δdeep excursion pre-block to post-block		−43.4% (−57.7∼−6.8%)		
ΔFVC pre-block to post-block			−11.9% (−31.2∼−4.2%)	
ΔFVC% pre-block to post-block			−11.9% (−31.2∼−4.3%)	
ΔFEV_1 pre-block to post-block			−13.2% (−30.7∼−5.1%)	

SCBPB, supraclavicular brachial plexus block; ΔCMAP, percent reduction in diaphragm compound muscle action potential pre-block to post-block; ΔPNCT, percent reduction in phrenic nerve conduction time pre-block to post-block; Δquiet excursion, percent reduction in quiet breath excursion pre-block to post-block; Δdeep excursion, percent reduction in deep breath excursion pre-block to post-block; ΔFVC, percent reduction in forced vital capacity pre-block to post-block; ΔFVC%, percent reduction in predicted FVC pre-block to post-block; ΔFEV1, the percent reduction in forced expiratory flow in the first second.

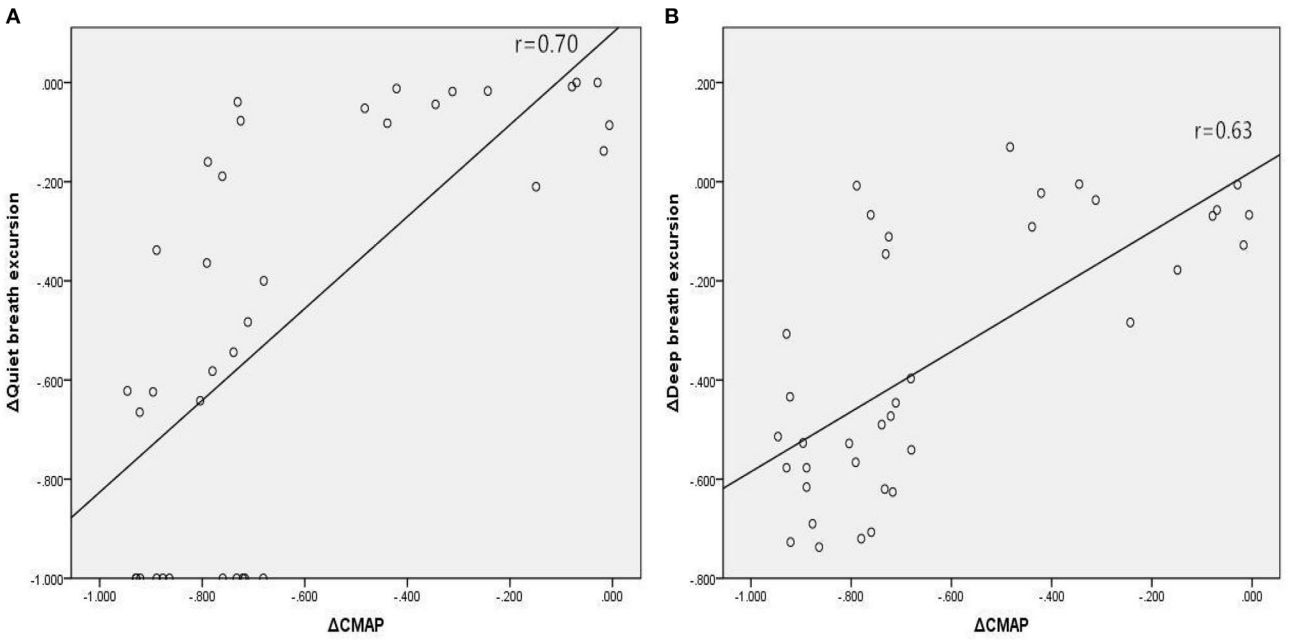

FIGURE 2 | Associations between relative changes (Δ) in diaphragm CMAP amplitude and diaphragmatic excursions during quiet breathing **(A)** and deep breathing **(B)** before and 30 min after SCBPB in Group A. CMAP, compound muscle action potential.

Group A (−73.1%) and Group B (−74.6%), the amplitude of diaphragm CMAP was decreased 30 min after SCBPB compared to baseline. In Group A, hemidiaphragmatic excursions during quiet breathing (−40.0%) and deep breathing (−43.4%) were decreased 30 min after SCBPB compared to baseline. In Group B, FVC (−11.9%), FVC% (−11.9%), and FEV1 (−13.2%) were decreased 30 min after SCBPB compared to baseline (**Table 2**).

Five patients (13.5%) in Group A vs. three patients (8.1%) in Group B reported transient dyspnea and oxygen desaturation 10 min after SCBPB. Symptoms were significantly improved after oxygen inhalation through a nasal catheter.

In Group A, there were significant associations between the reduction in amplitude of diaphragm CMAP and reductions in diaphragmatic excursion during quiet breathing (**Figure 2A**; $r = 0.70$, $p < 0.001$) and deep breathing (**Figure 2B**; $r = 0.63$, $p < 0.001$), when expressed as a percentage of baseline values. In Group B, there were significant associations between the reduction in amplitude of diaphragm CMAP and reductions in FVC (**Figure 3A**; $r = 0.67$, $p < 0.001$), FVC% (**Figure 3B**; $r = 0.67$, $p < 0.001$), and FEV1 (**Figure 3C**; $r = 0.62$, $p < 0.001$), when expressed as a percentage of baseline values.

Diagnostic Efficacy of Pulmonary Function for Hemidiaphragmatic Paralysis

In Group B, 24 of the 37 (64.9%) patients experienced hemidiaphragmatic paralysis. ROC curve analysis was used to assess the diagnostic efficacy of pulmonary function for hemidiaphragmatic paralysis evidenced by diaphragm CMAP as an assessment of diaphragm strength (**Figure 4**). The area under the ROC curve for FVC was 0.86. At a cut-off of 0.084, FVC predicted hemidiaphragmatic paralysis (determined by diaphragm CMAP) with sensitivity and specificity of 79.2 and 100%, respectively.

DISCUSSION

This study assessed the associations between diaphragm CMAP, hemidiaphragmatic excursion, and pulmonary function after SCBPB. The efficacy of pulmonary function for diagnosing hemidiaphragmatic paralysis evidenced by diaphragm CMAP as an assessment of diaphragm strength was evaluated. To date, the pulmonary function has not been used to indicate diaphragmatic paralysis. A decrease of >8.4% in FVC compared to pre-block has predicted hemidiaphragmatic paralysis (determined by diaphragm CMAP) with sensitivity and specificity of 79.2 and 100%, respectively.

The brachial plexus nerve is anatomically adjacent to the phrenic nerve and originates from the anterior rami of C5 ≈ 8 and T1. The phrenic nerve originates from the anterior rami of C3 to C5 and passes over the anterior surface of the anterior scalene. The accessory phrenic nerve originates from the anterior rami of C5 ≈ 6. The accessory phrenic nerve is a common anatomic variant that is present in up to 75% of individuals. Local anesthetic blockade of the accessory phrenic nerve may lead to diaphragmatic paralysis during brachial plexus block. Diaphragmatic paralysis may be a temporary or persistent phenomenon. The incidence of diaphragmatic paralysis after SCBPB in our patient population was high. Eight patients reported transient dyspnea and oxygen desaturation 10 min after SCBPB. These data highlight the unmet clinical need to raise

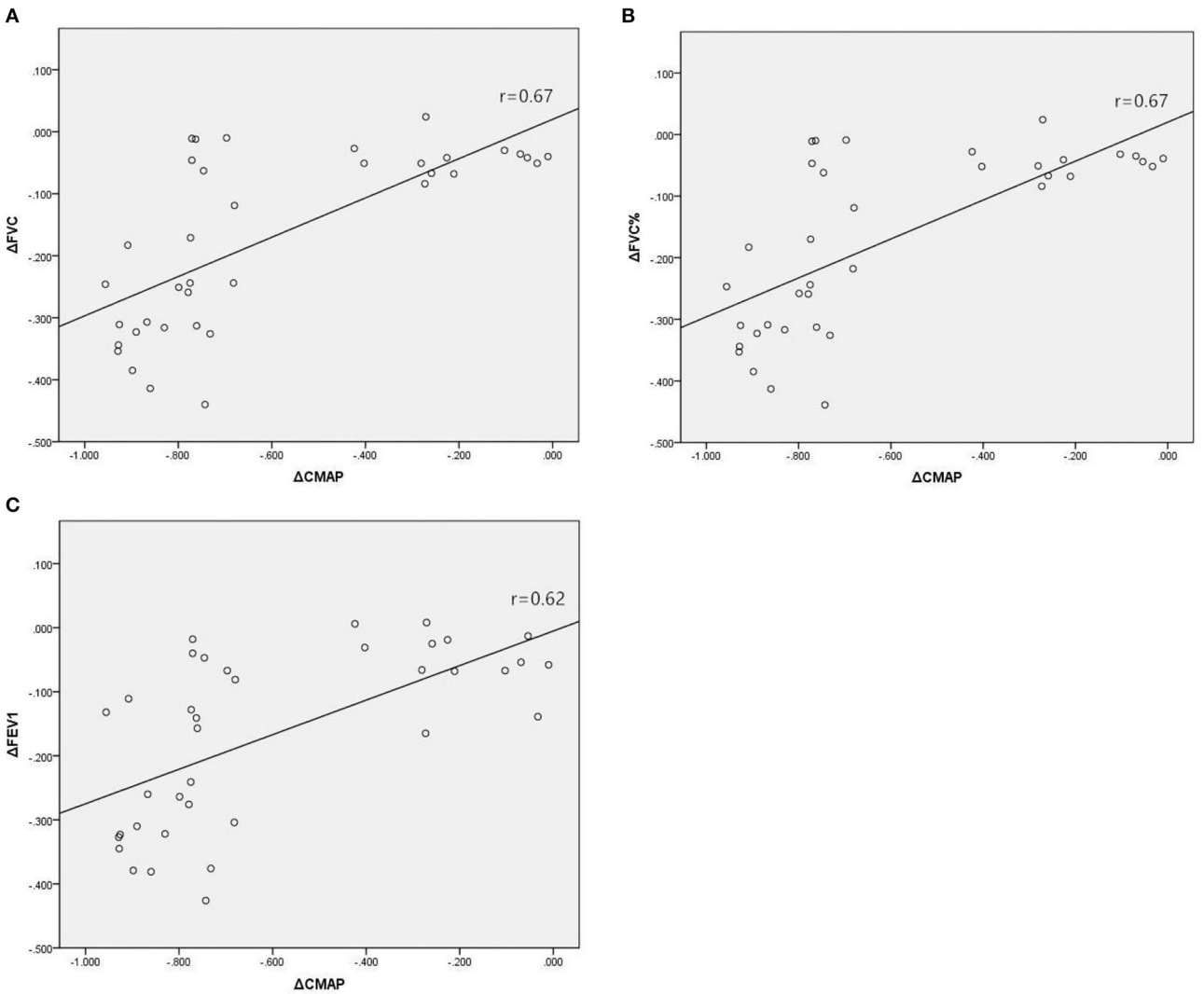

FIGURE 3 | Associations between relative changes (Δ) in diaphragm CMAP amplitude and FVC **(A)**, FVC% **(B)**, and FEV1 **(C)** before and 30 min after SCBPB in Group B. CMAP, compound muscle action potential; FVC, forced vital capacity; FVC%, predicted value of FVC; FEV1, forced expiratory flow in the first second.

awareness about diaphragmatic paralysis as a complication of brachial plexus block and identify novel methods to measure diaphragmatic paralysis. Previously, we explored the effect of 20 and 30 ml of 0.375% ropivacaine on electromyography of the diaphragm and pulmonary function before and after ultrasound-guided SCBPB (21).

In the present study, we showed a significant association between the reduction in amplitude of diaphragm CMAP after SCBPB, measured by PNCS, and the reductions in a diaphragmatic excursion on quiet and deep breathing, visualized on the ultrasound, when expressed as a percentage of baseline values. The association was better during quiet breathing compared to deep breathing. The diaphragm is responsible for 75% of tidal volume during quiet inspiration (22). This percentage is reduced during deep breathing due to the assistance of extrinsic musculature, which increases the volume of the thorax and expands the lungs (23).

In addition, there was a significant association between the reduction in amplitude of diaphragm CMAP after SCBPB and the reduction in pulmonary function (FVC, FVC%, and FEV1), when expressed as a percentage of baseline values. It is well-established that diaphragmatic paralysis is related to a restricted breathing pattern and reduced ability of the lung to expand, which is characterized by reduced FVC and FVC% (24). There was a moderate association between the pre-block and post-block changes in diaphragm CMAP and FVC, FVC%, and FEV1. During unilateral diaphragmatic paralysis, the contralateral diaphragm may compensate for the dysfunction of the paralyzed hemidiaphragm, and patients can maintain normal ventilation at rest and during mild exercise. Patients may not have signs or symptoms until FVC falls to 38% of predicted due to physiological compensation (25, 26).

Results of our ROC curve analysis imply that hemidiaphragmatic paralysis after SCBPB, evidenced by diaphragm CMAP as an assessment of diaphragm strength,

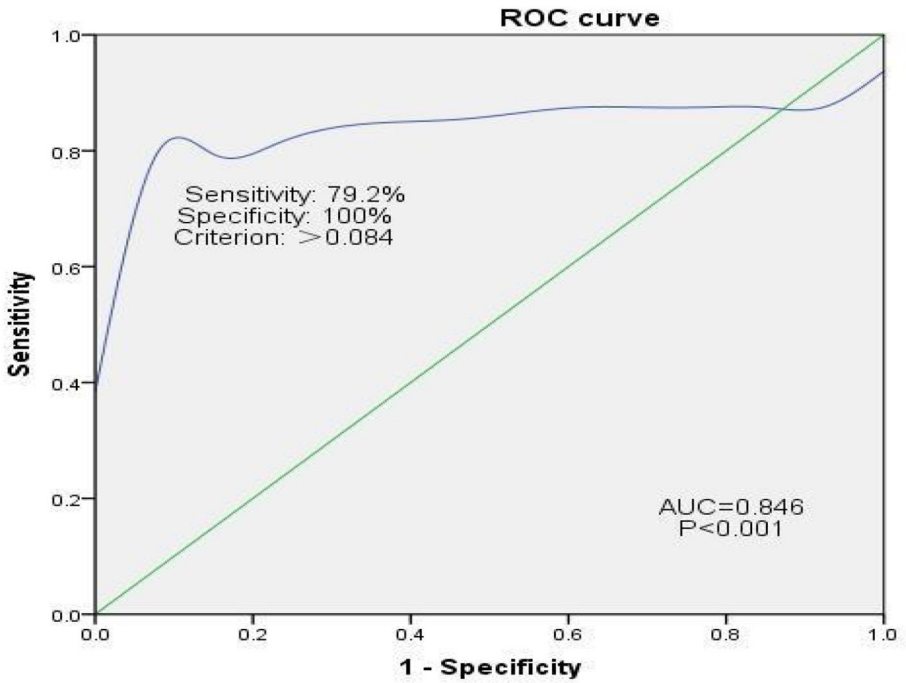

FIGURE 4 | ROC curve analysis assessing the efficacy of pulmonary function for diagnosing hemidiaphragmatic paralysis evidenced by diaphragm CMAP as an assessment of diaphragm function. CMAP, compound muscle action potential. Sensitivity = True Positives/(True Positives + False Negatives). False Positive Rate = 1 – Specificity, where specificity = True Negatives/(True Negatives + False Positives).

may be diagnosed when FVC falls below 0.084. The area under the ROC curve was 0.85, and sensitivity and specificity were 79.2 and 100%, respectively. Consistent with this, Ming-Lung et al. reported that phrenic nerve transfer for the repair of avulsed brachial plexus injury resulted in permanent ipsilateral diaphragmatic paralysis accompanied by an estimated 8% decrease in inspiratory capacity, FVC, and total lung capacity (27).

Several methods have been used to diagnose hemidiaphragmatic paresis after the brachial blockade, such as fluoroscopy, maximum transdiaphragmatic pressure, PNCS CMAP amplitude, and ultrasound of the diaphragm. Each method has its advantages and disadvantages. Ultrasound of the diaphragm has advantages over diaphragm CMAP as it is easy to apply. However, ultrasound does not account for the influence of the contralateral diaphragm and accessory respiratory muscles. PNCS can overcome these deficiencies. In this study, we used needles instead of surface electrodes to collect diaphragm CMAP. As surface electrodes are distant from the deep target muscle, the signal can be influenced by factors, such as lung inflation, skin thickness, the amount of subcutaneous tissue, and the depth of lung tissue separating the recording electrode from the diaphragm. Needles are not prone to interference from activity in neighboring muscles and are considered safe (17); however, this approach can be technically challenging. Inaccurate placement of needles can make it difficult to collect diaphragmatic needle electromyographic recordings, providing inaccurate measurements of the latency and amplitude of the diaphragm CMAP.

Pulmonary function is widely used to evaluate the effect of brachial plexus block on respiratory function. It is simple and easy to perform, but the quality of the spirometry test is influenced by patient efforts and cooperation, and there are no diagnostic criteria for diaphragmatic paralysis. Diaphragm CMAP has been applied in the diagnosis of respiratory insufficiency due to amyotrophic lateralizing sclerosis and other neuromuscular disorders (28), but data describing the use of diaphragm CMAP to diagnose diaphragmatic paralysis after SCBPB are limited. The majority of previous reports have used ultrasound to diagnose hemidiaphragmatic paralysis after SCBPB. With the rapid development and decreasing cost of ultrasound technology, real-time 2-dimensional visualization of the diaphragm can be easily performed at the bedside (29). However, ultrasound may misdiagnose complete paralysis of the phrenic nerve as diaphragmatic movement on the ipsilateral side can be affected by movement from the contralateral diaphragm and accessory respiratory muscles (30). PNCS of the affected hemidiaphragm is needed to confirm complete paralysis of the phrenic nerve. The phrenic nerve is the only source of motor innervation to the diaphragm (24). Therefore, the electrophysiological function of the diaphragm can be analyzed without being affected by other respiratory muscles.

This study was associated with several limitations. First, the latency and amplitude of the diaphragm CMAP were extremely atypical in several patients. This may have been due to interference caused by stimulation of the brachial plexus. Second, patients with a high BMI or cardiopulmonary disease were excluded from this study. This may limit the generalizability

of our findings, as these patients are most likely to be affected by diaphragmatic paralysis. Third, the left hemidiaphragm is lower than the right hemidiaphragm, but the motion of the left hemidiaphragm is greater than the right hemidiaphragm (24); therefore, measurements from the right hemidiaphragm cannot be extrapolated to the left. Fourth, it takes 2 min to measure diaphragmatic excursions and 3 min to measure pulmonary function. As we could not adjust for the differences in the time it takes to obtain these measurements, we did not explore the relationship between diaphragmatic excursion and pulmonary functions. Although diaphragm dysfunction (CMAP) was identified by two different approaches [ultrasound and pulmonary function tests (PFTs)] in two groups of patients, the characteristics of the patients in Groups A and B were similar, allowing for a relevant extrapolation of data.

CONCLUSIONS

Diaphragm CMAP, determined by PNCS, diaphragmatic excursion during quiet and deep breathing, and pulmonary function (FVC, FVC%, and FEV1) were decreased after SCBPB, implying these three methods can be used to diagnose diaphragmatic paralysis. The pulmonary function can directly reflect the influence of diaphragmatic paralysis on respiratory function after SCBPB. A decrease of $>8.4\%$ in FVC compared to pre-block has predicted hemidiaphragmatic paralysis (determined by diaphragm CMAP). The relative reduction in diaphragm CMAP amplitude was correlated well with the relative reductions in diaphragmatic excursion and pulmonary functions. These data establish diaphragm CMAP as an accurate and objective index of diaphragmatic paralysis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of 98th Hospital of PLA. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XB is responsible for conducting this study and the manuscript writing. Data collection and analysis were completed by TL and YZ. YW is responsible for implementation of clinical trials. This study was designed by XK. After completing this clinical study and article writing, XW was responsible for the revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.744670/full#supplementary-material

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Association Between Platelet Levels and 28-Day Mortality in Patients With Sepsis: A Retrospective Analysis of a Large Clinical Database MIMIC-IV

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Background: This research focused on evaluating the correlation between platelet count and sepsis prognosis, and even the dose-response relationship, in a cohort of American adults.

Method: Platelet counts were recorded retrospectively after hospitalization for patients admitted to Beth Israel Deaconess Medical Center's intensive care unit between 2008 and 2019. On admission to the intensive care unit, sepsis patients were divided into four categories based on platelet counts (very low $< 50 \times 10^9$/L, intermediate-low 50×10^9–100×10^9/L, low 100×10^9–150×10^9/L, and normal $\geq 150 \times 10^9$/L). A multivariate Cox proportional risk model was used to calculate the 28-day risk of mortality in sepsis based on baseline platelet counts, and a two-piece linear regression model was used to calculate the threshold effect.

Results: The risk of 28-day septic mortality was nearly 2-fold higher in the platelet very low group when compared to the low group (hazard ratios [HRs], 2.24; 95% confidence interval [CI], 1.92–2.6). Further analysis revealed a curvilinear association between platelets and the sepsis risk of death, with a saturation effect predicted at 100×10^9/L. When platelet counts were below 100×10^9/L, the risk of sepsis 28-day death decreased significantly with increasing platelet count levels (HR, 0.875; 95% CI, 0.84–0.90).

Conclusion: When platelet count was less than 100×10^9/L, it was a strong predictor of the potential risk of sepsis death, which is declined by 13% for every 10×10^9/L growth in platelets. When platelet counts reach up to 100×10^9/L, the probability of dying to sepsis within 28 days climbs by 1% for every 10×10^9/L increase in platelet count.

Keywords: intensive care unit, mortality, platelet count, sepsis, MIMIC-IV

Abbreviations: BMI, body mass index; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; Charlson, Modified Charlson comorbidity index; SAPS II, Simplified Acute Physiology Score II; MV, mechanical ventilation; RRT, renal replacement therapy; WBC, white blood cell count; Cr, creatinine; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio; PTT, partial thromboplastin time; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

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INTRODUCTION

Low platelet counts are common in critically ill patients, both on admission to and during intensive care unit (ICU). The incidence of thrombocytopenia in ICU patients varies from 15 to 58%, depending on the value used to define thrombocytopenia (1, 2).

Thrombocytopenia is defined as a platelet count below 150×10^9/L [BCSH (3)] and severe thrombocytopenia is defined as a platelet count below 50×10^9/L. In a recent study, Yang et al. reported (4) that thrombocytopenia is common in patients with Coronavirus Disease-19 (COVID-19) and is associated with an increased risk of in-hospital mortality, with lower platelet counts associated with higher mortality. A study of 931 patients with sepsis collected from two ICUs in the Netherlands found that in-hospital thrombocytopenia was associated with increased mortality and a more disturbing host response during sepsis, independent of disease severity (5).

Sepsis is a life-threatening organ dysfunction that is caused by a dysregulated host response to infection (6). Sepsis and septic shocks are major medical problems that affect millions of people worldwide each year, with one-third to one-sixth of them dying from sepsis (7–9). Researchers have understood sepsis as a complex interplay of cytokine storm, systemic inflammation, endothelial dysfunction, capillary leakage, and pathological hemostasis (10–13). Microvascular thrombosis, microvascular occlusion, and hypoperfusion are the main causes of organ dysfunction during sepsis (14). In overwhelming sepsis, platelets contribute to activating the procoagulant cascade and subsequent complications associated with microvascular thrombosis and subsequent organ dysfunction (15). Platelets are the cornerstone of this process, and, therefore, thrombocytopenia may be a prognostic factor in infectious shock. Early detection and appropriate treatment within the first few hours after the onset of sepsis may improve the prognosis. The lowest point of platelet count that is assessed during the clinical course may represent an early warning to the clinician. Platelet counts are also readily available in routine practice. This simple bedside test can help in identifying critically ill patients without the need to calculate more complex and time-consuming scores.

There are conflicting data in the literature regarding the impact of thrombocytopenia on prognosis. To date, few studies have investigated the association between early platelet counts and mortality among the many identified risk factors for mortality (16). In addition, these studies have mainly considered ICU or in-hospital mortality (17, 18). Some authors (19–21) reported thrombocytopenia as an independent prognostic factor for ICU or hospital discharge mortality. In contrast, others did not find any such association or only found an effect depending on the instability of the patient's clinical status (22). In addition, these existing studies suffer from several limitations, such as small sample sizes, inconsistent definitions of platelet count subgroups, observed endpoints, and adjustment for some important covariates.

The dose-response relationship between baseline platelet count levels and the risk of sepsis death has not been elucidated. A prospective study revealed a persistent negative association between platelet count and sepsis 28-day death without a threshold effect analysis (22). Therefore, this study is aimed to examine the association between baseline platelet counts and the risk of sepsis 28-day death and describe in detail the nature of the dose-response relationship.

METHODS

Data Source

Data from the Marketplace for Medical Information in Intensive Care (MIMIC) database were used to conduct this study. Patients admitted to the ICU of Beth Israel Deaconess Medical Center from 2008 to 2019 were included in Johnson et al. (23). The raw data were extracted using Navicat using Structured Query Language (SQL) and further processed using R software. The MIMIC-IV database is publicly available from https://mimic.physionet.org/. The database is freely accessible, and any researcher who has accepted the data use agreement and completed the "Protection of Human Subjects" training can request to access to the data (24). We did not require patient consent or ethical approval, data information was de-identified, and patient identifiers were removed. Individuals who have completed the Collaborative Training Program exam (author Wang's certification number 27714078) can access the database. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed (25).

Study Population

All patients in the database were selected. The inclusion criteria for this study were as follows: (1) sepsis was identified through the MIMIC-IV database and (2) adults ($\geq$18 years) who were admitted to the ICU; exclusion criteria were as follows: (1) patients with the Sequential Organ Failure Assessment (SOFA) < 2; (2) patients with no vital signs or no platelets recorded were also excluded. The outcome cohort for the final analysis included 16,401 participants (**Figure 1**).

Data Extraction and Management

We extracted platelet counts measured within 24 h of admission to the monitoring unit from MIMIC-IV for each included patient. The lowest platelet count was retained if multiple measurements were taken within the first 24 h. Platelet counts were then divided into four groups based on five groups of the SOFA scores: platelet counts below 50×10^9/L, platelet counts between 50×10^9/L and 100×10^9/L, platelet counts between 100×10^9/L and 150×10^9/L, and platelet counts above 150×10^9/L (26).

We also considered covariates that may affect the relationship between platelets and sepsis. We extracted the following basic data for each patient from MIMIC-IV: age, sex, race, body mass index (BMI), Elixhauser comorbid conditions (27), site of infection, organ support therapy, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MBP). The following biochemical tests were also collected for each patient: partial thromboplastin time (PTT), international normalized ratio (INR), prothrombin time (PT), white blood cell count (WBC), hemoglobin, blood urea nitrogen (BUN), and creatinine (Cr). Simplified acute physiology score

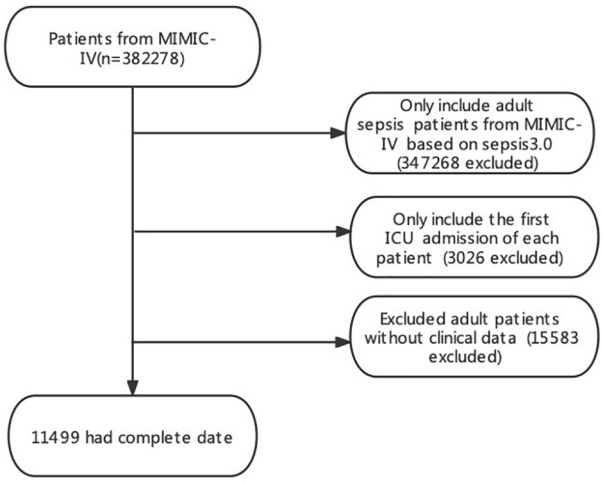

FIGURE 1 | Study flowchart. MIMIC, Medical Information Mart for Intensive Care.

(SAPS II) of patients was also recorded (28). The worst score and mean values of laboratory parameters and vital signs within 24 h of ICU admission were used in this study.

All scripts used for demographic characterization, severity score calculation, and comorbidity were obtained from the Github website (https://github.com/MIT-LCP/mimic-iv, access date. October 2021). Data extraction was performed with PostgreSQL tools (v9.6; PostgreSQL Global Development Group) using SQL.

Primary and Secondary Results

The primary outcome was 28-day mortality. Baseline platelet count and 28-day risk of mortality from sepsis have a dose-response relationship. Secondary outcomes included length of stay in the ICU (LOS ICU) and LOS in the hospital (LOS hospital).

Statistical Analysis

Data are expressed as mean ± standard deviation (SD) for continuous variables and frequencies or percentages for categorical variables. Statistical differences between platelet count subgroups were tested for baseline characteristic analysis with one-way ANOVA for continuous variables and chi-square tests for categorical variables. Cox proportional risk models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for the association between platelet counts and 28-day mortality from sepsis. Unadjusted and multivariable-adjusted models were used. To assess confounding, we entered covariates into the Cox regression model in the base model or removed each covariate from the full model and compared regression coefficients. Covariates with initial regression coefficients that changed by more than 10% were included. In the multivariate model, we adjusted for age (years), sex (male or female), race (white or other), and BMI (<18.5, 18.5–25, 25–30, or ≥ 30.0 kg/m^2) in model 1. In model 2, we further adjusted for congestive heart failure (CHF; yes or no), chronic obstructive pulmonary disease (COPD; yes or no), liver (yes or no), kidney (yes or no), diabetes (yes or no), metastatic tumors (yes or no), AIDS (yes or no), abdominal infections (yes or no), bloodstream infections (yes or no), respiratory infections (yes or no), and urinary tract infections (yes or no). In model 3, we further adjusted for catheter infection (yes or no), mechanical ventilation (MV; yes or no), renal replacement therapy (RRT; yes or no), SAPS II (≤40 or >40), ICU LOS, length of visit, hemoglobin, WBC, Cr, BUN, PT, INR, PTT, HR, SBP, DBP, and mean arterial pressure (MAP).

Trend tests were performed using linear regression by entering the median value of each subgroup of each platelet as a continuous variable in the model. A generalized additivity model was used to assess the non-linear relationship between platelets and sepsis prognosis. Based on the smoothed curves, we developed a two-segment linear regression model to identify threshold effects and adjust for potential confounders. Threshold levels of platelet counts were determined using a recursive approach that involved selecting turning points along with predefined intervals and selecting turning points that yielded a maximum likelihood model. The log-likelihood ratio test compared a two-segment linear regression model with a one-linear linear model. Using a stratified Cox regression model, subgroups were performed by age, gender, race, and severity scores. Interactions between subgroups were tested using likelihood ratio tests.

We used multiple imputation (MI), based on five replications and a chained equation approach method in the R MI procedure, to account for missing data. We performed several sensitivity analyses to assess the robustness of our findings. To ensure that our findings were not confounded by missing partial data, we further assessed whether indicator variables with missing data introduced bias in our results by performing multiple interpolation analyses. We also assessed the robustness of our main results by using a variety of analytical strategies, such as stratified analysis and multivariate Cox regression.

For all statistical analyses, we used the statistical packages R version 3.4.3 (R Foundation, Vienna, Austria). Two-sided values of $p < 0.05$ were considered statistically significant.

RESULTS

Demographics and Baseline Characteristics

Of the 16,401 adult patients with sepsis (mean age 66.0 ± 15.6 years; 39.8% men), a total of 8,612 patients (52.5%) had no thrombocytopenia, whereas 47.5% (7,789/16,401) had thrombocytopenia [4,403 (26.8%) had platelet counts of 100 × 10^9–150 × 10^9/L; 2,463 (15%) had platelet counts of 50 × 10^9–100 × 10^9/L; and 923 (5.6%) had platelet counts < 50 × 10^9/L]. The baseline characteristics of the study population according to platelet count are shown in **Table 1**. Participants with reduced platelet counts were more likely to be younger and more likely to have concurrent liver

TABLE 1 | Baseline and clinical characteristics of the study population according to platelet count.

	Total	Very low ($<50 \times 10^9$/L)	Intermediate-low (50×10^9–100×10^9/L)	Low (100×10^9–150×10^9/L)	Normal ($\geq 150 \times 10^9$/L)	*P*-value
Patients, n (%)	16,401	884 (5.4)	2,396 (14.6)	4,423 (27.0)	8,698 (53.0)	
Demographics						
Age (years)	66.0 ± 15.6	60.0 ± 14.8	65.3 ± 15.7	66.9 ± 14.9	66.3 ± 15.9	<0.001
Male (%)	6,535 (39.8)	366 (41.4)	883 (36.9)	1,482 (33.5)	3,804 (43.7)	<0.001
White (%)	11,118 (67.8)	582 (65.8)	1,582 (66)	3,054 (69)	5,900 (67.8)	0.043
BMI (kg/m^2)	29.1 ± 7.4	28.2 ± 6.4	28.5 ± 6.5	28.9 ± 6.6	29.4 ± 8.1	<0.001
Chronic comorbidity, n (%)						
CHF	5,267 (32.1)	179 (20.2)	620 (25.9)	1,390 (31.4)	3,078 (35.4)	<0.001
COPD	4,473 (27.3)	197 (22.3)	566 (23.6)	1,081 (24.4)	2,629 (30.2)	<0.001
Liver	2,521 (15.4)	447 (50.6)	785 (32.8)	552 (12.5)	737 (8.5)	<0.001
Renal	3,890 (23.7)	183 (20.7)	561 (23.4)	1,048 (23.7)	2,098 (24.1)	0.148
Diabetes	4,079 (24.9)	189 (21.4)	493 (20.6)	1,084 (24.5)	2,313 (26.6)	<0.001
Metastatic tumor	802 (4.9)	61 (6.9)	101 (4.2)	153 (3.5)	487 (5.6)	<0.001
AIDS	121 (0.7)	20 (2.3)	23 (1)	17 (0.4)	61 (0.7)	<0.001
Charlson	5.9 ± 2.9	6.4 ± 2.9	6.0 ± 2.9	5.7 ± 2.8	5.9 ± 2.9	<0.001
Primary source of infection, n (%)						
Abdomen	49 (0.3)	6 (0.7)	9 (0.4)	11 (0.2)	23 (0.3)	0.157
Bloodstream	1,175 (7.2)	128 (14.5)	204 (8.5)	284 (6.4)	559 (6.4)	<0.001
Catheter	43 (0.3)	2 (0.2)	7 (0.3)	8 (0.2)	26 (0.3)	0.657
Respiratory tract	1,576 (9.6)	97 (11)	225 (9.4)	364 (8.2)	890 (10.2)	0.001
Urinary tract	1,284 (7.8)	75 (8.5)	166 (6.9)	301 (6.8)	742 (8.5)	<0.001
Severity of disease						
SAPS II	40.9 ± 14.5	48.2 ± 17.7	43.0 ± 14.9	39.7 ± 13.9	40.2 ± 14.1	<0.001
Organ support therapy, n (%)						
RRT	1,070 (6.5)	127 (14.4)	204 (8.5)	232 (5.2)	507 (5.8)	<0.001
MV	10,709 (65.3)	10,709 (65.3)	605 (68.4)	1,666 (69.5)	2,874 (65)	<0.001
Clinical data						
Hemoglobin (g/dL)	9.7 ± 2.1	8.1 ± 2.0	8.9 ± 2.0	9.6 ± 2.0	10.0 ± 2.1	<0.001
WBC ($\times 10^9$/L)	15.8 ± 11.5	14.4 ± 26.3	14.7 ± 12.4	14.9 ± 9.6	16.7 ± 9.4	<0.001
Cr (mg/dL)	1.7 ± 1.8	2.1 ± 1.7	1.9 ± 1.8	1.7 ± 2.0	1.7 ± 1.8	<0.001
BUN (mg/dL)	31.8 ± 25.3	40.1 ± 28.4	33.1 ± 26.5	29.7 ± 24.0	31.6 ± 25.0	<0.001
PT (s)	18.7 ± 13.1	24.3 ± 16.1	20.8 ± 14.1	18.1 ± 11.9	17.8 ± 12.8	<0.001
INR	1.7 ± 1.3	2.3 ± 1.5	1.9 ± 1.3	1.7 ± 1.2	1.6 ± 1.3	<0.001
PTT (s)	45.2 ± 30.4	54.6 ± 34.7	50.7 ± 32.4	44.1 ± 29.1	43.2 ± 29.6	<0.001
HR (bpm)	87.0 ± 16.1	93.5 ± 17.7	87.3 ± 16.3	85.2 ± 15.2	87.1 ± 16.1	<0.001
SBP (mmHg)	114.5 ± 14.7	111.8 ± 14.4	112.5 ± 14.1	113.7 ± 13.3	115.7 ± 15.4	<0.001
DBP (mmHg)	60.8 ± 10.0	61.5 ± 10.0	60.1 ± 10.1	60.0 ± 9.6	61.4 ± 10.2	<0.001
MAP (mmHg)	76.2 ± 9.8	75.3 ± 9.9	75.4 ± 9.9	75.7 ± 9.1	76.7 ± 10.1	<0.001

Variables are presented as mean ± SD or N (%). BMI, body mass index; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; Charlson, Modified Charlson comorbidity index; SAPS II, Simplified Acute Physiology Score II; MV, mechanical ventilation; RRT, renal replacement therapy; WBC, white blood cell count; Cr, creatinine; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio; PTT, partial thromboplastin time; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

disorders, metastases, and AIDS; SAPS II scores averaged 40.9 ± 14.5, continuous renal replacement therapy accounted for 6.5%, and mechanical ventilation treatment accounted for 65.3%.

Univariate analysis of clinical outcomes calculated mortality and hospital length and ICU LOS stratified by platelet count are summarized in **Table 2**. Participants with reduced platelet count had longer hospital and ICU LOS. In addition, 37.5% of patients with very low platelet count were died during hospitalization ($p < 0.001$); 35.9% of patients with very low platelet count were killed during 28 days of hospitalization ($p < 0.001$); and 43.3% of patients with very low platelet count were died within 1 year ($p < 0.001$). Kaplan-Meier curves for 28-day survival by platelet count are shown in **Figure 2**.

Association Between Platelets and Prognosis of Sepsis

In our study, patients with sepsis recorded 2,736 (16.7) deaths at 28 days, 2,984 (18.2) deaths at 60 days, 3,086 (18.8) deaths

TABLE 2 | Outcome of sepsis patients stratified according to platelet counts.

	Total	Very low ($<50 \times 10^9$/L)	Intermediate-low (50×10^9–100×10^9/L)	Low (100×10^9–150×10^9/L)	Normal ($\geq 150 \times 10^9$/L)	*P*
Patients (%)	16,401	884 (5.4)	2,396 (14.6)	4,423 (27.0)	8,698 (53.0)	
Length of stay						
Length of ICU stay (d)	6.3 ± 7.6	7.0 ± 7.7	6.0 ± 6.8	5.5 ± 7.0	6.8 ± 8.0	<0.001
Length of hospital stay (d)	13.5 ± 17.0	17.7 ± 19.4	14.2 ± 16.8	11.9 ± 11.9	13.7 ± 18.8	<0.001
Mortality, n (%)						
In-hospital mortality	2,814 (17.2)	338 (38.2)	486 (20.3)	568 (12.8)	1,422 (16.3)	<0.001
28-day mortality	2,736 (16.7)	324 (36.7)	462 (19.3)	557 (12.6)	1,393 (16)	<0.001
Acute kidney injury	12,858 (78.4)	735 (83.1)	1,902 (79.4)	3,378 (76.4)	6,843 (78.7)	<0.001

Notes: Variables are presented as mean ± SD or N (%).

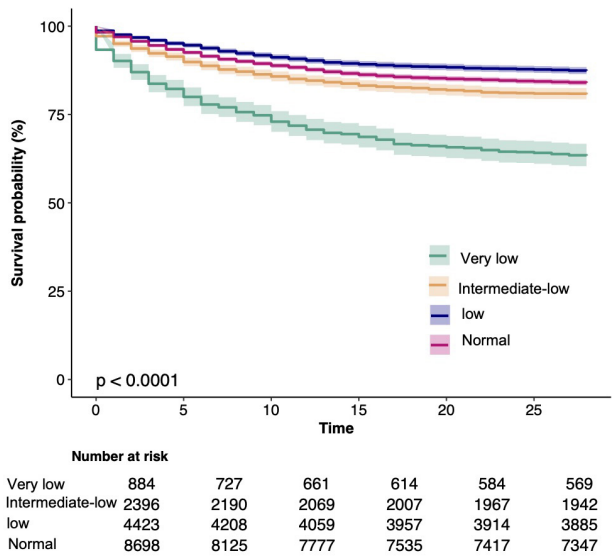

FIGURE 2 | Kaplan-Meier Survival Curves for day 28 of sepsis patients depending on the platelet count.

at 90 days, and 3,387 (20.7) deaths at 1 year. In the unadjusted model, an increased risk of 28-day death occurred with a decrease in platelet count when compared with participants with 100×10^9–150×10^9/L ($p < 0.066$ for trend). Participants with platelet counts at $\leq 50 \times 10^9$/L vs. 100×10^9 to 150×10^9/L had a 3-fold 3.31 (2.89–3.79) increase in the odds of developing 28-day death. We also found that as platelet counts were increased, the odds of 28-day mortality were increased 1-fold 1.28 (1.16–1.42) for participants with platelet counts $\geq 150 \times 10^9$/L vs. 100×10^9–150×10^9/L. After multivariate adjustment, such as age, sex, race, BMI, chronic disease, source of infection, interventions, severity score, and presence of laboratory findings, lower or higher platelet counts were significantly associated with lower long-term and short-term survivals in patients with sepsis (**Table 3**). The multivariable-adjusted HR and 95% CI from the platelet count categories ($<50 \times 10^9$/L, 50×10^9–100×10^9/L, 100×10^9–150×10^9/L, $\geq 150 \times 10^9$/L) were 2.31 (1.99–2.68), 1.34 (1.18–1.51), 1.00 (reference), and 1.17 (1.06–1.29) for 28-day mortality (p_{trend} = 0.262); 2.28 (1.98–2.63), 1.32 (1.17–1.49), 1.00 (reference), and 1.17 (1.06–1.28) for 60-day mortality, respectively (p_{trend} = 0.239); and 2.27 for 90-day mortality (1.97–2.6), 1.33 (1.18–1.49), 1.00 (reference), and 1.18 (1.08–1.3), respectively, (p_{trend} = 0.125); and 1-year mortality rates of 2.3 (2.01–2.62), 1.32 (1.18–1.48), 1.00 (reference), and 1.19 (1.09–1.3), respectively (p_{trend} = 0.085).

Threshold Effect Analysis of Platelet Count on Sepsis Mortality

We used smoothing function analysis to assess whether there is a dose-response relationship between platelets and 28-day mortality events in sepsis. After adjusting for potential confounders, a non-linear relationship was observed between serum platelet counts and sepsis 28-day mortality events. The results also applied to the long-term prognostic outcomes of septic patients (**Figure 3**). Further analysis revealed a curvilinear association between platelets and the occurrence of sepsis 28-day mortality events, with a saturation effect predicted at 100×10^9/L. The risk of developing sepsis 28-day mortality was significantly decreased with increasing platelet count levels when platelet counts were below 100×10^9/L (HR 0.875; 95% CI, 0.847–0.903). When platelet counts exceeded 100×10^9/L, the risk of sepsis 28-day death was increased by 1% for each 10×10^9/L increase in platelet counts (**Table 4**).

In the graph, the black line indicates the estimated risk of septic death, and the gray band indicates the point-by-point 95% CI adjusted for age, sex, laboratory results, etc.

Subgroup Analyses

We performed stratified and interaction analyses to see if the association between platelet count and sepsis 28-day mortality was stable in different subgroups. Consistent results were observed when the analysis was stratified by age, sex, race, BMI, associated comorbidities, source of infection, severity score, and intervention treatment (**Supplementary Table 2**). The data showed an interaction between SAPA II in the association between platelet count and 28-day mortality events in sepsis (interaction p = 0.004). For participants with SAPS II < 56, the risk of mortality increased with decreasing

TABLE 3 | Relationship between platelet count and 28-day mortality in patients with sepsis.

	Low (100×10^9–150×10^9/L)	Very low ($<50 \times 10^9$/L)	Intermediate-low (50×10^9–100×10^9/L)	Normal ($\geq 150 \times 10^9$/L)	P_{trend}
28-day mortality					
Number of deaths/total	557/4,423	324/884	462/2,396	1,393/8,698	
Crude Model	1.0	3.43 (2.99–3.93)	1.6 (1.42–1.81)	1.3 (1.18–1.43)	0.042
Model 1	1.0	3.79 (3.3–4.35)	1.61 (1.43–1.83)	1.28 (1.16–1.41)	0.132
Model 2	1.0	2.72 (2.35–3.14)	1.39 (1.22–1.57)	1.24 (1.12–1.37)	0.07
Model 3	1.0	2.24 (1.92–2.6)	1.35 (1.19–1.53)	1.21 (1.1–1.34)	0.041

Data presented are HRs and 95% CIs.
Model 1: adjusted for age, Sex, Ethnicity, and BMI;
Model 2: further adjusted (from Model 1) for CHF, COPD, Liver, Renal, Diabetes, Metastatic tumor, AIDS, Charlson, Abdomen, Bloodstream, Catheter, Respiratory tract, Urinary tract;
Model 3: further adjusted (from Model 2) for MV, RRT, SAPS II, Length of ICU stay, Length of hospital stay, Hemoglobin, WBC, Cr, BUN, PT, INR, PTT, HR, SBP, DBP, MAP.
BMI, body mass index; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; SAPS II, Simplified Acute Physiology Score II; MV, mechanical ventilation; RRT, renal replacement therapy; WBC, white blood cell count; Cr, creatinine; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio; PTT, partial thromboplastin time; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

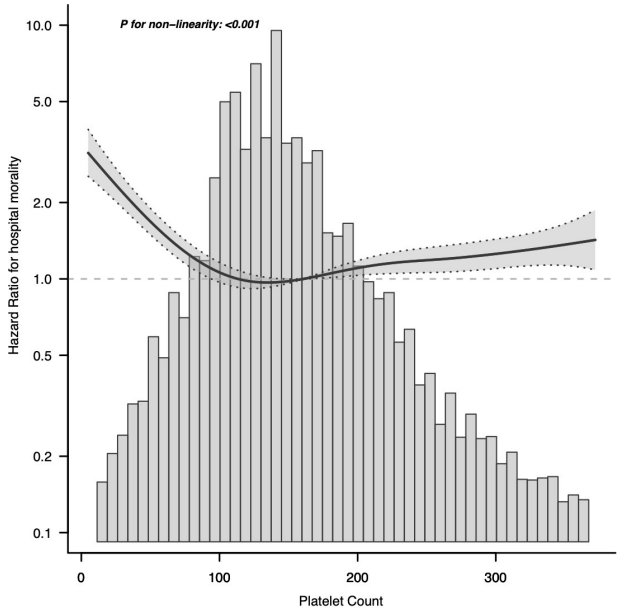

FIGURE 3 | Associations between platelet count concentrations with 28-day mortality among participants with sepsis in MIMIC-IV. HRs were adjusted for age, sex, ethnicity, BMI, CHF, COPD, liver, renal, diabetes, metastatic tumor, AIDS, Charlson, abdomen, bloodstream, respiratory tract, urinary tract, catheter, MV, RRT, SAPS II, length of ICU stay, length of hospital stay, hemoglobin, WBC, Cr, BUN, PT, INR, PTT, HR, SBP, DBP, and MAP. Both *p* linearity, 0.001. BMI, body mass index; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; SAPS II, Simplified Acute Physiology Score II; MV, mechanical ventilation; RRT, renal replacement therapy; WBC, white blood cell count; Cr, creatinine; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio; PTT, partial thromboplastin time; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

platelet levels, [$<50 \times 10^9$/L 2.57 (2.11–3.13); 50×10^9 to 100×10^9/L 1.38 (1.17–1.63); and $>150 \times 10^9$/L 1.24 (1.09–1.4) vs. 100×10^9 to 150×10^9/L 1.00, p for trend = 0.004]. Similarly, significant interactions were detected between platelet count and the presence of comorbid liver disorders, metastases, respiratory tract infections, urinary tract infections, and clinical interventions for treatment stratified variables (all $p_{trend} < 0.05$). There were no significant associations among those with or without comorbidity (chronic obstructive pulmonary, renal, and diabetes). Although the differences were not statistically significant ($p > 0.05$ for interaction), similar results were found across gender, race, and bloodstream infection status.

Sensitivity Analysis

We performed several sensitivity analyses to test the robustness of our findings. First, considering the presence of missing partial laboratory data, we further performed multiple interpolation of the missing data and performed multiple regression analysis that did not significantly alter our findings (**Supplementary Table 4**). Secondly, considering the possibility of organ impairment in patients, we assessed the presence of acute kidney injury and concomitant disseminated intravascular coagulation (DIC) in patients, which could still explain our findings (**Supplementary Tables 5**, **6**).

DISCUSSION

This large retrospective cohort study of the US adults with sepsis found that platelet count was significantly associated with a 28-day risk of death from sepsis. The association was independent of traditional risk factors, such as sex, race, BMI, comorbidities, and source of infection. Various sensitivity analyses and stratified analyses demonstrated the robustness of these findings. Our findings confirm a non-linear association between platelet count and 28-day risk of death. This relationship was characterized as follows: when platelet counts were below 100×10^9/L, the risk of 28-day death from sepsis was reduced by 13% for every 10×10^9/L increase in platelet count. When platelet counts were above 100×10^9/L, the risk of sepsis death at 28 days was increased by 1% for every 10×10^9/L increase in platelet

TABLE 4 | Threshold effect analysis for the relationship between Platelet Count and 28-day mortality.

Outcome:	HR (95% CI)	*P*-value
One-line linear regression model	1.0 (1.0, 1.0)	0.001
Two-piecewise linear regression model		
<100	0.875 (0.847, 0.903)	<0.001
≥100	1.014 (1.007, 1.021)	<0.001
Log-likelihood ratio test	<0.001	

HRs were adjusted for Age, Sex, Ethnicity, and BMI, CHF, COPD, Liver, Renal, Diabetes, Metastatic tumor, AIDS, Charlson, Abdomen, Bloodstream, Respiratory tract, Urinary tract, Catheter, MV, RRT, SAPS II, Length of ICU stay, Length of hospital stay, Hemoglobin, WBC, Cr, BUN, PT, INR, PTT, HR, SBP, DBP, MAP.

count. A platelet count that is sensitive to change may help strengthen the therapist's ability to assess prognosis a few days after a patient is admitted to the ICU, thereby improving treatment decisions.

In addition to their important role in hemostasis, platelets play an important role in inflammatory diseases. Studies have shown that platelets are also cells that have immunogenic capabilities. Like traditional congenital immune cells, platelets are immediately recruited into injured and inflamed tissues, they release immune media, express and fall off to immunoactive membrane receptors, which interact with other immunocytes, identify and remove pathogens (29). Sepsis is caused by a dysregulated host response to infection and can lead to organ dysfunction, permanent disability, or death. During sepsis, tissue damage results while uncontrolled complement, coagulation, inflammatory systems, and platelet dysfunction. The balance between systemic inflammatory response syndrome and compensatory anti-inflammatory response (CARS) regulates the outcome of sepsis. Persistent low platelet count is considered an independent risk factor for death in sepsis. In general, 20–58% of patients with sepsis develop a low platelet count, of which 10% develop a severely low platelet count (30). Variations in reported values may arise from patient heterogeneity, different inclusion criteria, pathogens, and other factors. In sepsis, low platelet counts may be regulated by altered platelet production or phagocytosis or by platelet clearance in the circulation due to platelet-leukocyte or platelet-pathogen interactions, vascular injury, or deoxygenation (31). Some studies have shown that thrombocytopenia is associated with the prognosis of sepsis death (5, 22). Our findings on platelet counts are consistent with these studies. They found that thrombocytopenia is an important predictor of short-term death in sepsis. However, what degree of platelet count depression has not been clearly stated. The current study did not describe its dose-response relationship in detail. Our research found that patients with thrombocytopenic infectious shock were more severely ill, had higher SAPS II scores, and required more organ function support and hospital stay than non-thrombocytopenic patients. Moreover, advanced age, men, cirrhosis, respiratory, urinary tract, and bloodstream infections were risk factors for thrombocytopenia. These findings are consistent with previous studies (16, 21, 32, 33).

Strengths of the current study include the relatively large sample size provided by MIMIC-IV and the use of a representative sample of United States adults with sepsis, which facilitates the generalization of our findings. Most previous studies on the prognostic impact of thrombocytopenia, the dose-response relationship between baseline platelet count levels and the risk of death from sepsis has not been elucidated. The aim of this study was to investigate the relationship between baseline platelet counts and the risk of death from sepsis at 28 days and to describe in detail the nature of the dose-response relationship. In addition, given the comprehensive data obtained in MIMIC-IV, we adjusted for a variety of potential confounders, such as race/ethnicity, BMI, comorbidity, source of infection, severity score, and level of laboratory indicators. However, our study also had the limitations of most retrospective studies; first, causality could not be determined due to the observational study design. Second, it was not designed to determine the cause of platelet count decline in ICU patients; therefore, we could not speculate on the pathophysiological mechanism of increased mortality in patients with decreased platelet counts. The most common cause of postoperative thrombocytopenia (33, 34) or bluntly increased platelet counts has been reported to be sepsis-related disseminated intravascular coagulation, which is more common than liver disease, blood disorders, massive transfusions, pharmacologic thrombocytopenia, and immune-mediated thrombocytopenia. Therefore, a decreased platelet count may be a powerful marker for assessing patient prognosis regardless of the mechanism. This study should serve as the basis for future well-designed studies to evaluate the impact of decreased platelet counts on mortality and causality.

CONCLUSION

A representative sample of the United States adults with sepsis found that lower platelet counts were significantly associated with higher 28-day mortality. These findings support the potential benefit of maintaining a normal platelet count status in the sepsis clinic in preventing premature death in septic patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

DW conducted data analysis and wrote the manuscript. SW conducted data analysis and modified the manuscript. HW and JG conducted the data collection. KH conducted the data collection and data interpretation. DX drew the figure. HR study manuscript design, data collection and analysis, manuscript preparation, and review. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.833996/full#supplementary-material

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SYSTEMATIC REVIEW
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Comparison of the Effectiveness of Various Drug Interventions to Prevent Etomidate-Induced Myoclonus: A Bayesian Network Meta-Analysis

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Background: Myoclonic movement is a very common but undesirable phenomenon during the induction of general anesthesia using etomidate. Such movement may cause unnecessary problems. Currently, there is an increasing number of drugs for preventing etomidate-induced myoclonus (EM). However, direct comparisons of various drugs are lacking, and this interferes with clinical decision-making. Our network meta-analysis (NMA) aimed to compare the efficacy of different drugs for the prevention of moderate-to-severe general myoclonus.

Methods: Using several biomedical databases, randomized controlled trials (RCTs) published in English from inception to August 22, 2021 were searched. Among the various interventions, we selected nine types of intervention drugs (dexmedetomidine, etomidate, lidocaine, NMDA receptor antagonist, κ opioid receptor agonist, μ opioid receptor agonist, muscle relaxant, gabapentin, and midazolam) for comparison, according to the number of studies. Bayesian NMA was performed using STATA16 and R softwares. The relative risk of EM was assessed using risk ratios (RRs) and the corresponding 95% confidence intervals (CI).

Results: A total of 31 RCTs (3209 patients) were included. NMA results showed that, compared with a placebo, etomidate (RR 4.0, 95%CI 2.1–7.8), κ opioid receptor agonist (RR 2.9, 95%CI 1.9–4.6), μ opioid receptor agonist (RR 3.1, 95%CI 2.3–4.3), NMDA receptor antagonist (RR 1.7, 95%CI 1.0–2.8), dexmedetomidine (RR 2.4, 95%CI 1.5–3.9), lidocaine (RR 2.1, 95%CI 1.2–3.9), and midazolam (RR 2.2, 95%CI 1.5–3.2) can significantly reduce the risk of EM. In contrast, the effects of muscle relaxants (RR 2.1, 95%CI 0.81–5.3) and gabapentin (RR 2.8, 95%CI 0.92–9.3) were inconclusive. Further subgroup analyses showed that preoperative low-dose etomidate, μ-opioid receptor agonist, and κ-opioid receptor agonist were significantly better than other interventions in the prevention of moderate to severe EM.

Conclusion: Preoperative use of small doses of etomidate or opioids may be the most effective way to avoid EM, especially moderate and severe EM, which makes anesthesia induction safer, more stable, and aligns better with the requirements of comfortable medicine.

Systematic Review Registration: [https://www.crd.york.ac.uk/prospero/], identifier [CRD4202127706].

Keywords: etomidate, myoclonus, anesthesia induction, network meta-analysis, Bayesian framework

INTRODUCTION

Etomidate, a compound containing an imidazole carboxyl group, was introduced to clinical practice in the 1970s. In addition to its strong anesthetic efficacy, rapid onset, and rapid recovery, etomidate has the advantages of stable cardiovascular profiles and minimal respiratory depression, making it an ideal substitute for propofol and the first-line anesthetic for many elderly people and patients with impaired hemodynamics and cardiac reserve (1, 2).

However, etomidate often induces spontaneous movements, especially myoclonic activities. Generalized convulsive seizures may occur in severe cases, with an incidence of 50%–80% (3). Myoclonus is also related to epileptiform activities. Therefore, epileptic activities may be enhanced in the EEG of some patients after etomidate injection (4). According to the classical definition, myoclonus is a sudden, brief, lightning-like muscle jerk arising from an abnormality of the nervous system, excluding short or prolonged movements caused by the muscle itself such as fasciculation, spasms, or cramps (5). Myoclonus can damage muscle fibers and cause serum potassium to rise. Transient mild myoclonus may not be pathologically significant in most patients, but severe myoclonus can have unintended consequences, especially in patients with a full stomach, malignant hypertension, open eye injury, aneurysms, and hyperkalemia (6, 7).

In the past few decades, many drugs have been used in clinical practice for the prevention and treatment of EM, including opioids, benzodiazepines, dexmedetomidine, ketamine, lidocaine, magnesium sulfate, muscle relaxants, antiepileptics, and preoperative low-dose etomidate. The variety of drugs available is appreciated by many anesthesiologists. Some traditional pairwise meta-analyses have evaluated the efficacy of two drugs or a drug versus a placebo to guide agent selection (8–15). However, when faced with a wide range of interventions, most anesthesiologists still struggle to choose the best option, and instead, use the drug empirically. Furthermore, traditional meta-analyses cannot clearly rank different classes of interventions based on their efficacy outcomes.

Owing to the limitations of standard pairwise meta-analyses, we adopted a network meta-analysis (NMA) to determine the most effective approach for preventing myoclonus. NMA integrates direct and indirect evidence and enables the evaluation of multiple treatments in a single analysis (16). In this study, we determined the effectiveness of all interventions, as well as their ranking probabilities in overall and subgroup networks by summarizing the available evidence. The results of this study will provide evidence for the best preventive measure of moderate to severe myoclonus, when using etomidate.

METHODS

Protocol and Registration

This systematic review followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (17), and was registered under the PROSPERO International prospective register of systematic reviews on October 6, 2021 (registration number CRD42021277063).

Search Strategy

The search strategy was first designed jointly by the two authors, and then, the search was conducted independently. PubMed, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), and NIH ClinicalTrials.gov were searched to find relevant articles from inception to August 2021 within the restriction limit of "randomized controlled trial" and "English-language." Some of the English literature from the CNKI database was supplemented. Using the combination of MeSH medical subject words and item words, the search terms were combined for literature retrieval through the logical characters "OR" and "AND." Relevant search strategies are provided in the Supplementary Material.

All citations were downloaded and imported into EndNote for management (18). First, duplicates were excluded from the analysis. The titles and abstracts were then reviewed, and studies that did not meet the inclusion criteria were excluded. Finally, the full text of any potential study was analyzed and further screened according to the exclusion criteria. The above tasks were also performed by two authors independently. The reasons for article exclusion were recorded for the preparation of the literature screening flowchart.

Inclusion and Exclusion Criteria

Inclusion criteria were formulated according to the PICOS framework (19), as follows: (1) adult patients who are purposed to surgical or invasive intervention under etomidate; (2) interventions including opioids, lidocaine, ketamine, dexmedetomidine, etomidate, muscle relaxant, magnesium sulfate, gabapentin, and midazolam; and (3) the control group could be a placebo or a comparison between the above drugs;

(4) the outcome was the incidence of myoclonus induced by etomidate, and the degree of myoclonus was divided into none, mild (mild myoclonus in the face and/or upper limbs and/or distal lower limbs), moderate (some movement in the face and/or limbs), and severe (movement in limbs and trunk); and (5) the study must be a randomized controlled trial and published in English.

Studies were excluded if they included the following characteristics: (1) patients who had neuropsychological disease; adrenal cortex dysfunction; heart failure; renal, pulmonary, hepatic, or endocrine diseases; history of allergic reaction to etomidate and other study drugs; (2) patients who had taken sedative and analgesic drugs on the day of operation; and (3) lack of necessary outcomes to be extracted, for example, incomplete data.

Data Extraction and Methodological Quality Assessment

We created a unified information extraction table in advance. Two authors independently screened the information, and any discrepancies were resolved through discussion. The following information was extracted: author's name, publication year, age distribution, type of surgery, American Society of Anesthesiologists (ASA) physical status, induction dose of etomidate, treatment, sample size, and outcome. The primary outcomes were the incidence of EM and moderate-to-severe EM.

For randomized controlled trials, two reviewers independently applied the Cochrane Review Manager (Version 5.4) to assess the risk of bias (ROB) in randomized trials (20). The Cochrane Collaboration's bias risk assessment tools are well-structured and mainly included random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. Each trial was independently performed by two reviewers and classified as low-, unclear-, or high-risk. The Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) Working Group recommended a four-step evidence quality grading for network meta-analyses (21). The certainty of the evidence was appraised as high, moderate, low, or very low.

Statistical Analysis

We first constructed a network evidence plot using Stata16.0, and conducted a traditional pairwise direct comparison meta-analysis. The network plot clearly showed whether there was a direct comparison, and whether the effect between interventions was the result of direct comparison, indirect comparison, or a combination of the two. Heterogeneity was assessed between studies using the Q test and I^2 statistic (22). If the P value of Cochran's Q test statistic was less than 0.05, or the I^2 statistic was greater than 50%, large heterogeneity between studies was determined, and the random-effects model was preferred. A pairwise meta-analysis was performed using the random-effects model. For binary outcomes, we reported the risk ratios (RR) and corresponding 95% confidence intervals (CI). If the 95%CI did not include 1, the difference between the two comparisons was considered statistically significant. A comparison-adjusted funnel plot was used to determine the possibility of a publication bias.

Owing to the existence of closed rings in the network evidence graph, we used a Bayesian network meta-analysis to compare the differences between different interventions (23). The "*gemtc*" package and the "*rjags*" package of R software that invoke the JAGS software[1] for NMA based on a Bayesian generalized linear model were used (24, 25). For each outcome, the fixed-effects model and random-effects model were used for evaluation. The fitting degree of the model was determined by the deviance information criterion (DIC), and a model with less DIC was generally selected (26). Four Markov chains were used to set the initial values. The iterations were set to 70000, and the initial 30000 iterations, with a thinning factor of 10. Furthermore, the convergence of the model was diagnosed using a trace plot, density plot, and Brooks-Gelman-Rubin diagnosis plot (25). Finally, we calculated the RR and corresponding 95%CI, and the surface under the cumulative ranking (SUCRA) probabilities were used to rank the efficacy of various interventions (27). The value of the SUCRA is between 0 and 1 ($0 \leq \text{SUCRA} \leq 1$). When the SUCRA was 1, the intervention was effective, whereas when the SUCRA was 0, the intervention was ineffective. Subgroup analysis was performed according to EM severity (mild, moderate, and severe). Only 29 of the 31 RCTs included had myoclonus classification; therefore, a subgroup analysis was performed.

Song et al. pointed out that indirect comparison and NMA often involved three basic assumptions: homogeneity, similarity, and consistency hypothesis (28). In this study, we used the "node-splitting technique" to evaluate network consistency (29). A P value > 0.05 indicated consistency, and as such, we combined the direct and indirect estimates in the comparison of mixed treatment.

RESULTS

Search Results

The literature retrieval results and screening process are shown in **Figure 1**. A total of 251 studies were initially retrieved. Of these, 89 duplicate studies were removed using the EndNote software. A total of 108 studies were excluded after reading the titles and abstracts. Based on the full-text reviews, 24 studies were further excluded for various reasons: 15 did not meet the inclusion criteria or had incomplete information, and the full text of 6 records was not available. Finally, 31 RCTs (3209 patients) were included in this study.

Characteristics of Included Studies

An overview of the selected studies is shown in **Table 1**. Most patients were scheduled for elective surgery under general anesthesia, with ASA physical status ranging from I to IV. During the induction of general anesthesia, the injection dosage of etomidate was 0.2–0.3 mg/kg, which is a commonly used

[1] http://mcmc-jags.sourceforge.net/

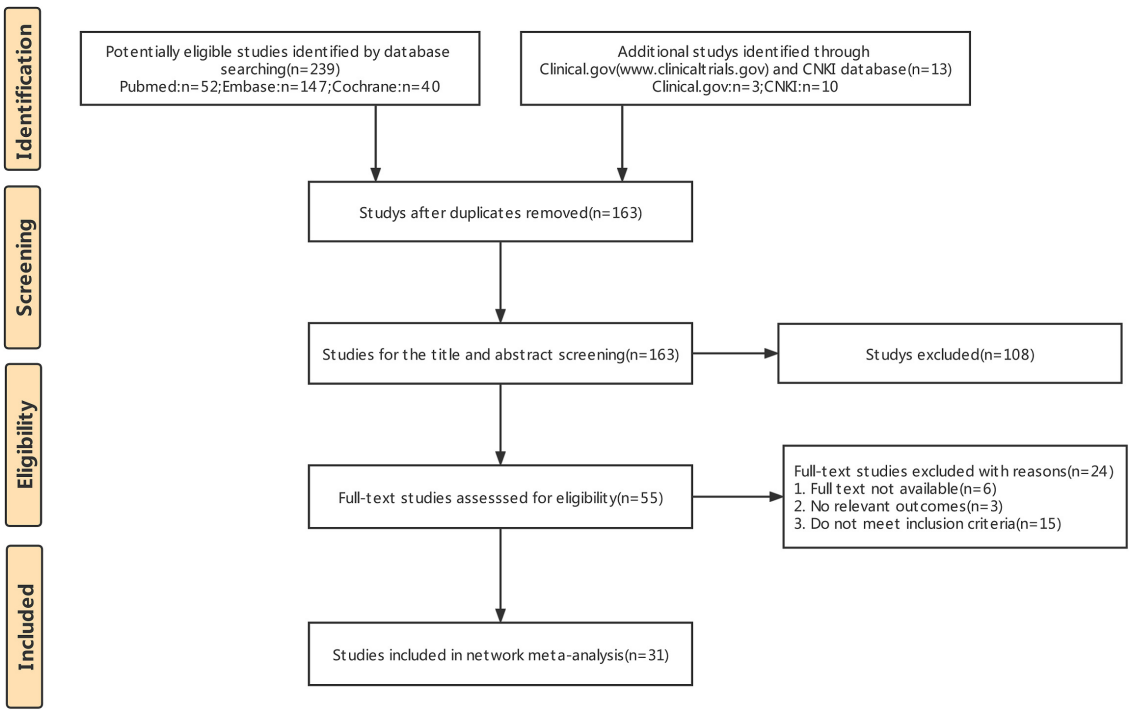

FIGURE 1 | Flow diagram of the literature search and study.

induction dose in clinical practice. While there was a wide variety of drugs studied, for drugs with similar pharmacological effects, we categorized them as a group for analysis. Oxycodone, fentanyl, sufentanil, and remifentanil are all μ opioid agonists (μ-R agonists). Butorphanol, dezocine, and nalbuphine are κ opioid agonists predominate (κ-R agonists), and magnesium sulfate and ketamine are N-methyl-D-aspartic acid receptor antagonists (NMDA-R antagonists). The sample size of the 31 studies ranged from 45 to 284.

Pairwise Meta−Analysis and Network Meta-Analysis Results

The network relationship between different treatment regimens and placebo is shown in **Figure 2**. In our NMA, there were 26 two-arm studies, 4 three-arm studies, and 1 four-arm study, and a comparison between 10 interventions-, including placebo, was performed. μ-R agonists were most frequently included for comparisons, followed by midazolam, and NMDAR antagonists. Regarding heterogeneity, we compared the fixed-effects model with the random-effects model, and the results showed that the latter had smaller DIC and I^2 values. Therefore, based on the heterogeneity analysis and DIC comparison, all data were analyzed using a consistent random-effects model. After 70000 iterations, the fluctuation of the four Markov chains was small, the trace plot and density plot tended to be stable, and the PSRF was close to 1, indicating satisfactory convergence of the model and relatively stable results (**Supplementary Figure 1**).

The results of the pairwise meta-analysis are shown in **Figure 3**. The results produced by NMA are illustrated in **Figure 4A**. In comparison with placebo, overall myoclonus incidence was significantly reduced after low-dose dexmedetomidine (RR 2.4, 95%CI 1.5–3.9), etomidate (RR 4.0, 95%CI 2.1–7.8), NMDA-R antagonist (RR 1.7, 95%CI 1.0–2.8), lidocaine (RR 2.1, 95%CI 1.2–3.9), midazolam (RR 2.2, 95%CI 1.5–3.2), μ-R agonist (RR 3.1, 95%CI 2.3–4.3), and κ-R agonist (RR 2.9, 95%CI 1.9–4.6) before induction of anesthesia. Gabapentin (RR 2.8, 95%CI 0.92–9.3) and muscle relaxants (RR 2.1, 95%CI 0.81–5.3) did not significantly reduce the overall incidence of EM. Additionally, etomidate (RR 2.35, 95%CI 1.11–5.06) was significantly better than the NMDAR antagonist, NMDAR antagonist (RR 0.56, 95%CI 0.31–0.96) was significantly worse than the μ-R agonist, and the differences among other drugs were not statistically significant. To further understand the results, the nine interventions were ranked by the SUCRA value. The higher the SUCRA value, the lower the incidence of myoclonus after etomidate induction. The corresponding SUCRA values are shown in **Figure 4B**. The results suggest that preoperative administration of low doses of opioids and etomidate is preferable to other regimens.

Study Quality

Node-splitting technology was used to test the consistency of indirect and direct evidence, and the results are shown in **Supplementary Figure 2**. In the vast majority of comparisons, there was no statistically significant inconsistency between the direct and indirect estimates ($P > 0.05$). Publication bias was visually inspected using comparison-adjusted funnel plots (**Supplementary Figure 3**). Most studies were distributed on

TABLE 1 | Characteristics of included studies.

Author,year	Type of surgery	Age	ASA status	Induction dose of etomidate	Treatment	Case	No EM	Mild EM	Moderate-Severe EM
Wu, (55)	Elective surgery	18-65	I-II	0.3mg/kg	Placebo	52	13	8	31
					Ketamine 0.5mg/kg	52	40	7	5
Wang, (58)	Elective surgery	22-64	I-II	0.3mg/kg	Placebo	54	15	10	29
					Oxycodone 0.1mg/kg	54	54	0	0
					Fentanyl 1ug/kg	54	37	4	13
Sedighinejad, (42)	Orthopedic surgery	19-59	I-II	0.3mg/kg	Etomidate 0.03mg/kg	71	41	12	18
					Remifentanil 1ug/kg	71	30	12	29
					Midazolam 0.015mg/kg	71	20	3	48
					Magnesium sulfate 30mg/kg	71	10	4	57
Hwang, (51)	Elective surgery	Adults	I-II	0.3mg/kg	Placebo	30	7	6	17
					Remifentanil 1ug/kg	29	24	3	2
					Midazolam 0.5mg/kg	30	25	5	0
Mullick, (43)	Elective surgery	18-60	I-II	0.3mg/kg	Placebo	63	10	10	43
					Etomidate 0.03mg/kg	63	25	15	23
Mizrak, (59)	Day case surgery	18-60	I-II	0.3mg/kg	Placebo	30	11	7	12
					Dexmedetomidine 0.5ug/kg	30	20	5	5
Miao, (60)	Elective surgery	Adults	I-II	0.3mg/kg	Placebo	50	18	8	24
					Dexmedetomidine 0.5ug/kg	50	37	7	6
Alipour, (61)	Elective eye surgery	Adults	II-III	0.3mg/kg	Sufentanil 0.2ug/kg	25	18	2	5
					Midazolam 0.015mg/kg	25	4	0	21
An, (62)	Elective surgery	18-65	I-II	0.3mg/kg	Placebo	60	22	22	16
					Oxycodone 0.05mg/kg	60	45	10	5
Luan, (63)	Elective surgery	18-60	I-II	0.3mg/kg	Placebo	30	11	10	9
					Dexmedetomidine 0.5ug/kg	30	19	9	2
					Dexmedetomidine 1ug/kg	30	21	8	1‘
He[1], (47)	Elective surgery	20-65	I-II	0.3mg/kg	Placebo	54	11	7	36
					Butorphanol	54	47	3	4
Guler, (64)	Elective surgery	Adults	I-III	0.2mg/kg	Placebo	25	7	6	12
					Ketamine 0.2mg/kg	25	9	10	6
					Ketamine 0.5mg/kg	25	7	10	8
					Magnesium sulfate 60mg	25	19	1	5
Gultop, (65)	Elective surgery	Adults	I-II	0.3mg/kg	Placebo	30	5	3	22
					Lidocaine 20mg	30	13	2	15
Gupta[1], (46)	Laparoscopic cholecystectomy	20-60	I-II	0.3mg/kg	Placebo	50	14	6	30
					Nalbuphine,0.2mg/kg	50	40	6	4
Gupta[2], (66)	Elective surgery	20-60	I-II	0.3mg/kg	Placebo	50	12	6	32
					Lidocaine 0.5mg/kg	50	20	5	25
					Lidocaine 1.0mg/kg	50	29	7	14
					Lidocaine 1.5mg/kg	50	23	9	18
He[2], (48)	Elective surgery	20-65	I-II	0.3mg/kg	Placebo	54	13	7	34
					Dezocine 0.1mg/kg	54	54	0	0
Hüter, (67)	Elective cardioversion	Adults	III-IV	0.3mg/kg	Placebo	20	10	6	4
					Midazolam 0.015mg/kg	20	18	2	0
Aktolga, (68)	Not mentioned	Adults	I-III	A sleep dose of etomidate	Placebo	51	5	28	18
					Midazolam 0.5mg/kg	51	32	16	3
					Dexmedetomidine 1ug/kg	50	35	11	4

(Continued)

TABLE 1 | (Continued)

Author,year	Type of surgery	Age	ASA status	Induction dose of etomidate	Treatment	Case	No EM	Mild EM	Moderate-Severe EM
Ko, (69)	Elective surgery	65-74	I-II	0.2mg/kg	Placebo	30	16	8	6
					Fentanyl 1ug/kg	30	28	2	0
					Remifentanil 1ug/kg	30	29	1	0
Lv[1], (49)	Elective surgery	Adults	I-II	0.3mg/kg	Placebo	40	14	3	23
					Dezocine 0.1mg/kg	40	28	5	7
Lv[2], (57)	Elective hysteroscopy	20-55	I-II	0.3mg/kg	Placebo	43	5	13	25
					Sufentanil 0.1ug/kg	43	17	12	14
Woo, (70)	Plastic surgery	Adults	I	0.3mg/kg	Placebo	30	5	5	20
					Remifentanil 0.5ug/kg	30	27	3	0
					Remifentanil 1ug/kg	30	25	5	0
Prakash, (71)	Elective surgery	Adults	I-II	0.3mg/kg	Fentanyl 2ug/kg	70	36	11	23
					Midazolam 0.03mg/kg	70	15	10	45
Ilke, (72)	Various operations under general anesthesia	Adults	I-II	0.3mg/kg	Placebo	20	3	4	13
					Fentanyl 1ug/kg	20	12	2	6
					Midazolam 0.03mg/kg	20	6	1	13
Singh, (73)	Elective surgery	Adults	I-II	0.3mg/kg	Placebo	25	6	8	11
					Lidocaine 20mg	25	14	6	5
					Midazolam 1mg	25	18	4	3
Boztug, (74)	Not mentioned	Adults	I-II	0.3mg/kg	Placebo	15	3	1	11
					Remifentanil 0.5ug/kg	15	13	1	1
					Remifentanil 1ug/kg	15	14	1	0
Yukselen, (75)	Not mentioned	Adults	III	0.3mg/kg	Placebo	20	2	6	12
					Remifentanil 1ug/kg	20	19	1	0
					Fentanyl 1ug/kg	20	6	8	6
Yılmaz Çakirgöz, (33)	Elective surgery	18-60	I-II	0.3mg/kg	Placebo	25	6	7	12
					Gabapentin 400mg	25	11	8	6
					Gabapentin 800mg	25	18	2	5
					Gabapentin 1200mg	25	17	3	5
Choi, (53)	Elective surgery	Adults	I-III	0.3mg/kg	Placebo	54	20	18	16
					Rocuronium 0.06mg/kg	56	42	12	2
Un, *(56)	Elective surgery	Adults	I-II	0.3mg/kg	Placebo	50	22	TNM = 28	
					Magnesium sulfate 60mg	50	37	TNM = 13	
Mutlu, *(76)	Minor surgery or procedures	Adults	I-II	0.3mg/kg	Placebo	30	26	TNM = 4	
					Remifentanil 1ug/kg	30	30	TNM = 0	
					Remifentanil 0.75ug/kg	30	30	TNM = 0	
					Remifentanil 0.5ug/kg	30	25	TNM = 5	

ASA status = American Society of Anesthesiologists physical status; EM = Etomidate- induced myoclonus; TNM = Total number of myoclonus cases.
**The presence of myoclonus was only reported as "present/absent", and no gradation was performed.*

both sides of the midline, and the left and right distributions were roughly symmetrical, suggesting that there was little possibility of publication bias and a small sample effect.

The risk of bias for the 31 RCTs is presented in **Supplementary Figure 4**. A total of 21 studies described the generation of random sequences, 23 trials described concealment details, 29 studies blinded subjects, 28 trials blinded evaluators of outcomes, and all the included studies showed complete data. One study was judged to be high-risk because of the different baseline data (the induction dose of etomidate was statistically different between different groups). The GRADE assessment showed that the quality of evidence for etomidate compared to other interventions was "high," indicating that the effect of using small doses of etomidate pre-induction to prevent EM is likely supported. The quality of evidence for the other comparisons is detailed in **Supplementary Table 1**.

Subgroup Analysis of Myoclonus of Different Degrees

Mild myoclonus is a brief movement of a part of the body, such as the fingers and wrist. Moderate myoclonus is usually

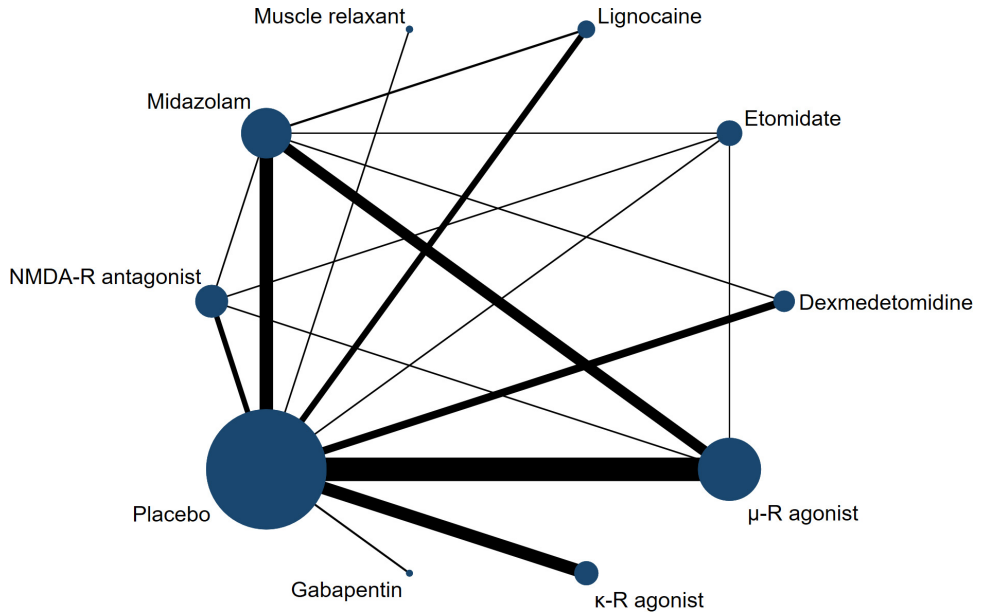

FIGURE 2 | Network plot of treatment comparisons. The width of the line represents the number of RCTs per pairwise comparison, and the size of each node is proportional to the number of sample size.

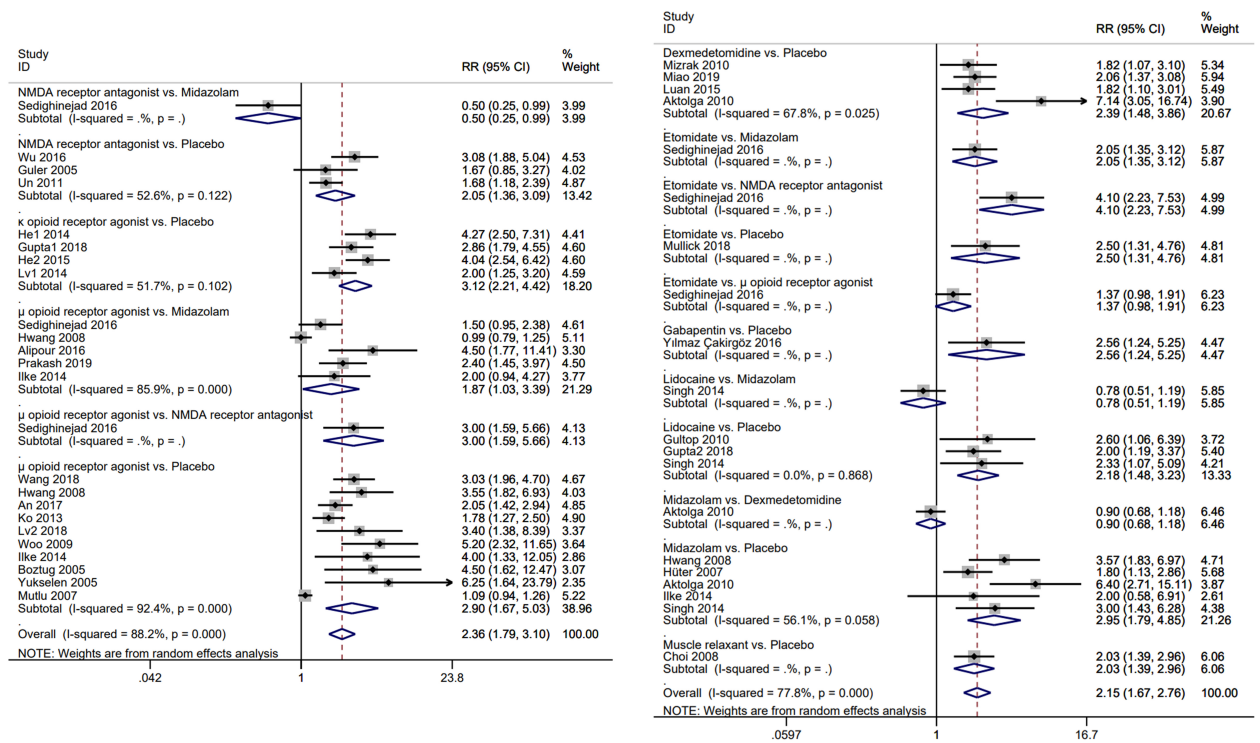

FIGURE 3 | Forest plot for direct comparison of each pair of interventions. Meta-analysis use RR and 95%CI for the incidence of etomidate-induced myoclonus. *RR*, risk ratio; *CI*, confidence interval.

a movement of two different muscles, such as the face, leg, shoulder or elbow, with pronounced tremors. Severe myoclonus is the intense movement or rigidity of two muscles; for example, the body undergoes fast abduction (30). Subgroup analysis was conducted based on the severity of myoclonus. Here, we mainly divided the patients into two groups: mild

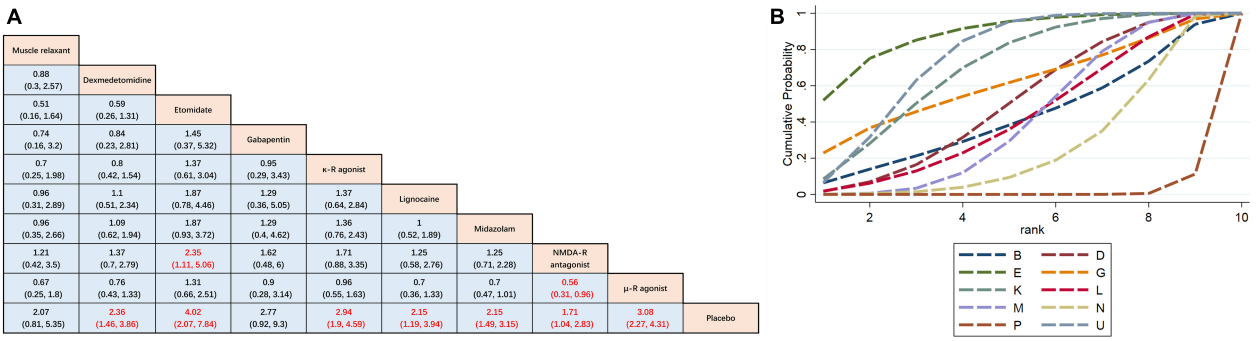

FIGURE 4 | Network meta-analysis comparison. **(A)** The incidence of etomidate-induced myoclonus was analyzed by using RR and 95%CI. Data in each cell are RR (95%CI) for the comparison of column-defining treatment versus row-defining treatment. Significant results are highlighted in red. *RR*, risk ratio; *CI*, confidence interval. **(B)** Graphical ranking based on SUCRA values (Incidence of total EM). The numbers on the X-axis represent the rankings. As the numbers increases, the effectiveness of the interventions decreases. *B* muscle relaxant, *D* dexmedetomidine, *E* etomidate, *G* gabapentin, *P* placebo, *K* κ opioid receptor agonist, *L* lidocaine, *M* midazolam, *U* μ opioid receptor agonist, *N* NMDA receptor antagonist.

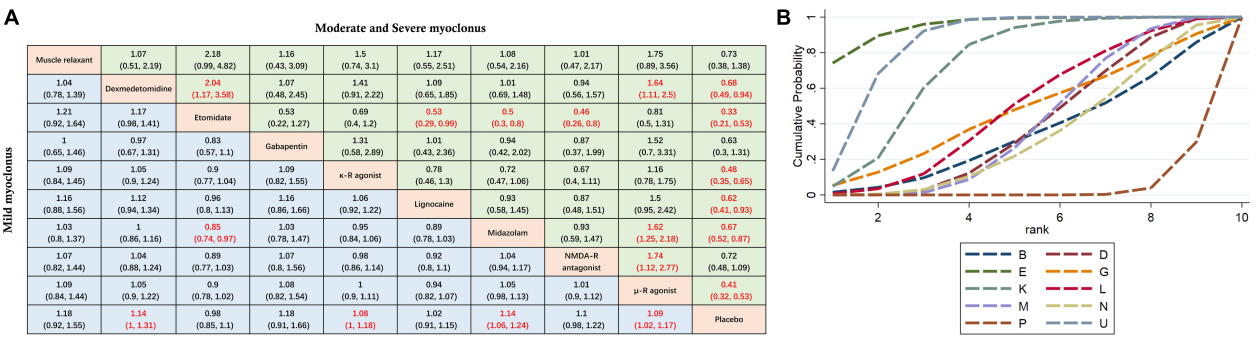

FIGURE 5 | Subgroup analysis of myoclonus of different degrees. **(A)** The incidence of myoclonus of different severity after etomidate induction was analyzed by using RR and 95%CI. Data in each cell are RR (95%CI) for the comparison of column-defining treatment versus row-defining treatment. Significant results are highlighted in red. *RR*, risk ratio; *CI*, confidence interval. **(B)** Graphical ranking based on SUCRA values (Incidence of moderate to severe EM). The numbers on the X-axis represent the rankings. As the numbers increases, the effectiveness of the interventions decreases. *B* muscle relaxant, *D* dexmedetomidine, *E* etomidate, *G* gabapentin, *P* placebo, *K* κ opioid receptor agonist, *L* lidocaine, *M* midazolam, *U* μ opioid receptor agonist, *N* NMDA receptor antagonist.

myoclonus and moderate-to-severe myoclonus. The results showed that preoperative low doses of etomidate (RR 0.33, 95%CI, 0.21–0.53), μ-R agonist (RR 0.41, 95%CI 0.32–0.53), κ-R agonist (RR 0.48, 95%CI 0.35–0.65), dexmedetomidine (RR 0.68, 95%CI 0.49–0.94), midazolam (RR 0.67, 95%CI 0.52–0.87), and lidocaine (RR 0.62, 95%CI 0.41–0.93) significantly reduced the incidence of moderate to severe myoclonus compared with placebo, but only dexmedetomidine (RR 1.14, 95%CI 1–1.31), midazolam (RR 1.14, 95%CI 1.06–1.24), κ-R agonist (RR 1.08, 95%CI 1–1.18), and μ-R agonist (RR 1.09, 95%CI 1.02–1.17) reduced the incidence of mild myoclonus. The results of the subgroup analysis are shown in **Figure 5A**. Since moderate-to-severe myoclonus is the most common clinical problem, we focused on the prevention and treatment effects of various interventions on moderate-to-severe myoclonus. For effectiveness in preventing moderate to severe EM, **Figure 5B** shows the corresponding ranking based on SUCRA values: etomidate > μ-R agonist > κ-R agonist > lidocaine > gabapentin > midazolam > dexmedetomidine > muscle relaxant > NMDA-R antagonist.

DISCUSSION

Our NMA attempted to summarize the available data using direct and indirect evidence to conclude that pre-induction of anesthesia with low-dose opioids and etomidate is the best intervention to reduce the incidence and severity of etomidate-induced myoclonus. However, further research is warranted.

As a fast-acting intravenous anesthetic, etomidate has a low risk of hemodynamic damage. Compared with propofol, etomidate is especially suitable for anesthesia, procedural sedation and analgesia (PSA) in the emergency department for patients with trauma, shock, and acute abdomen, with hemodynamic instability. However, in some areas, etomidate use is partially limited by its ability to cause adrenocortical depression, myoclonus, and injection pain (31). Etomidate can induce myoclonus which may increase the risk of aspiration in satiated patients and patients with a decreased cardiac reserve and increased cardiac oxygen consumption (32). During severe myoclonus, electrocardiogram electrode shifts and pulse oxygen saturation measurements often show desaturation (33).

Myoclonus has been reported to be associated with hypoxemia during spontaneous ventilation when etomidate was used in the emergency department for PSA (34). In summary, myoclonic events may be large enough to delay patient monitoring and evaluation of intervention success.

The anesthetic effects of etomidate and its derivatives are generally thought to occur *via* $GABA_A$ receptors (35). Our current understanding of the mechanisms of etomidate-induced myoclonus is fragmented, contradictory, and confusing. Modica and Gancher et al. noted that etomidate is an electroencephalogram drug that has been shown to cause epileptic activities in non-epileptic patients (36–38). Therefore, etomidate-induced myoclonus may occur as epileptic activities, similar to the mechanisms underlying epilepsy. In contrast, Doenicke et al., in their study, reported that after giving etomidate, part of myoclonus patients only can be observed in EEG amplitude smaller, isolated, rapid, sharp transient wave, different from the typical epileptic EEG activity (30). Epilepsy is a clinical event with a definitive EEG diagnosis accompanied by a widespread, diffuse wave of EEG activities (39). It is prudent to say that anesthetics usually induce epileptiform activity, but rarely seizures. Epileptiform activity differs from epilepsy in that it primarily refers to the hypersynchrony of neurons in a small area (< 1 cm^2), and is considered an indicator of an incipiently unstable neocortex, with a weak association with clinically meaningful seizures (40). Another theory is that etomidate-induced myoclonus is a disinhibitory phenomenon (41). It may be that there are differences in local cerebral blood flow or in the affinity of receptors in the central nervous system that cause the action of etomidate to become unsynchronized. For example, large quantities of etomidate tend to inhibit cortical activity before they inhibit subcortical activity (30). Subsequently, subcortical disinhibition makes the pathways associated with controlling skeletal muscles more sensitive to spontaneous neurotransmitters, causing spontaneous myoclonus. GABAergic synaptic excitation and subgroup specificity between interneurons, which control the output of pyramidal cells, also partly explain this remarkable neurophysiological phenomenon (40). Although myoclonic excitation is not thought to be caused by epileptic foci, drugs such as dexmedetomidine (α-2 agonist-mediated reduction of convulsion severity) and gabapentin (antiepileptic agents that increase the inhibitory effect of GABA) effectively reduce EM (33).

Although knowledge gaps still remain, it seems that implementing effective prevention is crucial and of the most practical value. As a single large dose of etomidate inhibits cortical activity earlier than subcortical activity, myoclonus can be prevented by prior suppression of subcortical neuronal activity with known drugs. Among the results of our analysis, seven interventions (μ-R agonist, κ-R agonist, etomidate, dexmedetomidine, midazolam, NMDA-R antagonist, and lidocaine) showed statistically significant improvements in preventing the incidence of EM compared with placebo, and six interventions (μ-R agonist, κ-R agonist, etomidate, dexmedetomidine, midazolam, and lidocaine) showed statistically significant improvements in preventing the incidence of moderate to severe EM.

The distinct distribution of $GABA_A$ receptor subunits (mainly β subunits) explains the dose-dependent effects of etomidate on the central nervous system. Etomidate can inhibit subcortical inhibitory circuits earlier and at lower doses, and when large doses are administered simultaneously, this mismatch is exaggerated, producing clinically visible myoclonus (32, 42, 43). Therefore, small doses of etomidate pre-induction can reduce the incidence of myoclonus.

The role of the κ-opioid receptor as a neuronal excitatory modulator is well known. Activation of the κ receptor reduces glutamate release, produces postsynaptic hyperpolarization, and inhibits seizure activity (44). κ-opioid receptor agonists also interact with a variety of neurotransmitter systems (μ opioid receptor, δ opioid receptor, γ-aminobutyric acid-benzodiazepine-chloride ion channel, GABA receptors, and NMDA receptor). Dezocine, butorphanol, and nalbuphine mainly bind to and regulate κ-opioid receptors; therefore, the mechanism by which these drugs reduce etomidate-induced myoclonus may lie in their activation through κ receptor regulation as agonists (45–49).

Benzodiazepines and opioids, such as fentanyl, are known to inhibit subcortical neuronal activity (38). Many randomized controlled trials have shown that multiple opioids, including fentanyl, sufentanil, remifentanil, and oxycodone, are effective in reducing the incidence and severity of EM. However, apnea, nausea, vomiting, and bradycardia are possible (46, 50, 51). In the study by Su et al., intramuscular injection of midazolam (0.05 mg/kg) 30 min before etomidate injection did not reduce the incidence of myoclonus, which was not significantly different from the previously reported incidence of myoclonus (50). Since opioid receptors are widely distributed in the brain, the mechanism by which opioid agonists inhibit myoclonus remains unknown. It may be that μ-opioid receptors are stimulated in the basal ganglia, which changes the function of GABA receptors and reduces the release of GABA, thus inhibiting subcortical neuronal activity. In Parkinson's-related studies, opioid neuropeptides have been reported to strongly regulate synaptic transmission and striatal projection neuron (SPNs) activity (52). High opioid levels occur in parallel with abnormal dopaminergic transmission, producing symptoms similar to increased dopamine levels, thus attenuating the onset of muscle fibrillation.

Although other studies were included in a supplementary analysis, the reduction in myoclonic symptoms was not as significant as opioid and etomidate preconditioning, as indicated by our results. Non-depolarizing muscle relaxants are associated with blocking nerve conduction at neuromuscular junctions (53). Lidocaine reduces the activity of the nerve centers that cause myoclonus (54). Magnesium sulfate and ketamine are non-competitive N-methyl-D-aspartate receptor antagonists, and their myoclonic inhibition is thought to be related to the inhibition of NMDA receptor activity in the central nervous system (42, 55). However, the efficacy varies from study to study. In the study by Un et al., the incidence of EM after magnesium sulfate pretreatment was 26%, whereas in the study by Sedighinejad et al., the incidence was as high as 86% (42, 56).

Regarding the response rates before and after the entire NMA, the hierarchical order of total myoclonus incidence differed

slightly from the results of the subgroup analysis, but opioid or etomidate preconditioning still showed a significant advantage. The latter seems to be the more important result.

There were some limitations to our study. First, fewer than 60% of the studies included more than 100 participants, which could have contributed to the risk of bias. Second, SUCRA was used to estimate the rank probability of comparative efficacy between different interventions. However, it has limitations, all of which are subject to uncertainties, and thus, the results need to be interpreted with caution. Third, in the past, most studies had different drug dosages according to the different curative effects. However, in this study, we only limited the category of drugs. We did not limit the dosage. This may have caused a bias. Fourth, this study did not consider the safety of using these drugs because only a few studies reported adverse reactions and there was not a large amount of data available. Fifth, although transcutaneous acupoint electrical stimulation has been previously reported to reduce the incidence and severity of etomidate myoclonus (57), non-pharmacological or other interventions were not considered in our study. Sixth, 31 RCTs were included, including nine interventions. However, only seven were studied in two or more trials. Only two studies reported on both muscle relaxants and gabapentin, respectively, which explains the wide 95% confidence interval for the ultimate RR for both drugs.

CONCLUSION

Based on the currently available evidence, we used the NMA approach to compare the impact of different interventions on EM for the first time. Taken together, preoperative low-dose etomidate is the best intervention for preventing severe general myoclonus. Although opioids also have a prophylactic effect on general myoclonus, side effects should not be ignored. Our study provides strong evidence that implicates clinical practice. In particular, Etomidate may have an even more important role in clinical intravenous anesthesia.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

K-DZ proposed the research idea, developed a retrieval strategy, and drafted the manuscript. L-YW and K-DZ conducted a literature search, literature selection, and bias risk assessment. D-XZ and Z-HZ performed data extraction. H-LW reviewed and revised the manuscript. All authors contributed to the analysis and interpretation of the data, revised the manuscript, and approved the final version prior to submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.799156/full#supplementary-material

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CASE REPORT
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Case Report: Anesthetic Management of Cesarean Section in a Patient With Paraplegia

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Background: With the advancement of medical science and rehabilitative care, more women with spinal cord injury (SCI) can conceive. However, autonomic hyperreflexia due to SCI complicates anesthesia management during cesarean sections.

Case Presentation: This study reports the anesthesia management in a woman with paraplegia with a T6 SCI lesion who underwent a cesarean section. It also reviews the anesthesia strategies used in other studies. Spinal anesthesia with a low concentration of ropivacaine was administered along with dexmedetomidine for sedation. Stable hemodynamics were achieved without complications.

Conclusions: Based on the reported case and literature review, we conclude that the intrathecal block is the preferred choice for women with paraplegia who require cesarean section if the lumbar bone structure allows puncture attempts.

Keywords: anesthesia, cesarean section, paraplegia, autonomic hyperreflexia, spinal cord injury

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INTRODUCTION

The global incidence of spinal cord injury (SCI), both traumatic and non-traumatic, is approximately 40–80 cases/million (1). Pregnancy is rare in patients with SCI. However, in recent times, pregnancies in patients with paraplegia have a good prognosis because of advanced care and better knowledge. Yet, more complications, such as autonomic hyperreflexia (AHR), may occur during labor and delivery due to the pathophysiological changes induced by SCI. In women with paraplegia, AHR is the most serious complication, and 85% of mothers with paraplegia may develop AHR if the level of SCI exceeds T6; AHR manifests as sudden and severe hypertension or even cardiovascular and cerebrovascular incidents.

There are very few reports on anesthesia management during cesarean section in patients with SCI. Studies documenting perioperative anesthetic management of pregnant women with SCI are equally rare. In this study, we report our experience of intraoperative anesthesia management in a parturient with paraplegia with SCI above T6 using spinal anesthesia with a low concentration of ropivacaine combined with dexmedetomidine for sedation. This case study has been approved by the Ethics Committee of the Chongqing Health Center for Women and Children, and informed consent was obtained.

CASE DESCRIPTION

The patient was a 29-year-old woman, G_1 P_0, at 37+6 weeks of gestation. She had a history of traumatic thoracolumbar and cervical injuries (cervical compression fractures and thoracic burst fractures) that had occurred in 2009; she had received a blood transfusion of ∼4,000 ml at that time. The current level of paraplegia was T6, and the sensory and motor abilities of the limbs below the xiphoid process were completely lost. The patient complained of occasional flushing, fever, and sweating above the xiphoid process. In 2019, she decided to undergo *in-vitro* fertilization with the implantation of two frozen embryos, and one had survived. She visited the anesthesia clinic 1 month before the due date. She was diagnosed with Hashimoto's thyroiditis in 2003 and consumed levothyroxine sodium tablets during pregnancy for hypothyroidism. Thyroid function was checked regularly with recent administration of levothyroxine 100 μg/day. Diagnoses at admission were pregnancy with hypothyroidism, 37+6 weeks of gestation, G_1 P_0 waiting for delivery, high paraplegia, and embryo transplantation.

ANESTHETIC MANAGEMENT

Because of the high paraplegia and the condition of the pelvis, a cesarean section was scheduled at 38 weeks of gestation. Preoperative physical examination revealed a pregnant woman (height, 160 cm; and weight, 64 kg) with light peripheral edema, Mallampati class 2 airway with a 6-cm thyromental distance, and a total absence of sensation below T6, including spinal cord reflexes and temperature sensation. Her vital signs were as follows: body temperature, 37°C; pulse, 87 bpm; respiration rate, 20 breaths/min; and blood pressure, 129/71 mm Hg. Preoperative laboratory examinations, including routine blood and urine tests, liver and kidney function, electrolytes, coagulation function, immunity, and thyroid function, showed no obvious abnormalities. There were no abnormalities on the ultrasound of the chest or lower limbs.

Electrocardiogram, pulse rate, oxygen saturation, and non-invasive cuff blood pressure were closely monitored in the operating room. Venous access to the left and right forearms was established. A radial artery puncture was also performed on the right side to monitor the internal arterial blood pressure. Baseline vital signs were as follows: heart rate, 78 bpm, SpO_2, 99%; blood pressure, 148/75 mm Hg, and respiration rate, 21 breaths/min. Fetal heart rate was 140–150 bpm with minimal long-term variability. The patient was placed in the right knee-chest position. After successfully completing the L3/4 epidural puncture, a 25G lumbar puncture needle was inserted through the opening of the epidural puncture needle, guided by an 18G needle. A total of 3 ml of 0.3125% ropivacaine (heavy specific gravity, 0.75% ropivacaine 1.25 ml + 5% glucose 1.75 ml) was administrated into the subarachnoid space after the arachnoid membrane was punctured. Considering the possibility of epidural adhesion caused by a previous surgery, no epidural catheter was placed before the withdrawal of the lumbar puncture needle. The level of the sensory block did not exceed T4.

The neonate (3,050 g) was removed 5 min later, and the Apgar score was 10–10–10 at 1–5–10 min. The parturient was followed up with dexmedetomidine (initial dose 0.5 ug/kg/10 min, maintenance dose 0.3 ug/kg/h) intravenously. No obvious hemodynamic response was observed during the procedure. Her blood pressure remained within 15% of her baseline pressure (148/75 mmHg), and her heart rate fluctuated between 60 and 80 bpm throughout. No other analgesia was administered after the operation and during the postoperative period between surgery and discharge. The maternal vital signs were stable, and there were no complaints of discomfort except for slight pain in the surgical area.

DISCUSSION

With socioeconomic development and individual preference, more women with paraplegia would want to become pregnant. For delivery, the cesarean section is suitable for some women with paraplegia considering specific conditions, although paraplegia itself is not a contraindication for vaginal delivery (2). Another factor and indication to perform an elective cesarean section is the patient's inability to feel labor pains (1). A retrospective cohort study of 15 patients with paraplegia reported a cesarean section rate of 47% (indications were obstetric reasons) (2).

The pathophysiological changes of SCI make anesthesia management more complicated during cesarean sections in a parturient with paraplegia, and AHR triggered by noxious and distension stimuli below the level of the injury during childbirth is the most severe life-threatening complication (3). As a result of uterine contractions, any parturient with SCI whose injury is at T6 or above is at a risk of acute AHR, which is usually mistaken for preeclampsia at onset (4).

In women with paraplegia, AHR may occur during cesarean sections either by general or regional anesthesia, and analgesic insufficiency is a strong inducing factor in AHR (5). The impact of introducing various methods of anesthesia on patients with paraplegia and SCI has been investigated in previous studies, including the general anesthesia (6–8), epidural anesthesia (4, 9), spinal anesthesia (1, 5, 10), transversus abdominis plane block (11), and even no anesthesia (12).

Cross et al. (12) reported that the cesarean section was performed in 43% of 22 SCI patients with 30 deliveries. Among the 13 deliveries that involved cesarean section, epidural anesthesia was used in seven patients, general anesthesia in five patients, and no anesthesia in one patient. Fantin et al. (11) reported the case of a patient with high thoracic flaccid paraplegia (T3/4 lesion) who underwent elective cesarean delivery by ultrasound-guided lateral TAP blocks; the authors believe that these blocks lowered the risk of AHR during surgical incision.

In fact, epidural or spinal anesthesia is recommended as an intrathecal block for parturients with paraplegia undergoing cesarean delivery (7). Spinal anesthesia, which prevents AHR better by achieving a more predictable neural blockade at the T4 level, is preferred.

The optimal level of sensory block for cesarean section is T4. The patient presented in this study had a cervical and thoracic spine fracture due to trauma and had a history of surgery. A part of the lumbar vertebral gap was still available for induction of spinal anesthesia. Therefore, spinal anesthesia with low-concentration analgesia (0.3125% gravity ropivacaine) was used, and the epidural catheter was not placed because of possible postoperative adhesions (from previous surgeries) in the epidural space. However, it is also necessary to prepare for the possibility of spinal anesthesia not reaching the expected level of efficacy (13).

Preoperative anxiety is more severe in parturients with paraplegia than in ordinary parturients. Women with SCI who decide to get pregnant need special healthcare services (14). Therefore, auxiliary sedation is necessary to relieve parturient tension and anxiety during surgery if spinal anesthesia is selected. Excellent sedation was achieved in the reported case using a dexmedetomidine intravenous pump after the fetus was delivered. The stability of vital signs, blood pressure, and heart rate of the mother was maintained in this case, and no complications occurred during the entire anesthesia process.

In conclusion, spinal anesthesia, which likely prevents AHR better, is preferred for cesarean delivery in parturients with paraplegia in cases that allow puncture attempts. Preoperative evaluation and pre-arranged plans are necessary and important to prevent related complications as AHR. Dexmedetomidine can achieve the desired sedation effect in conscious SCI parturients selected for neuraxial anesthesia.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Chongqing Health Center for Women and Children. The patients/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

YS collected the patient's information and wrote the manuscript. XL and JY performed the anesthesiology procedures. All authors approved the final manuscript.

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ORIGINAL RESEARCH
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Protocol for a Sepsis Model Utilizing Fecal Suspension in Mice: Fecal Suspension Intraperitoneal Injection Model

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Background: Various animal models of sepsis have been developed to optimize sepsis treatment. However, therapeutic agents that were successful in animal models were rarely effective in human clinical trials. The cecal ligation and puncture (CLP) model is currently the gold standard for sepsis studies. However, its limitations include the high variability among researchers and the difficulty in comparing animals with different cecum shapes and sizes. In this study, we established a protocol for the creation of a simple and accessible sepsis rodent model using fecal suspensions that minimized differences in technical effects among researchers and individual differences in animals.

Methods: A mouse model of sepsis using fecal suspension intraperitoneal injection (FSI) was created using fresh stool excreted within 24 h. The collected fresh stool was dissolved in saline solution and filtered. The obtained fecal suspension was injected intraperitoneally into the mice. Moreover, fecal suspensions with different concentrations were prepared, and the survival rates were compared among the fecal suspensions for each concentration. To assess the validity of the FSI as a sepsis model, CLP and FSI with similar mortality rates were compared pathologically, physiologically, immunologically, and bacteriologically. Histopathological comparison was evaluated by hematoxylin-eosin and Gram staining of the parenchymal organs. Physiological evaluation was performed by comparing the respiratory rate, body temperature, and blood gas analysis results. Immunological assessment was performed using multiplex analysis. Bacteriological comparisons were performed by culturing ascites fluid.

Results: The FSI model increased mortality in proportion to the fecal suspension concentration. The mortality rate was reduced with antibiotic administration. In various comparative experiments conducted using the FSI and CLP models, both models showed findings consistent with sepsis. Furthermore, the FSI model showed less variability among the individuals in each test.

Conclusion: This is the first detailed and accurate report of a protocol for creating a sepsis model using fecal suspension. The FSI model is a minimally invasive and accessible sepsis rodent model. Its clinical validity as a sepsis model was proven via histological, physiological, microbiological, and immunological evaluation methods. The FSI model minimizes individual differences between mice and helps to conduct accurate studies after the onset of sepsis.

Keywords: sepsis, mice, cytokine, cecal ligation and puncture, peritonitis, inflammation

INTRODUCTION

Sepsis is a common disease with a mortality rate of 28–48% (1–3). To develop superior sepsis treatments, various studies using animal models of sepsis have been conducted. However, even if an animal model demonstrates the efficacy of a therapeutic agent, it rarely leads to success in human clinical trials. The ideal animal model used to develop sepsis therapeutics should mimic the course of human diseases. However, there are major differences among the current animal sepsis models, and there is no optimal model for drug discovery of sepsis thus far. There are many possible causes for this setback. To date, a variety of sepsis models have been developed (4–13). Each sepsis model has its own specific advantages and disadvantages, which have been discussed in previous reviews (6–8, 14, 15).

At present, the cecal ligation and puncture (CLP) model is considered the gold standard for sepsis studies (8, 9). The main feature of the CLP model is that it recreates the hemodynamic and metabolic phases of human sepsis (8); further, apoptosis of selected cell types and the host immune response also resemble the course of human sepsis (16, 17). However, it suffers from such limitations as the high variability among researchers (18) and the difficulty in making comparisons among animals with different cecum shapes and sizes (4). Another known model of sepsis using fecal suspensions has been reported in mice (4), rats (11), and sheep (12, 13). However, its position as a sepsis model is not well established because their preparation methods are not clearly described (11) or are complicated (4, 12, 13).

In this study, we devised a simple and easy animal model of sepsis using fecal suspensions that minimizes the effects of differences in techniques used by researchers, and individual differences in animals. We prepared two different concentrations of fecal suspensions (thin and thick) to confirm the effects of antimicrobial agents. The survival rates were compared according to various concentrations of each fecal suspension. The validity of the model was evaluated using bacteriological, pathological, physiological, and immunological methods.

MATERIALS AND METHODS

Ethics Statement

All experiments were conducted in accordance with the Hokkaido University Animal Experiment Regulations. The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation from the Institutional Ethical Review Board of Hokkaido University (Approval number: 20-0163).

Experimental Animals

Seven-week-old Institute of Cancer Research male mice were obtained from Japan SLC Inc., (Hamamatsu, Japan). Mice were specific-pathogen-free and weighed 32–34 g.

Housing and Husbandry

All animals were housed and treated in accordance with the guidelines for performing animal experiments at Hokkaido University. Following an acclimation period of a minimum of 3 days in the animal breeding quarters, the animals were subjected to experimentation. Mice were housed in a facility with a stable room temperature and humidity (24 ± 2°C, 0–20%) and a regular light/dark cycle (12 h of light from 6 a.m. to 6 p.m.). The mice were fed a standard diet (Labo MR Stock®, Japan Nosan Corporation, Kanagawa, Japan) and given free access to water from a water bottle placed above the cage. The bottom of the cage was covered with paper matting (Paper clean® , Japan SLC Inc., Hamamatsu, Japan) that was changed every few days to keep the cage clean. The number of mice in each cage was limited to five.

Experimental Procedures (Creating a Model Mouse)

The CLP model was created using the procedure of Rittirsch et al. (19) with adipose tissue resection (20). A 10 mm incision was made in the midline of the shaved and sterilized abdomen. A 21-gauge needle was used for the puncture, and the ligation site was half the distance between the distal pole and the base of the cecum. Silk thread (2-0) was used to ligate the cecum, and a 4-0 nylon thread was used to suture the peritoneum and skin. Anesthesia was administered with ketamine (125 mg/kg) and xylazine (10 mg/kg), and buprenorphine 0.05 mg/kg was administered for adequate analgesia. After surgery, 1 mL of saline was administered subcutaneously for fluid resuscitation. To prevent post-surgical infection, the mice were placed in the supine position in the cage until the effect of the anesthesia wore off. The cage was warmed with a heater to prevent hypothermia after surgery. Postoperative antibacterial agents of imipenem/cilastatin at a dose of 25 mg/kg twice daily for 3 days (21) were used to prevent infections. The first antibiotic administration was performed 2 h after surgery.

The details of the FSI model preparation are shown in **Figure 1**; the thin and thich fecal suspensions are shown in **Figures 1A,B**, respectively. The cage was replaced 24 h before

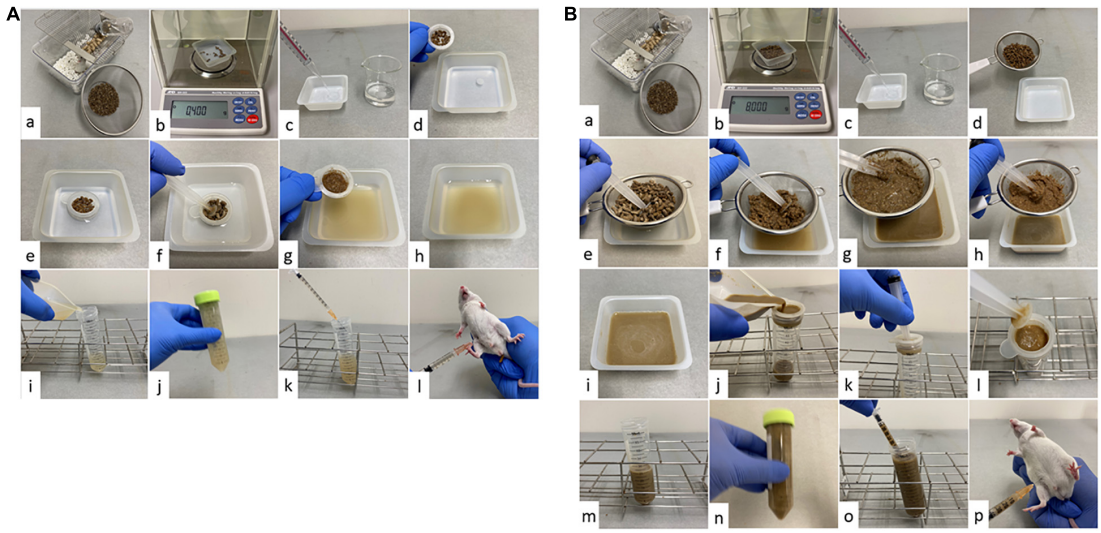

FIGURE 1 | Procedure for creating the fecal suspension intraperitoneal injection (FSI) model. **(A)** FSI model with thin fecal suspension. (a) Collect the required amount of feces from the cage. Spread enough paper matting to prevent urine from mixing with feces. Eliminate as much non-fecal waste as possible and be careful not to let water drip from the water bottle. (b) Weigh out 400 mg (450 mg for making 30 mg/mL) from the collected feces. (c) Weigh out 40, 20, 10, 15, or 8 mL of saline solution into a tray, depending on the concentration of fecal suspension to be prepared. (d) Putting feces on a Falcon® 70 μm cell strainer. (e) Soak the feces in the saline solution through the filter and macerate it thoroughly. (f) Filter the feces using a Falcon® 70 μm cell strainer and grind stick (we used a syringe pusher as a grind stick). (g) Grind the feces well until it becomes a paste (until the feces is no longer gritty). After filtration, drain the water from the paste feces well. (h) The obtained fecal suspension is impurity-free and can be aspirated without resistance with a 25-gauge needle. (i) Transfer the fecal suspension to a container that can be stirred. (j) Shake the tube well to mix sufficiently. Step (j) is performed once for each procedure (k). (k) Immediately after Step (j), aspirate 1 ml of fecal suspension with a syringe. (l) Administer 1 ml of fecal suspension intraperitoneally to the mice with a 25-gauge needle. **(B)** FSI model with thick fecal suspension. (a) Collect the required amount of feces from the cage. Spread enough paper matting to prevent urine from mixing with feces. Eliminate as much non-fecal waste as possible and be careful not to let water drip from the water bottle. (b) Weigh out 8,000 mg from the collected feces. (c) Weigh out 80, 60, 53, 40, or 27 mL of saline solution into a tray, depending on the concentration of fecal suspension to be prepared. (d) Putting feces on a tea strainer. (e) Soak the feces in the saline solution through a tea strainer and macerate it thoroughly. (f) Filter the feces using a tea strainer and grind stick (We used a syringe pusher as a grind stick). (g) Grind the feces well until it becomes a paste (until the feces is no longer gritty). (h) After rough filtration, drain the water from the paste feces well. (i) The obtained fecal suspension is full of impurities and still cannot be aspirated with a 25-gauge needle. (j) Pour the fecal suspension onto a Falcon® 70 μm cell strainer placed over a 50 ml tube. (k) Use a grind stick when necessary to promote filtration. (l) If the filter is clogged, remove the residue as needed. (m) The obtained fecal suspension is impurity-free and can be aspirated without resistance with a 25-gauge needle. (n) Shake the tube well to mix sufficiently. Step (n) is performed once for each procedure (o). (o) Immediately after Step (n), aspirate 1 mL of fecal suspension with a syringe. (p) Administer 1 ml of fecal suspension intraperitoneally to the mice with a 25-gauge needle.

TABLE 1 | Results of bacterial species identification in ascites culture.

	FSI model					CLP model					Feces in the cecum		Fecal suspension	
	1	2	3	4	5	1	2	3	4	5	1	2	1	2
Enterococcus gallinarum										◎	◎	◎	◎	
Enterococcus faecalis	○	◎	○		○	◎	○		◎		◎			
Lactobacillus species			○		○	◎	△		◎	◎	◎	◎		
Escherichia coli		○		○	○		△		○		◎	◎	◎	◎
Bacteroides vulgatus						◎	○		○	◎	◎	◎	◎	◎
Lactococcus garvieae			○										◎	◎
Proteus mirabilis		○			△	◎				△			◎	◎
Klebsiella oxytoca						○				◎				
Stenotrophomonas maltophilia	◎	◎	○	◎	◎									

Ascites was evaluated with samples taken 20 h after the onset of peritonitis. Results of identification of bacterial numbers and species in ascites culture of 5 FSI models and 5 CLP models. Bacterial numbers are evaluated by colony forming unit (CFU) per 1 mL and displayed in three stages (◎:10∧5 CFU/ml or more, ○:10∧3 or more and less than 10∧5 CFU/ml, △:less than 10∧3 CFU/ml). One of the CLP model sample had no bacterial growth. Bacterial identification tests were also performed on stool collected from the cecum of two non-antibiotic mice and fecal suspension (two samples) used to create the FSI model.

surgery to remove the old stool. Two types of suspensions were prepared, the thin and thick fecal suspension, depending on whether antimicrobial agents were used after surgery. Filtration of the thick fecal suspension was performed in two stages: coarse filtration using a commercial 630 μm tea strainer (product number DH7086, Kai Industries Co., Ltd., Tokyo, Japan) and

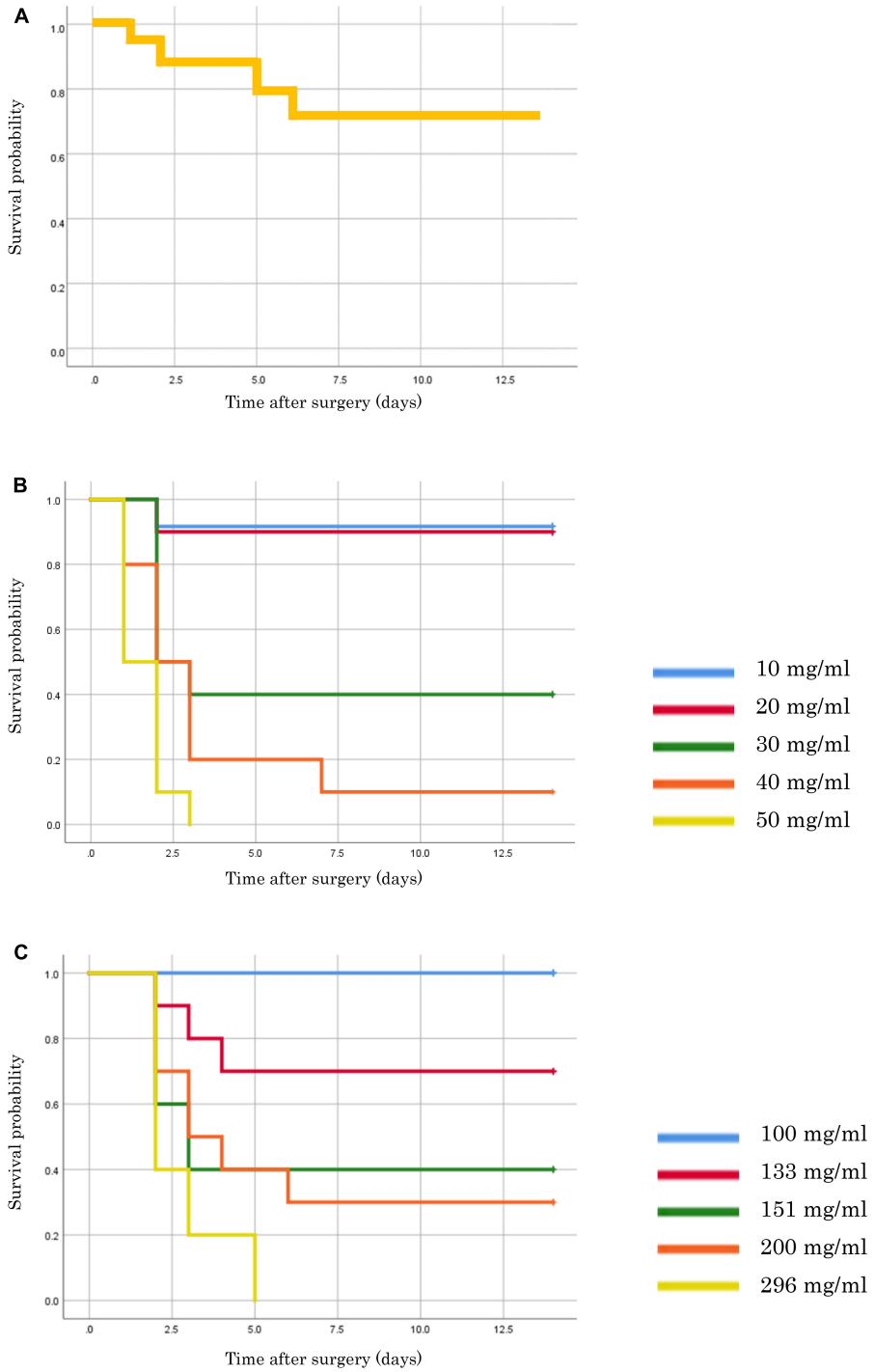

FIGURE 2 | Survival rates of each model mouse. **(A)** CLP model. **(B)** FSI model of thin fecal suspension (without antimicrobial administration). **(C)** FSI model of thick fecal suspension (with antimicrobial administration).

fine filtration using a Falcon 70 μm Cell Strainer (product number 352350; Corning, New York, United States). Thin fecal suspension was prepared by suspending 400 mg of feces in 40, 20, 10, and 8 mL of saline solution, or 450 mg of feces in 15 mL of saline solution. The concentrations of the prepared thin fecal suspensions were 10, 20, 30, 40, and 50 mg/mL. Thick fecal suspensions were prepared by suspending 8,000 mg of feces in 80, 60, 53, 40, and 27 mL of saline solution. The concentrations of the prepared thick fecal suspensions were 100, 133, 151, 200, and 296 mg/mL. The prepared fecal suspensions were intraperitoneally administered to each mouse at a volume of 1 mL. Buprenorphine 0.05 mg/kg was administered for

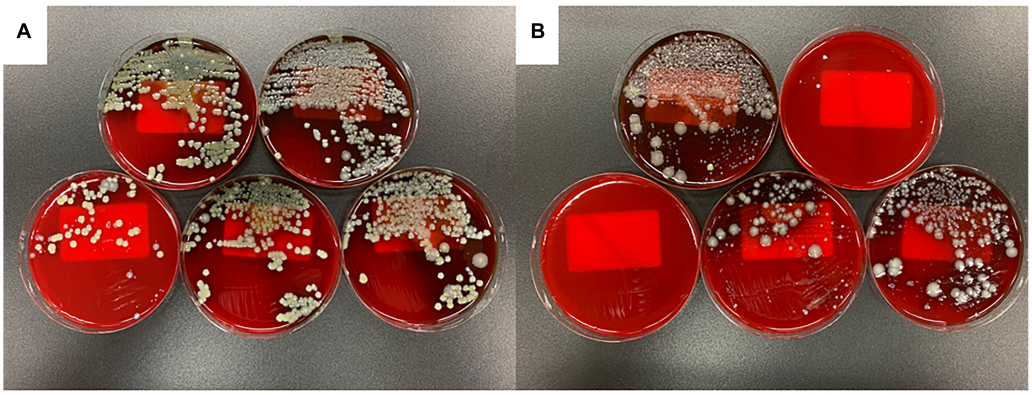

FIGURE 3 | Results of ascites culture of FSI and CLP models 20 h after surgery. Results, 24 h after the start of capneic incubation with Sheep Blood Agar **(A)** FSI model, **(B)** CLP model.

analgesia. Since there was enough water in the fecal suspension, fluid resuscitation was not performed as in postoperative CLP. As with CLP, the postoperative antibacterial agents were imipenem/cilastatin at a dose of 25 mg/kg twice a day for 3 days. The first antibiotic administration was performed 2 h after the injection of fecal suspension.

Experimental Outcomes

The survival rate was evaluated for 14 days by setting the day 0 from 0 to 24 h after surgery and continuing observation until day 14. Experiments comparing the CLP and FSI models [experiments (i) through (iv) below] were performed before the mice began to die, that is, 20 h after surgery.

Study Design

We compared the survival rates of the FSI models at various severities. Survival rates were verified in groups of 10 animals each. Next, the following experiments were performed using the FSI model (i.e., 133 mg/mL FSI model), which had a mortality rate similar to that of the CLP model: (i) histopathological comparison of parenchymal organs; (ii) bacteriological comparison by ascites culture; (iii) physiological comparison by respiratory rate, body temperature, and blood gas analysis; and (iv) immunological comparison by multiplex analysis by Luminex® 100/200™ (R&D Systems Inc., Minneapolis, MN, United States). Comparison (i) was performed between groups of three animals each. Comparisons for (ii) to (iv) were performed between groups of five animals each. The control group consisted of three animals each for (i) and five animals for (iii) and (iv).

Allocating Animals to Experimental Groups

Survival comparison experiments and comparison experiments between the CLP model and FSI model [experiments (i) to (iv)] were conducted independently on different days. To reduce the number of mice used, the same mice were used for experiments (i) and (iii) and for experiments (ii) and (iv). Mice were randomly assigned to the required number and groups.

Experimental Procedures (Comparison of Cecal Ligation and Puncture and Fecal Suspension Intraperitoneal Injection Models)

Histopathological comparisons were performed using the kidneys, spleen, lungs, and liver. Organs were taken 20 h after surgery from three animals each from the CLP and FSI models and placed in a 10% formalin neutral buffer solution. For euthanasia prior to organ removal, ketamine 250 mg/kg and xylazine 20 mg/kg were used. These were almost twice the doses used for surgical anesthesia. Tissue sections were then prepared from these organs using a microtome. The tissue sections were assessed using hematoxylin and eosin (HE) staining. Gram stain was added to the lung, kidney, spleen, and liver sections. In addition, post-tonsillectomy and post-adenoidectomy hemorrhage stain was added to the kidney sections. Gitter staining was performed for the liver sections. Elastica–Masson staining was performed on the lung sections for examination.

Bacteriological comparisons were assessed by culturing the ascites fluid 20 h after surgery. In addition, two sets of culture tests were performed on the cecal feces of mice that were not treated with antibiotics as controls and on the fecal suspension used to create the FSI model. Ascites fluid, collected after injecting 5 mL of saline into the abdominal cavity of mice with a 25-gauge needle and agitated well, was extracted using a clean syringe. Since multiple lavages could increase the errors, once the 5 mL of saline was injected, the saline was collected as much as possible for bacteriological evaluation. During the collection of the ascites fluid, the body surface of the shaved mice and the instruments used were disinfected with alcohol to prevent contamination, and the entire procedure was a clean operation. For ascites collection, 250 mg/kg ketamine and 20 mg/kg xylazine were administered subcutaneously to the backs of the mice as anesthesia. Samples were cultured on Nissui Plate Sheep Blood Agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and DHL agar (Becton Dickinson and Company, Tokyo, Japan) for capneic incubation and Anaero Columbia Agar with rabbit blood and

PEA/BBE agar (Becton Dickinson and Company, Tokyo, Japan) for anaerobic culture. After incubation, the number of bacteria was determined semi-quantitatively based on the number of colonies. Bacterial species were identified by mass spectrometry using a MALDI Biotyper® (Bruker Japan K.K., Kanagawa, Japan).

Respiratory rate and body temperature were compared for physiological evaluation. Blood gas analysis was performed. Respiratory rate was measured visually, and body temperature was determined as the average of three measurements taken with a high-performance cutaneous infrared thermometer (product number CTD711, Citizen Co., Ltd., Tokyo, Japan). Cardiac blood from mice anesthetized with ketamine 250 mg/kg and xylazine 20 mg/kg was collected using a heparin-coated syringe. Blood samples were analyzed using the ABL800 FLEX® (Radiometer Medical ApS, Kobenhavn, Denmark).

Immunological evaluation was performed by multiplex analysis using Luminex® 100/200™ (R&D Systems Inc., Minneapolis, MN, United States). Blood from mice was collected in the same way as in the physiological evaluation, and the obtained blood was centrifuged at 2,000 g for 10 min to separate the plasma. Plasma was subjected to multiplex analysis with a Luminex® 100/200™ using MILLIPLEX® MAP kit (Merck Millipore Corporation, Darmstadt, Germany) as a reagent.

Statistical Analysis

All data are expressed as the median and standard error (SE). Data were analyzed using the Mann–Whitney test using IBM SPSS software (version 25; IBM Japan, Tokyo, Japan). Statistical significance was set at $P < 0.05$.

RESULTS

Survival Rate

The survival curves for each model are shown in **Figure 2**. Fourteen CLP models were created using 15 mice, as one mouse died due to anesthesia. The survival rate of the CLP model after 14 days was 71.4% (**Figure 2A**). The FSI model showed an increased mortality rate in proportion to the concentration of fecal suspension. Without antimicrobial administration, mice often died early (up to 3 days) after fecal suspension injection (**Figure 2B**). In the FSI model with antibiotic administration, the mice did not die from fecal suspensions of up to 100 mg/mL. Therefore, it was necessary to create the thick fecal suspensions (100–296 mg/mL) to validate the mortality rate following antimicrobial use. Results from the model with a thick fecal suspension showed that antibiotics reduced mortality and prolonged survival (**Figure 2C**).

Bacteriological Evaluation

Figure 3 shows the results of bacterial culture of ascites at 20 h after the onset of peritonitis. In the FSI model, 10∧3 colony forming units (CFU)/mL or more of multiple bacterial species were cultivated from all animals (**Figure 3A** and **Table 1**). However, in the CLP model, there were animals with negative cultures or very small numbers of bacteria, and individual

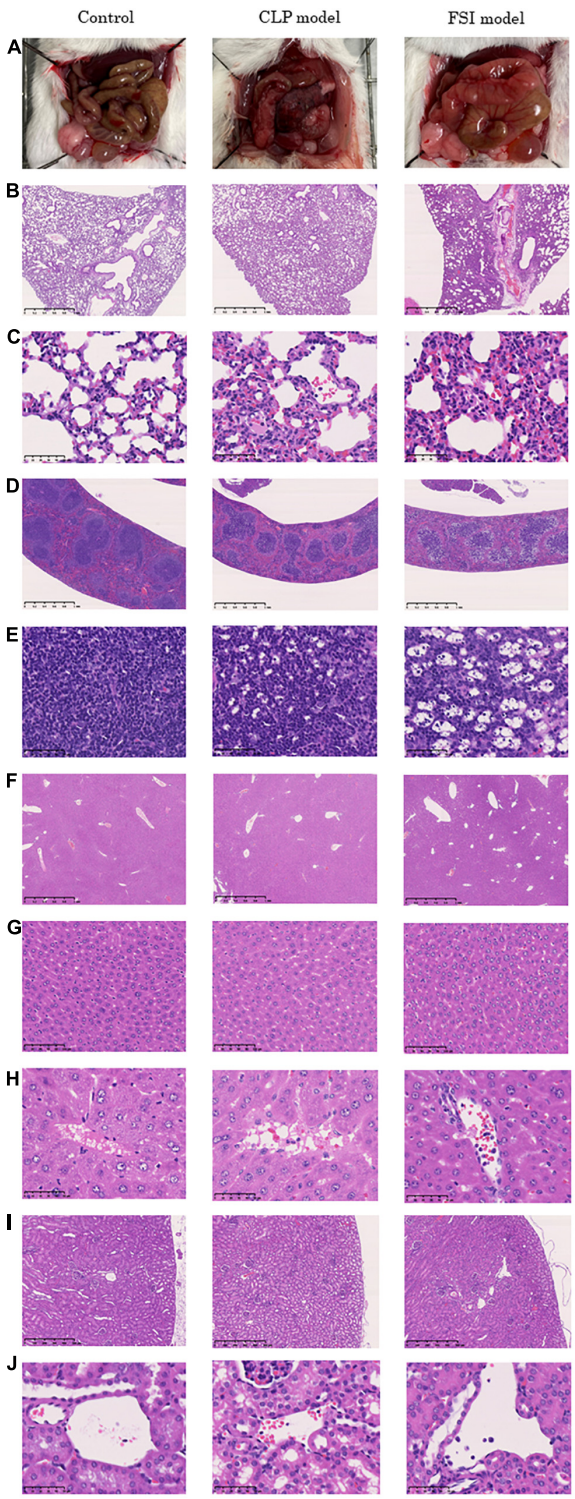

FIGURE 4 | Histopathological findings 20 h after onset of peritonitis in FSI model, CLP model and control cases. **(A)** Microscopic findings of the abdominal cavity. **(B)** 2.5×(Objective lens) lung tissues. **(C)** 40×(Objective lens) lung tissues. **(D)** 2.5×(Objective lens) spleen tissues. **(E)** 40×(Objective lens) spleen tissues. **(F)** 2.5×(Objective lens) liver tissues. **(G)** 20×(Objective lens) liver tissues. **(H)** 40×(Objective lens) liver tissues. **(I)** 5×(Objective lens) kidney tissues. **(J)** 40×(Objective lens) kidney tissues.

differences were large (**Figure 3B**). Although there was a slight difference in the bacterial species between the stool culture in the cecum and the fecal suspension culture, no significant difference was observed in ascites cultures between the FSI and CLP models. *Stenotrophomonas maltophilia* was detected at an equal rate in the ascites culture of the FSI model, which was the most significant difference between the FSI and CLP models (**Table 1**).

Histopathological Evaluation

The pathological findings at 20 h after surgery for the FSI and CLP models are shown in **Figure 4**. The microscopic findings of the abdominal cavity are shown in **Figure 4A**. The FSI model showed significant edema of the intestine, while the CLP model showed necrosis in addition to edema of the intestine. In the lungs, congestion and neutrophil infiltration were observed in both groups. Neutrophil infiltration into the alveolar space was not observed in any case, and no traces of pneumonia were noted in any of the lung samples (**Figures 4B,C**). In the spleen, hemophagocytosis was found both in the FSI and CLP model cases, but not in the control case (**Figures 4D,E**). Histopathological findings in the lung and spleen supported that the FSI model caused systemic inflammation that was of the same intensity or more severe than that in the CLP model cases. In the liver, the sinusoids were dilated, reflecting congestion findings (**Figure 4G**). There were no remarkable morphological changes in the kidneys (**Figures 4I,J**). In particular, microthrombi indicating disseminated intravascular coagulation were not detectable in the glomerulus, and centrilobular degeneration of hepatocytes, which reflects shock, was not found in any case. A few neutrophil infiltrates were found in the portal veins, central veins of the liver, and renal small vessels in the FSI model case, while a few neutrophil infiltrates were found in the central veins of the liver in the CLP model case (**Figures 4H,J**). Bacteria were not detected in any organ in any of the cases.

Physiological Evaluation

The respiratory rate, body temperature, and blood gas analysis results at 20 h after surgery for FSI and CLP models are shown in **Table 2**. Both the FSI and CLP models exhibited tachypnea, hypothermia, and hypoglycemia. In addition, hyperchloremia was observed in both models, which was thought to be due to the bolus administration of saline. In addition, the FSI model had severe metabolic acidosis, which was different from the CLP model. The CLP model showed no significant acidosis findings compared to the control group. In the CLP model, the standard error was greater than that in the FSI model for most variables.

Immunological Evaluation

Comprehensive measurements of inflammatory-related cytokines and chemokines revealed a high inflammatory response in both the FSI and CLP models at 20 h after surgery (**Figure 5** and **Table 3**). In the FSI model, a strong inflammatory reaction was observed. Because of this difference, levels of IL-1β and RANTES were high only in the FSI model, while IL-1α and IL-7 levels were high only in the CLP model. No increase was observed in IL-9 levels in any model. Although it differs slightly depending on the measurement parameters, the standard error of the FSI model was smaller overall.

DISCUSSION

We established a protocol for a murine sepsis model (FSI) using fecal suspensions made from excreted feces. Comparison between the FSI and CLP (gold-standard) models physiologically, pathologically, bacteriologically, and immunologically indicated that the FSI model showed almost similar changes as the CLP model. In addition, the FSI model showed less variation than the CLP model. These results suggest that the FSI sepsis model may be better than the conventional model.

The rodent CLP model is known to present with typical symptoms of sepsis or septic shock, such as hypothermia, tachycardia, and tachypnea (16), and it is also associated with hypoglycemia (22). Therefore, the results of the physiological evaluations in the FSI and CLP models were consistent with those observed in sepsis. Pathological evaluation revealed neutrophil infiltration into the parenchymal organs in both the CLP and FSI models. Although Gram staining of organs did not identify the bacteria and could not directly prove bacteremia, bacteriological evaluation confirmed that similar bacteria were cultivated in the ascites culture in both models. As a result of these detailed comparisons, both the FSI and CLP models were considered as being consistent with sepsis. Immunological evaluation using Luminex® showed that all cytokines and chemokines, except IL-7, RANTES, and IL-9, were elevated in both the FSI and CLP models (**Figure 5** and **Table 3**). In particular, the FSI model showed higher levels of inflammation with subsequent higher levels of anti-inflammatory cytokines, such as IL-4 and IL-10, than the CLP model. RANTES is elevated in sepsis and promotes neutrophil infiltration into the lungs (23). In this study, only the FSI model showed elevated RANTES levels, supporting the sepsis-like characteristics of the FSI model rather than the CLP model. IL-7 is a potent anti-apoptotic cytokine that is essential for lymphocyte survival and expansion (24, 25). Only the CLP model showed significantly higher IL-7 levels in our study, but there was no correlation between plasma IL-7 levels and the severity of sepsis (26). Therefore, although there are some differences between the FSI and CLP models, which may be due to the difference in time phase, the results are immunologically consistent with sepsis.

The major features of the FSI model are that peritonitis can be easily created without the need for anesthesia or surgical procedures and that mice models with uniform severity of sepsis can be developed. The results obtained by physiological, bacteriological, and immunological evaluations showed that there was little variation among individuals in the FSI model, suggesting that the influence of individual differences in mice during model creation could be minimized. A previous study demonstrated a cecal slurry preparation protocol similar to the current FSI model. As shown in this study, this protocol requires preparation of cecal slurry with glycerol-PBS, freezing of the cecal slurry at −80°C, and thawing (4). These suggest that the procedures for cecal slurry are considerably more complicated

TABLE 2 | Results of the respiratory rate, body temperature, and blood gas analysis 20 h after surgery for FSI and CLP models.

	Control	FSI model	CLP model
Respiratory rate (/min)	136 ± 4.476	204 ± 11.123*	160 ± 6.849*
Body temperature (°C)	37.0 ± 0.085	36.1 ± 0.044*	36.0 ± 0.164*
pH	7.241 ± 0.0034	6.974 ± 0.0110*#	7.148 ± 0.0300
pCO_2 (mmHg)	57.2 ± 2.276	82.4 ± 3.991*	63.3 ± 3.523
pO_2 (mmHg)	49.1 ± 4.161	30.6 ± 2.724	42.8 ± 2.705
K (mmol/L)	3.7 ± 0.061	4.6 ± 0.171*	3.9 ± 0.203
Na (mmol/L)	148 ± 0.219	150 ± 0.456#	154 ± 1.308*
Cl (mmol/L)	118 ± 0.867	127 ± 0.780*	125 ± 2.028*
Hemoglobin (g/dL)	13.0 ± 0.461	14.3 ± 0.614	13.7 ± 1.141
Hematocrit (%)	39.9 ± 1.385	43.8 ± 1.846	42.1 ± 3.449
HCO3 (mmol/L)	23.4 ± 0.999	18.2 ± 1.089*#	25.0 ± 1.592
Base excess (mmol/L)	−2.9 ± 0.946	−12.0 ± 1.102*#	−2.8 ± 1.837
Glucose (mg/dL)	298 ± 8.112	125 ± 11.229*	112 ± 17.035*
Lactate (mmol/L)	2.8 ± 0.118	3.2 ± 0.481	2.1 ± 0.275

*Data presented as median l' standard error. *Significantly different from the control group (p-value < 0.05). #Significantly different from the CLP group (p-value < 0.05).*

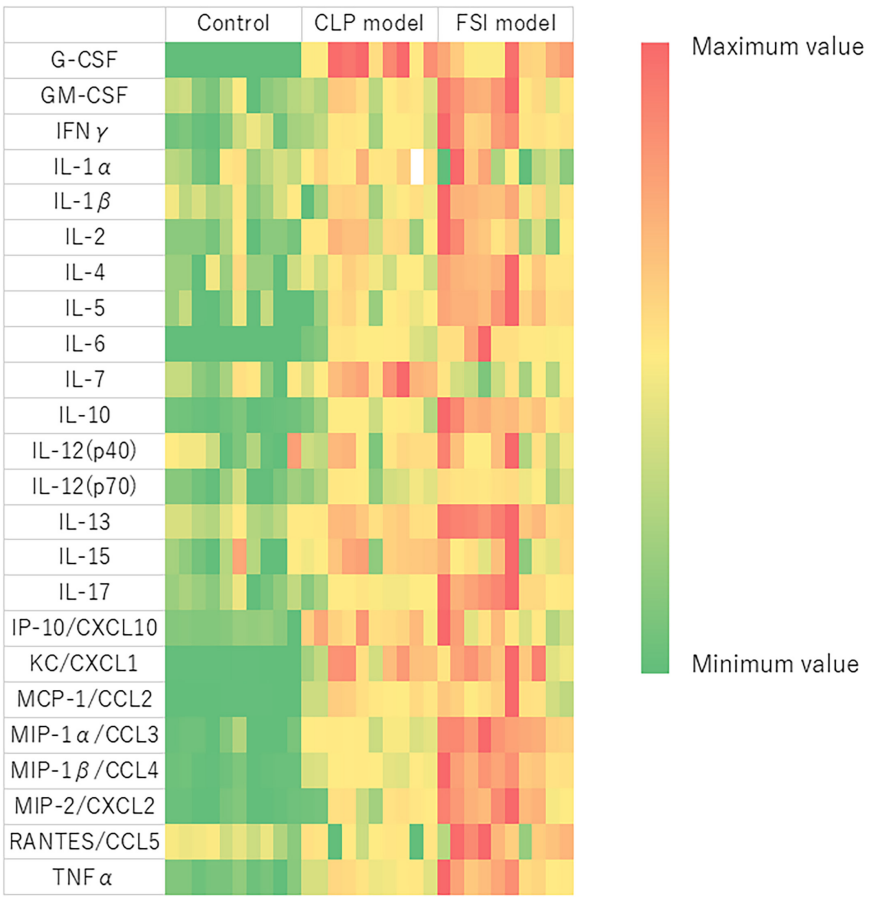

FIGURE 5 | Heatmap analysis of cytokine and chemokine measurements performed by multiplex analysis for FSI and CLP models. Blood was evaluated with samples taken 20 h after the onset of peritonitis. Heat map showing relative evaluation of cytokine and chemokine values. The maximum value of each variable is red, the minimum value is green, and the intermediate value corresponds to the gradation scale from red to green. G-CSF, Granulocyte-colony stimulating factor; GM-CSF, Granulocyte macrophage colony-stimulating factor; IFNγ, Interferon gamma; IL, Interleukin; IP, Interferon gamma-induced protein; CXCL, C-X-C motif chemokine ligand; KC, Keratinocyte-derived chemokines; MCP, Monocyte chemotactic protein; CCL, C-C motif chemokine; MIP, Macrophage inflammatory protein; RANTES, Regulated on activation, normal T cell expressed and secreted; TNF, tumor necrosis factor.

TABLE 3 | Cytokine and chemokine measurements performed by multiplex analysis in FSI and CLP models 20 h after onset of peritonitis.

	Control	FSI model	CLP model
G-CSF	46.9 ± 5.0	89451.3 ± 734.2*	92031.9 ± 629.0*
GM-CSF	54.1 ± 7.3	196.7 ± 10.6*#	107.0 ± 23.4*
IFNγ	3.3 ± 1.0	37.6 ± 1.5*#	11.7 ± 13.1*
IL-1α	66.0 ± 13.5	73.3 ± 16.9	136.4 ± 51.3*
IL-1β	5.8 ± 0.6	17.9 ± 1.4*#	8.1 ± 2.7
IL-2	3.2 ± 0.8	12.9 ± 1.5*	11.4 ± 3.1*
IL-4	0.6 ± 0.1	3.0 ± 0.1*#	1.1 ± 0.4*
IL-5	1.9 ± 0.7	22.2 ± 1.2*#	8.4 ± 2.7*
IL-6	4.6 ± 0.7	17553.0 ± 1126.7*#	2720.4 ± 14972.8*
IL-7	4.9 ± 1.2	6.1 ± 4.6#	26.8 ± 0.9*
IL-9	151.7 ± 35.0	160.7 ± 141.9	93.4 ± 52.3
IL-10	10.4 ± 2.1	1594.9 ± 16.4*#	133.1 ± 347.4*
IL-12(p40)	12.2 ± 4.0	21.8 ± 3.1*	23.4 ± 5.1*
IL-12(p70)	12.7 ± 4.1	94.8 ± 6.9*#	48.5 ± 12.7*
IL-13	40.5 ± 5.3	560.8 ± 28.4*#	168.5 ± 67.0*
IL-15	47.1 ± 22.1	139.9 ± 18.1*	182.7 ± 24.0*
IL-17	3.0 ± 0.6	73.6 ± 1.1*#	8.2 ± 14.6*
IP-10/CXCL10	94.1 ± 11.7	408.4 ± 300.0*	484.7 ± 61.2*
KC/CXCL1	158.2 ± 25.0	18761.0 ± 2137.1*	17657.4 ± 2032.9*
MCP-1/CCL2	58.1 ± 4.5	6618.7 ± 468.3*	5992.5 ± 1182.5*
MIP-1α/CCL3	42.3 ± 4.9	599.1 ± 5.0*#	138.4 ± 58.5*
MIP-1β/CCL4	68.7 ± 5.8	4123.3 ± 26.7*#	424.8 ± 667.3*
MIP-2/CXCL2	32.0 ± 15.4	4732.8 ± 192.7*#	966.2 ± 871.9*
RANTES/CCL5	47.7 ± 3.7	185.0 ± 9.0*#	65.7 ± 42.0
TNFα	6.4 ± 1.0	113.5 ± 5.2*#	39.7 ± 23.2*

*Data presented as median ± standard error. All units are picogram per milliliter (pg/ml). *Significantly different from the control group (p-value < 0.05). #Significantly different from the CLP group (p-value < 0.05). G-CSF, Granulocyte-colony stimulating factor; GM-CSF, Granulocyte macrophage colony-stimulating factor; IFNγ, Interferon gamma; IL, Interleukin; IP, Interferon gamma-induced protein; CXCL, C-X-C motif chemokine ligand; KC, Keratinocyte-derived chemokines; MCP, Monocyte chemotactic protein; CCL, C-C motif chemokine; MIP, Macrophage inflammatory protein; RANTES, Regulated on activation, normal T cell expressed and secreted; TNF, tumor necrosis factor.*

than for the FSI. The fact that most of the other models using fecal suspensions require surgical intervention (12, 13) also implies that the FSI has a stronger advantage in terms of the simplicity of its creation process. In addition, sacrificial deaths of multiple mice are needed just to obtain the cecal slurry (4). Thus, we believe that the simplicity and uniformity of the FSI model allowed us to reduce the strain on the mice.

Existing animal sepsis models can be divided into three categories: exogenous administration of a toxin, exogenous administration of a viable pathogen, or alteration of the animal's endogenous protective barrier (14). Injection of specific toxins or pathogens alone are problematic in that they are different from the clinical pathology, although accurate information with few errors can be obtained. On the other hand, the model that induces sepsis by changing the endogenous protective barrier of animals, represented by the CLP model, is problematic in that it is difficult to obtain genuine information due to large individual differences; however, it is the closest to the clinical pathology of sepsis. The FSI model facilitates the homogenous development of peritonitis due to multiple causative organisms, much like a model injecting a specific toxin or pathogen. In the FSI model, the detection rate of *S. maltophilia* in ascites culture was very high, and the concentration was also high (**Table 1**). *S. maltophilia* is an emerging multidrug-resistant, global opportunistic pathogen (27). Although it is not a highly pathogenic pathogen, it is an important in-hospital pathogen associated with bloodstream mortality (28, 29). In the FSI model, administration of antibiotics may have increased the occupancy of *S. maltophilia* because there was no influx of new feces from the intestinal tract.

In the CLP model, the severity of disease varies greatly depending on the degree of fecal outflow from the cecum perforation site, the degree of filming (localization) of the perforation site, or the degree of necrosis of the distal cecum (14, 30–32). This makes it difficult to examine the pathophysiology of sepsis, especially in periods other than the hyperacute phase. In the present study, adipose tissue was resected to prevent localized inflammation (20), but bacteriological evaluation showed that there were individual mice with negative ascites cultures and very low bacterial growth, thus prompting the hypothesis that adipose tissue resection during a CLP procedure cannot completely prevent localized abscess formation. Localization of peritonitis due to abscess formation has been a major problem in the CLP model as drugs that have proven to be beneficial during experiments using the CLP model with abscess formation can be

ineffective in clinical sepsis treatment (14). The FSI model can overcome these problems inherent in the CLP model.

Limitations

This study had some limitations. The experimental results were obtained at a single institution, and the protocol and experimental results of the FSI model need to be verified at other institutions. The results may vary depending on the facility, operator, condition of the stool, and degree of filtration and agitation of the stool suspension. It may be necessary to arrange the fecal dosage and filtration method, depending on the facility, to create mice with the desired severity. However, in any case, it is expected that there will be less variability when using the FSI model than with the CLP model.

As regards the physiological evaluation, the blood gas analyzer is designed for human blood samples; thus, the results may be inaccurate. The results of the immunological evaluation may also be affected by the administration of the anesthetic agent. During the bacteriological evaluation, there were samples with many species of bacteria, and it is possible that not all bacteria were identified. The FSI model progresses to severe disease more rapidly than the CLP model because a large amount of the pathogen is administered intraperitoneally at once. Therefore, even if the comparison is between models with the same mortality rate over the same time course, it may reflect a different pathological condition.

CONCLUSION

In this study, we presented a protocol for an easy-to-create animal model of sepsis, the FSI model, and proved its clinical validity as a sepsis model by histological, physiological, bacteriological, and immunological methods. We believe that the FSI model, which can minimize individual differences among animals, will help produce new findings in sepsis research and contribute to the improvement of clinical sepsis outcomes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All experiments were conducted in accordance with the Hokkaido University Animal Experiment Regulations. The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation from the Institutional Ethical Review Board of Hokkaido University (Approval number: 20-0163).

AUTHOR CONTRIBUTIONS

TT contributed to the research conception, analysis, experimentation, and manuscript preparation. TW contributed to the research concept and oversaw the entire study. AM contributed to the performance of the experiments. YO and ST contributed to the provision of histopathological photographs and pathological evaluation. KY contributed to immunological evaluation through sample measurements. KK contributed to the revision of the intellectual content. All authors have read and approved the final version of the manuscript prior to submission.

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Differentiating Phenotypes of Coronavirus Disease-2019 Pneumonia by Electric Impedance Tomography

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Introduction: Coronavirus disease-2019 (COVID-19) pneumonia has different phenotypes. Selecting the patient individualized and optimal respirator settings for the ventilated patient is a challenging process. Electric impedance tomography (EIT) is a real-time, radiation-free functional imaging technique that can aid clinicians in differentiating the "low" (L-) and "high" (H-) phenotypes of COVID-19 pneumonia described previously.

Methods: Two patients ("A" and "B") underwent a stepwise positive end-expiratory pressure (PEEP) recruitment by 3 cmH_2O of steps from PEEP 10 to 25 and back to 10 cmH_2O during a pressure control ventilation of 15 cmH_2O. Recruitment maneuvers were performed under continuous EIT recording on a daily basis until patients required controlled ventilation mode.

Results: Patients "A" and "B" had a 7- and 12-day long trial, respectively. At the daily baseline, patient "A" had significantly higher compliance: mean ± SD = 53 ± 7 *vs*. 38 ± 5 ml/cmH_2O ($p < 0.001$) and a significantly higher physiological dead space according to the Bohr–Enghoff equation than patient "B": mean ± SD = 52 ± 4 *vs*. 45 ± 6% ($p = 0.018$). Following recruitment maneuvers, patient "A" had a significantly higher cumulative collapse ratio detected by EIT than patient "B": mean ± SD = 0.40 ± 0.08 *vs*. 0.29 ± 0.08 ($p = 0.007$). In patient "A," there was a significant linear regression between the cumulative collapse ratios at the end of the recruitment maneuvers ($R^2 = 0.824$, $p = 0.005$) by moving forward in days, while not for patient "B" ($R^2 = 0.329$, $p = 0.5$).

Conclusion: Patient "B" was recognized as H-phenotype with high elastance, low compliance, higher recruitability, and low ventilation-to-perfusion ratio; meanwhile patient "A" was identified as the L-phenotype with low elastance, high compliance, and lower recruitability. Observation by EIT was not just able to differentiate the two phenotypes, but it also could follow the transition from L- to H-type within patient "A."

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT04360837.

Keywords: acute lung injury, compliance, Coronavirus-COVID-19, electric impedance tomography, recruitment maneuver

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INTRODUCTION

Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2)-associated pneumonia can deteriorate into acute respiratory distress syndrome (ARDS). However, severe Coronavirus disease-2019 (COVID-19) pneumonia fulfills the Berlin criteria of ARDS (1), this type of acute lung injury is exceptionally specific. The SARS-CoV-2 acquired ARDS is characterized by near normal respiratory mechanics associated with hypoxemia in almost half of the cases (2). Even more a significant dissociation was observed as distinct lung mechanics were detailed with the same level of grievous oxygenation disturbance. This perception led to the distinction of two phenotypes of COVID-19 pneumonia by Gattinoni et al. (3) whereas, low (L-) type is featured by lower elastance and almost normal compliance (>50 ml/cmH_2O), low ventilation-to-perfusion ratio (V_A/Q), lower recruitability, and estimated lung weight. On the contrary, high (H-) type is characterized by higher elastance and low compliance (<40 ml/cmH_2O), high right-to-left shunt, higher recruitability, and lung weight. Of note, L-type can transit into H-type by advancing time.

The two different phenotypes require differing mechanical ventilation and therapeutic approach. According to the recommendations, due to the various characteristics in pathophysiology, H-type can profit from the standard settings for ARDS: lower tidal volume, higher positive end-expiratory pressure (PEEP) level and prone positioning. While H-type benefits from an excursive management: more permissive tidal volume, lower PEEP setting and applying prone positioning just as a rescue therapy (3).

The patient individualized approach can be challenging for the attending physician taking care of the COVID-19 pneumonia patients. Even more, as the clinical state of the critically ill patient is fluctuating and phenotype can alter while the leading symptom, the hypoxia is equally profound. However, bedside measurement implemented by the ventilator supports the follow-up, the gold standard of ARDS diagnostics is still an imaging technique, the computed tomography (CT) (4). However, the CT scan of the chest is at high resolution and it gives information not just about the complete lungs but about all organs situated in the thorax, CT examination performed on a daily basis is not feasible. Meanwhile, electrical impedance tomography (EIT) is a radiation-free functional imaging technique providing continuous information about the lungs at the bedside (5). EIT is capable of estimating not just lung aeration, but also the ratio of collapse and overdistension during a PEEP trial (6).

Our objective was to investigate the different phenotypes of COVID-19 pneumonia patients under a stepwise PEEP incremental and decremental recruitment trial performed on a daily basis under continuous EIT monitoring to estimate if impedance tomography is capable of differentiating the various phenotypes of COVID-19 pneumonia.

MATERIALS AND METHODS

Study Registration

The study was approved by the Human Investigation Review Board of the University of Szeged. The trial was registered in a public registry under the registration number NCT04360837 on ClinicalTrials.gov. Informed consent was obtained from the legal representatives of the patients.

Study Population

All patients admitted to the COVID-19 intensive care unit (ICU) of the University of Szeged, diagnosed with SARS-CoV-2 pneumonia, following a positive polymerase chain reaction were considered for investigation during the first wave of the pandemic in Hungary. Further inclusion criteria were orotracheal intubation and pressure-controlled ventilation mode at a sedation level of minimum-4 on the Richmond Agitation Sedation Scale (RASS). Exclusion criteria were age under 18, pregnancy, pulmonectomy, and lung resection in the past medical history, clinically end-stage chronic obstructive pulmonary disease, severe hemodynamic instability with vasopressor refractory shock, severe bullous emphysema, and chest drainage *in situ* due to pneumothorax and/or bronchopleural fistula. During the first national surge of the epidemic, seven patients were admitted to the ICU. Out of them, one patient was not intubated, two patients were lightly sedated at RASS-3 level and were ventilated in a pressure support mode, one of them was excluded because of severe bullous emphysema and one of them because of end-stage chronic obstructive pulmonary disease regarding to the exclusion criteria of the investigation. Finally, two patients could undergo the research protocol, patient "A" on 7 and patient "B" on 12 consecutive days.

Experimental Protocol

Following orotracheal intubation and initiation of deep sedation to at least RASS-4 with continuous intravenous infusion of propofol and sufentanil patients were ventilated in pressure-controlled mode with a tidal volume of 6 ml/kg. Patients underwent a once-daily PEEP incremental and decremental recruitment maneuver until controlled ventilation mode was required according to their clinical stage. During the repeated interventions pressure-controlled ventilation mode was applied with a constant pressure control of 15 cmH_2O by a Mindray SV300 respirator (Mindray Bio-Medical Electronics Co.,

TABLE 1 | Patient characteristics.

	Patient "A"	Patient "B"
Age (years)	67	81
Sex	Female	Female
Body-mass index (kg/m^2)	30	31
APACHE II score	17	18
PEEP trials (N)	7	12
Comorbidities	Hypertension, Hypothyroidism	Hypertension
Days of symptomes before intubation	8	2
Hours of hospitalization before intubation	18	6
PaO_2/FiO_2 at inclusion (mmHg)	258	142
Outcome on ICU	Died	Died

APACHE II, acute physiology and chronic health evaluation II; PEEP, positive end-expiratory pressure; ICU, intensive care unit.

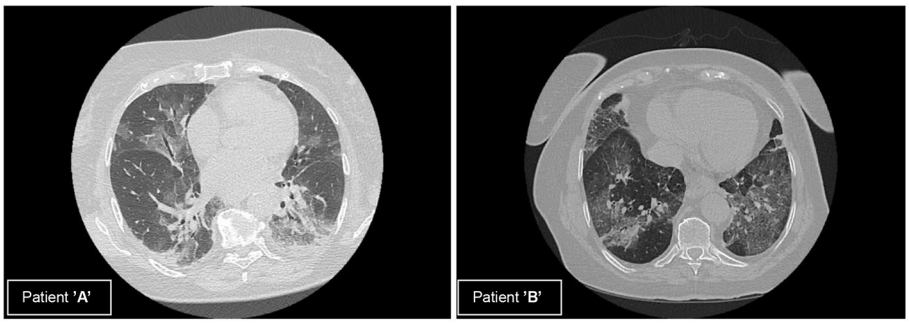

FIGURE 1 | Computed tomography (CT) scans of patients **(A)** and **(B)**. **(A)** Multifocal, subpleural, bilateral ground glass opacities with subpleural traction in the dorsal regions. **(B)** Multifocal, subpleural, bilateral ground glass opacities, crazy paving dominantly on the left side, consolidation in the right basal region.

Shenzen, China). The fraction of inspired oxygen (FiO_2) and respiratory rate were set according to the discretion of the attending physician. Pending the incremental limb PEEP was increased in 3 cmH_2O steps from basal PEEP 10 to top 25, reaching a peak pressure of 40 cmH_2O. On the descending limb, PEEP was decreased in 3 cmH_2O steps back to the initial level of 10. Each PEEP step were kept constant for 2 min and at each level, an inspiratory hold maneuver was performed to detect plateau pressure and static compliance (C_{stat}). Vital parameters and volumetric capnography measurements of the ventilator were recorded throughout the intervention. Arterial blood gas samplings were performed at basal and at terminating PEEP levels of 10. Physiological dead space (V_D) representing the sum of the anatomical plus the alveolar dead space was calculated by the Bohr–Enghoff equation.

Electrical impedance tomography measurements were recorded continuously during the increasing and decreasing limb of the PEEP recruitment maneuver by the Dräger PulmoVista 500 impedance tomography (Dräger Medical, Lübeck, Germany). The device has 16 electrodes equidistantly placed on the chest circumference in a transverse plane between the 5^{th} and 6^{th} intercostal space. EIT monitoring data were measured with adjacent injection current and adjacent voltage measurement with 50 frames per second. Time difference EIT images were reconstructed using the Newton–Raphson algorithm. It is demonstrated that the regional tidal volume correlates well with the pixel-wise conductivity variation (ΔZ) indicated by the EIT tidal image (7). Hence, the pixel compliance, required for the evaluation of global overdistension and collapse, can be calculated as:

$$\text{Compliance}_{pixel} = \frac{\Delta \mathbf{Z}}{\text{P}_{plateau} - \text{PEEP}} \quad (1)$$

The complete estimation of collapse and overdistension was presented previously (8). Optimal PEEP can be determined by EIT (5) which was established for both patients. During the decremental PEEP trial phase, regional lung hyper distension and collapse ratios were estimated at each step. The crossover point between the curves of the decreasing line of overdistension and the increasing line of collapse indicated the optimal PEEP where the level of the two opposing factors was meeting.

Statistical Analysis

For statistical analysis, Sigmaplot 14 (Systat Software Inc., San Jose, CA, USA) was used. Following the Shapiro–Wilk normality test data between two groups were tested by the *t*-test or the Mann–Whitney rank sum test, matched data were tested by the paired *t*-test or the Wilcoxon signed-rank test in a group. For multiple-comparison one-way repeated measures analysis of variance (ANOVA) with the Brown–Forsythe equal variance test, for comparison vs. a control group Bonferroni *t*-test or Dunnett's method was used. For regression analysis, simple linear regression was applied. The *p*-value was considered significant if <0.05.

RESULTS

Patient Characteristics

Patient "A" was ventilated for 7 days and patient "B" was ventilated for 12 days in a controlled mode. Both of them underwent a PEEP intervention on a daily basis (**Table 1**). CT scans of the chest were recorded right before the admission to ICU (**Figure 1**).

Respiratory Mechanics

At the daily baseline and following the intervention patient "A" had significantly higher C_{stat} than patient "B". Following the PEEP recruitment trial C_{stat} significantly improved both with patients "A" and "B" (**Figure 2**). On days 4, 5, and 7 C_{stat} was significantly lower as compared to day 1 with patient "A." With patient "B" C_{stat} was significantly lower on days 2, 3, 4, 6, 8, and 12 as compared to day 1. There was significant decreasing linear regression in C_{stat} with patient "A" by moving forward in days but not for patient "B" (**Figure 3**).

Blood Gas and Capnography

With patient "A," pH significantly increased and partial pressure of carbon dioxide in the arterial blood (P_aCO_2) significantly decreased following the recruitment maneuver. In the meantime, a not significant decrease in pH and a not significant increase in

P_aCO_2 were detected with patient "B." There was no significant alteration in partial pressure of oxygen in the arterial blood (P_aO_2)/FiO_2, oxygen saturation (SO_2), base excess (BE), and lactate with neither of the patients (**Table 2**).

Patient "A" had a significantly higher V_D at baseline measurements than patient "B". Comparing the two patients, there was no significant difference in V_D following the recruitment maneuver. With patient "A," V_D significantly decreased and with patient "B," V_D did not significantly increase following the intervention. There was no significant alteration in end-tidal carbon dioxide ($EtCO_2$) in neither of the patients following the PEEP trial (**Table 2**).

Overdistension and Collapse by EIT

Regarding the cumulative overdistension ratio at the top-level PEEP of 25 cmH_2O, there was no significant difference between the two subjects. Following recruitment maneuvers, patient "A" had a significantly higher cumulative collapse ratio detected by EIT than patient "B" (**Table 3**). With patient "A," the cumulative collapse ratio at the end of the recruitment maneuvers revealed a significant decreasing linear regression by moving forward in days, while not with patient "B" (**Figure 4**)" Overdistended and collapsed regions were reconstructed at the top PEEP 25 and final PEEP 10 cmH_2O (**Figure 5**). Optimal PEEP gradually decreased with patient "A" while it was fluctuating over time with patient "B" (**Figure 5**).

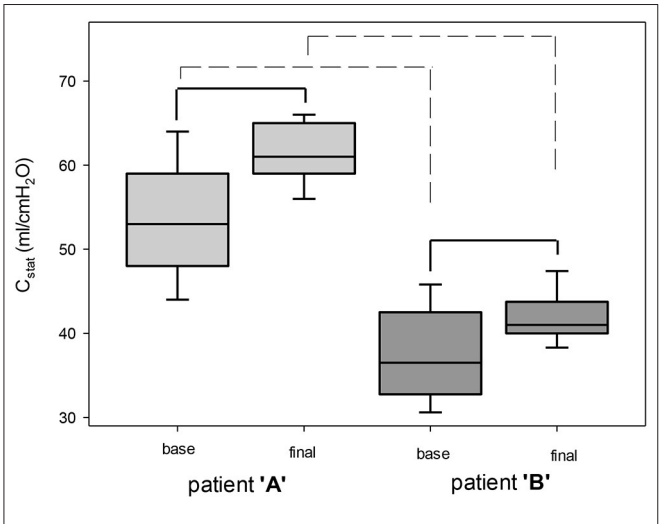

FIGURE 2 | Compliance at baseline and following positive end-expiratory pressure (PEEP) trial. C_{stat}, compliance. Solid line, significant difference within a patient. Dashed line, significant difference between patients. Box plots represent mean, ± *SD* and 5th-95th percentile, $p < 0.05$.

TABLE 2 | The average of blood gas results and physiological dead space ratios at the initial (base) and at the terminating (final) positive end-expiratory pressure (PEEP) levels of the daily trials.

	Patient "A"		Patient "B"	
	Base	**Final**	**Base**	**Final**
pH	7.45 ± 0.02	7.46 ± 0.02[a]	7.40 ± 0.05[b]	7.38 ± 0.04[b]
$PaCO_2$ (mmHg)	44±4	42±4[a]	40±5	40±4
PaO_2/FiO_2	166 ± 34	184 ± 37	120 ± 30[b]	124 ± 26[b]
SO_2 (%)	97 ± 2	97 ± 1	95 ± 2	95 ± 2[b]
BE	5.1 ± 2.4	4.7 ± 2.7	−1.3 ± 2.5[b]	−1.5 ± 2.6[b]
Lactate (mmol/L)	1.2 ± 0.2	1.3 ± 0.3	1.7 ± 0.9	1.7 ± 0.8
V_D	0.53 [0.48–0.56]	0.47[0.44–0.50][a]	0.45 ± 0.06[b]	0.47 ± 0.09
$EtCO_2$ (mmHg)	39 ± 5	37 ± 4	36 ± 3	37 ± 3

$PaCO_2$, partial pressure of carbon dioxide in the arterial blood; PaO_2/FiO_2, partial pressure of oxygen in the arterial blood to fraction of inspired oxygen; SO_2, oxygen saturation; BE, base excess; V_D, physiological dead space; $EtCO_2$, end-tidal carbon dioxide, [a] significant difference within a patient, [b] significant difference as compared to patient "A", $P < 0.05$. Data are presented as mean ± SD and median [25th-75th].

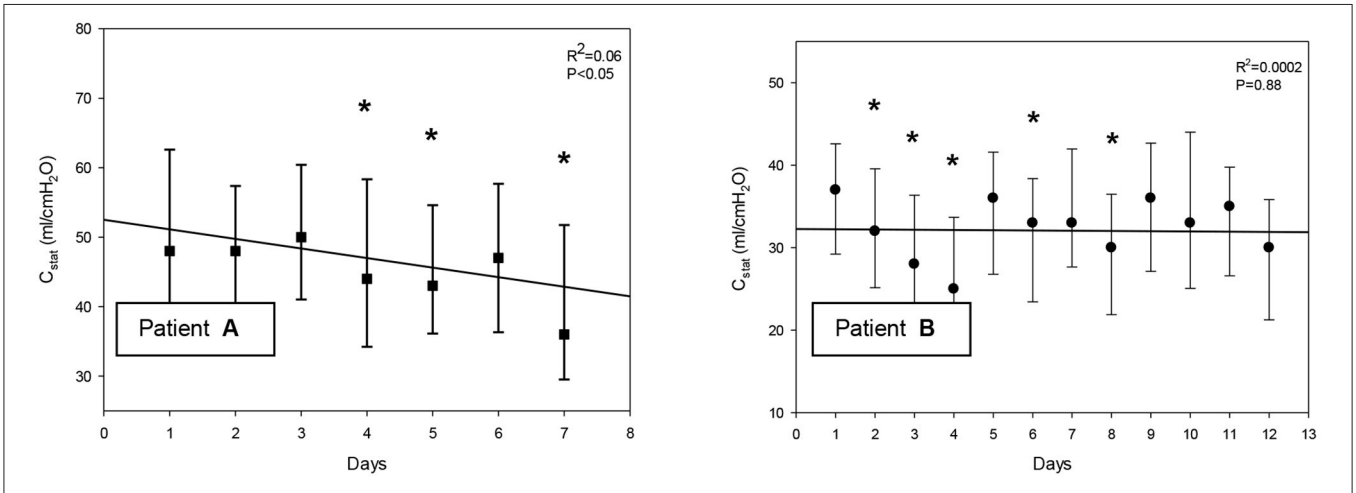

FIGURE 3 | (A,B) Alteration in compliance by moving forward in days. C_{stat}, compliance. Bars represent mean and ± *SD*. *significant difference as compared to day 1, $p < 0.05$.

DISCUSSION

Identification of L- and H-Phenotype

Under the investigation two severe, intubated and mechanically ventilated patients were assessed and recognized as L- and H-phenotype according to the Gattinoni classification (3). Based on the respiratory mechanics and volumetric capnography, patient "A" was revealed as L-type with low elastance, fairly normal compliance, and low V_A/Q. On the contrary, patient "B" was identified as H-type with high elastance, low compliance, and high right-to-left shunt. On the other hand, real-time, bedside, EIT-based overdistension and collapse ratio measurements were capable to distinguish the two phenotypes. Patient "A" had a more pronounced tendency for collapse following the PEEP recruitment; while at the end of the PEEP trials, patient "B" was presented with a lower cumulative collapse ratio. Meanwhile, EIT demonstrated the transition from L- to H-type with patient "A" as the cumulative collapse ratio decreased over time at the end of the daily PEEP trials in addition to the deterioration in C_{stat}.

Respiratory Mechanics

However, the gold standard CT scan is the most valuable method to identify the pathophysiological mechanisms not just in any ARDS but also in all, severe COVID-19 pneumonia patients, assessment of respiratory mechanics serves as a surrogate (3). A simple end-inspiratory hold maneuver is capable to determine the plateau pressure and with the charted tidal volume C_{stat} can be recognized. Throughout the first days with patient "A," an almost normal C_{stat} was revealed in line with a deep level of hypoxia. However, severe COVID-19 pneumonia meets the criteria of Berlin definition of ARDS (1), the disease acts in a very specific way which was rarely apparent in severe ARDS previously. Meanwhile, patient "B" was recognized with a considerably damaged C_{stat}, the same level of grievous hypoxia was presented as with patient "A." These observations testify the heterogeneity of COVID-19 pneumonia and the necessity of the identification of various phenotypes.

Nevertheless, L-type can transit into H-type (3, 9). This transformation was clearly demonstrable with patient "A" as C_{stat} significantly deteriorated by time cascading down to around 40 ml/cmH_2O. As patient "A" was the same ventilated as patient "B" in pressure control mode throughout the investigation days self-inflicted lung injury (P-SILI) cannot have a share in evolving the deterioration. However, esophageal pressure measurement for estimating transpulmonary pressures was not applied. The P-SILI mechanism is much more characteristic in patients breathing spontaneously on non-invasive ventilation with an increased swing in transpulmonary pressure leading to enhance the stress at alveolar level (10). With patient "A," the transition can be explained by the evolution of COVID-19 pneumonia on itself.

TABLE 3 | Overdistension ratio at top and collapse ratio at final PEEP levels.

	Patient "A"	Patient "B"
Cumulative overdistension ratio at PEEP 25 cmH_2O	0.37 ± 0.08	0.35 ± 0.06
Cumulative collapse ratio at final PEEP 10 cmH_2O	0.40 ± 0.08	0.29 ± 0.08*

*PEEP, positive end-expiratory pressure, *significant difference as compared to patient "A", $P < 0.05$. Data are presented as mean ± SD.*

Ventilation-to-Perfusion

In parallel with the hypothesis of V_A/Q mismatch in L-type patients, Santamarina et al. (11) detailed the possible underlying pathomechanisms with the help of subtraction iodine mapping CT. With L-type patients, just like with our patient "A," the low V_A/Q is possibly secondary to loss of compensatory hypoxic pulmonary vasoconstriction leading to increased blood flow through the injured lung areas. In addition to vasoplegia around the damaged alveoli, hypoperfusion can develop in apparently healthy areas. The downregulation of angiotensin-converting enzyme 2 (ACE2) has an utmost importance in the mechanisms formerly detailed. With H-type

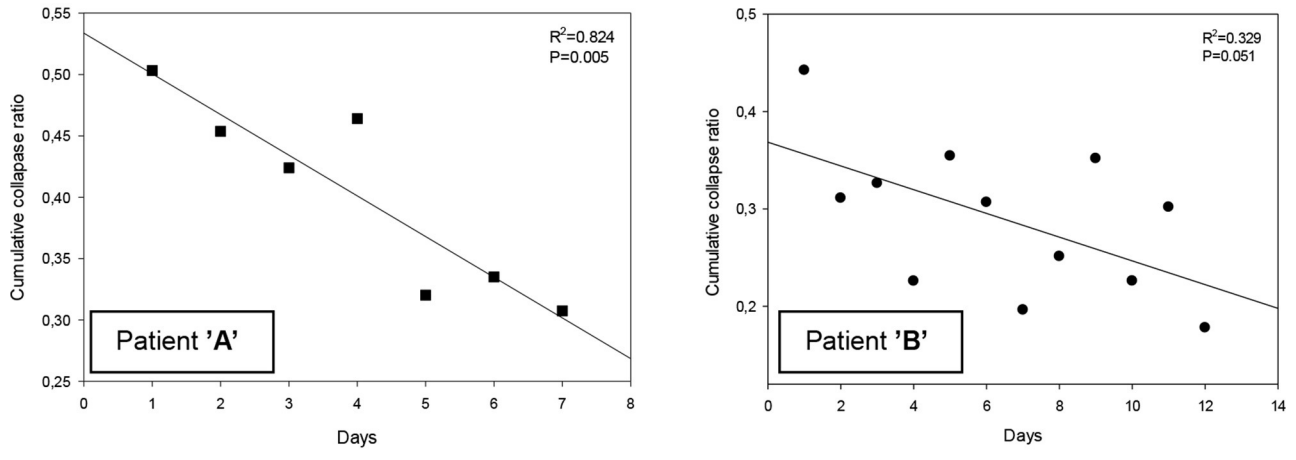

FIGURE 4 | (A,B) Cumulative collapse ratio following PEEP trial.

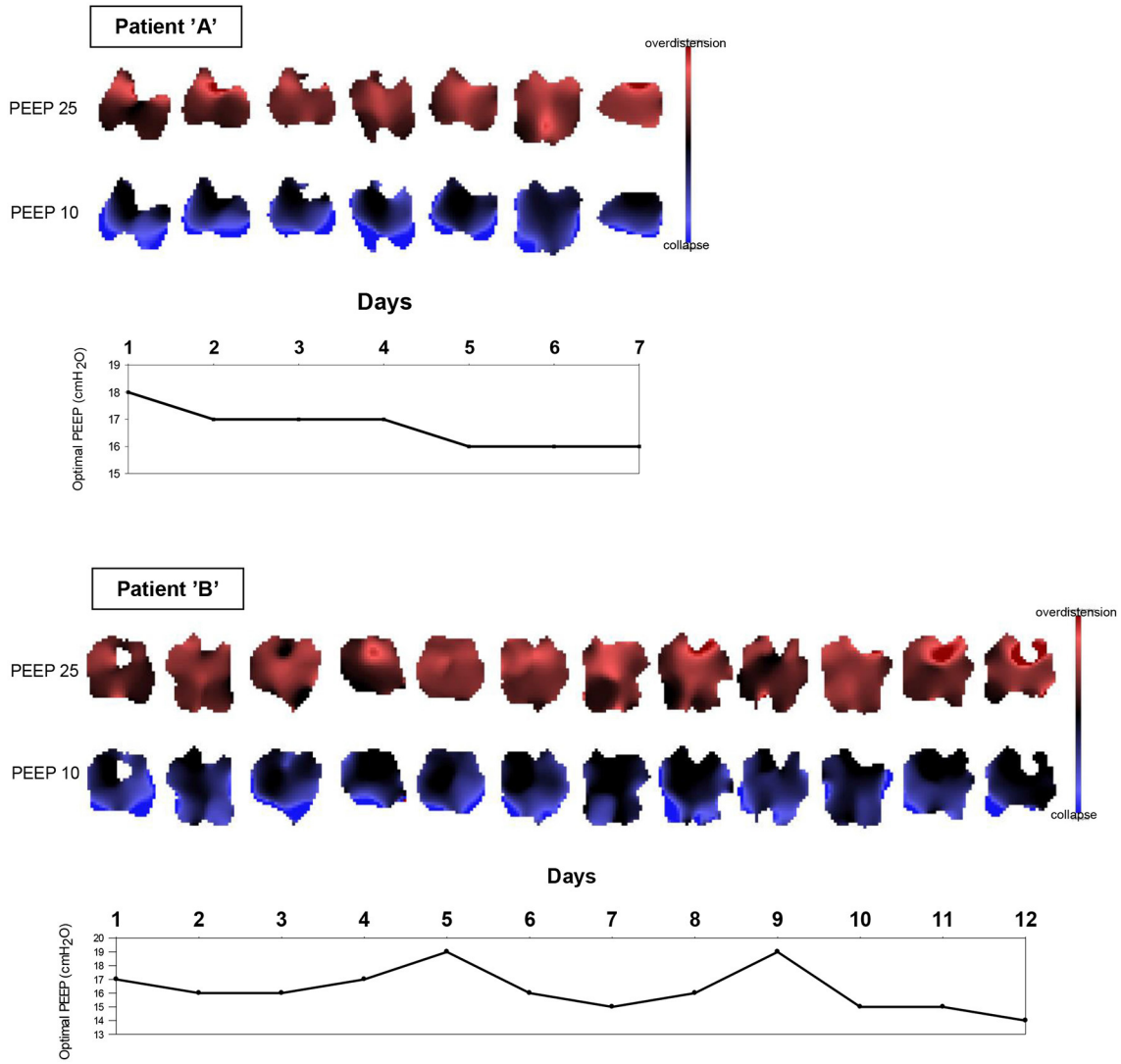

FIGURE 5 | Pixel overdistension and collapse images on each day with patients **(A,B)** at top PEEP 25 and final PEEP 10 cmH_2O and optimal PEEP levels. PEEP, positive end-expiratory pressure.

patient "B," the increased right-to-left shunt probably can be explained by the substantial perfusion through the atelectatic parenchyma, which is precipitated by the extended edema and the associated increase in lung weight. However, without direct lung ventilation/perfusion imaging and extravascular lung water index assessment, we could not validate our findings so they remain hypothetical.

The V_D measurements indicated these disturbances with both patients "A" and "B." However, Bohr–Enghoff equation is not capable to distinguish anatomically and the alveolar dead space as it can present only their sum (12). With L-type patient "A," a significant decrease in V_D was presented following the recruitment parallel to a significant decrease in $PaCO_2$ and pH. Tough C_{stat} improved, one cannot exclude the possibility of development in V_A/Q. However, this would have required an assessment of perfusion. While a significant improvement was detected in C_{stat} in both patients there was no improvement in PaO_2/FiO_2 which coincides with the previous observations (13, 14).

Electric Impedance Tomography

Numerous investigations applied CT scans to assess the recruitabilty in COVID-19 pneumonia patients. However, a CT scan has an outstanding resolution; this imaging method is not suitable for serial evaluation as it requires high doses of radiation and in-hospital transportation of the critically ill. It is no far to seek that a radiation-free, bedside, real-time, functional imaging like EIT can significantly aid the follow-up in the evolution of pathophysiology (15). However, scarce literature can be hit

considering the application of impedance tomography in the evaluation of COVID-19 pneumonia.

Kotani and Shono (16) followed the homogeneity of ventilation distribution by EIT following prone positioning. The investigation of Tomasino et al. (17) with ventilation distribution assessed by EIT could indicate the usefulness of prone positioning in an L-type patient. EIT can be beneficial in personalizing respiratory therapy leading to setting a higher level of PEEP in COVID-19 patients (18) than recommended by previously developed FiO_2/PEEP tables for ARDS. Respectively, the recruitment-to-inflation ratio observed by EIT could determine recruitability with COVID-19 pneumonia (19).

Our series of PEEP trials investigated the alteration in global overdistension and collapse. During a decremental PEEP trial, the ease of the overdistended regions is characterized by an increase, while the collapse of previously open areas by a decrease in pixel compliance. For the estimation of these processes, the algorithm designed by Costa et al. (6) was applied. As expected, the ratio of overdistension was the highest at the top PEEP level in both patients. The cumulative collapse ratio was a useful tool to differentiate L-type (patient "A") from H-type (patient "B"). Furthermore, the alteration in collapse ratio by time was capable to pursue the transition from L- to H-type with patient "A."

L-type patient "A" had a higher tendency for collapse during the decremental PEEP phase in the first few days. Parallel with this tendency, higher optimal PEEP levels were revealed on the same days. This recognition can lead to an opposing PEEP recommendation than suggested by Gattinoni et al. (3) according to which higher PEEP settings can be advocated in L-type patients with respect to our observations, at least during the first few days following orotracheal intubation. This perception complies with the article of van der Zee et al. (18). Unambiguously with patient "B," the H-phenotype labeling was persisting. As the condition in the ratio of overdistension and collapse was fluctuating day by day, EIT was a useful tool to individualize ventilator settings.

Limitations

One of the main limitations of the research is the type of case series investigation. A higher number of PEEP trial instances with more COVID-19 pneumonia patients would have significantly strengthened the observations. The other limitation of the research is that a completely aerated lung was estimated following the incremental phase of the PEEP trial. With this estimation, the relative ratio of the recruitable alveolar collapse was calculated. This is limited by the time difference in EIT protocol as an absolute amount of collapse cannot be procured. However, the protocol still provides a collapse ratio related to the minimal potential collapse, but can still render information about the condition of the lungs. Comparing the EIT observations with lung CT scans performed before and after the PEEP trials would significantly promote the results. However, transferring critically ill patients for such a high radiation dose investigation on a daily basis is unethical and unfeasible. Finally, the absence of muscle paralysis could affect the lung mechanics.

CONCLUSION

This is the first investigation that followed up COVID-19 pneumonia patients under EIT observation during PEEP trials on a daily basis. The estimated ratio of global collapse and overdistension defined by EIT can be a potential bedside device to differentiate L- from H-phenotype. EIT was a feasible tool to monitor the transition of L-phenotype into the other. EIT monitoring provides sufficient information about the evolution of COVID-19 pneumonia, hence promoting the daily, patient individualized settings on the mechanical ventilator. As SARS-CoV-2-associated pneumonia has a slow tendency of regression and requires long-term respiratory therapy, optimizing ventilator parameters has an utmost importance in the prevention of ventilator-associated lung injury. However, the short case number of PEEP trials and the lack of CT validation of the observations require further investigations to promote these findings.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Investigation Review Board of the University of Szeged. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KM, BB, and AL conceived of the presented idea. AL carried out the experiment. TM, FH, and ÁS recorded the data. RC and SK-Z performed the numerical calculations derived by impedance tomography. KM supervised the impedance tomography calculations. TM, FH, and AL performed the statistical analysis. AL took the lead in writing the manuscript. All authors provided critical feedback about the article and contributed to the article and approved the submitted version.

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Vasopressors and Risk of Acute Mesenteric Ischemia: A Worldwide Pharmacovigilance Analysis and Comprehensive Literature Review

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Vasodilatory shock, such as septic shock, requires personalized management which include adequate fluid therapy and vasopressor treatments. While these potent drugs are numerous, they all aim to counterbalance the vasodilatory effects of a systemic inflammatory response syndrome. Their specific receptors include α- and β-adrenergic receptors, arginine-vasopressin receptors, angiotensin II receptors and dopamine receptors. Consequently, these may be associated with severe adverse effects, including acute mesenteric ischemia (AMI). As the risk of AMI depends on drug class, we aimed to review the evidence of plausible associations by performing a worldwide pharmacovigilance analysis based on the World Health Organization database, VigiBase®. Among 24 million reports, 104 AMI events were reported, and disproportionality analyses yielded significant association with all vasopressors, to the exception of selepressin. Furthermore, in a comprehensive literature review, we detailed mechanistic phenomena which may enhance vasopressor selection, in the course of treating vasodilatory shock.

Keywords: septic shock (MeSH), vasopressors, pharmacovigilance, systematic review, mesenteric ischemia

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BACKGROUND

Vasodilatory shock, among which the first cause is septic shock, rank first in the causes of mortality in intensive care units (1). Upkeeping organs perfusion is one of the main goals of shock management, and to that end, volume resuscitation and vasopressors administration are key to maintaining adequate blood pressure (1).

Yet, the variety of molecules which may be used as vasopressors has been increasing over the years, and their efficacy and drawbacks have been compared in numerous meta-analyses and reviews. Among adverse effects related to use of vasopressors, acute mesenteric ischemia (AMI) is an uncommon (with a below than 1% incidence) but lethal (2, 3) and its diagnosis difficult (4).

In this review, we especially focused on AMI related to vasopressors use. In the first part, we performed a worldwide pharmacovigilance analysis based on the World Health Organization database, VigiBase® to assess the potential association between the different vasopressors and AMI. In the second part, we performed a comprehensive literature review on the mechanisms of action of the available vasopressors at bedside and their respective adverse effects, with a focus on AMI.

METHODS

Study Design

This is a worldwide observational case-non-case cross-sectional study focusing on AMI related to vasopressors use, from the international pharmacovigilance database, VigiBase® (5). VigiBase® is the WHO global individual case safety reports (ICSR) deduplicated database, managed by the Uppsala-Monitoring-Centre (Uppsala, Sweden, accessible at www.vigiaccess.org). It contains over 23 million ICSR received from over 130 countries since 1967 with over 25,000 drugs and vaccines. ICSR originate from different sources, such as healthcare professionals, patients, and pharmaceutical companies, and are generally notified post-marketing. ICSR include administrative information (country, type of report and reporter), patient data (age, sex) and nature of the outcome, using the latest version (currently v22.1) of MedDRA (Medical Dictionary for Regulatory Activities) terms (6). Drug(s) involved (name, drug start and stop dates, indication, dose) are also indicated. Drugs are coded using the WHO drug dictionary and categorized using the Anatomical Therapeutic Chemical (ATC) classification (7). Each event is characterized as "serious" or "non-serious" according to the WHO definition. Seriousness corresponds to death, life-threatening situations, hospitalization, hospitalization prolongation, persistent incapacity or disability, and situations judged clinically serious by the physician reporting the case.

Analysis in VigiBase

VigiBase® is a spontaneous reporting system, which allows for more robust and rigorous analyses than isolated case reports or case series, due to the possibility of performing quantitative comparisons, such as disproportionality analysis (case–non-case) to identify drugs significantly associated with AMI (8). We identified cases of AMI by searching in VigiBase® all ICSR flagged with the MedDRA preferred-term level referring to AMI (a composite of "*Intestinal ischemia,*" "*Mesenteric arterial occlusion,*" "*Mesenteric artery stenosis,*" "*Mesenteric vascular insufficiency*" and "*Mesenteric vascular occlusion*") from inception to June 2021; with a drug declared as "suspect" or "interacting" with AMI reaction.

Disproportionality analysis compares the proportion of a selected specific adverse-drug-reaction (ADR) reported for a single drug with the proportion of the same ADR for a control group of drugs (i.e., full database with all drugs). The denominator in these analyses is the total number of ADR reported for each group of drugs. If the proportion of cases associated with a specific drug is greater than in patients without this ADR (non-cases), there is a disproportionality association (signal identification) between the ADR and the drug. In the present work, the calculated Bayesian disproportionality estimate was the information component (IC) (8). Herein, we also performed for selected previously unknown liable drugs a sensitivity analysis excluding from full database the ICSR in which drugs already known to be associated with AMI were reported.

Calculation of the IC using a Bayesian confidence propagation neural network was developed and validated by the Uppsala Monitoring Centre as a flexible, automated indicator value for disproportionate reporting that compares observed and expected ADR associations to find new drug-ADR signals with identification of probability difference from the background data (full database) (9). Probabilistic reasoning in intelligent systems (information theory) has proved to be effective for the management of large datasets, is robust in handling incomplete data, and can be used with complex variables. The information theory tool is ideal for finding drug-ADR combinations with other variables that are highly associated compared with the generality of the stored data (9). Several examples of validation with the IC exist, showing the power of the technique to find signals sooner after drug approval than by a regulatory agency, and to avoid false positives, whereby an association between a common drug and a common ADR occurs in the database only because the drug is widely used and the ADR is frequently reported (i.e., between digoxin and rash) (9, 10). Like others, our team published several studies using VigiBase® and disproportional reporting calculation to characterize and identify new drug-ADR associated signals, which were subsequently corroborated by preclinical mechanistic studies or prospective cohorts (8, 11–14). This later element requires to be emphasized, as IC value should be interpreted only as means to perform clinical reviews of plausible associations and do not signify causality in any way. The IC_{025} is the lower end of the 95% credibility interval for the IC. A positive value of the IC_{025} is deemed significant (8, 15).

For description of ICSR, continuous data were reported in median (interquartile range). All data were available, otherwise specified. Data management was performed using Python software v3.0 (Python software foundation, Wilmington, Delaware, USA).

RESULTS

Overall, 23,937,083 ICSR were screened and 104 ICSR of AMI were retained. All vasopressors, to the exception of selepressin were significantly associated with AMI ($IC_{025} > 0$ and ROR > 1, see **Figure 1**). Affected patients were >65 years old in 48%, with men representing 61% of cases. Death was concomitantly reported in 49% of cases. Reports originated from standard of care in 91% and 6 from investigational drug studies. The summary of ICSR characteristics by vasopressor is detailed in **Table 1**.

DISCUSSION AND REVIEW OF THE DIFFERENT AVAILABLE VASOPRESSORS

Vasopressors are indicated for patients with persistent arterial hypotension after appropriate fluid resuscitation. While some vasopressors are natural hormones that exert a vasopressor activity through specific receptor activation (norepinephrine, epinephrine, vasopressin, angiotensin II), most recent vasopressors, such as selepressin, are modifications of

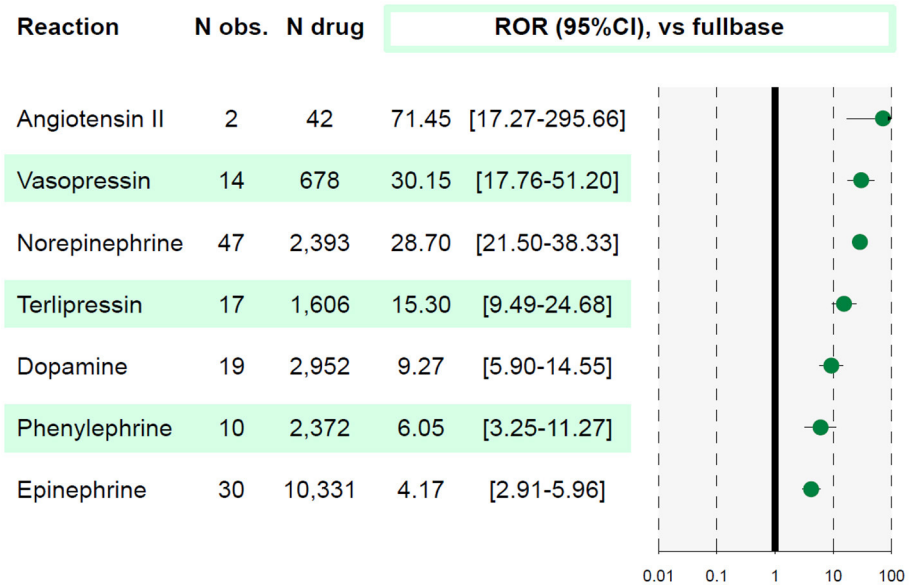

FIGURE 1 | Association between acute mesenteric ischemia and each molecule. A reporting odds ratio (ROR) value is considered significant when lower bound of 95% confidence interval (95%CI) is above 1. The studied reaction is acute mesenteric ischemia (AMI). *N obs*., number of observed AMI reports; *N drug*, number of reports involving the studied drug in VigiBase.

natural hormones. All vasopressors have adverse effects such as ischemia, cardiac arrhythmias and/or metabolic changes. Here, we confirmed in this worldwide pharmacovigilance study, an association between use of all vasopressors and AMI, to the exception of selepressin.

Pharmacovigilance disproportionality analyses using IC and ROR have long been considered relevant toward building case for delving deeper into associations between incriminated drugs and specific ADR, using spontaneous reports as material. As for any other measures of disproportionality, the need for caution to interpret quantitative results is paramount and IC values primarily serve to triage which drugs or drug categories require scrutiny while building case reviews (16). Hence, the primary aim of such methods is to look at plausible drug-ADR associations, before delving deeper using combined *in vitro* and *in vivo* translational methods to assess causality (13).

Gastrointestinal Side Effects of Vasopressors, an Overview

In critically-ill patients with shock, vasopressors are used to restore vasoconstriction and enhancement of mean arterial pressure. However, a higher cumulative vasopressor dose is associated with organ dysfunction and mortality (17, 18). Vasopressors are also associated with digestive side effects when used inappropriately or in high doses.

Gastrointestinal complications are frequent in critically ill patients (19). Mechanisms underlying vasopressors use and gastrointestinal complications are not fully understood yet, but splanchnic blood flow seems to be a major factor. Firstly, the restoration of microcirculatory blood flow is not distributed evenly when vasopressors are used, especially in the digestive organs. In pigs who were exposed to fecal peritonitis-induced septic shock; norepinephrine and epinephrine failed to increase microcirculatory blood flow in most abdominal organs, despite increased perfusion pressure and systemic blood flow (20). These both drugs appeared to divert blood flow away from the mesenteric circulation and decrease microcirculatory blood flow in the jejunal mucosa and pancreas (20). In late 90s, the effects of vasopressors on increasing the splanchnic perfusion, principally assessed by gastric intramucosal pH, was found to be unpredictable (21). Secondly, in critically-ill patients, the use of catecholamines and degree of motility disturbance were found to be associated (22), although the severity of illness and use of sedative drugs disturbs motility and also associates with catecholamine use (22).

Splanchnic vasoconstriction, secondary to the vasopressors use, could lead to non-occlusive AMI, characterized by gastrointestinal ischemia with "normal" vessels. Overall, non-occlusive AMI is associated with a high mortality rate in critically-ill patients (2, 23). The mechanisms underlying non-occlusive AMI are incompletely understood and include macrovascular vasoconstriction, hypoperfusion of the tips of the villi and shunting (4). However, causality link between vasopressors use and AMI was not established on randomized studies (24).

Angiotensin II

The main angiotensin II cardiovascular effects are the regulation of arterial blood pressure with short-term vasoconstriction, the regulation of aldosterone synthesis and vasopressin release and the regulation of the water and salt balance. All these effects are primary mediated through the binding of angiotensin II

TABLE 1 | Descriptive statistics by molecule of all reports of acute mesenteric ischemia in VigiBase.

Drug of interest	Overall	Norepinephrine	Epinephrine	Phenylephrine	Dopamine	Vasopressin	Terlipressin	Angiotensin II
Nb of cases	104	47	30	10	19	14	17	2
Male	59 (60.8%) [97]	28 (59.6%) [47]	15 (53.6%) [28]	3 (30.0%) [10]	10 (62.5%) [16]	9 (69.2%) [13]	9 (64.3%) [14]	2 (100.0%) [2]
Age > 65 years-old	44 (47.8%) [92]	24 (53.3%) [45]	15 (53.6%) [28]	3 (37.5%) [8]	7 (43.8%) [16]	3 (25.0%) [12]	5 (35.7%) [14]	1 (50.0%) [2]
Serious adverse event	96 (100.0%) [96]	46 (100.0%) [46]	30 (100.0%)	7 (100.0%) [7]	17 (100.0%) [17]	14 (100.0%)	14 (100.0%) [14]	2 (100.0%)
Deaths	47 (49.0%) [96]	22 (47.8%) [46]	15 (50.0%)	6 (85.7%) [7]	8 (47.1%) [17]	9 (64.3%)	8 (57.1%) [14]	0 (0.0%)
Region of reporting								
Africa	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
America	27 (26.0%)	10 (21.3%)	12 (40.0%)	3 (30.0%)	8 (42.1%)	7 (50.0%)	0 (0.0%)	2 (100.0%)
South-East Asia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Europe	42 (40.4%)	23 (48.9%)	12 (40.0%)	5 (50.0%)	1 (5.3%)	2 (14.3%)	12 (70.6%)	0 (0.0%)
East Meditterranean	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
West Pacific	34 (32.7%)	14 (29.8%)	6 (20.0%)	2 (20.0%)	9 (47.4%)	5 (35.7%)	5 (29.4%)	0 (0.0%)
Type or reporting								
Spontaneous	94 (91.3%) [103]	42 (89.4%) [47]	27 (90.0%) [30]	9 (90.0%) [10]	18 (94.7%) [19]	13 (92.9%) [14]	14 (87.5%) [16]	2 (100.0%) [2]
Report from study	6 (5.8%) [103]	4 (8.5%) [47]	2 (6.7%) [30]	0 (0.0%) [10]	1 (5.3%) [19]	0 (0.0%) [14]	2 (12.5%) [16]	0 (0.0%) [2]
Other	3 (2.9%) [103]	1 (2.1%) [47]	1 (3.3%) [30]	1 (10.0%) [10]	0 (0.0%) [19]	1 (7.1%) [14]	0 (0.0%) [16]	0 (0.0%) [2]
Category of reporter								
Physician	75 (79.8%) [94]	34 (82.9%) [41]	21 (75.0%) [28]	4 (66.7%) [6]	14 (93.3%) [15]	6 (54.5%) [11]	15 (88.2%) [17]	1 (50.0%) [2]
Pharmacist	2 (2.1%) [94]	1 (2.4%) [41]	0 (0.0%) [28]	0 (0.0%) [6]	0 (0.0%) [15]	1 (9.1%) [11]	0 (0.0%) [17]	0 (0.0%) [2]
Other health professional	13 (13.8%) [94]	6 (14.6%) [41]	6 (21.4%) [28]	2 (33.3%) [6]	0 (0.0%) [15]	4 (36.4%) [11]	0 (0.0%) [17]	0 (0.0%) [2]
Lawyer	0 (0.0%) [94]	0 (0.0%) [41]	0 (0.0%) [28]	0 (0.0%) [6]	0 (0.0%) [15]	0 (0.0%) [11]	0 (0.0%) [17]	0 (0.0%) [2]
Consumer or non-health professional	4 (4.3%) [94]	0 (0.0%) [41]	1 (3.6%) [28]	0 (0.0%) [6]	1 (6.7%) [15]	0 (0.0%) [11]	2 (11.8%) [17]	1 (50.0%) [2]

Number of available data are indicated in brackets.

to its type 1 receptor, which belongs to the G protein-coupled receptor superfamily, in blood vessels, kidney, brain and heart (25). Besides its classical cardiovascular effects, angiotensin II might also exert inflammatory, pro-proliferative and pro-fibrotic effects, involved in oncologic and transplantation pathways (26).

Given its vasopressor activity which allows the restoration of vascular tone and arterial pressure through both venous and arterial constriction, (27) the interest of angiotensin II administration has been studied in vasodilatory shock and especially in septic shock, since a relative renin-angiotensin system failure has been evidenced in sepsis, illustrated by a relative decrease in angiotensin II plasma levels combined with a decrease in sensitivity to angiotensin II stimulation (26). Thus, some experimental (28–30) and human studies (31–35) have shown that angiotensin II administration allowed improvements to arterial pressure and even a catecholamine-sparing effect (36). In 2017, the multicentric randomized double-blind, placebo-controlled ATHOS-3 trial conducted in 334 patients with high-output catecholamine-resistant vasodilatory shock, defined by persistent vasodilatory shock despite adequate fluid resuscitation and administration of high doses of norepinephrine for a minimum of 6 h and a maximum of 48 h, showed that low-dose of angiotensin II allowed to achieve a predefined mean arterial pressure target along with a decrease in catecholamine dosage, but did not reduce the mortality rate (37). *Post-hoc* analyses showed that patients who benefited most from angiotensin II administration were those with the most severe shocks with a relative angiotensin II deficiency, (38) those with markedly elevated serum renin concentrations at baseline (39) and those with acute kidney injury requiring renal replacement therapy (40). In this latter group of patients, the administration of angiotensin II was associated with a lower 28-day mortality rate, a better correction of hypotension, and a faster recovery of kidney function (40).

Despite these encouraging results, angiotensin II is currently not recommended in patients with septic shock (1), since its safety is still matter of debate. Indeed, the marked vasopressor activity of angiotensin II could result in AMI and microvascular thrombosis in experimental models of septic shock (41, 42). Nonetheless, in the ATHOS-3 trial, the proportion of serious ischemic adverse effects (digital, gut, myocardial) and cardiac arrhythmias were similar in patients receiving angiotensin II or placebo (37). Especially, AMI occurred in <1% of patients receiving angiotensin II (37). A systematic review also concluded that angiotensin II-induced side effects were infrequent, with ≤300 reported adverse effects, and no AMI was reported (43). It must be noted that only 13 of the included studies were conducted in patients with vasodilatory shock, making the external validity of these results questionable in the case of critically-ill patients.

Non-catecholaminergic Vasopressors: Vasopressin, Selepressin, Terlipressin

Non-catecholaminergic vasopressors rely on an alternate pathways, which all depend on three receptors, responsive to plasma osmolality, blood volume and pressure (44, 45):

- V1a receptors, located on vascular smooth muscular cells, allowing vasoconstriction,
- V1b receptors, mostly located in the anterior-pituitary gland and in the pancreas. Their activation leads to the endocrinological role of vasopressin (AVP), especially by the induction of corticotropic axis stimulation,
- V2 receptors, located on basolateral surface of renal tubular cells, inducing aquaporin 2 recruitment leading to water reabsorption.

AVP has pleiotropic effects, by stimulating all three receptors. Selepressin is a selective agonist of V1a receptors, which may mitigate sepsis-induced vasodilatation, vascular leakage and tissue edema. Finally, terlipressin is mostly a V1a receptor agonist, but also interacts with V1b and V2 receptors. To be noted, selepressin and terlipressin use are justified by the potential toxicity of V2 activity on endothelial and renal cells of vasopressin (46).

Vasopressin (AVP)

AVP is a nine-amino-acid peptide that is produced by the hypothalamus and then stored in the post-pituitary gland. Its vasoconstrictive effect is very low in healthy individuals (47, 48). In patients with vasodilatory shock such as septic shock, AVP has a much more potent effect and is released at the very early phase of shock (49). Its effect is then potentiated within the first 2 h after septic injury (i.e., endotoxinemia), with an increase in plasma levels concentration (50). Interestingly, if the injury lasts more than a few hours, the levels of AVP drop under baseline level, leading to a hormonal paradoxical level that can be observed during critical illnesses (51). This decrease may be due to the depletion of pituitary stores (52), although, only a third showed this feature in a cohort of patients with septic shock (53). Autonomic dysfunction with impaired baroreflex loop and osmoregulation may also participate to the low levels of AVP observed during septic shock (52–56). Furthermore, increased neuronal apoptosis in the autonomic centers may also contribute to the observed deficiency in AVP during septic shock (57, 58).

In contrast to norepinephrine, AVP may cause less vasoconstriction in mesenteric, coronary, and cerebral circulations (59). In several *in vivo* models, AVP was associated with improved recovery from mesenteric ischemia. The upregulation of endothelin 1 (ET-1) gene expression, with subsequent increased plasma levels of ET-1 and intestinal fatty acid binding protein, has been previously associated with mesenteric ischemia, and use of vasopressin in porcine models reversed these observations (60). Interestingly, endothelin receptor antagonists have been identified as potential protectors against ischemia-reperfusion injury in small intestine, and it was suggested in rats that AVP may have cross-path effect (61).

The largest trial compared in 778 patients with septic shock, the effect of vasopressin to those of norepinephrine (62). There was no difference in overall survival (35.4 and 39.3% 28-day mortality, respectively; difference, −3.9%; 95% CI, −10.7 to 2.9), however, the less severe patients who received AVP tended to show lower mortality, but interaction tests between severity of shock and mortality did not confirm this observation. AMI occurred in similar proportion in both treatment arms (3.4% in the norepinephrine arm vs. 2.3% the AVP arm, $p = 0.39$). In a recent meta-analysis, AVP use in septic shock was associated with an increase of digital ischemia (RR 4.85, 95% CI 2.81–8.39, $I_2 = 26\%$), but not AMI (RR 0.83, 95% CI 0.44–1.55, $I_2 = 0\%$) and diarrhea (2.47, 95% CI 0.77–7.96, $I_2 = 49\%$) (63). Hence, to date, no prospective studies in human showed differences in AMI when comparing AVP to another vasopressor, but the scarcity of data does not allow any conclusion, yet. Currently AVP is recommended as a second-line vasopressor in patients with septic shock (1).

Terlipressin

Terlipressin is mainly used in patients with hepatic failure, hepatorenal syndrome (64), and esophageal varices rupture bleeding (65). Only few trials assessed the benefits of terlipressin compared to norepinephrine in vasodilatory shock. Moreover, terlipressin was either evaluated alone or in combination with other vasopressors, with various dosage, and patients' profiles.

The largest randomized controlled trial comparing terlipressin and norepinephrine in patients with septic shock was stopped due to futility (66). In 526 patients, neither difference in 28-day mortality was observed nor vasopressor-free days or change in SOFA score during the first week after randomization. While the investigators reported a greater prevalence of adverse effects (30 vs. 12%, $p < 0.01$) including diarrhea in the terlipressin group, they did not find more AMI (1.02 vs. 0.35%, $p = 0.62$) (66).

Since then, several meta-analyses were published. In 2019, Huang et al. (67) assessed only randomized controlled trials specifically evaluating terlipressin vs. norepinephrine, each in single-therapy in the management of septic shock. With 6 studies included in their analysis, the authors showed no difference in 28-day mortality, urine output, liver and kidney functions as well as in adverse effects between groups (67). More recently, Yao et al. (68) compiled trials evaluating terlipressin alone or with norepinephrine compared with norepinephrine alone or with dopamine. They observed a significant lower 28-day or 30-day mortality rate among patients with septic shock who received terlipressin [RR = 0.87 (95%CI, 0.77–0.98)]. Adverse effects, including AMI, were not different between groups (68).

Selepressin

Selepressin is a pure V1a agonist. *In vitro* and *in-vivo* studies on animals showed it can reduce endothelial barrier dysfunction, vasodilatation, capillary leakage, lung edema and pro-inflammatory cytokines generated by sepsis (69–73).

Only two major studies focused on selepressin in addition to norepinephrine in patients with septic shock. A phase IIa trial highlighted that patients receiving selepressin compared to placebo, received less catecholamine while maintaining mean

arterial pressure and reducing net fluid balance (74). A larger study, SEPSIS-ACT, published in 2019 by Laterre et al. (75), evaluated the value of adding to norepinephrine, selepressin compared to placebo in patients with septic shock in a phase 2b/3 trial. The trial was stopped for futility, finding no difference on the primary endpoint: ventilator- and vasopressor-free days within 30 days nor in any of the secondary end points (90-day mortality, kidney replacement therapy-free days, intensive care unit-free days). However, beneficial effects were observed in the selepressin group: decreased norepinephrine doses and positive fluid balance, increased urine output. Focusing on adverse effects, the proportion of AMI was no greater in the selepressin group than in the placebo group (3.2 vs. 2.6%) (75).

Catecholaminergic Vasopressors: Norepinephrine, Epinephrine, Phenylephrine and Dopamine

Norepinephrine

Norepinephrine is a potent α- and β1-adrenergic agonist, with little activity on β2 receptors. By binding to its receptors, norepinephrine increases cytosolic calcium concentration into smooth muscle, leading to vasoconstriction and some positive inotropic activity. Through its β-adrenergic effect, norepinephrine exerts its vasopressor activity with arterial and venous vasoconstriction. Besides the increase in arterial pressures, left ventricular afterload and cardiac filling pressures, norepinephrine also increases the venous return, resulting in an increase in right atrial pressure and cardiac preload (76, 77). This increase in venous return results from the increase in mean systemic filling pressure (78, 79) and thus in venous return pressure gradient. Through its β1 stimulation, norepinephrine also exerts a positive inotropic effect and an increase in stroke volume. It has been recently demonstrated in 38 patients with septic shock who had been resuscitated for <3 h and whose mean arterial pressure remained <65 mmHg, in whom norepinephrine administration increased the left and right systolic function and the cardiac output despite the increased left ventricular afterload (76, 77, 80). Interestingly, the potential chronotropic effect is counteracted by baroreflex stimulation following vasoconstriction. Consequently, norepinephrine increases cardiac output without increasing heart rate or myocardial oxygen consumption (81). Finally, norepinephrine enhances the coronary blood flow because of coronary vasodilation secondary to enhanced cardiac metabolism and the normalization of diastolic blood pressure when low.

Besides to its effects on macrocirculation, norepinephrine administration might also improve microcirculation, especially in case of septic shock, which is characterized by microcirculatory abnormalities even in patients with preserved or corrected microcirculation (82). To this end, Georger et al. (83) demonstrated in severely hypotensive patients with septic shock that norepinephrine administration improved muscle tissue oxygenation and microcirculatory reserve capacities. In addition, the assessment of tissue oxygenation might be of interest to personalize mean arterial pressure target and thus the dosage of norepinephrine in patients with septic shock (84). Finally, it has also been suggested that norepinephrine might have some immune effects (85).

While norepinephrine is currently recommended as the first-line vasopressor in patients with septic shock (1), hemorrhagic shock (86), and cardiogenic shock (87–89), some potential adverse effects of high-dose of norepinephrine should be nonetheless kept in mind. First, high-dose of norepinephrine may induce oxidative stress and myocardial cells insult but also alter sepsis-associated immunomodulation (90). Furthermore, high-dose of norepinephrine may impair the splanchnic circulation with an increase in systemic and mesenteric vascular resistances (91). Nevertheless, a large randomized trial, the SEPSISPAM study, assessed the effects of two levels of mean arterial pressure in 776 patients with septic shock (92). To achieve the high mean arterial pressure level, norepinephrine doses were significantly increased. The 28-day mortality rate was not different between both groups. Serious adverse effects related to norepinephrine use were ventricular arrhythmias, bleeding, as well as digital ischemia and AMI. Except atrial fibrillation which was more frequent in patients receiving higher dose of norepinephrine, the incidence of other adverse effects, including AMI, was similar in both groups of patients and AMI occurred in 2% of patients (92).

Epinephrine

Epinephrine is the first adrenergic hormone of the adrenal medullar gland which was identified and is a potent agonist of α, β1 and β2 receptors. Through its α-adrenergic effect, epinephrine exerts its vasopressor activity with marked arterial and venous vasoconstriction. However, the epinephrine effects on vasculature is partly counteracted by β2-mediated vasodilation. Thus, epinephrine administration results clinically in a marked increase in systolic arterial pressure while diastolic arterial pressure only slightly increased. Consequently, the increase in mean arterial pressure is less than that with norepinephrine. Through its β1 stimulation, which is more marked than that of norepinephrine (81, 93), epinephrine also exerts positive inotropic and chronotropic effects, resulting in an increase in cardiac output. Epinephrine also facilitates ventricular relaxation and enhanced coronary blood flow through the increase in myocardial oxygen consumption. Finally, as with norepinephrine, it has also been suggested that epinephrine might have some immune effects (85).

Two large trials evaluated the effects of epinephrine administration in critically-ill patients (94, 95). In the CAT study, Myburgh et al. showed in 280 patients with shock (mainly septic shock) that the median time to achieve a predefined mean arterial pressure target was similar with epinephrine and norepinephrine administration. The 28- and 90-day mortality rate was also similar and there was no difference in vasopressor-free days (94). However, epinephrine administration was associated with more frequent lactic acidosis and arrhythmia, which led to the discontinuation of the administration of epinephrine in 13% of patients. Of note, no AMI or others ischemic adverse effects were reported (94). In the multicentric and randomized CATS study, Annane et al. (95) compared in 330 patients with septic shock epinephrine alone to the association norepinephrine and dobutamine. The different mortality rates were not different

as the time to achieve hemodynamic success and the time to vasopressors withdrawal (95). Once again, epinephrine was associated with more frequent lactic acidosis, but the incidence of the other severe adverse effects (arrhythmias, ischemic events, bleeding) was similar between epinephrine and the association norepinephrine and dobutamine and AMI was reported (95). Epinephrine-induced lactic acidosis is a well-known metabolic effect (96, 97), which is assumed to be independent of tissue hypoxia and related to the activation of the β2-adrenergic receptors located at the surface of the skeletal muscle cells (98). This β2-activity stimulates the skeletal muscle cell Na+/K+-ATPase and accelerates the aerobic glycolysis and thus the production of pyruvate and hence of lactate into the cell (99).

Regarding the microcirculation, while it has been suggested in patients with septic shock that epinephrine administration increased more gastric mucosal perfusion than norepinephrine alone for the same mean arterial pressure level (100), other experimental (20, 101) and human studies (96, 100, 102–104) suggested that epinephrine might impair splanchnic circulation. Finally, compared to other vasopressors, epinephrine has the most negative inhibitory effect of propulsive gut motility (105). Thus, because of its more marked metabolic and cardiac adverse effects than norepinephrine (81, 93), and its potential deleterious effects on microcirculation, epinephrine is currently considered as a second-line vasopressor in patients with septic shock (1) and is no longer recommended as a vasopressor therapy in patients with hemorrhagic shock (86).

In patients with cardiogenic shock, there is no evidence of superiority of one vasopressor over another in terms of mortality (106). However, although epinephrine and the association norepinephrine and dobutamine has similar hemodynamic efficiency, patients with cardiogenic shock receiving epinephrine experience more lactic acidosis and arrhythmias and have inadequate gastric mucosa perfusion (107). More recently, Levy et al. (108) showed in a randomized trial including 57 patients with cardiogenic shock after myocardial infarction confirmed that epinephrine and norepinephrine had similar hemodynamic efficacy, but epinephrine was associated with higher incidence of refractory shock. Currently, it is recommended to favor norepinephrine over epinephrine in patients with cardiogenic shock requiring vasopressors (89).

Phenylephrine

Phenylephrine is a pure α-adrenergic receptor agonist with marked vasopressor activity (81, 93). The effects of phenylephrine on cardiac output are complex and difficult to predict, depending on its venous and arterial modulation (109). Phenylephrine-induced venous vasoconstriction might exert opposite effects on systemic venous return. On one hand, it decreases the unstressed venous volume, which in turn increases the mean systemic filling pressure, the venous return pressure gradient and thus the systemic venous return. On the other hand, it also increases the resistance to venous return, which consequently decreases the systemic venous return. Thus, phenylephrine may either increase or decrease cardiac output in patients with preload reserve (110). Moreover, phenylephrine-induced arterial vasoconstriction results in a marked increase in left ventricular afterload with an important rise in systolic arterial pressure and thus may induce a decrease in cardiac output in patients with impaired cardiac contractility (111). This marked increase in systolic arterial pressure may also result in baroreceptor-mediated reflex bradycardia and contribute to the decrease in cardiac output (81, 93). Recently, Kalmar et al. (112) showed in patients with preload reserve and anesthesia-induced hypotension that a single phenylephrine administration increased the systemic venous return and thus cardiac preload, which in turn increased cardiac output while the left ventricular afterload increased. Besides its hemodynamic effects, phenylephrine might also have some immune effects (85).

While phenylephrine is widely used to restore arterial pressure in anesthesia-induced hypotensive patients in the operating theater (113), its use is no longer recommended in critically-ill patients with septic shock from 2016 (1, 101). First, phenylephrine induced a more pronounced global α1-mediated splanchnic vasoconstriction than norepinephrine (114), with a potential risk of splanchnic ischemia (115), even though the adverse effects of phenylephrine on microcirculatory blood flow in the gastrointestinal tracts might be less marked than those of epinephrine and norepinephrine (20). Second, phenylephrine has a lower efficacy than norepinephrine when continuously administered due to the absence of β-adrenergic effects (116). Third, phenylephrine use could be associated with a higher mortality rate in critically-ill patients (117).

Dopamine

Dopamine is the immediate physiologic precursor of norepinephrine and epinephrine. Its effects depend on the activated receptors, which in turn depend on the dose administered (81, 118, 119). At low dose (<5 μg/kg/min), dopamine activates D1 receptors located in cerebral, coronary renal and mesenteric vessels and induces vasodilation with no effect on arterial pressures. At intermediate dose (5–10 μg/kg/min), dopamine has chronotropic and inotropic effects by activating the β1-adrenergic receptor. At high dose (10–20 μg/kg/min), by activating the α-adrenergic receptor, dopamine has a vasopressor activity similar to that of norepinephrine, with arterial and venous vasoconstriction, which results in an increase in systemic venous return and left ventricular afterload. As with other catecholaminergic vasopressors, dopamine might also have some immune effects (120, 121).

Regarding microcirculation, dopamine has similar effects than norepinephrine in splanchnic circulation (102). Yet, gastroduodenal motility was found to be adversely impacted by the use of low-dose dopamine (4 μg/kg per minute) as compared to placebo in mechanically ventilated critically ill patients (122). Currently, dopamine is no longer recommended in critically-ill patients for the following reasons. First, there is a great inter-individual variability of the dopamine effects, because of unpredictable relationship between infusion rate and plasma levels (81). Second, in a multicenter and randomized trial, De Backer et al. (123) compared dopamine to norepinephrine as first-line vasopressor therapy to restore and maintain blood pressure in 1,679 patients with shock. While there was no difference in mortality rate, except in the subgroup of patients

with cardiogenic shock, dopamine was associated with a two-fold incidence of cardiac arrhythmias (123), confirming findings of a previous study conducted in patients with septic shock (124). Conversely, the incidence rate of ischemic complications (skin ischemia and arterial occlusions) with dopamine was similar to that observed with norepinephrine. In particular; AMI was reported in <1% of patients (123).

LIMITATIONS

We acknowledge several and important biases due to the nature of the pharmacovigilance database. The first being underreporting, associated with halo bias and lack of information on the exposed population for calculation of incidence, which would require sales data from the industry. Indeed, acute mesenteric ischemia may be caused by shock, which is a major confusion bias. Moreover, as mesenteric ischemia remains a rare entity, true incidence remains elusive, due to numerous factors. The lack of consensual definition, with multiple criteria possible, as well as hardship to have definite diagnosis makes underreporting plausible. Moreover, not being able to return to each report to ensure that an exhaustive search for etiologies and concomitant drugs intake has been carried out leads to an information bias, which leads to the fact that the likelihood of a causal relationship is not the same in all reports. It is of importance to underline that the association we found between vasopressors and acute mesenteric ischemia is not necessarily synonymous with causality and, given the low quality of evidence that can be inferred from the analysis of large databases such as the pharmacovigilance database, our results should be interpret with caution. Yet, with all these elements, disproportionality analysis methodology allows to focus the attention of clinical physicians, and to assess plausibility of the incrimination of a drug toward a singular adverse effect. Although all vasopressors except Dopamine do not have dose-dependent physiological effects and were used in the same indications in the different studies included in our literature review, it should be kept in mind that the incidence of acute mesenteric ischemia may be influenced by the dose of vasopressors administered, especially in patients with impaired vascularity (elderly patients, smokers…). However, as the dose ranges of vasopressors used in the different studies are very similar, it is not possible to analyze a potential dose-dependent effect. The numerous models and mechanisms, yielded from *in vivo* models, which we presented in this review of literature bring support to these findings, and warrant further scrutiny in the field.

CONCLUSION

In this pharmacovigilance analysis combined with literature review, we observed a significant association between the use of all vasopressors but not selepressin and AMI in patients with vasodilatory shock. The development of new-generation of vasopressors activating different receptors and intracellular pathways, the individualization of a vasopressor therapy based on specific biomarkers and the development of artificial intelligence to better adjust in real-time vasopressor therapy may help in the future to avoid vasopressor-related AMI in critically ill patients ad help improve the management of patients with vasodilatory shock.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: www.vigiaccess.org.

AUTHOR CONTRIBUTIONS

CD allowed statistical analyses on VigiBase® extraction. LN wrote the final manuscript and supervised the study. All authors actively participated to the manuscript writing and provided critical insight to its revision. All authors contributed to the article and approved the submitted version.

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The supplied data from VigiBase® come from various sources. The likelihood of a causal relationship is not the same in all reports.

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Factors Associated With Deep Sedation Practice in Mechanically Ventilated Patients: A *Post hoc* Analysis of a Cross–Sectional Survey Combined With a Questionnaire for Physicians on Sedation Practices

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Purpose: The study aimed to explore factors associated with deep sedation practice in intensive care units (ICUs).

Materials and Methods: A *post hoc* analysis was conducted for a cross–sectional survey on sedation practices in mechanically ventilated (MV) patients, combined with a questionnaire for physicians regarding their preferences for light sedation (P–pls Score) in 92 Chinese ICUs.

Results: There were 457 and 127 eligible MV patients in the light and deep sedation groups respectively. A multivariable logistic regression analysis demonstrated that the control mode of mechanical ventilation, plasma lactate level, and the Sequential Organ Failure Assessment (SOFA) score were independent risk factors for deep sedation practice ($p < 0.01$). Notably, the adjusted odds ratio (95% CI) of the average P–pls score in the ICU ≤ 2 for deep sedation practice was 1.861 (1.163, 2.978, $p = 0.01$). In addition, the areas under curves of receiver operating characteristics (AUC–ROC) of the model to predict the probability of deep sedation practice were 0.753 (0.699, 0.806) and 0.772 (0.64, 0.905) in the training set and the validation set, respectively. The 28–day mortality was increased in patients with exposure to deep sedation practice but not significantly.

Conclusion: Both factors related to stressful stimuli and the ICU physicians' perception of patient tolerability in mechanical ventilation were likely associated with deep sedation practice in MV patients.

Keywords: deep sedation practice, patient tolerability, stressful stimuli, physician's perception, mechanical ventilation

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INTRODUCTION

It was previously documented that mechanically ventilated (MV) and critically ill patients were deeply sedated (defined as the Richmond Agitation–Sedation Scale equal to or < -3, RASS ≤ -3) very frequently (1–3). Compared with light levels of sedation (i.e., RASS ranged from −2 to 1, largely), significantly, deep sedation has been associated with poor outcomes including prolonged duration of mechanical ventilation, increased incidence of ventilator–associated pneumonia (VAP), declined cognitive ability, and even increased long–term mortality (1–5). Moreover, it was recently demonstrated that the implementation of no sedation protocol resulted in more days free from coma or delirium than the light sedation strategy during the stay in the ICUs (6). Therefore, deep sedation is mostly unnecessary and should be avoided by the implementation of a minimal sedation strategy, including light sedation protocol and the early Comfort using Analgesia, minimal Sedatives, and maximal Humane care (eCASH) concept in ICU MV patients (7–9).

However, at present, the frequency of deep sedation remains high in clinical practice. Fuller et al. reported that the prevalence of deep sedation was 52.8% (171/324) in a consecutive cohort of MV patients in the emergency department (ED) (10). Significantly, deep sedation was continued in 75% of the patients (92/171) on ICU day 1 in this cohort. Moreover, the depth of sedation was determined as RASS < -2 (mean RASS $= -2.3$) on day 1 in the light sedation group of Olsen's randomized control trial (RCT) on no sedation or light sedation in critically ill and MV patients (6). Low adherence to the minimal sedation strategy was previously attributable to inadequate assessments due to a shortage of nurses, lack of multidisciplinary cooperation, and even misperception (11–14). However, there is a paucity of research to comprehensively interpret the fact that care providers deepen sedation at RASS < -2 for MV patients frequently. It was proposed that several factors, including the severity of pathophysiological alternations, the intensity of supportive therapies, and ICU physicians' perception of patients' tolerability to light sedation, were involved in the care providers' decision–making for sedation depth in MV patients. Therefore, as an extension of the previous study, we did a *post hoc* analysis of a nationwide cross–sectional study combined with a questionnaire survey to investigate the factors associated with deep sedation practice in MV patients.

METHODS

Study Design and Setting

The *post hoc* analysis, which included a 24–h survey on real sedation practices in MV patients and a questionnaire for physicians regarding their preferences for light sedation, was conducted on 92 Chinese ICUs on 11 May 2016. Ethical committee approval was obtained from each participating hospital. Informed consent was waived by the ethics committees of all the participating hospitals because of the observational nature of this study. A site investigator was responsible for this study in each recruited ICU. Additionally, a clinical research coordinator (CRC) was assigned to each ICU to ensure the quality of data collection and to perform the questionnaire survey simultaneously. This study was registered on the website of www.chictr.org.cn (registration number: ChiCTR–EOC−16008444).

Patient Recruitment, Data Collection, and Questionnaire Survey

All patients on invasive mechanical ventilation were eligible to be enrolled in this study. The exclusion criteria were people aged younger than 18 or over 90 years, those with a Glasgow Coma Scale (GCS) score ≤ 7, and people with history of alcoholism, drug abuse, psychiatric illness, severe acute respiratory distress syndrome (ARDS), or use of neuromuscular blockade (**Figure 1**). Patients who died within 24 h were eliminated. Intensive care was provided as usual for all the recruited patients in each participating center.

The observation was initiated at 6:00 AM on the study day until 5:00 AM the next day. Demographics and characteristics of the patients were collected. Three measurements of RASS were required for each enrolled patient, at 6:00–7:00, 13:00–14:00, and 22:00–23:00, no matter how frequently RASS was assessed in usual care. Body temperature, ventilator settings, and the Sequential Organ Failure Assessment (SOFA) score were repeatedly evaluated while assessing RASS. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score within 24 h of ICU admission was recorded. Agitation was recorded within this 24–h observational period. The confusion assessment method for the intensive care unit (CAM–ICU) was used to evaluate delirium while the patients were agitated. Pain assessment was reviewed by the CRC the next day.

Patients recorded with all required RASS assessments ≥ -2 composed the light sedation group; otherwise, they were classified into the deep sedation group (i.e., patients with one record of RASS < -2 at least).

Up to 6 physicians in each recruited ICU were surveyed simultaneously. The questionnaire was developed based on the results of Delphi processing in a panel of experts and testing on 63 doctors. ICU physicians' preference for light sedation (P–pls score) was calculated by selected answers for 10 specific items of this questionnaire, which was described in detail in our previous study (15). The average P–pls score of each recruited ICU was calculated by the sum of P–pls score divided by the number of physicians completing this survey in the ICU in this *post hoc* analysis.

Statistical Analysis

The number of events per variable (EPV) in logistic regression analysis was used to estimate the sample size of this study (16). According to our previous publications (15, 17), there were no more than 12 variables that would be considered to potentially impact decision–making for the depth of sedation and included in the logistic regression model. Accordingly, at least 120 events (patients with deep sedation) should be collected. With a deep sedation rate of about 23%, a total number of 522 eligible patients should be enrolled in this study.

In fact, 80% of the patients were randomly selected as the training set and twenty percent as the validation set.

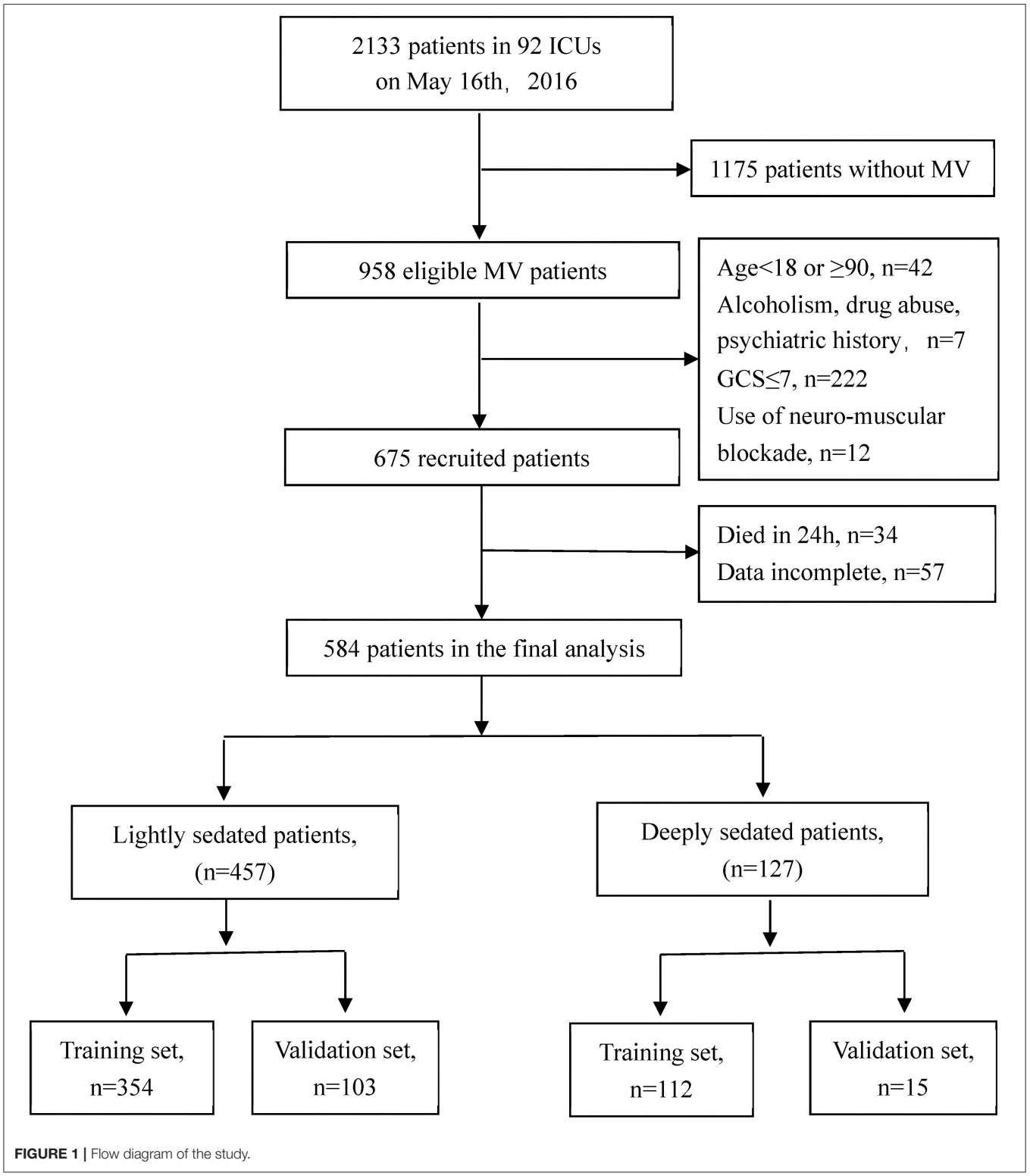

FIGURE 1 | Flow diagram of the study.

Patients' characteristics, such as age, gender, highest body temperature, and disease type, were described according to data distributions. Continuous variables that followed a Gaussian distribution were described as means and standard deviation and compared by Student's *t*–test. When continuous variables were not normally distributed, they were expressed as median (minimum, maximum) and were compared using rank–sum tests. Categorical variables were described as numbers and

proportions and were compared using the Chi–square test or Fisher's exact test as appropriate.

Based on the training set, we tried to establish a multivariable logistic regression model to confirm the associate factors that

TABLE 1 | Baseline characteristics of mechanically ventilated (MV) patients in the training set and the validation set.

	Training set			Validation set		
	Light sedation (*n* = 354)	**Deep sedation (*n* = 112)**	**P**	**Light sedation (*n* = 103)**	**Deep sedation (*n* = 15)**	**P**
High–T (C°), Mean (SD)	36.9 (0.9)	37.0 (0.9)	0.474	37.0 (0. 9)	36.7 (0.8)	0.192
Gender, n(%)						
Male	243 (68.6%)	74 (66.1%)	0.611	64 (62.1%)	7 (46.7%)	0.253
Female	111 (32.0%)	38 (33.9%)		39 (37.9%)	8 (53.3%)	
Age Mean (SD)	61.7 (17.7)	61.5 (15.6)	0.920	63.7 (17.5)	67.2 (15.5)	0.462
Category of disease, *n*(%)						
Surgical	200 (56.5%)	64 (57.1%)	0.904	51 (49.5%)	5 (33.3%)	0.241
Medical	154 (43.5%)	48 (42.9%)		52 (50.5%)	10 (66.7%)	
Septic shock, *n*(%)	40 (11.3%)	22 (19.6%)	0.023	9 (8.7%)	5 (33.3%)	0.006
SOFA, Median (range)	4.0 (0.0, 18.0)	7.0 (0.0, 18.0)	<0.001	4.0 (0.0, 17.0)	8.0 (2.0, 15.0)	<0.001
Mean (SD)	5.0(3.2)	7.2(3.6)	<0.001	4.8(3.2)	8.1(3.2)	<0.001
APACHE II, Median (range)	14.0 (1.0, 37.0)	15.5 (4.0, 33.0)	0.351	15.0 (3.0, 39.0)	22.0 (8.0, 33.0)	0.008
Mean (SD)	15.3(7.4)	16.0(7.1)	0.405	16.2(7.3)	22.1(7.9)	0.005
Mode of MV, *n*(%)						
Assisted	285 (80.5%)	59 (52.7%)	<0.001	83 (80.6%)	7 (46.7%)	0.004
Control	69 (19.5%)	53 (47.3%)		20 (19.4%)	8 (53.3%)	
PEEP (cmH_2O), Median (range)	5.0 (0.0, 20.0)	5.0 (0.0, 14.0)	0.081	5.0 (0.0, 14.0)	5.00 (2.0, 10.0)	0.363
Mean (SD)	5.3(2.2)	5.8(2.6)	0.050	4.9(2.2)	5.5(2.2)	0.312
Plat–p (cmH_2O), Median (range)	17.0 (9.0, 36.0)	17.0 (7.1, 38.0)	0.774	16.0 (7.0, 33.0)	20.0 (10.0, 32.0)	0.047
Mean (SD)	17.8(5.1)	18.3(6.6)	0.415	17.0(5.0)	20.2(6.7)	0.027
PaO_2(mmHg), Median (range)	101.0 (52.6, 410.0)	96.5 (46.0, 240.0)	0.165	100.0 (54.0, 267.0)	86.9 (62.5, 169.0)	0.039
Mean (SD)	109.4(36.0)	105.3(36.4)	0.300	111.4(38.6)	92.9(27.1)	0.076
FiO_2 (%), Mean (SD)	43.5 (10.9)	49.1 (15.3)	<0.001	43.5 (9.9)	43.7 (7.4)	0.955
Mean (SD)	43.5(10.9)	49.1(15.3)	<0.001	43.5(9.9)	43.7(7.4)	0.963
P/F ratio,Median (range)	250.0 (59.2, 487.5)	210.6 (68.8, 495.0)	<0.001	245.0 (106.5, 473.3)	211.5 (125.0, 338.0)	0.039
Mean (SD)	260.4(83.8)	230.5(95.3)	0.002	259.9(80.7)	216.8(63.6)	0.050
RR (breaths/min), Median (range)	17.0 (6.0, 41.0)	16.0 (11.0, 35.0)	0.118	18.0 (11.0, 37.0)	18.0 (13.0, 34.0)	0.694
Mean (SD)	18.1(5.3)	17.7(5.5)	0.453	18.7(5.0)	20.1(6.8)	0.328
Min–vent (L/min)**,** Median (range)	8.00 (4.30, 19.20)	7.90 (4.80, 17.60)	0.881	8.20 (4.30, 18.90)	8.69 (4.93, 16.70)	0.340
Mean (SD)	8.40(2.24)	8.55(2.61)	0.583	8.71(2.61)	10.04(3.99)	0.227
NE dosage,Median (range)	0.0 (0.0, 0.7)	0.0 (0.0, 3.0)	0.001	0.0 (0.0, 2.0)	0.0 (0.0, 0.5)	0.025
Mean (SD)	0.04(0.10)	0.10(0.30)	0.070	0.06(0.23)	0.13(0.17)	0.307
Lac (mmol/L), Median (range)	1.3 (0.3, 10.8)	1.9 (0.5, 15.8)	<0.001	1.5 (0.3, 8.6)	1.2 (0.7, 6.4)	0.622
Mean (SD)	1.6(1.2)	2.8(2.9)	<0.001	1.6(1.1)	1.9(1.5)	0.451
RASS, Median (range)	0.0 (−2.0, 4.0)	−3.0 (−5.0, −3.0)	<0.001	0.0 (−2.0, 4.0)	−3.0 (−5.0, −3.0)	<0.001
Mean (SD)	−0.4(1.2)	−3.4(0.6)	<0.001	−0.5(1.3)	−3.3(0.62)	<0.001
Agitation, n(%)	131 (37.0%)	11 (9.8%)	<0.001	37 (35.9%)	1 (6.7%)	0.023
Pain assessment, n(%)	112 (31.6%)	40 (35.7%)	0.423	29 (28.2%)	9 (60.0%)	0.014
P–pls score, Median (range)	3.1 (−5.0, 7.0)	2.0 (−2.0, 7.0)	0.193	2.8 (−4.0, 6.5)	3.2 (−2.0, 5.8)	0.598
Mean (SD)	2.6(2.6)	2.3(2.5)	0.398	2.7(2.5)	3.0(2.3)	0.633
>2, n(%)	221 (62.4%)	52 (46.4%)	0.003	69 (67.0%)	10 (66.7%)	0.980
≤2, n(%)	133 (37.6%)	60 (53.6%)		(33.0%)	5 (33.3%)	

High–T means highest body temperature within the observation period; SOFA, Sequential Organ Failure Assessment score; APACHE II, Acute Physiology And Chronical Health Evaluation II score; Plat–p, plateau pressure; P/F ratio was calculated by PaO_2 divided by FiO_2; RR, respiratory rate; MV, mechanical ventilation; Min–vent, minute ventilation; NE, norepinephrine; PEEP, positive end–expiratory pressure; M (range), median (minimal, maximal); P–pls score was the average score of P–pls (physician's preference to light sedation) in the ICU where the patient was admitted.

impact the decision–making of deep sedation. Variables with p–values <0.1 and factors that were considered as a potential impact factor in previous studies, such as highest body temperature, were included in the logistic regression model. For this purpose, logistic regression with backward selection was conducted. The receiver operating characteristics (ROC) curve of the training set and the verification set were drawn, and areas under the ROC (AUC–ROC) curves were calculated to assess the accuracy of the prediction model. All statistical analyses were performed in SPSS v. 25.0. A two–sided p–value <0.05 was regarded as statistically significant.

RESULTS

Patients

A total of 2,133 patients were screened in this study. After applying the inclusion and exclusion criteria, 584 eligible MV patients from 92 ICUs were finally enrolled, including 457 in the light sedation group and 127 in the deep sedation group (**Figure 1**). Baseline comparisons between the light and deep sedation groups in either the training set or the validation set are shown in **Table 1**. The distribution of gender, category of diseases, and age was similar between the light and deep sedation groups. Characteristics of patients such as with/without pain assessment, highest body temperature, positive end–expiratory pressure (PEEP) level, plateau pressure (Plat–p), PaO_2, respiratory rate (RR), and minute ventilation (Min–vent) within the observation period, APACHE II score, and average P–pls score in the recruited ICU were not found to be significantly different in the deep sedation group compared with those in the light sedation group (p >0.05, **Table 1**). Meanwhile, there were significant differences in the proportion of patients with septic shock and those receiving the control mode (referenced to the assisted mode) of mechanical ventilation, SOFA score, level of variables related to oxygenation [FiO_2 and P/F ratio (PaO_2/FiO_2)], and circulatory function (norepinephrine dosage and plasma lactate level) between the light and deep sedation groups in the training set (**Table 1**). Additionally, the proportion of patients recruited from the ICUs characterized with an average of P–pls score $\geq$ 2 was significantly higher in the light sedation group than in the deep sedation group (62.4% vs. 46.4%, p = 0.005) in the training set.

Outcomes Associated With Sedation Depth

The 28–day mortality and the prevalence of delirium during ICU stay are listed in **Table 2**. The 28–day mortality was significantly lower in the light sedation group than in the deep sedation group [10.7% vs. 19.7%, crude OR = 2.218 (1.251, 3.62)]. However, the prevalence of delirium within the observation day increased in the light sedation group in comparison with the deep sedation group [4.2% vs. 0.8%, crude OR (95% CI) = 0.269 (0.035, 2.046)]. By multivariable logistic regression, the adjusted OR (95% CI) of deep sedation for 28–day mortality and delirium was 1.492 (0.828, 2.688) and 0.273 (0.031, 2.382), respectively. Differences between the light sedation group and the deep sedation group were not statistically significant.

Factors Associated With Deep Sedation Practice

The multivariable logistic regression analysis demonstrated that the control mode of mechanical ventilation, plasma lactate level, and SOFA score were independent risk factors associated with deep sedation practice (p < = 0.01, **Table 3**). In addition, the adjusted odds ratio (95% CI) of the average ICU P–pls score $\leq$ 2 for deep sedation practice was 1.861 (1.163, 2.978, p = 0.01).

After establishing the prediction model of the training set, prediction probabilities were estimated in the validation set. The ROC curves of both the training set and the validation set are shown in **Figure 2**. The AUCs of ROC were 0.753 (0.699, 0.806)

TABLE 2 | Risk potential of deep sedation for outcomes in MV patients(n = 584).

	Light sedation	Deep sedation	Crude OR (95% CI)	Adjusted OR (95% CI)
Delirium	19/457 (4.2%)	1 /127(0.8%)	0.269 (0.035, 2.046)	0.273 (0.031, 2.382)
28–day death	49/457 (10.7%)	25/127 (19.7%)	2.128 (1.251, 3.620)	1.492(0.828, 2.688)

Adjusted odds ratios (ORs) were adjusted for gender, age, category of disease, septic shock, SOFA (Sequential Organ Failure Assessment) score, APACHE II (Acute Physiology And Chronical Health Evaluation II) score, P/F ratio (calculated by PaO_2 divided by FiO_2), infused norepinephrine dosage, and plasma lactate level. Light sedation was used as the reference for estimation of OR.

TABLE 3 | Independent risk factors for deep sedation practice (training set, n = 466).

	β	Odds Ratio (95% CI)	p
P–pls (≤2 vs. >2)	0.621	1.861 [1.163,2.978]	0.010
Control Mode of mechanical ventilation	0.958	2.608 [1.591,4.275]	<0.001
Lactate(mmol/L)	0.245	1.278 [1.108,1.472]	0.001
SOFA	0.139	1.149 [1.071,1.231]	<0.001

Multivariable logistic regression was conducted for analyzing independent risk factors for deep sedation practices in the training set. P–pls score was the average score of P–pls (physician's preference for light sedation) in the ICU where the patient was admitted. SOFA, Sequential Organ Failure Assessment score.

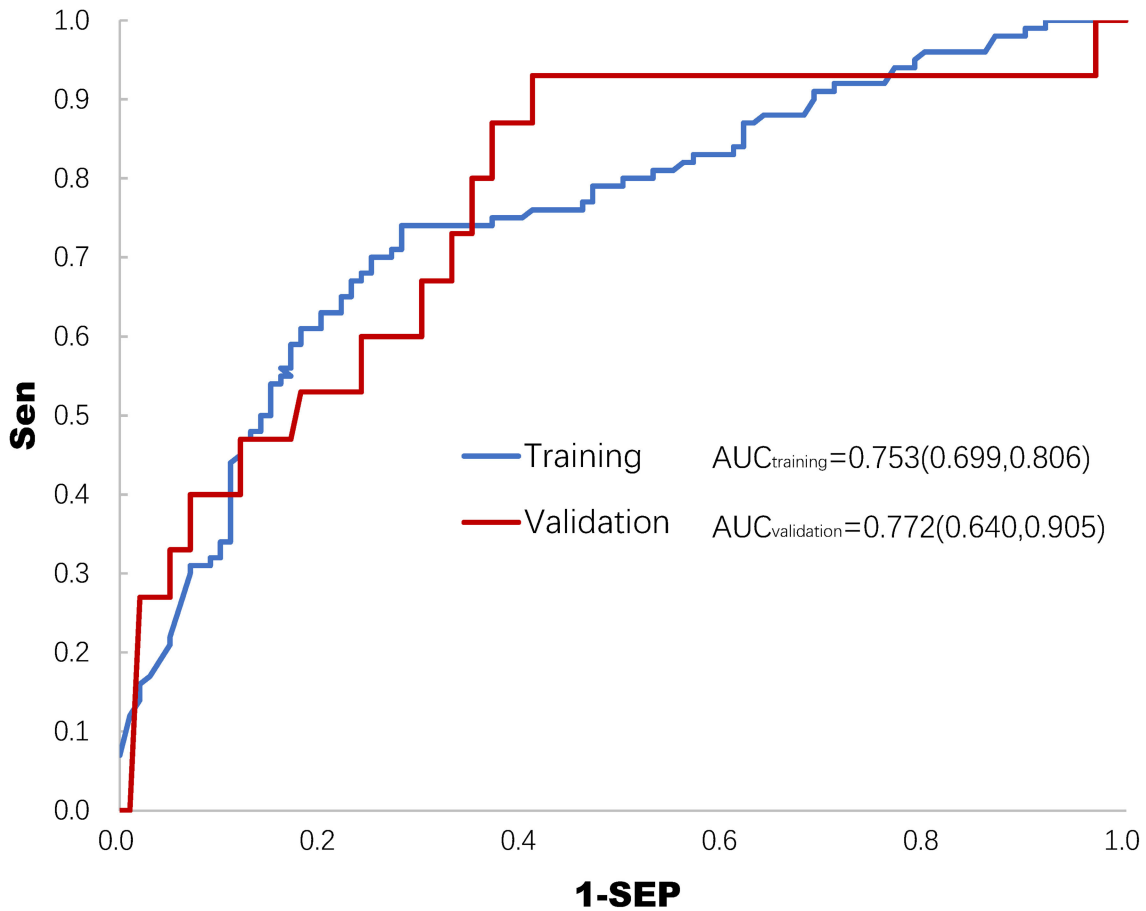

FIGURE 2 | Receiver operating characteristics (ROC) curve of the training set and the validation set for predicting deep sedation practice. AUC, area under the curve of ROC.

and 0.772 (0.640, 0.905) in the training set and the validation set, respectively (**Figure 2**).

DISCUSSION

The main results of this study demonstrated that factors including the SOFA score, plasma lactate level, mode of mechanical ventilation, and average P–pls (physician's preference to light sedation) score in the ICU were significantly associated with deep sedation practice (usually defined as RASS< −2), which likely worsened the 28–day mortality in MV patients. In addition, a logistic regression model was developed and validated to predict the probability of deep sedation practice despite the AUC–ROC being 0.753 (0.699, 0.806) and 0.772 (0.640, 0.905) in the *post hoc* analysis (**Figure 2**). These findings provided important information that either the intensity of stressful stimuli or the ICU physicians' perception of patient tolerability in mechanical ventilation was involved in decision–making for deep sedation practice in MV patients.

The use of analgesics and sedatives is aimed at regulating patient discomfort (18), which is caused by stressful stimuli including both physiological stresses induced by pathophysiological abnormalities or/and intensive care and mental stress (18–21). Meanwhile, overuse of sedatives and analgesics is harmful to host defenses and leads to worse outcomes for MV patients (22, 23). Up to now, increasing data, including the results in this study, have revealed a direct relationship between sedation depth and clinical outcomes (24, 25). These results indicated that MV patients were largely at risk potential for overuse of sedatives and analgesics while RASS was scored below −2. Based on the opinions of experts, deep sedation was recommended only for managing a few specific situations in MV patients, such as severe acute respiratory distress syndrome (ARDS) with ventilator–patient asynchrony or use of neuromuscular blocking agents, severe brain injury with severe intracranial hypertension, and status epilepticus (26–29). Meanwhile, indications for deep sedation or contraindications for maintaining MV patients at light levels of sedation remain unexplained (30). One of the important barriers is how to define the intensity of stimuli–induced discomfort requiring deep levels of sedation. Although a variety of stressful stimuli was reported (20, 21, 31), tools to scale the intensity of stimuli are yet to be developed. In fact, few research studies previously provided evidence regarding the estimation of stressful stimuli and investigated the dose responses of either sedatives or analgesics (or the combined use of both drugs) against the

stimuli. A strength of this study was that some stressful stimuli, in terms of high plasma lactate level, increase in SOFA score, and use of the control mode of mechanical ventilation (shown in **Table 3**), were demonstrated as independent risk factors for deep sedation practice in the management of MV patients. This finding suggested that the intensity of these stressful stimuli should be considered while care providers are titrating the depth of sedation for MV patients. Based on these variables, we recently developed an ensemble model for the prediction of agitation in MV patients who were sedated at light levels of sedation in the same cohort (17). The results indicated that the stressful stimuli could not be successfully attenuated by titrating sedatives as well as analgesics while the MV patients who were predicted with a high risk of agitation were lightly sedated (RASS $\geq$ 2). Therefore, an investigation on the relationship between the intensity of stressful stimuli and the levels of sedation depth is necessary for defining the stimuli–based indications of deep sedation in further study, which will help to promote sedation practices in MV patients.

In addition, it was demonstrated that the physicians' perception of patient tolerance to stressful stimuli was involved in the decision–making for deep sedation practice. In fact, assessment of patients' tolerability during mechanical ventilation remains problematic. By titrating analgesics and sedatives, in clinical practice, a calm and cooperative status was estimated as patient tolerable in mechanical ventilation. Among assessment tools, RASS, which offers broader discrimination in the mild–to–moderate sedation range, is the most commonly used and reliable one to evaluate patient tolerability in mechanical ventilation (32–34). However, the result of the RASS assessment is the transient tolerability of MV patients regulated by the infused analgesics and sedatives. While the intensity of nociceptive stimuli changes because of the occurrence or disappearance of fever, thirst, drainage tube pain, andintestinal colic as well as upregulation or downregulation of supportive therapies such as changes in ventilator settings, significantly, MV patients would become over—or under–sedated as the dosages of sedatives and analgesics were unchanged (30). This partially at least accounts for the frequent and unpredictable agitation as well as oversedation in the real practice. Lacking a reliable tool to scale patient tolerance to stressful stimuli instantly, care providers always face a big challenge in titrating sedatives and analgesics to regulate patient discomfort during mechanical ventilation. The decision–making for the depth of sedation was more likely dependent on their own recognitions and experiences. As reported in previous studies, care providers' concern about patient intolerance to mechanical ventilation such as agitation was an important barrier to the implementation of a minimal sedation strategy (15, 35). Therefore, the development of evidence–based deep sedation indications is critical to avoiding unjustified deep sedation practices in MV patients. As the basis for this task, it is necessary to investigate stressful stimuli, sedative choice (either type or dosage), and patient tolerability as well as their interactions in further research studies (36).

Some limitations to our study should be addressed. First, this is a *post hoc* analysis of a study aimed at developing a model for the prediction of agitation in MV patients maintained under light sedation (30). Some important measurements such as anxiety score and pain score were not collected in this study. In addition, the instant changes of variables before patients were deeply sedated, such as agitation and asynchrony, which would help to spot if the deep sedation was appropriate or not, were not systematically considered in the primary study protocol. Lack of data on these factors was the most important limitation of this study, accounting for partially, at least, the AUC–ROC of the logistic regression model being less powerful [0.753 (0.699, 0.806)] to predict deep sedation. However, the findings of this study demonstrated the feasibility of a prospective, multicentered, large–scale cohort study to define stimulus–based indications for deep sedation in further research. Second, several factors contributed to the low rate of delirium in this study, including excluding patients with delirium assessment reporting positive before the cross–sectional survey, delirium being assessed only within the 24–h observational period, and CAM–ICU assessment being problematic in the deeply sedated MV patients. These are the reasons for the low incidence of delirium in this cohort. Significantly, the lower delirium rate in the deep than in the light sedation group was largely caused by the failure in CAM-ICU assessment in most of the deeply sedated MV patients. Finally, the total dosage of sedatives and analgesics was not provided, which could be used as direct evidence of oversedation.

CONCLUSION

The results of this study demonstrated that, in the 24–h survey, deep sedation practice was frequent and likely worsened 28–day mortality in MV patients. Factors related to the intensity of stressful stimuli such as severity of pathophysiological alternations and intensity of supportive therapies were significantly associated with the probability of deep sedation practice in MV patients. Additionally, the ICU physicians' perception of patients' tolerability in mechanical ventilation was involved in decision–making for deep sedation practice. Besides our findings in this study, meanwhile, several factors could contribute to the behavior of intensive care providers toward deepening sedation (the primary outcome) in clinical practice. These findings suggest that the development of evidence–based deep sedation indications is feasible and, notably, critical to avoid unjustified deep sedation practices in MV patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Our study involving human participants was reviewed and approved by the Ethical Committees of all participating hospitals (approval number from the principal center: 309201906171118).

Informed consent was waived by the ethics committees. The list of 80 participating hospitals (recruiting 92 ICUs) was as follows: 1. The 8th Medical Center of General Hospital of Chinese People's Liberation Army, 100091, Beijing, P. R. China; 2. Peking University Third Hospital; 3. Peking University First Hospital; 4. Peking University People's Hospital; 5. Peking Union Medical College Hospital; 6. Fuwai Hospital Chinese Academy of Medical Sciences; 7. Beijing Tsinghua Changgung Hospital; 8. Beijing Anzhen Hospital of Capital Medical University; 9. Beijing Chaoyang Hospital of Capital Medical University; 10. Beijing TianTan Hospital of Capital Medical University; 11. Beijing Fuxing Hospital of Capital Medical University; 12. General Hospital of Chinese People's Liberation Army; 13. Beijing Youyi Hospital of Capital Medical University; 14. China-Japan friendship hospital; 15. The Fourth Clinical Hospital affiliated to Harbin Medical University; 16. The Third Clinical Hospital affiliated to Harbin Medical University; 17. The Second Clinical Hospital affiliated to Harbin Medical University; 18. The First Clinical Hospital affiliated to Harbin Medical University; 19. Bethune First Hospital Of Jilin University; 20. The Second Hospital of Jilin University 21. The First Affiliated Hospital of Dalian Medical University; 22. The First Affiliated Hospital of Liaoning Medical College; 23. General Hospital of the northern theater Military Region; 24. The First Hospital of China Medical University; 25. Shengjing Hospital of China Medical University; 26. First Hospital of China Medical University; 27. Hebei General Hospital; 28. Hebei Kailuan General Hospital; 29. The fourth hospital of Hebei Medical University; 30. The third hospital of Hebei Medical University; 31. Tianjin Third General Hospital; 32. Fujian Provincial Hospital; 33. The First Affiliated Hospital of Fujian Medical University; 34. The First Affiliated Hospital of Xiamen University; 35. Xiamen Cardiovascular Hospital of Xiamen University; 36. Shanghai Changzheng Hospital; 37. Zhongshan Hospital Fudan University; 38. Renji Hospital Affiliated to Shanghai Jiaotong University School of Medicine; 39. Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine; 40. Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine; 41. The First Affiliated Hospital of Zhejiang University; 42. The Second Affiliated Hospital of Zhejiang University; 43. Zhejiang Provincial People's Hospital; 44. The Institute of Gerontology of Guangdong Provincial People's Hospital; 45. General Hospital of Southern Treater Command; 46. The First Affiliated Hospital of Guangzhou Medical University; 47. The First Affiliated Hospital of Sun Yat-sen University; 48. The First Affiliated Hospital of Guangxi Medical University; 49. The Affiliated Hospital of Guizhou Medical University; 50. Hainan General Hospital; 51. Qingdao Municipal Hospital 52. Qilu Hospital of Shandong University; 53. Shandong Provincial Hospital; 54. Liaocheng People's Hospital; 55. Yantai Yuhuangding Hospital; 56. Zibo Central Hospital; 57. The First Affiliated Hospital of Bengbu Medical College; 58. Anhui Provincial Hospital; 59. Zhongda Hospital Southeast University; 60. General Hospital of Eastern Treater Command; 61. Suzhou Municipal Hospital; 62. Henan Provincial People's Hospital; 63. The First Affiliated Hospital of Zhengzhou University; 64. Xijing Hospital; 65. Wulumuqi General Hospital of Chinese PLA; 66. First Affiliated Hospital, School of medicine, Shihezi University; 67. The First Affiliated Hospital of Xingjiang Medical University; 68. Sichuan Provincial People's Hospital; 69. West China Hospital of Sichuan University; 70. The First Affiliated Hospital of Kunming Medical University; 71. Army Medical Center of Chinese PLA; 72. The First Affiliated Hospital of Chongqing Medical University; 73. Renmin Hospital of Wuhan University; 74. Zhongnan Hospital of Wuhan University; 75. Union Hospital, Tongji Medical College, Huazhong University of Science and Technology; 76. The Second Affiliated Hospital of South China University; 77. The Second Xiangya Hospital of Central South University; 78. The Third Xiangya Hospital of Central South University; 79. The Xiangya Hospital of Central South University; 80. The First Affiliated Hospital of Nanchang University.

AUTHOR CONTRIBUTIONS

PM, LZ, and WS were the major contributors in designing, conducting this study and writing the manuscript. TW, YG, and JL participated in conducting this study and helped to revise the manuscript. All authors read and approved the submitted manuscript.

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REVIEW
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Recent Advances in Videolaryngoscopy for One-Lung Ventilation in Thoracic Anesthesia: A Narrative Review

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Since their advent, videolaryngoscopes have played an important role in various types of airway management. Lung isolation techniques are often required for thoracic surgery to achieve one-lung ventilation with a double-lumen tube (DLT) or bronchial blocker (BB). In the case of difficult airways, one-lung ventilation is extremely challenging. The purpose of this review is to identify the roles of videolaryngoscopes in thoracic airway management, including normal and difficult airways. Extensive literature related to videolaryngoscopy and one-lung ventilation was analyzed. We summarized videolaryngoscope-guided DLT intubation techniques and discussed the roles of videolaryngoscopy in DLT intubation in normal airways by comparison with direct laryngoscopy. The different types of videolaryngoscopes for DLT intubation are also compared. In addition, we highlighted several strategies to achieve one-lung ventilation in difficult airways using videolaryngoscopes. A non-channeled or channeled videolaryngoscope is suitable for DLT intubation. It can improve glottis exposure and increase the success rate at the first attempt, but it has no advantage in saving intubation time and increases the incidence of DLT mispositioning. Thus, it is not considered as the first choice for patients with anticipated normal airways. Current evidence did not indicate the superiority of any videolaryngoscope to another for DLT intubation. The choice of videolaryngoscope is based on individual experience, preference, and availability. For patients with difficult airways, videolaryngoscope-guided DLT intubation is a primary and effective method. In case of failure, videolaryngoscope-guided single-lumen tube (SLT) intubation can often be achieved or combined with the aid of fibreoptic bronchoscopy. Placement of a DLT over an airway exchange catheter, inserting a BB *via* an SLT, or capnothorax can be selected for lung isolation.

Keywords: videolaryngoscope, one-lung ventilation, double-lumen tube, bronchial blocker, difficult airways

INTRODUCTION

In thoracic anesthesia, lung isolation techniques are often required to achieve one-lung ventilation. Double-lumen tubes (DLTs) are widely used in adult thoracic surgery because they provide reliable isolation and separate ventilation. However, owing to their large diameter, high rigidity, and complex configuration, it is not easy to intubate a DLT compared with a routine endotracheal tube (ETT); this difficulty is increased in the case of a difficult airway (1–3). In such cases, it is necessary to secure the airway and maintain oxygenation, and then consider the possibility of lung collapse (2, 3).

Since their advent, videolaryngoscopes have played an important role in airway management, including expected and unexpected difficult airways (4, 5). There are many reports on using videolaryngoscopes in DLT intubation, but their advantages in thoracic anesthesia are not as distinct as in other specialties (6–8). This review summarizes the techniques of videolaryngoscope-guided DLT intubation and discusses the roles of videolaryngoscopy in DLT intubation in normal airways compared with direct laryngoscopy. The different types of videolaryngoscopes for DLT intubation are also compared. In addition, we highlight several strategies to achieve one-lung ventilation in difficult airways using videolaryngoscopes.

OVERVIEW OF VIDEOLARYNGOSCOPY

Videolaryngoscopy is a new type of laryngoscopy that incorporates video systems using micro-camera technology and optical or fiber optical guided transmission (9). GlideScope, invented by John A. Pacey, was the first videolaryngoscope and was officially introduced into clinical practice in 2001 (10). It was first published for resolving difficult intubation in 2003 (11). Various videolaryngoscopes have been developed since 2006. Most videolaryngoscopes contain a light source and an image sensor close to the blade tip. Instead of line of sight with a direct laryngoscope, the videolaryngoscope does not require alignment of the oral, pharyngeal, and laryngeal axes and enables the operator to visualize the glottis on the video screen. Videolaryngoscopy has several advantages over direct laryngoscopy (12, 13), such as a better view of the larynx, increased success rate of intubation, short intubation time, and less force required for intubation (14, 15). The videolaryngoscopy technique is also easy to learn especially for novices (16, 17).

In the past 20 years, videolaryngoscopy has played important roles in airway management (18). It has not only managed expected difficult airways (13, 19), such as in the case of morbid obesity, limited mouth opening, cervical immobility, and oropharyngeal masses, but also unexpected difficult airways (20). This method has also been successfully applied in obstetric anesthesia (21), pediatric anesthesia (22), emergent intubation (23), nasotracheal intubation (24), and awake intubation (25). Therefore, guidelines for difficult airway management have indicated that videolaryngoscopy can be applied as an initial approach for difficult intubation (26) or a rescue technique for unanticipated difficult intubation (18), and all anesthetists should be proficient with the use of a videolaryngoscope (5).

FIGURE 1 | Classfication of videolaryngoscopes. GlideScope image courtesy of Verathon, USA. McGrath series 5 image courtesy of Aircraft Medical, UK. Airtraq image courtesy of Prodol Meditec, Spain. C-MAC image courtesy of KARL STORZ Endoscopy, Germany. UEscope image courtesy of UE Medical Corp, China. Pentax AWS, King Vision image courtesy of Ambu USA. Part of this figure is taken from Healy et al BMC Anesthesiol. 2012; 12: 32. ©2012 Healy et al.; licensee BioMed Central Ltd. Reproduced under the terms of its Creative Commons Attribution License (2.0)

Videolaryngoscopes can be classified as non-channeled or channeled. For example, GlideScope (Verathon Inc, Bothell, WA, USA) (27), McGrath (Aircraft Medical, Edinburgh, UK) (28), Storz (Karl Storz, Tuttlingen, Germany) (29), UEscope (UE Medical Corp, Zhejiang, China) (30), and non-channeled King Vision (Ambu A/S, Ballerup, Denmark) (31) are non-channeled videolaryngoscopes. They are similar to a standard laryngoscope (**Figure 1**). However, it may be difficult to place the tube into the glottis despite obtaining a good laryngeal view when using these devices (18). This difficulty can be resolved by a rigid stylet. A pre-shaped tracheal tube is recommended to facilitate intubation (10, 20). Channeled videolaryngoscopes, such as Airtraq (Prodol Meditec, Vizcaya, Spain) (32, 33), Pentax Airway Scope (Nihon Kohden, Tokyo, Japan) (34), and King Vision (Ambu A/S, Ballerup, Denmark) (35), have a guiding channel. The lubricated ETT is preloaded into the guiding channel. The videolaryngoscope is inserted to obtain a glottic

view in the midline, and the tube is then placed into the trachea through the channel.

In addition, based on the blade angle, videolaryngoscopes can be classified as standard or hyperangulated (36). The Storz V-Mac (37), Storz C-Mac (38), and McGrath MAC devices were designed using a standard laryngoscope blade but with a camera incorporated into the distal tip. After insertion of the blade into the mouth, the glottis can be viewed alongside the blade following the traditional method or on the monitor. GlideScope, McGrath Series 5, Storz D-blade, and Airtraq have hyperangulated blades. They can increase the field and angle of view with less neck flexion and improve the glottic view compared with direct laryngoscopy (27, 39).

Videostylets, such as Optiscope, Trachway, Shikani optical stylet, and Light Wand, are other types of videoscope (36). They differ from traditional laryngoscopes in design and intubating techniques, and they are not considered the first rescue choice after failed intubation with a direct laryngoscope (18). Although they have extensive advantages in limited mouth opening and cervical fixation cases, they are outside the scope of this review.

KEY POINTS OF VIDEOLARYNGOSCOPE-GUIDED DLT INTUBATION

Many studies have reported the application of different types of videolaryngoscopes in DLT intubation, such as GlideScope (40, 41), McGrath (42), Airtraq (43, 44), CEL-100 (45), Pentax Airway Scope (46, 47), C-Mac D blade (48), King Vision (KVL) (49).

Generally, DLT intubation comprises four steps: glottis exposure using a videolaryngoscope, guiding the tip of the DLT into the glottis, advancing the tube through the glottis until it enters the appropriate main bronchus, and confirming the position of the DLT by fibreoptic bronchoscopy (FOB) (50). In the next section, we discuss GlideScope and Airtraq as typical examples of non-channeled and channeled videolaryngoscopes, respectively, to illustrate videolaryngoscope-guided DLT placement.

GlideScope: A Non-channeled Videolaryngoscope

Since its introduction in 2001, GlideScope has been one of the most extensively studied videolaryngoscopes for DLT intubation. GlideScope provides superior glottis views to direct laryngoscopy; however, it can often be difficult to place the DLT into the glottis and advance the tube into the trachea when using GlideScope (40, 51) and other non-channeled videolaryngoscopes (7, 45). Several tips can be used to facilitate DLT intubation using GlideScope.

1. A pre-shaped DLT with a malleable stylet following the curve of the GlideScope should be used (40, 52). An appropriate stylet angle may lead to fewer failed intubation attempts and less airway trauma. Stylets with a 90° angle resulted in easier and faster intubation than those with a 60° angle using the GlideScope in single lumen tube (SLT) intubation (53, 54). Owing to the thick diameter of the DLT, manipulation inside the oropharyngeal space is restricted. Adjusting the DLT into the glottis was more difficult than the placement of the SLT. Hernandez and Wong (40) first described the successful use of GlideScope for DLT placement in a patient with a potentially difficult airway. They recommended bending the stylet of the DLT at 16–20 cm proximal to the tip to follow the curve of the GlideScope. Bustamante et al. (52) and Hsu et al. (41) recommended pre-curving the tube at the distal 10–12 cm of the DLT. The GlideRite DLT Stylet is a semi-rigid intubating stylet recommended by Bussieres et al. (55, 56). However, the optimal shape and angle of the stylet required for successful DLT intubation with GlideScope have not been determined in a randomized controlled study.
2. Sequentially rotate the tube to the desired depth when advancing the DLT. After the distal DLT passes through the vocal cord, difficulty can arise when attempting to advance it with GlideScope, because the distal concavity is directed anteriorly to the tracheal wall, and the axis of the bronchial lumen is nearly perpendicular to the axis of the trachea. Bustamante et al. (52) recommended that after the tip of the bronchial lumen engages in the glottis, the stylet should be removed, and the DLT should be rotated 180° counter-clockwise to facilitate passage of the bronchial cuff. An additional 90° clockwise rotation should then be performed to align the DLT with the left main bronchus. Hsu et al. (57) also reported a modified technique. After the DLT was inserted through the vocal cords, the DLT was advanced gently by rotating in a 90° clockwise direction until resistance was noted. The modified technique saves intubation time and reduces the severity of post-intubation complications, compared with the 180° clockwise rotation of the DLT during the placement of a DLT using GlideScope.
3. Sequential rotation could increase the risk of incorrect tube positioning during videolaryngoscope-guided DLT placement (58). For example, the tip of left-sided DLT could be migrated into the right bronchus. It is important to confirm the DLT position using FOB.
4. Take care to avoid the tracheal cuff scraping by the teeth during DLT insertion.

Airtraq: A Channeled Videolaryngoscope

Hirabayashi and Seo (43) first reported the successful use of Airtraq for DLT intubation in 2007 and recommended Airtraq as an alternative approach for DLT placement.

The Airtraq videolaryngoscope with a guiding channel offers multiple options for visualizing the glottis, including a direct view, AWDR video system, A-360 Wi-Fi camera, universal adapter for smartphones, or Endo cam connection (59–61). The side channel can accommodate a tube with an external diameter of $\leq$19 mm, which allows for the placement of DLTs of 28–41 Fr. The inner surface of the side channel is treated with a concavo-convex pattern to reduce tube friction.

For Airtraq-guided DLT intubation, remove the original stylet, lubricate the DLT and channel, then preload the tube into the channel before intubation. Airtraq is inserted into the midline of the patient's mouth to slide it over the center of the

TABLE 1 | Randomized controlled trials on videolaryngoscope vs. the Macintosh laryngoscope for double lumen tube intubation.

Classification	Videolaryngoscope	Number of studies	References
Non-Channeled	GlideScope	9	Bensghir et al. (65) Hsu et al. (41) Russell et al. (51) Yi et al. (66) El-Tahan et al. (67) Wei and Tian (68) El-Tahan et al. (64) Huang et al. (6) Risse et al. (69)
	McGrath	4	Kido et al. (70) Yao et al. (7) Yoo et al. (71) Bakshi et al. (72)
	CEL-100	1	Lin et al. (45)
	C-MAC D-blade	2	Shah et al. (48) Huang et al. (6)
	King Vision	2	El-Tahan et al. (67) El-Tahan et al. (64)
Channeled	Airtraq	6	Jiang et al. (73) Wasem et al. (74) Hamp et al. (75) El-Tahan et al. (67) El-Tahan et al. (64) Feng et al. (76)

tongue until the glottis structures are identified (16). Once the bronchial cuff passes through the glottis, the DLT is rotated 90° counter-clockwise and further advanced until resistance is felt. The tube is separated from the channel, and the laryngoscope is removed from the mouth (62, 63). Finally, the position of the DLT is checked using FOB. As the DLT was loaded in the channel during Airtraq-guided DLT intubation, one distinct advantage is that Airtraq has zero tracheal cuff rupture, compared with the Macintosh, GlideScope and non-channel KVL (64).

COMPARISON OF VIDEOLARYNGOSCOPY AND DIRECT LARYNGOSCOPY FOR DLT INTUBATION IN NORMAL AIRWAYS

To date, 19 prospective randomized controlled studies have reported the efficacy of six videolaryngoscopes for DLT intubation compared with the Macintosh laryngoscope (**Table 1**) in patients with predicted normal airways. The main outcomes included glottic view, intubation time, success rate, intubation difficulty score, incidence of malposition, postoperative sore throat, hoarseness, related complications, and intubation-related stress response. Except that videolaryngoscopy can provide a better view of the glottis than the Macintosh, other findings were inconsistent between different studies. This heterogeneity may be attributed to the performer's experience, type of videolaryngoscope used, and primary outcome definition.

Two studies demonstrated that GlideScope provided a shorter intubation time and a lower incidence of sore throat and hoarseness than the Macintosh laryngoscope (41, 65). In contrast, Russell et al. (51) reported that GlideScope for DLT intubation resulted in a longer intubation time, increased intubation difficulty, and increased incidence of intubation-related complications. These contradictory results may be explained by the diversity of operator experience in using GlideScope for DLT intubation. Yi et al. (66) also reported that GlideScope prolonged the DLT intubation time compared with Macintosh, although it improved the exposure of the glottis because the method is more complex.

Wasem et al. (74) found no significant differences between Airtraq and Macintosh regarding intubation time or the number of attempts required for successful DLT insertion; however, a higher incidence of hoarseness was observed with Airtraq. Other studies have also reported that videolaryngoscopes are not superior to the conventional Macintosh laryngoscope for DLT intubation in patients with anticipated normal airways (7, 44, 64, 75, 77).

In a systematic review and meta-analysis, Liu et al. (58) found that videolaryngoscopy provided a higher success rate of DLT intubation at the first attempt and lower incidences of intubation-related injuries and sore throat. However, videolaryngoscopy increased the incidence of DLT mispositioning. According to the performer's experience, the subgroup analysis showed the success rate at the first attempt with a videolaryngoscope was higher than that with a Macintosh laryngoscope for experienced performers. However, this advantage was not observed in novices. Additionally, the time to DLT intubation was comparable between the videolaryngoscope and the Macintosh laryngoscope. However, the reported outcomes were highly heterogeneous, likely due to the different definitions and types of videolaryngoscopes used in the studies.

Kim et al. (78) performed a network meta-analysis of 23 studies. The patients were classified into four groups according to the types of the laryngoscopes: channeled videolaryngoscope, non-channeled videolaryngoscope, videostylet, and Macintosh laryngoscope. They found that most videoscopes showed higher success rates in the first attempt but an increased risk of DLT malposition than Macintosh. Videolaryngoscopes, particularly non-channeled videolaryngoscopes, seemed time-consuming compared to Macintosh. Channeled videolaryngoscope was also associated with a higher risk of oral mucosal injury but did not increase the risk of sore throat.

Compared with the Macintosh laryngoscope, the lifting forces on the base of the tongue were reduced with the videolaryngoscope (15). However, the cardiovascular response following DLT intubation was not analyzed in the meta-analysis. There are three studies on the cardiovascular response following DLT intubation, but the conclusions differ. Hamp et al. (75) found that arterial blood pressure, heart rate, and catecholamine levels were comparable after DLT intubation using Airtraq and Macintosh. Wei and Tian (68) reported that GlideScope induced milder circulatory fluctuations than did Macintosh according to the change in systolic blood pressure. Feng et al. (76) found that Airtraq-guided DLT

TABLE 2 | Random controlled studies comparing different types of videolaryngoscopes for double lumen tube intubation.

References	Groups	Patients' characteristics	Main outcomes*
Yi et al. (62)	Airtraq ($n = 36$) GlideScope ($n = 35$)	Predicted normal airways	Airtraq provides shorter intubation time, better glottic view, less MAP, HR than GlideScope
El-Tahan et al. (67)	Airtraq ($n = 21$) GlideScope ($n = 21$) Non-channeled KVL ($n = 21$)	Manikin: simulated easy and difficult airways	In easy airway, GlideScope provides shorter intubation time and less intubation difficulty scores than Airtraq and KVL; In difficult airway, KVL had higher intubation difficulty scores than GlideScope and Airtraq
El-Tahan et al. (64)	Airtraq ($n = 35$) GlideScope ($n = 34$) Non-channeled KVL ($n = 32$)	Predicted normal airways	Compared with GlideScope, the Airtraq resulted in shorter times for DLT intubation, a lower score of difficult intubations and fewer optimization maneuvers
Wan et al. (63)	Airtraq ($n = 45$) McGrath Series 5 ($n = 45$)	Predicted normal airways	Airtraq provides shorter intubation time than McGrath Series 5
Belze et al. (79)	Airtraq ($n = 36$) GlideScope ($n = 36$)	Predicted or known difficult airway	No significant difference in outcomes
Ajimi et al. (80)	Airtraq ($n = 30$) AWS-200 ($n = 30$)	Predicted normal airways	Airtraq provides shorter intubation time than AWS-200
Chang et al. (81)	Lighted Stylet ($n = 32$) GlideScope ($n = 32$)	Predicted normal airways	Lighted stylet allowed easier advancement of the DLT toward the glottis and reduced time for DLT intubation compared with GlideScope.
Huang et al. (6)	C-MAC(D) ($n = 30$) GlideScope ($n = 30$)	Predicted normal airways	C-MAC(D) provides better glottic view, shorter intubation time and less difficulty score of DLT delivery and insertion than GlideScope

**This table just lists the significantly different outcomes between groups. If the outcomes were comparable between groups, they were not listed.*
DLT, double lumen tube; HR, heart rate; MAP, mean arterial pressure.

intubation required a higher EC50 of remifentanil for inhibiting cardiovascular responses compared to Macintosh when induced with a target-controlled infusion of propofol. This indicated that the cardiovascular response during DLT intubation was more intense with Airtraq videolaryngoscopy than with direct laryngoscopy.

Taken together, compared with a direct laryngoscope, the significant advantage of videolaryngoscope-guided DLT intubation is that it improves glottis exposure and increases the success rate at the first attempt. It does not show any advantage in intubation time; moreover, it increases the incidence of DLT mispositioning in patients with normal airways. Thus, videolaryngoscopy is suitable for DLT intubation. However, it is not considered as the first choice for patients with anticipated normal airways, particularly for anaesthesiologists with limited experience in videolaryngoscope-guided DLT intubation.

COMPARISON OF DIFFERENT TYPES OF VIDEOLARYNGOSCOPES FOR DLT INTUBATION

There are some differences among the different types of videolaryngoscopes. A channeled videolaryngoscope provides an adjacent passage to advance the DLT toward the glottis but limits the ability to manipulate the tube. A non-channeled videolaryngoscope, particularly one with an angulated blade, requires a stylet preshaped to follow the curve of the blade, and the extreme angulation of these blades may complicate tube delivery into the trachea. Eight studies have compared videolaryngoscopes for DLT intubation, mainly focusing on the comparison between channeled and non-channeled, such as Airtraq vs. GlideScope or McGrath Series 5 (**Table 2**). There is no consensus regarding which videolaryngoscope is optimal for DLT intubation.

Consistently, Yi et al. (62), El-Tahan et al. (64), and Wan et al. (63) reported that Airtraq provided more rapid intubation of DLT than GlideScope or McGrath; however, the success rates at the first attempt, intubation difficulty score, DLT malpositioning, and intubation-related complications were comparable. The authors attributed the longer intubation time to the use of a molded stylet and a steering technique. These results are different from a manikin study (67) in which they found a longer intubation time and greater intubation difficulty with Airtraq than with GlideScope. The authors attributed the contrary findings to the diversity of prior operator experience and the inherent problems (e.g., high resistance because of the simulator material) in all manikin studies. In addition, El-Tahan et al. study (64) has shown that the Airtraq had an advantage in avoiding tracheal cuff ruptures during insertion of the DLT, compared with GlideScope and non-channel KVL, but a network meta-analysis showed the oral mucosal damage occurred most frequently with the channeled videolaryngoscope (78).

Both Airtraq and AWS-200 are channeled videolaryngoscopes. A recent study (80) described that DLT intubation was quicker with Airtraq than with AWS-200. The

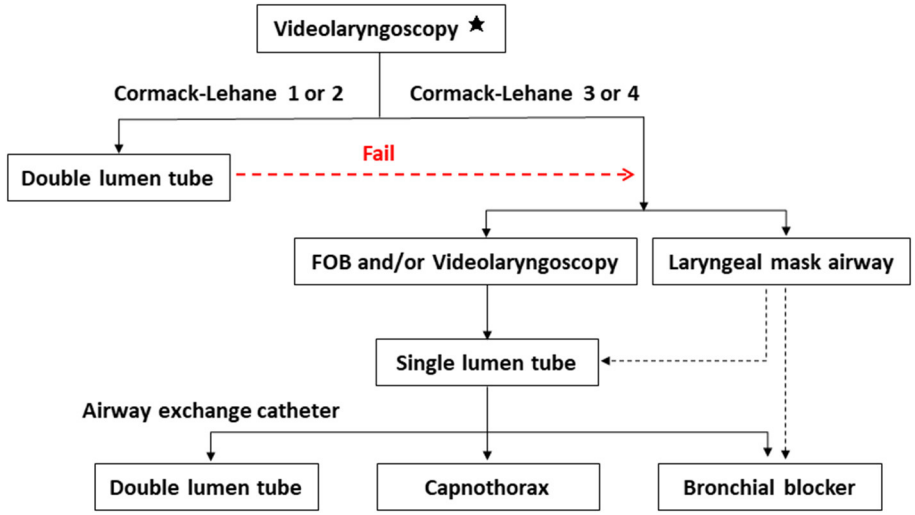

FIGURE 2 | Several strategies to achieve one-lung ventilation in difficult airways using videolaryngoscopes. *Backward, upward and rightward pressure of larynx (BURP) maneuver is used to improve glottic exposure if required. Using videolaryngoscopy, the glottic view is determined according to Cormack-Lehane classification. Grade 1, most of the glottis is visible; grade 2, partial glottis is visible; grade 3, only the epiglottis is visible; grade 4, not even the epiglottis can be seen. If the glottic view is adequate, a double lumen tube is placed with the guide of videolaryngoscope. In case of failure or inadequate glottic view, a single lumen tube is placed with FOB and/or videolaryngoscope, or the patient is secured with a laryngeal mask airway. Then one-lung ventilation is achieved through exchange of a double lumen tube over an airway exchange catheter, inserting a bronchial blocker, or capnothorax. FOB: fibreoptic bronchoscopy.

authors explained that the difference was attributed to the shape and special treatment of the side channel with Airtraq.

Both GlideScope and C-MAC(D) are non-channeled videolaryngoscopes. The blade of GlideScope has an angulation of 60°, while the C-MAC(D) videolaryngoscope has an angulation of 40°. In the study by Huang et al. (6), the DLT insertion time was shorter with C-MAC(D) than with GlideScope. However, they found no differences in the success rate, DLT malposition, and incidences of intubation-related complications.

All of the above studies were performed in normal airways. Belze et al. (79) enrolled elective thoracic patients with a predicted difficult intubation score of ≥7 and compared the efficacy of GlideScope and Airtraq for DLT intubation. They found no differences in the overall success rate, visualization of the glottis, intubation time, and side effects between the two videolaryngoscopes. Thus, the success rate of DLT intubation for difficult airways is not dependent on the videolaryngoscope used.

Taken together, current evidence does not indicate which videolaryngoscope is superior to others for DLT intubation in glottic view, intubation success, first attempt intubation, and incidence of mispositioning. Although a slight difference in intubation time can be observed between different videolaryngoscopes, there was not much clinical significance.

CHOICES OF VIDEOLARYNGOSCOPE IN MANAGING DIFFICULT AIRWAYS IN THORACIC SURGERY

Difficult airways can be divided into the following five categories: difficult facemask or supraglottic airway (SGA) ventilation, difficult SGA placement, difficult laryngoscopy, difficult tracheal intubation, and failed intubation (26). In thoracic surgery, difficult airways are very challenging. The first challenge lies in securing the airway and maintaining oxygenation; the second involves achieving one-lung isolation using DLT or a bronchial blocker (BB) (3). Due to the distinct advantages of videolaryngoscopy, several options can be considered for managing difficult thoracic airways with videolaryngoscopes (**Figure 2**).

Videolaryngoscope-Guided DLT Intubation

Several case reports (40, 46, 49, 60) have reported that videolaryngoscopes successfully managed the DLT intubation for patients with difficult airways (**Table 3**). Lin et al. (89) reported that in cases of difficult laryngoscopy and failed DLT intubation with Macintosh, using the CEL-100 videolaryngoscope improved the glottic view with a ∼90% success rate for DLT insertion. A recent randomized clinical trial (71) reported that the McGrath videolaryngoscope provided a better glottic view and decreased the overall DLT intubation difficulty score in patients with simulated difficult airways through manual in-line stabilization of the cervical spine. Belze et al. (79) reported no difference in the overall success rates, glottic visualization, intubation time, and side effects between GlideScope and Airtraq for DLT intubation in difficult airways.

Based on a detailed evaluation of the airway history, physical examination, and additional evaluation in cases of anticipated difficult laryngoscopy or difficult intubation, if a DLT is applicable, the performer may reasonably choose a videolaryngoscope as a primary intubation tool. The choice of videolaryngoscope is based on individual experience, preference, and availability. Studies have demonstrated a correlation between

intubation success and the operator's proficiency in the device rather than the device used (58, 79).

If the glottic view is adequate, but guiding the tip of the DLT into the glottis or advancing the tube pass through the glottis is difficult, videolaryngoscopy combined with FOB may be an effective approach. In the study by Imajo et al. (87), Lai and Wu (90), a fibreoptic bronchoscope was used as a stylet and placed through the bronchial lumen. Under videolaryngoscopy observation, they guided the tip of the tube to the glottis using FOB and facilitated DLT intubation. This hybrid method has an additional benefit of real-time visualization of all procedures, allowing the anaesthesiologist to ensure successful and safe intubation. For patients with restricted neck movement or limited mouth opening, a videostylet may be a useful alternative tool for DLT placement (91, 92).

Although awake fibreoptic DLT intubation is a good airway management option for an anticipated difficult airway, it has several disadvantages, such as the difficulty of the technique, and anatomical structures, such as upper airway soft tissue resistance, which can make it difficult to intubate. Previous reports (83, 86, 93) have described several alternatives to FOB, such as GlideScope, Airtraq, and videostylets that can be effectively used in patients with difficult airways for awake DLT intubation.

Videolaryngoscope-Guided SLT Intubation

It is more difficult to intubate a DLT than a standard SLT. For anaesthesiologists with limited experience in thoracic anesthesia, although videolaryngoscopes can improve glottic exposure in patients with difficult airways, videolaryngoscope-guided DLT intubation can fail (82). Videolaryngoscope-guided SLT placement is familiar to most anaesthesiologists. In this situation, the first choice is to secure the airway with an SLT under videolaryngoscope guidance. Otherwise, the combined use of videolaryngoscopy and FOB can be used for SLT intubation (94). Therefore, we must now consider how to achieve lung isolation. In the following section, several strategies are considered for lung isolation after successful SLT insertion.

SLT Exchange for DLT Using an Airway Exchange Catheter

Airway exchange catheters (AECs) are an important airway-assisted tool for thoracic anesthesia. For patients with difficult airways who require one-lung ventilation, one commonly used option is to intubate with an SLT and then exchange it with a DLT over an AEC. If postoperative ventilation is necessary, the AEC is also used to exchange the DLT for an SLT.

Chen et al. (82) reported a case of an unanticipated difficult airway in which GlideScope-guided DLT intubation failed in two attempts, while the SLT was successfully inserted on the first attempt. The DLT was then successfully placed over an AEC under the guidance of GlideScope. Poon and Liu (84) described using an AEC alongside AWS-S100 guidance for DLT placement in two cases of difficult laryngoscopy. Although the tracheal tube guiding channel of the disposable rigid blade (PBlade) used in this study cannot accommodate DLTs, Airway Scope can guide bougies or AECs into the trachea and then railroad a DLT over them.

McLean et al. (95) reported a failure rate for exchanging an SLT with DLT of 40%. Tube impingement on the arytenoids or epiglottis is often encountered during the exchange of the DLT over an AEC. The fit of the AEC in the DLT is vital and should be checked before use (82). Further, videolaryngoscopy can be used to visualize the railroading process of the DLT over an AEC. It can also lift the supraglottic tissue to avoid resistance while advancing the DLT into the trachea. Mort and Braffett (96) compared conventional and videolaryngoscopes for ETT exchange in high-risk, difficult airways; they observed that videolaryngoscopy-based ETT exchange over an AEC provided efficient and timely ETT passage and fewer attempts due to improved glottic visualization.

Although McLean et al. (95) reported that the failure rate for postoperative DLT to SLT exchange was 0%, this result should be interpreted cautiously. If DLT placement is extremely difficult, it can be continued to maintain ventilation after surgery until the patient is fully awake. Suzuki et al. (97) reported that using two AECs reduced the risk of tube impingement into the trachea during DLT to SLT exchange.

SLT With BBs

Tube exchange has a failure risk not only from DLT to SLT, but also from SLT to DLT. This could also be associated with pneumothorax (95). It is more convenient to use BBs in such cases, another commonly used option for one-lung ventilation (98). BBs are advantageous when a DLT cannot be used, such as in pediatric patients undergoing thoracic surgery, in whom only nasotracheal intubation is possible, and patients with tracheal tumors or abnormalities. Another advantage is that BBs can provide a selective block of the pulmonary segment and postoperative tube exchange is unnecessary.

There are two methods for BB placement: intraluminal and extraluminal. Briefly, in intraluminal placement, the BB is typically inserted into the lumen of the SLT, along with an FOB to guide the BB to an optimal position. Thus, intraluminal placement of BBs requires a large SLT diameter. Intraluminal BB placement seems easy after SLT insertion, but it is difficult to control the BB and FOB simultaneously, even when a large tube is used (99).

Extraluminal BB placement has been described in adults and children (100, 101). Templeton et al. (99) reported that extraluminal BB placement was safe, adequate to excellent surgical exposure, and faster than intraluminal placement. However, it may be difficult to place the BB in cases of poor glottic exposure using the Macintosh laryngoscope. Recently, several reports described successful extraluminal BB and SLT placement via videolaryngoscopy (102, 103). As the BB is very thin and long, it is difficult to control the direction during extraluminal placement under videolaryngoscopy. A guiding tube can be self-made from a routine tracheal tube using an Airtraq videolaryngoscope (103). In children aged <2 years, a 5F Arndt BB was bent at a 35–45° angle at 1.5 cm proximal to

TABLE 3 | Videolaryngoscope-guided double lumen tube intubation in difficult airways.

References	Device	Patients' characteristics	Awake (yes/no)	Note
Hernandez and Wong (40)	GlideScope	Anticipated difficult airway (BMI 34, Mallampati 3)		
Chen et al. (82)	GlideScope	Unanticipated difficult airway		GlideScope guided SLT placement, then exchange DLT with AEC
Onrubia et al. (83)	GlideScope	Predicted difficult airway and broncho aspiration risk	Yes	
Suzuki et al. (46)	Pentax AWS	Cormack-Lehane grade 2b with Macintosh 4 blade		Remove the back plate of the tube channel
Poon and Liu (84)	Pentax AWS	Two patients with difficult conventional laryngoscopy		AWS guided placement of AEC or bougie first
Sano et al. (85)	Pentax-AWS	A patient with severe rheumatoid arthritis with restricted mouth opening and head tilting		With the newly developed Intlock for DLT
Salazar Herbozo et al. (86)	Airtraq	Two expected difficult patients	Yes	
Ajimi et al. (60)	Airtraq	A case of intubation difficulty (micrognathia)		With the universal adapter for smartphones
El-Tahan et al. (49)	Non-channeled King Vision	A morbidly obese patient (BMI 41.7), a short thyromental distance and a limited mouth opening		
Imajo et al. (87)	Broncho fiberscope combined with McGRATH MAC	Previous upper cervical spine surgery, a small jaw and restricted mouth opening		
Goh and Kong (88)	McGrath	A known difficult airway, bronchopleural fistula, and acute respiratory distress syndrome	Yes	
Lin et al. (89)	CEL-100	Failed DLT intubation with Macintosh		48 Cases
Belze et al. (79)	GlideScope vs.Airtraq	Patients with a predicted difficult intubation score of at least 7		RCT
Yoo et al. (71)	McGrath vs. Macintosh	Patients with a simulated difficult airway		RCT

AEC, airway exchange catheter; BMI, body mass index; DLT, double lumen tube; SLT, single lumen tube; RCT, randomized controlled trial.

the balloon when using Storz C-MAC (104). These tips are very useful for extraluminal BB placement under videolaryngoscopy.

The VivaSight™ SLT, in combination with a BB, is a new method for one-lung ventilation. VivaSight™ SLT is a new generation ETT that incorporates a high-resolution imaging camera and a light source at its tip. It has been reported for BB placement without the aid of FOB and can provide real-time and continuous monitoring of the BB position (105–107).

Rapid lung collapse with a BB is not associated with the type of device used but with the method of use (108). For example, when using an F_IO2 of 1.0 before one-lung ventilation, an apnoeic period of 30–60 s at the time of the pleural incision by disconnection of the breathing circuit and transient deflation of the BB balloon results in rapid lung collapse (109).

SLT With Capnothorax

Traditionally, lung isolation offers excellent surgical exposure during thoracic surgery. However, some complications are associated with one-lung ventilation, such as hypoxemia, bronchoalveolar injury, and postoperative pulmonary complications (110, 111). SLT intubation and CO_2 insufflation of artificial pneumothorax were introduced into thoracoscopy very early. In 1994, Wolfer et al. (112) studied the effects of CO_2 insufflation on haemodynamic parameters during thoracoscopy and reported promising results. SLT intubation and CO_2 insufflation have been previously described in various thoracoscopic procedures (113, 114), including thoracoscopic esophagectomy (115, 116), and have proven to be feasible, efficient, and safe. SLT intubation and CO_2 insufflation can be an alternative to one-lung ventilation for minimally invasive thoracic surgery, particularly when expertise for DLT placement is unavailable or when an operation is very short and simple, such as pleural effusion drainage and pleural biopsies (117). Yeh and Hsu reported an alternative method to achieve lung isolation using artificial pneumothorax under spontaneous breathing with ETT placement in patients with limited mouth opening (118).

When dealing with unanticipated difficult airways, if videolaryngoscopy fails, a laryngeal mask airway or surgical airway can be considered to secure the airway (5). An SLT can be inserted via the intubating laryngeal mask airway, and then a BB can be used to achieve one-lung ventilation. Alternatively, a BB can be directly inserted via the laryngeal mask airway (119, 120). For patients with tracheostomy, shortened DLTs or BBs can be considered to achieve lung isolation (2, 121).

CONCLUSION

Videolaryngoscopy plays an important role in thoracic airway management. Either non-channeled or channeled videolaryngoscope is suitable for DLT intubation. It can improve

glottis exposure and increase the success rate at the first attempt. However, it has no advantage in saving intubation time and increases the incidence of DLT mispositioning for patients with normal airways. Thus, it is not considered as the first choice for patients with anticipated normal airways. Current evidence does not indicate which videolaryngoscope is super to another one for DLT intubation. The choice of videolaryngoscope is based on individual experience, preference, and availability. For patients with difficult airways, videolaryngoscope provides multiple options to achieve one-lung ventilation. Due to the distinct advantages in glottic view, videolaryngoscope-guided DLT intubation is a primary and effective method. Nevertheless, it requires training, particularly for novices and anaesthesiologists with limited experience in videolaryngoscope-guided DLT intubation. In case of failure, videolaryngoscope-guided SLT intubation can be achieved because it is familiar to every anesthetist or combined with the aid of fibreoptic bronchoscopy. Placement of a DLT over an AEC, inserting a BB via an SLT, or capnothorax can be selected for lung isolation.

AUTHOR CONTRIBUTIONS

WY and CZ: conception or design of the work. ML and WY: literature review and draft of the manuscript. CZ and AL: critical revision of the manuscript. WY: take responsibility for data integrity and accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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Developmental Trends and Research Hotspots in Bronchoscopy Anesthesia: A Bibliometric Study

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Background: This study discusses the developmental trends and research hotspots in bronchoscopy anesthesia in the past six decades.

Methods: The original and review articles published from 1975 to June 2021 related to bronchoscopy anesthesia were retrieved from the Web of Science Core Collection (WoSCC). Three different scientometric tools (CiteSpace, VOSviewer, and Bibliometrix) were used for this comprehensive analysis.

Results: There was a substantial increase in the research on bronchoscopy anesthesia in recent years. A total of 1,270 publications were retrieved up to June 25, 2021. Original research articles were 1,152, and reviews were 118, including 182 randomized controlled trials (RCTs). These publications were cited a total of 25,504 times, with a mean of 20.08 citations per publication. The US had the largest number of publications (27.6%) and the highest H-index of 44. The sum of publications from China ranked second (11.5%), with an H-index of 17. Keyword co-occurrence and references co-citation visual analysis showed that the use of sedatives such as dexmedetomidine in the process of bronchoscopy diagnosis and treatment was gradually increasing, indicating that bronchoscopy anesthesia was further progressing toward safety and comfort.

Conclusion: Based on a bibliometric analysis of the publications over the past decades, a comprehensive analysis indicated that the research of bronchoscopy anesthesia is in a period of rapid development and demonstrated the improvement of medical instruments and surgical options that have significantly contributed to the field of bronchoscopy anesthesia. The data would provide future directions for clinicians and researchers in relation to bronchoscopy anesthesia.

Keywords: VOSviewer, CiteSpace, bibliometric, bronchoscopy, anesthesia

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BACKGROUND

Bronchoscopy is the main means for the examination, diagnosis, and treatment of various airway diseases and conditions involving the tracheobronchial tree (1–3). Fiberoptic bronchoscopy (FB) examination is an invasive examination, causing more harmful irritations such as severe reflux and aspiration in patients than gastroscopy. Pain and fear make some patients reject the FB procedure

and not cooperate (4). However, the application of anesthesia can relieve the pain and nervousness in patients and promote the wide use of painless FB. Therefore, anesthesia is also an important link in the diagnosis and treatment process using bronchoscopy (5). Not only the complexity and risk of the operation but also the safety, comfort, and feelings of the patient need to be factored in when planning the anesthesia.

Bibliometrics is a quantitative and qualitative analysis method that uses mathematical and statistical tools to measure the interrelationships and impacts of publications within a given area of research (6, 7). This method can assess the publications and developmental trends in a scientific field and reveal key research directions by analyzing databases and characteristics of the publications. Particularly, bibliometric analysis can be used to summarize developmental trends and research hotspots of various diseases, such as acute lung injury, sacral fracture surgery, and pain treatment (8–10). However, there are few quantitative studies that analyze the research on bronchoscopy anesthesia. In this study, we used bibliometrics to analyze the development, trends, and hotspots of research on bronchoscopy anesthesia, hoping that the results could provide clinical anesthesiologists and related researchers with useful information in this field.

MATERIALS AND METHODS

Data Sources and Retrieval Strategies

The original data used in this study were downloaded from the SCI-expanded database in the Web of Science Core Collection on 25 June 2021. The search was finished on the same day to eliminate any bias caused by the database update. The search strategies used in this study were as follows: [TS = (bronchoscopies OR bronchoscopic OR bronchoscopy OR tracheoscopic OR tracheoscope OR tracheobronchoscope) AND TS = (anesthesia OR anesthesia OR anesthesi* OR anaesthesi*)]. Only original articles and reviews written in English were included, and the detailed screening process is shown in **Figure 1**.

Data Collection

Raw data were initially extracted from the Web of Science SCI-expanded database. Two authors (KM and YW) independently searched information online and set the primary database, including the number of papers, titles, keywords, journals, institutions, Hirsch index (H-index), countries/regions, and citations, and reached a consensus after making comparisons. Although inaccurate analysis might not be avoided completely due to multiple versions of cited references and different forms of cited journals, we believe that most original data are reliable. Before data analysis by VOSviewer v.1.6.11, a thesaurus file was used for merging some duplicates into one word, correcting the misspelled elements, and deleting irrelevant words. Finally, the cleansed data were used for bibliometric analysis.

Statistical Analysis

Microsoft Excel 2016 was used to analyze the total number of publications (Np), citation frequency, the number of citations without self-citations (Nc), countries, institutes, journal sources, H-index, and impact factor (IF). The H-index is a mixed quantitative indicator used to evaluate the academic productivity and the academic contribution of a researcher, and it could also be used to describe the publication output of an institution, a nation, or a journal (11, 12). The IF is an important indicator that measures the quality and impact of medical journals (13).

Furthermore, literature keywords, co-cited frequencies, and research hotspots were evaluated in the network analysis. VOSviewer (1.6.11) was used to identify productive countries/regions, institutions, and keywords, and to construct related visual networks. In the VOSviewer network maps, different nodes indicate components, such as countries/regions and institutions (14). The size of the nodes reflects the number of studies or co-occurrence frequencies. The larger the node, the more times the term appears. Links between nodes represent the co-occurrence relationships, and the size of the links indicates the co-occurrence frequencies of the two nodes. The link strength between nodes reflects the co-occurrence frequency of terms. The total link strength is the sum of the link strengths of a term relative to all other terms. CiteSpace (5.7.R5W), a software tool for creating visual maps and exploring maps based on bibliographic data (15), was used to detect the references with strong citation burstness to identify emerging topics. R (Version 4.0.2) is the language and environment for statistical computing and graphics. The Bibliometrix package in R was used to further illustrate the changes in the annual document (16).

RESULTS

Overview of Publications on Bronchoscopy Anesthesia

A total of 1,270 publications were extracted from Web of Science. Based on the inclusion criteria, all the publications related to tracheoscopy anesthesia were extracted from Web of Science. The total number of citations was 25,504 (23,136 without self-citation), and the average citing frequency was 20.08 times per article. The H-index of all the publications related to bronchoscopy anesthesia was 70.

The Annual Trends of Bronchoscopy Anesthesia-Related Publications

The annual trends of the publications related to bronchoscopy anesthesia was shown in **Figure 2A**. It was found that the first article in this field appeared in 1975. From then on, the years could be divided into two periods using the exponential growth model: period one, covering 15 years between 1975 and 1990, and period two, from 1990 to the present. Period one was a rudimentary stage in the research on bronchoscopy anesthesia.

Abbreviations: FB, fiberoptic bronchoscopy; DLT, double-lumen tube; EBUS, endobronchial ultrasound; COPD, chronic obstructive pulmonary disease; LVR, lung volume reduction; HFJV, high-frequency jet ventilation; LA, local anesthesia; TEF, tracheoesophageal fistula; NO, nitrous oxide; LMA, larynx mask airway; GA, general anesthesia; VBN, virtual bronchial navigation, ECMO, extracorporeal membrane oxygenation; APC, argon plasma coagulation; ENB, electromagnetic navigation bronchoscopy; OSA, obstructive sleep apnea; SEMS, self-expandable metallic stent; OSA, obstructive sleep apnea; CT, computed tomography; BAL, bronchoalveolar lavage; TBNA, transbronchial needle aspiration; PDT, percutaneous dilatational tracheostomy; TIVA, total intravenous anesthesia; TBLB, transbronchial lung biopsy.

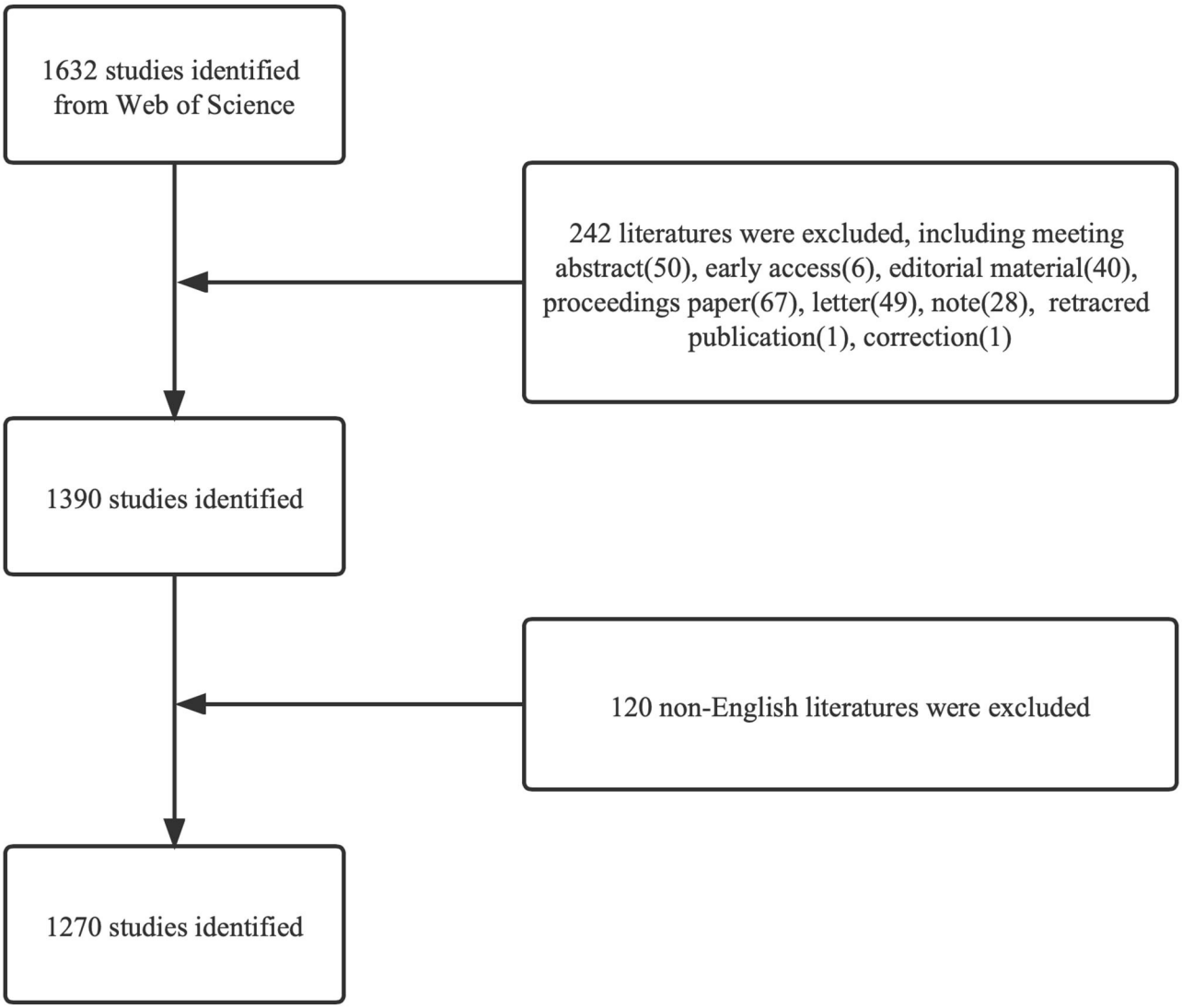

FIGURE 1 | Flowchart of the screening process.

The number of publications per year was less than ten, with a mean of 2.4, showing no significant increase because the theories concerning bronchoscopy anesthesia were not well developed at that time. In period two, the Np increased gradually, and especially since 2014, the number of articles issued each year exceeded 60 reaching the peak (75, 6%) in 2018 because of the rapid development of professional theories in this field during this period. In general, the Np increased from 5 in 1975 to 74 in 2020, demonstrating that bronchoscopy anesthesia had attracted increasing attention and interest among global researchers.

Global Contribution to Publications on Bronchoscopy Anesthesia

As shown in **Figure 3A**, the color block of the US, China, and Europe was darker than that of other countries and regions, representing greater contributions to the research of bronchoscopy anesthesia. **Figure 3B** showed the top 10 countries/regions of all publications in order of the number of publications. The US ranked first in the number of publications (351, 27.6%), followed by China (146, 11.5%) and the UK (90, 7.1%). According to the growing trend in different countries (**Figure 2B**), the US took the lead in the research of bronchoscopy anesthesia and contributed the most with a steady upward trend. It is worth noting that China began research in this field relatively late in 2000, but it has shown an apparent growing trend in this field since 2009. The growth trend in the UK was more fluctuating compared with the US and China. **Figure 4** showed the top 10 countries with Np, Nc, and H-index, which were considered key measures designed to evaluate the quality of papers. The published literature in the US was cited 7,422 times, ranking first in all countries/regions, and achieved the highest value of H-index of 44 as well. The second most-cited country was the UK,

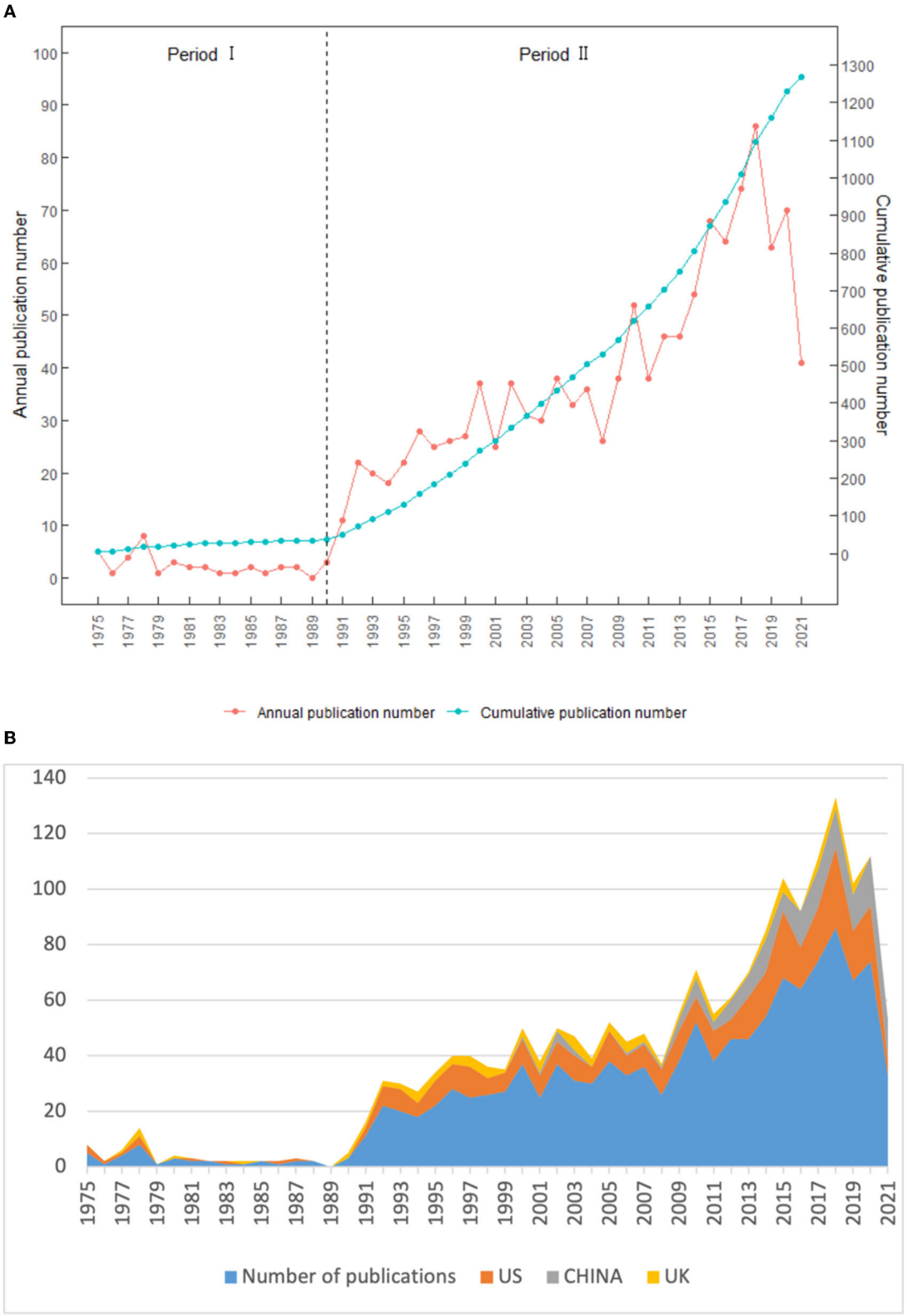

FIGURE 2 | (A) Number of publications by year. **(B)** Number of publications by year of US, China, and UK.

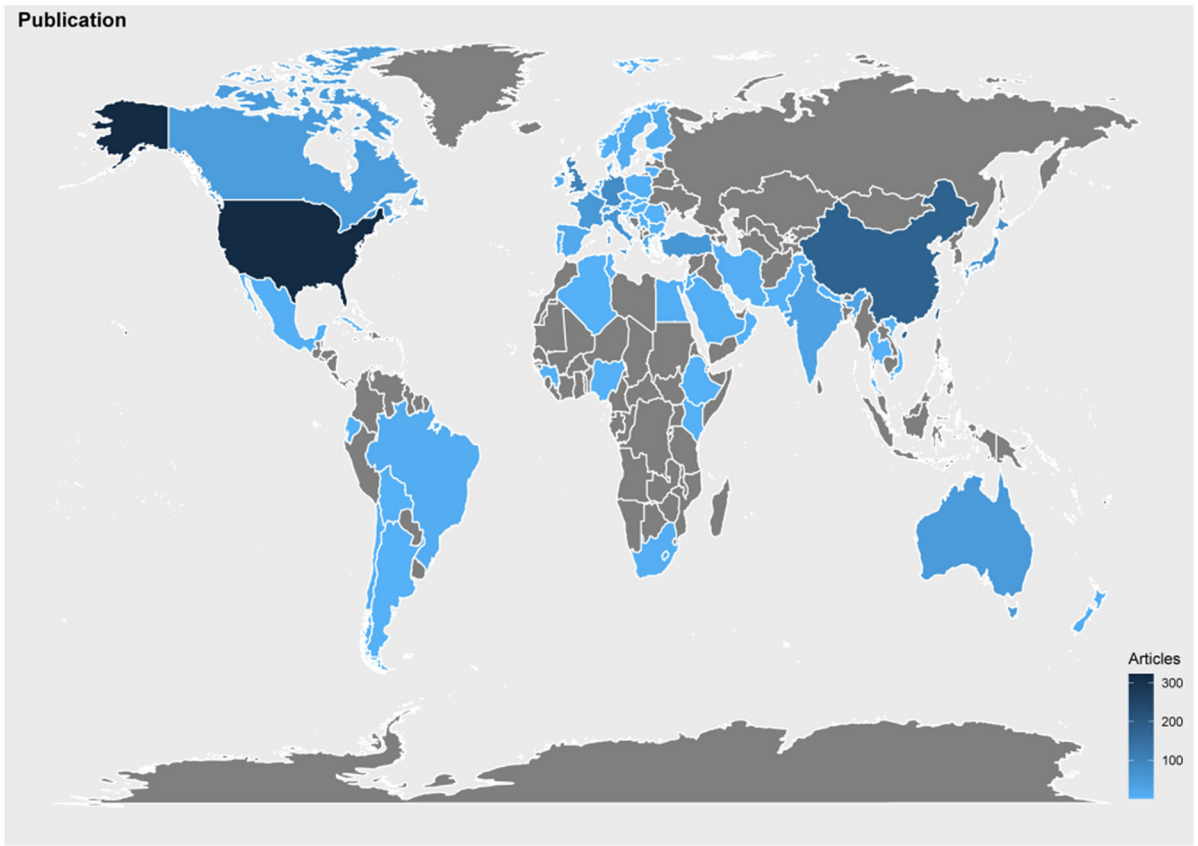

FIGURE 3 | (A) The regional distribution. **(B)** Distributions and percentages of countries/regions of publications.

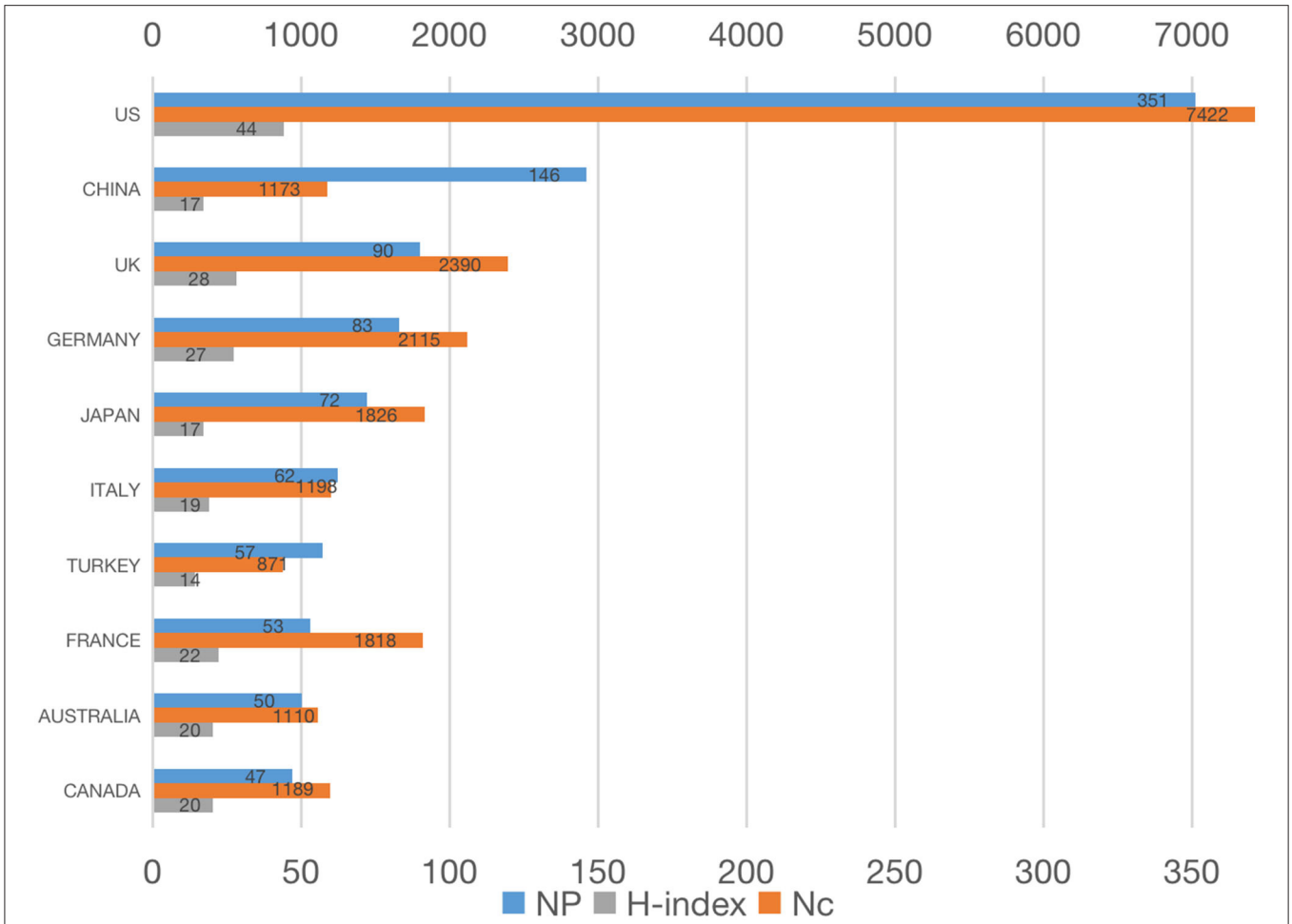

FIGURE 4 | Total publications, H-index, and the total citation of top 10 countries/regions.

with 2,390 citations and an H-index of 28. Germany ranked third with 2,115 citations and an H-index of 27.

Institutions Publishing Papers on Bronchoscopy Anesthesia

Figure 5 listed the top 10 institutions that published the most papers in this field. The most productive institution on bronchoscopy anesthesia was Harvard University (18), followed by Duke University (14) and the University of Pennsylvania (13). Citation frequency of papers from the University of Texas MD Anderson ranked first (619 citations), followed by Harvard University (587 citations) and Duke University (468 citations). In terms of the H-index, Harvard University ranked first (11), followed by Duke University (9) and Johns Hopkins University (9). Most of the top 10 institutions were from the US, excluding Sichuan University, the Capital Medical University of China, and the All India Institute of Medical Sciences of India. Although the number of publications from Chinese institutions was on the top 10 list, their H-index and Nc lagged behind those of other top 10 universities. Undoubtedly, the US took the lead in bronchoscopy anesthesia research.

Journals Publishing Papers on Bronchoscopy Anesthesia

The top 10 journals contributing to bronchoscopy anesthesia were listed in **Table 1**. They accounted for 29.84% (379) of all papers on bronchoscopy anesthesia. *CHEST* (IF 9.41) ranked first with 72 publications, and *Anesthesia and Analgesia* (IF 5.108) with 46 articles came next. In addition, *Respiration* (IF 3.58), *Pediatric Anesthesia* (IF 2.556), and *International Journal of Pediatric Otorhinolaryngology* (IF 1.675) ranked third, fourth and fifth place, respectively. Among the top 10 journals, other than *Pediatric Anesthesia, International Journal of Pediatric Otorhinolaryngology,* and *Acta Anaesthesiologica Scandinavica*, the IF of other journals was higher than 3. According to *Journal Citation Reports*, *CHEST* and the *British Journal of Anaesthesia* were listed under Q1, the highest-ranked journals in a category.

Highly Cited Publications on Bronchoscopy Anesthesia

To analyze the most influential papers in this field, we shortlisted the top 10 publications with the most citations and listed them

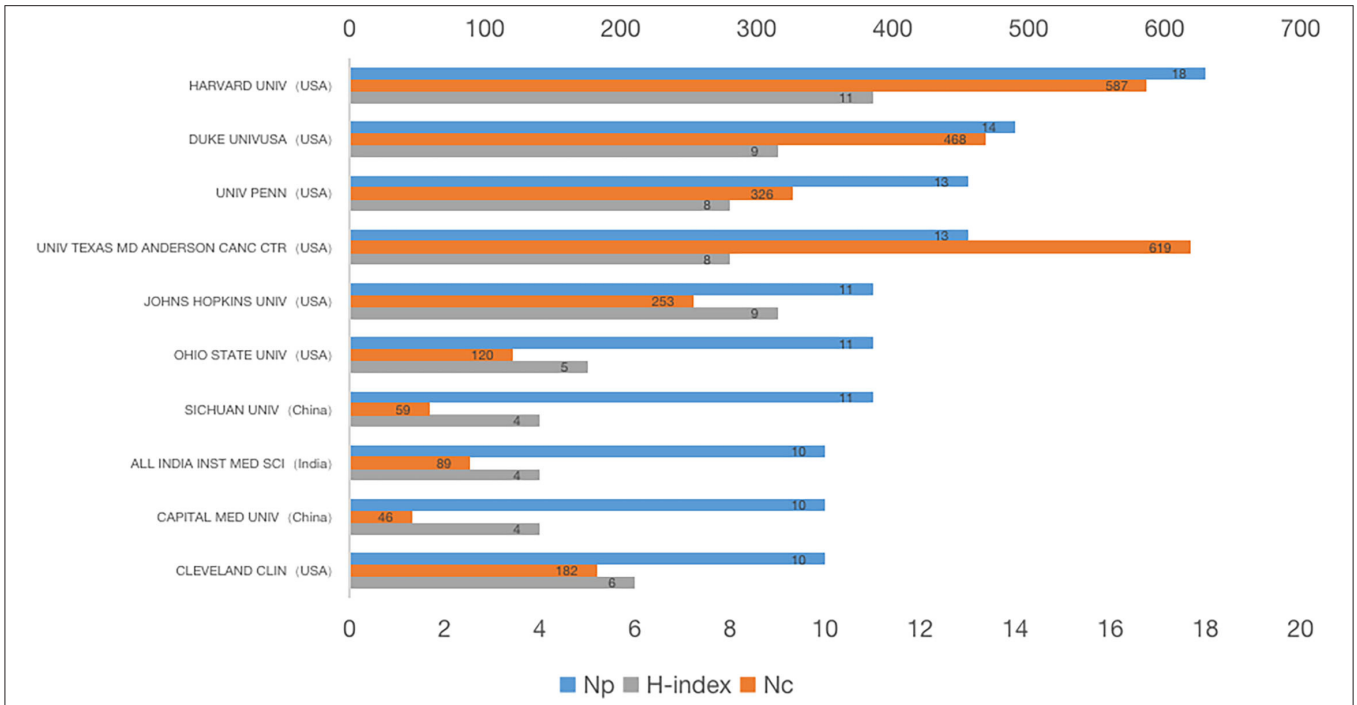

FIGURE 5 | Total publications, H-index, and the total citation of top 10 institutes.

TABLE 1 | The top 10 most active journals.

Rank	Journal	Np	H-index	Nc	IF (2020)	JCR
1	Chest	72	35	3,717	9.41	Q1
2	Anesth Analg	43	22	1,646	5.108	Q2
3	Respiration	42	18	878	3.58	Q2
4	Pediatr Anesth	38	16	604	2.556	Q2
5	Int J Pediatr Otorhi	36	13	566	1.675	Q3
6	J Thorac Dis	34	10	225	2.895	Q3
7	Brit J Anaesth	32	20	913	9.166	Q1
8	Acta Anaesth Scand	29	13	464	2.105	Q3
9	Eur J Anaesth	28	12	332	4.33	Q3
10	Can J Anaesth	25	14	673	5.063	Q2

in **Table 2** in terms of the title, first author, journal, publication year, and citation numbers. Two of the top 10 papers were published between 1991 and 2000, six between 2001 and 2010, and two between 2011 and 2017. As we mentioned above, the research on bronchoscopy anesthesia increased in period two from 1990 onward, and all the top 10 papers were published during this period, symbolizing the rapid progress in this field. The work of Yasufuku et al. ranked first with the highest Nc (478). In this paper, the authors evaluated the clinical efficacy of the newly developed ultrasound puncture bronchoscopy to visualize real-time transbronchial needle aspiration (TBNA) of the mediastinal and hilar lymph nodes under direct endobronchial ultrasonography (EBUS) guidance (17). The paper, "*Morbid obesity and tracheal intubation*", ranked second. It concluded that obesity alone was not a predictive factor of difficult tracheal intubation (18). The paper written by Tanaka et al., comparing the clinical efficacy of surgical stabilization and internal pneumatic stabilization in severe flail chest patients after receiving prolonged ventilatory support, ranked third (19).

Keywords Analysis

Considering the abundance of keywords, the minimum number of occurrences of keywords was set as 11. Of the 3,056 keywords, 131 were selected for co-occurrence analysis. As shown in **Figure 6A**, they were divided into six clusters. The first one, composed of 34 keywords (red circles), was mainly about applying sedatives and analgesics in bronchoscopy anesthesia. The second cluster was composed of 29 keywords (green circles) and mainly about difficult airway intubation and anesthesia management. The third cluster, consisting of 28 keywords (blue circles), was mainly about the application of bronchoscopy in the examination and treatment of various lung diseases. The fourth cluster of 22 keywords (yellow circles) concentrated on the treatment of airway stenosis and obstruction and intraoperative ventilation. The fifth cluster, with 11 keywords (purple circles), related to pediatric anesthesia during the removal of airway foreign bodies (AFBs) using rigid bronchoscopy. The sixth cluster was composed of 7 keywords (cyan circles) and focused on percutaneous dilatational tracheostomy with bronchoscopic guidance in the intensive care unit (ICU).

The top 10 keywords were listed in **Table 3**. The top frequent occurrences of keywords were "bronchoscopy," "fiberoptic bronchoscopy," "anesthesia," and "management", suggesting that

TABLE 2 | The top 10 most active papers.

Rank	Title	First Author	Year	Journal	Nc	IF (2020)
1	Real-time endobronchial ultrasound-guided transbronchial needle aspiration of mediastinal and hilar lymph nodes.	Yasufuku K.	2004	Chest	478	9.41
2	Morbid obesity and tracheal intubation.	Brodsky J. B.	2002	Anesth Analg	296	5.108
3	Surgical stabilization of internal pneumatic stabilization? A prospective randomized study of management of severe flail chest patients.	Tanaka H.	2002	J Trauma	280	2.512
4	Exhaled nitric oxide correlates with airway eosinophils in childhood asthma.	Warke T. J.	2002	Thorax	210	7.892
5	The laryngeal mask airway.Its uses in anesthesiology	Pennant J. H.	1993	Anesthesiology	210	7.892
6	Thoracoscopy–state of the art.	Loddenkemper R.	1998	Eur Respir J	193	16.671
7	Complications,consequences,and practice patterns of endobronchial ultrasound-guided transbronchial needle aspiration:Results of the AQuIRE registry.	Eapen G. A.	2013	Chest	192	9.41
8	The incidence of aspiration associated with the laryngeal mask airway-a metaanalysis of published literature	Brimacombe J. R.	1995	J Clin Anesth	185	9.452
9	Diagnostic yield and complications of bronchoscopy for peripheral lung lesions results of the aquire registry	Ost David E	2016	Am J Resp Crit Care	181	21.405
10	Incidence of and risk factors for pulmonary complications after non-thoracic surgery	McAlister F. A.	2005	Am J Resp Crit Care	178	21.405

studies related to bronchoscopy anesthesia mainly focused on airway management of various anesthesia methods in the process of bronchoscopy diagnosis and treatment. The colors of all keywords were divided by VOSviewer according to the year of publication. In **Figure 6B**, the keywords in purple appeared earlier than those in yellow. Yellow circles indicated the newest occurring keywords, including clinical trials of a combination of remifentanil and dexmedetomidine or other methods of sedation (20–22). For example, it was safer and more comfortable to use deep sedation when removing airway foreign bodies in children, indicating that the application of a combination of remifentanil and dexmedetomidine in painless bronchoscopy might be the priority and hotspot issue in future research on bronchoscopy anesthesia (23). Besides, electromagnetic navigation bronchoscope (ENB), video-laryngoscope, and lung volume reduction (LVR) were the newest occurring keywords.

References With Citation Burstness

For further study of the related co-citation references, we conducted CiteSpace to investigate citation burstness. Citation burstness indicates references that attract more attention from scholars in a specific field during a particular period. **Figure 7** illustrated the top 10 references with the strongest citation bursts. The minimum duration of the burst was set for 2 years, and a red line segment represented the initial and final years of the burst duration associated with academic circles for a specific period (24).

Among the top 10 references, the strongest burstness ($n = 6.97$) was caused by the paper, "*American College of Chest Physicians consensus statement on the use of topical anesthesia, analgesia, and sedation during flexible bronchoscopy in adult patients,*" authored by Wahidi et al. with citation burstness from 2013 to 2016 (25). This was followed by the paper, "*The anesthetic considerations of tracheobronchial foreign bodies in children: a literature review of 12,979 cases,*" published in 2010 by Fidkowski et al. (26). The research titled "*Complications Following Therapeutic Bronchoscopy for Malignant Central Airway Obstruction: Results of the AQuIRE Registry*" (27), also published in 2010, emerged third.

DISCUSSION

In this study, we undertook a bibliometric analysis to investigate the developmental trends and hotspots of research on bronchoscopy anesthesia using the data extracted from the SCI-expanded database using VOSviewer, CiteSpace, and Bibliometrix software. We retrieved a total of 1,270 original articles and reviews published since the establishment of the database. As noted in **Figure 2A**, there was an overall upward trend in the annual number of publications, especially after 1990. The growth rate peaked in 1991, and one of the main reasons for this was the application of local anesthetic lidocaine for fiberoptic bronchoscopy. This not only facilitated the diagnosis and treatment on the part of the doctor but also improved the comfort of the patient. Owing to the rapid development of professional theories in period two, more researchers focused on bronchoscopy anesthesia. An analysis of citation bursts is shown in **Figure 7**. These citation bursts mainly focused on 2009–2015, and the number of articles published worldwide also increased rapidly during this period (**Figure 2A**). We speculated that it was related to the issuance of authoritative guidelines. With the publication of "*The American College of Chest Physicians consensus statement on the use of topical anesthesia, analgesia, and sedation during flexible bronchoscopy in adult patients,*" in 2011 (25), all physicians performing bronchoscopy were asked

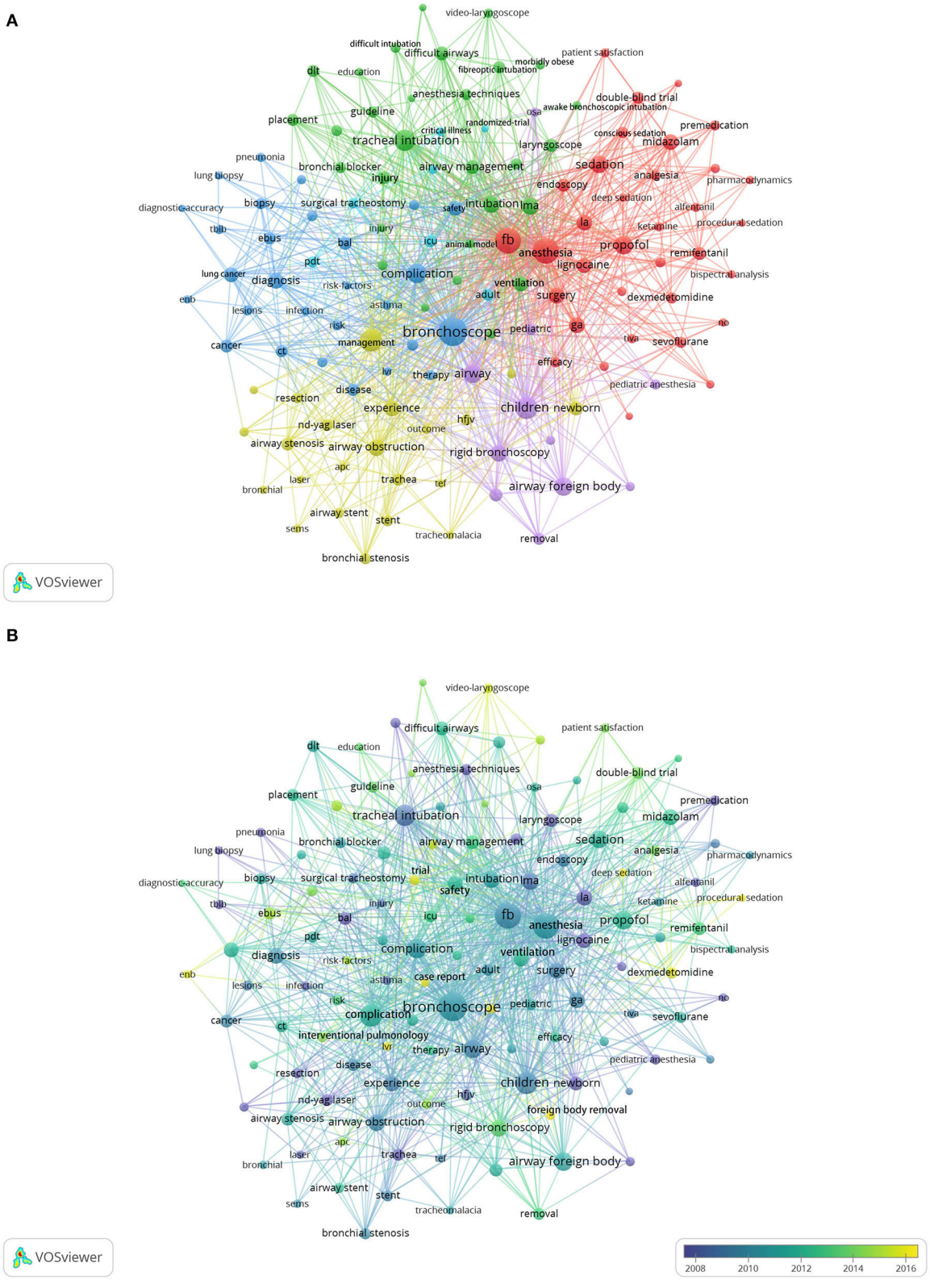

FIGURE 6 | (A) Analysis of keywords. **(B)** Visualization of time when a keyword appears.

TABLE 3 | The top 10 most frequently used keywords.

Rank	Keywords	Occurrences
1	Bronchoscope	408
2	Fiberoptic bronchoscopy	306
3	Anesthesia	287
4	Management	199
5	Children	196
6	Tracheal intubation	163
7	Complication	135
8	Airway	121
9	Propofol	112
10	Airway foreign body	110

to consider using topical anesthesia, analgesics, and sedative agents. Subsequently, the "*British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE*" published in 2013 gave anesthesiologists and bronchoscopists authoritative guidance (28).

The publications from various countries were then selected from the total list of publications. Among the top 10 countries/regions, the US ranked first in Np (27.6%), demonstrating that the US was the most productive country in research on bronchoscopy anesthesia. Seven of the top 10 affiliations in the research on bronchoscopy anesthesia were from the US. The H-index and Nc of the US also ranked first among the top countries/regions, and one of the reasons for this was that the US scholars started the research in this field earlier. As a developed country, the US allocates a large budget to research, has a vast number of research centers, and the quality of publications from the US is more likely to be acclaimed internationally. Although China ranked second in Np, the H-index and Nc of the papers from China were not high enough when compared with the other countries/regions in the top 10 category. The reason for this phenomenon may be a late start by China in this field. To improve this situation, more conscientious efforts need to be made by Chinese scholars in this field. The rapid growth of high-quality literature from China over the last decade also indicates that the research quality in China has improved quickly.

When it comes to journals, high IF journals reflect the quality of articles. Remarkably, *CHEST* ranked first in terms of the number of publications, citations, and H-index, indicating that it had a huge impact on bronchoscopy anesthesia. Three of the top 10 papers were from *CHEST*, demonstrating that it occupied an important status in the field of bronchoscopy anesthesia. This reminded scholars interested in this field to pay more attention to this journal. Among the top ten articles, nine were published in high-IF journals, which meant that publishing research on bronchoscopy anesthesia in high-quality journals was possible.

Keywords can provide information about the core content of an article, and the most frequent and newest keywords can be used to identify the dynamics of research trends and hotspots in a particular domain during the study period. As seen in **Table 3**, the keywords "anesthesia," "bronchoscope," and "management" were the most common and showed the greatest increase over time. Furthermore, all the top keywords with the strongest citation burst were related to the application of fiberoptic bronchoscopy in the removal of foreign bodies in the airway of children, as well as airway management and related complications during the diagnosis and treatment using bronchoscopy. Comparing **Figures 6A,B**, dexmedetomidine applied to procedural sedation (cluster 1) has become a hotspot over the last 5 years. Dexmedetomidine was associated with fewer incidents of oxygen desaturation and a reduced need for oral cavity suction than remifentanil during flexible bronchoscopy (21). Compared with midazolam, pre-injection of dexmedetomidine before induction significantly decreased the incidence of dreaming in patients undergoing flexible bronchoscopy during general anesthesia; as most dreams were pleasant, patients rarely kept painful memories. The application of dexmedetomidine improved sedative effectiveness with fewer procedural interruptions, shorter time to ambulation, and higher bronchoscopist satisfaction (20, 22). Therefore, we need to pay more attention to precision anesthesia and comfort in the future.

With progress in society, medical technology has also developed rapidly. As seen in **Figure 6B**, video laryngoscopy, ENB, and LVR were research hotspots over the last 5 years. The invention of video laryngoscopy reduced the risk of difficult airways. Research showed that video laryngoscopy for awake tracheal intubation was associated with a shorter intubation time, thus increasing the intubation effectiveness. Its success rate and safety profile seemed comparable to those of fiberoptic bronchoscopy (29, 30). In addition, ENB was used to locate small peripheral pulmonary nodules and guide surgical resection safely and accurately. ENB is safe and feasible with a high diagnostic success rate in interventional pulmonology in low resource settings under moderate sedation, which is beneficial to early detection and early treatment of small lung lesions (31, 32). Bronchoscopic lung volume reduction (BLVR) coil treatment is an alternative and promising treatment modality for selected severe emphysema patients (33). Therefore, improvements in surgical methods and advancements in surgical instruments have made it possible to treat intractable diseases.

Among the top productive 10 articles (**Table 2**), five articles addressed the progress in bronchoscopic examination, diagnosis, and treatment methods, and four articles highlighted airway assessment and management of anesthesia during the operation. In general, the advancement in surgical equipment and methods is inseparable from appropriate anesthesia. The synergy between improvements in medical equipment and precise anesthesia methods will become the focus of future research.

LIMITATIONS

To the best of our knowledge, this is the first bibliometric analysis of hotspots and dynamic pilot study on bronchoscopy anesthesia over the past decade. In addition, VOSviewer, Citespace, and Bibliometrix were applied to perform the survey simultaneously,

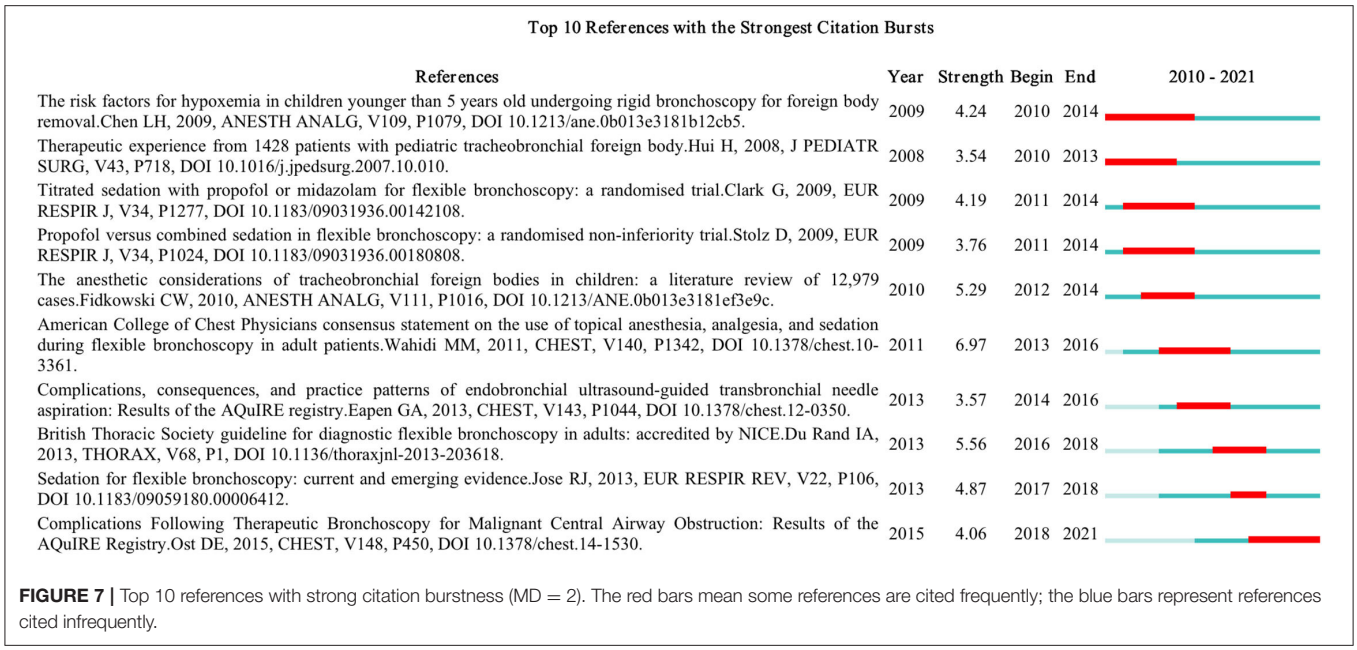

FIGURE 7 | Top 10 references with strong citation burstness (MD = 2). The red bars mean some references are cited frequently; the blue bars represent references cited infrequently.

enabling our research results to be more accurate and objective. Nevertheless, the present research has some limitations. First, we only chose Web of Science Core Collection and ignored other search engines such as PubMed and Scopus, knowing that different databases may produce different numbers of publications and citation counts. Second, only English articles were included, which may have decreased the number of retrieved articles. Finally, as our research was temporary and the count of relevant articles would also change as time goes on, the findings of this study are valid as of June 25, 2021. With the rapid updating of hot topics and research frontiers in bronchoscopy anesthesia, we might have missed some research hotspots.

CONCLUSION

In this study, we discuss the developmental trends and research hotspots in bronchoscopy anesthesia and find that there has been a surge of interest in this field in recent decades. The US dominates this field, represented by the largest number of publications (351), the highest H-index (44), and extensive international collaborations. The selection and dosage of sedatives have always been the focus of disease research for the sake of improving comfort and safety. The clinical application of ENB has increased the diagnosis rate of minimal changes, and the diagnosis rate of the early lung tissue has increased. The invention of video laryngoscopy has reduced the risk of difficult airways, which not only shortens the intubation time but also increases the success rate. Medical development requires technological progress. In summary, our results revealed a comprehensive scientometric analysis of research on bronchoscopy anesthesia from a global perspective and may provide useful clues for future research directions and scientific decision-making in this domain.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

KM, YW, and SW did this bibliometrics analysis and wrote manuscript. HY, HD, JW, WZ, HZ, XZ, and YG participated in experimental design and manuscript writing. XS and XL designed this study and organized the manuscript writing. All authors read and approved the final manuscript.

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New integrated weaning indices from mechanical ventilation: A derivation-validation observational multicenter study

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Background: To develop ten new integrated weaning indices that can predict the weaning outcome better than the traditional indices.

Methods: This retrospective-prospective derivation-validation observational multicenter clinical trial (Clinical Trial.Gov, NCT 01779297), was conducted on 1,175 adult patients admitted at 9 academic affiliated intensive care units (ICUs; 4 surgical and 5 medical), from Jan 2013 to Dec 2018. All patients, intubated and mechanically ventilated for at least 24 h and ready for weaning were enrolled. The study had two phases: at first, the threshold values of each index that best discriminate between a successful and an unsuccessful weaning outcome was determined among 208 patients in the derivation group. In the second phase, the predictive performance of these values was prospectively tested in 967 patients in the validation group. In the prospective-validation set we used Bayes' theorem to assess the probability of each test in predicting weaning.

Results: In the prospective validation group, sensitivity, specificity, diagnostic accuracy, positive and negative predictive values, and finally area under the receiver operator characteristic curves and standard errors for each index (ten formulae) were calculated. Statistical values of ten formulae for aforesaid variables were higher than 87% (0.87–0.99).

Conclusion: The new indices can be used for hospitalized patients in intensive care settings for accurate prediction of the weaning outcome.

KEYWORDS

mechanical ventilation, weaning indices, cut-off values, likelihood ratio, receiver-operating characteristic curve

Introduction

Mechanical ventilation (MV) is a life-supporting modality that is used in many critically ill patients and it aims to support ventilation, optimize oxygenation, and protect the airway (1). Weaning from MV is a particularly important issue because early and late extubation will put pressure on the patient's health, increase the risk of infection and length of stay in hospital (2). As many as 20% of mechanically ventilated patients may fail their first attempt to disconnect from mechanical ventilation. Weaning can account for more than 40–50% of the total duration of MV (3–5). Long-term MV is associated with many complications such as ventilator-associated pneumonia (VAP), ventilator-induced lung injury, airway injury, ventilator-induced diaphragmatic dysfunction, barotraumas, and prolonged immobility sequelae (6–8). According to the Sixth International Consensus Conference on Intensive Care Medicine (9), patients who meet the following criteria should be considered as potentially ready for liberation from the ventilator, a frequency to tidal volume ratio (f/Vt) less than 105 breaths/min/L, respiratory rate (f) of 35 breaths/min or less, maximal inspiratory pressure (MIP) of equal to or more negative than -20/-25 cm/H_20, spontaneous tidal volume (Vt) more than 5 mL/kg, vital capacity more than 10 mL/kg, and arterial oxygen saturation (SaO_2) > 90% with a fraction of inspired oxygen (FiO_2) of 0.4 or less (or partial pressure of arterial oxygen (PaO_2)/FiO_2 ratio of 150 mmHg or more) (10–13).

Challenges to weaning indices development include differences in patient populations and pathophysiologic conditions, variable techniques of measurements, and lack of objective criteria to define the weaning outcomes (14–16). Discontinuation of mechanical ventilation, spontaneous breathing trial (SBT), and extubation are carried out under the attending clinician's evaluation, arterial blood gas analysis, and observation of the patient's clinical condition. For example, the majority of clinicians use the measurement of F/Vt ratio after sustained low or absent pressure support and Positive End-Expiratory Pressure (PEEP), the so-called rapid shallow breathing index (RSBI) for both restrictive and obstructive patients (17, 18). In addition, diagnostic tools to evaluate respiratory muscle function could be helpful to guide the start of the weaning process (19, 20). Despite RSBI has been validated in so many studies and found to have an excellent accuracy, but the RSBI value is questionable in medical critically ill patients and during sedation with some drugs like propofol. In addition, single measurement of RSBI can be misleading and repeated measurement of RSBI increase its accuracy in weaning failure and an increasing RSBI was noted in weaning failures. Also, RSBI that measured during spontaneous breathing trial with spirometry has different accuracy from RSBI that measured during pressure support ventilation. So, it can be suggested that RSBI may not have adequate accuracy to be used routinely in the weaning process and the quest to obtain an accurate way to predict success when weaning a patient from mechanical ventilation continues (21–24).

This study was conducted to introduce and compare ten weaning indices in predicting successful weaning from MV. In the search for an index with better predictive power for MV liberation we considered the combination of parameters. The idea of formulation of the new integrative indices that evaluate different pathophysiology and weaning failure mechanisms can improve the predictive power of simple weaning indices.

Materials and methods

Study design

This was a retrospective-prospective derivation-validation observational multicenter clinical trial conducted in 9 academic affiliated intensive care units (ICUs; 4 surgical and 5 medical) from Jan 2013 to Dec 2018 develop ten new integrated weaning indices that can predict the weaning outcome better than the traditional indices. All parts of study were reviewed according to the Strengthening the Reporting of Observational Studies in Epidemiology for Respondent-Driven Sampling Studies: "STROBE-RDS" Statement. The study protocol was approved by the investigational review boards at each of all participating centers in Iran, and written informed consent from each patient or their legal representative was obtained before any study procedures.

Participants

This study enrolled all adult patients admitted to 9 academic affiliated intensive care units (ICUs; 4 surgical and 5 medical), from Jan 2013 to Dec 2018. Patients were eligible for study participation if: (a) age $\geq$ 18 years, (b) admitted to the ICU, (c) endotracheal intubated and on mechanical ventilation for $\geq$ 24 h, (d) full-code status, and if (e) informed consent was provided by the patient, legal guardian, or healthcare surrogate (before ventilator weaning). Patients were excluded for: (a) declining consent, (b) death without ventilator weaning, (c) cardiopulmonary arrest on the ventilator, (d) permanent ventilator dependence, (e) tracheostomy placement for long-term weaning, (f) self-extubation, (g) aspiration during the wean, (h) copious secretions and mucus plugging precluding wean, and (i) incomplete data.

Setting of study

All patients were intubated with tracheal tubes mechanically ventilated for at least 24 h and ready for weaning. The ventilators used were the Evita XL and Evita 4 edition ventilators (Draeger, Lubeck, Germany). All intubated patients in this study were divided into two groups, derivation, and validation groups. The

study had two phases: at first, retrospectively the threshold values of each index that best discriminate between a successful and an unsuccessful weaning outcome was determined among the derivation group. In the second phase, prospectively the predictive performance of these values was tested in the validation group.

New integrated weaning indices

We developed ten indices by combining different respiratory variables and different simple weaning indices. For the determination of the best performance of these variables, we invited three-expert of panels. The members of these panels included pulmonary diseases consultants, specialists in anesthesiology, and intensivists from different country regions. Sections one and two were held by posting indices on the internet looking for their predictive values among different studies and after all, opinions were collected for section three, we invited them to participate in the 120-min focus group. Finally, ten new indices emerged as follow:

$$\text{Index 1} = (\text{PPR})/(\text{RSBI} \times F_iO_2)$$
$$\text{Index 2} = (\text{PPR})/(\text{RSBI} \times F_iO_2 \times \text{P0.1})$$
$$\text{Index 3} = (\text{PPR} \times \text{NIF})/(\text{RSBI} \times F_iO_2)$$
$$\text{Index 4} = (\text{PPR} \times \text{NIF})/(\text{RSBI} \times F_iO_2 \times \text{P0.1})$$
$$\text{Index 5} = (\text{NIF})/(\text{P0.1})$$
$$\text{Index 6} = (S_aO_2)/[(\text{P (A-a) } O_2 \times \text{RSBI} \times F_iO_2]$$
$$\text{Index 7} = (S_aO_2)/[(\text{P (A-a) } O_2 \times \text{RSBI} \times F_iO_2 \times \text{P0.1}]$$
$$\text{Index 8} = (S_aO_2 \times \text{NIF})/[(\text{P (A-a) } O_2 \times \text{RSBI} \times F_iO_2]$$
$$\text{Index 9} = (S_aO_2 \times \text{NIF})/[(\text{P (A-a) } O_2 \times \text{RSBI} \times F_iO_2 \times \text{P0.1}]$$
$$\text{Index 10} = (S_aO_2)/[(\text{P (A-a) } O_2 \times \text{P0.1}]$$

(N.B: Where PPR = PaO2: PAO2 ratio)

For computing new indices, a calculator is designed to calculate new indices. Our recommended indices use four essential parameters that lend themselves to easy measurement and are independent of the patient's cooperation. The scores, in a single equation, the respiratory system dynamics, the respiratory drive, the oxygenation/ventilation, and the respiratory pattern, through NIF, P0.01, PPR-P(A-a) O2, SaO2 and RSBI ratio respectively. The operation with this calculator was so simple because seven variables should enter into the calculator for computing ten formulae (FiO2, PaO2, SaO2, PaCO2, RSBI, P0.1, and NIF). Baseline demographics, initial diagnosis, and pre-extubation clinical data are collected for each patient.

Weaning procedure

Liberation of MV was attempted when the primary physician judged that the patient was ready for a spontaneous breathing trial (SBT), according to the following criteria: competent airway, good cough reflex, absence of sedation, or excessive tracheal secretions, and hemodynamic stability. Sedation was discontinued before the evaluation of weaning. Patients who met these criteria were initially placed on SBT (continuous positive airway pressure of 5 mmHg, FiO2 $\leq$ 0.4) for 3 min to obtain weaning variables at the end of SBT. If Oxygen saturation $\geq$ 92% on pulse oximetry with FiO2 $\leq$ 0.4 and RSBI $<$ 105 breaths/min/L, patients were continued on SBT for 30 min during which clinical variables and ventilator variables were monitored closely for signs of respiratory distress (respiratory rate $>$ 30 breaths/min, SaO2 $<$ 90%, heart rate $>$ 140 breaths/min, or a sustained increase or decrease of heart rate of $>$ 20%, blood pressure $>$ 200 mm Hg or $<$ 80 mm Hg, and agitation, diaphoresis, or anxiety). At the end of the SBT, the RSBI was measured again, arterial blood gas (ABG) was obtained, and the predetermined values are calculated and measured. The decision to reinstitute MV was made based on airway competence (cough, sputum production, neurologic status, level of consciousness, and MIP) (25). Patients who remain extubated for 24 h are classified as successful extubation without extra helping including more oxygenation, reintubation, or using Non-invasive MV. Weaning failure considered if patients need more support during 24 h after extubation including more oxygenation, reintubation, or using Non-invasive MV.

Statistical analyses

Data were presented using mean $\pm$ standard division (SD) or medians (inter-quartile range, IQR), for continuous variables and frequencies with percentages (%) for categorical characteristics. The whole data was split into two subsamples: derivation data and validation data. To compare the differences in terms of demographic characteristics, clinical data, incidence of successful and failure weaning between derivation and validation groups *t*-test and Chi-square test were used for distributed continuous and categorical variables, respectively. Association with the success weaning was tested by univariate and multivariate logistic regression analysis using the "Enter" method as the independent variable of primary interest. In models, the odds ratio (OR) and their 95% confidence interval (CI) were reported as the effect size of the association. Based on the coefficients derived from the model in the derivation dataset, the scores were computed for the validation data set. The model validation was assessed in the validation data set utilizing diagnostic accuracy measures and their 95% CI by receiver operating characteristic (ROC) curves analysis and calculate their area under the curves (AUC), alongside sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR−), accuracy and Youden score to find appropriate cut-offs. In the tables with zero counts, likelihood ratios were estimated using the substitution formula and 0.5 was added to all cell frequencies before calculation.

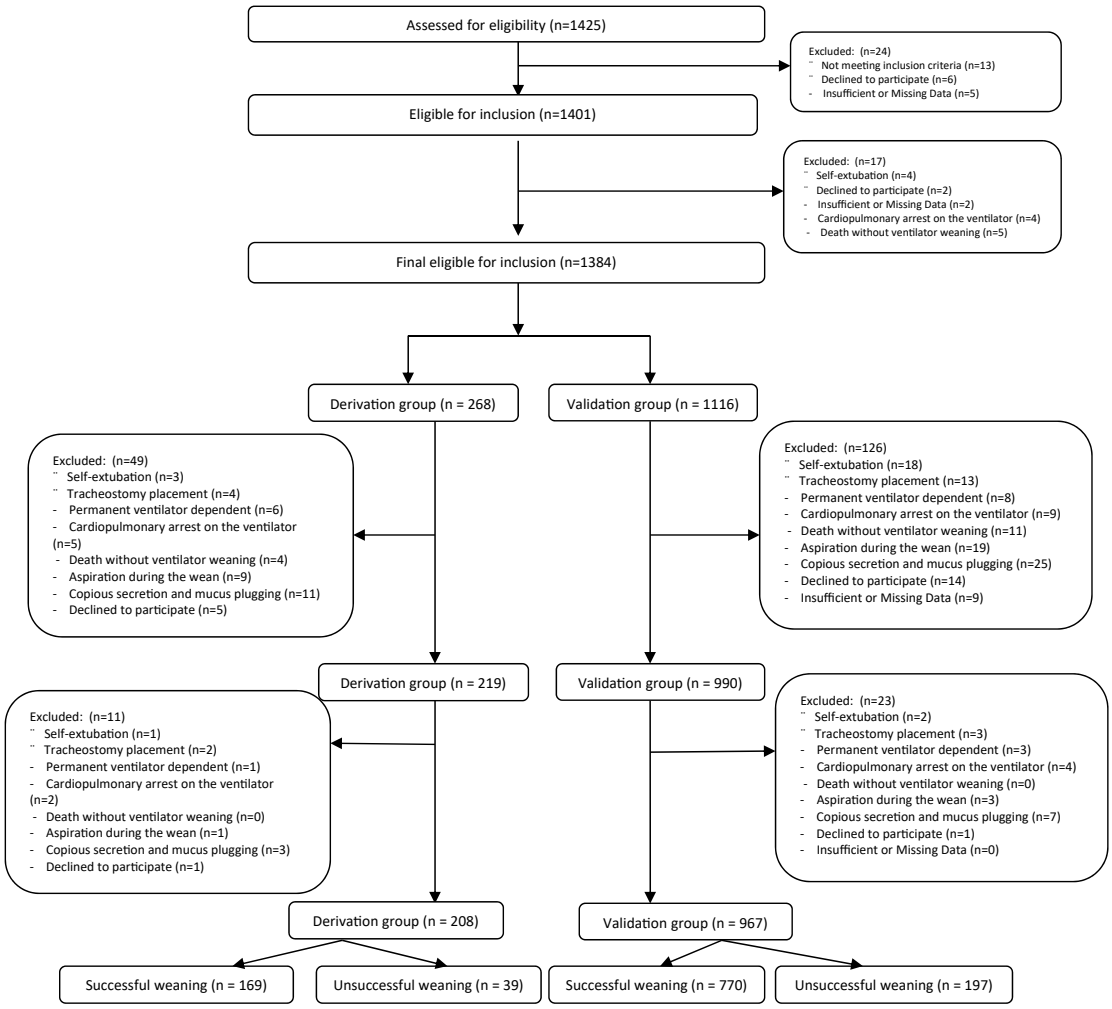


FIGURE 1
The study population flowchart.

According to general guide for the discriminative power of a test based on ROC, AUC between (0.9–1.0), (0.8–0.9), (0.7–0.8), and (0.6–0.7) was considered as excellent, good, fair, and poor, respectively. In addition, the AUCs was compared by DeLong test. All analyses were conducted using STATA software ver.13 (Stata Corp., College Station, TX, United States), SPSS software (ver.21) (SPSS Inc., Chicago, IL, United States) and MedCalc for ROC analysis. In all analyses, *P*-values less than 0.05 were considered significant.

Results

Clinical characteristics and outcomes of the study population

From Jan 2013 to Dec 2018, a total of 1,175 patients were screened at 9 academic affiliated intensive care units (ICUs; 4 surgical and 5 medical) in Iran. All 1,175 intubated patients in this study were divided into two groups, derivation (n = 208) and validation (n = 967) groups (Figure 1). Demographic data, clinical characteristics, incidence of successful and failure weaning in both derivation, and validation groups and total population are presented in Table 1. The mean ± SD of all participant ages was 58.36 ± 7.94 years. There were 523 (44.5%) male patients and 652 (55.5%) female patients. According to the results, the mean age of validation group was significantly higher than the derivation group (58.82 ± 7.57 vs. 56.19 ± 9.16, $P < 0.001$). However, no significant difference was observed between groups according to gender (P = 0.929). Patients were admitted to ICUs for various reasons including cancer, acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), multiple traumas, abdominal surgery, pneumonia, and sepsis. However, there was no significant difference between groups according to cause of ICU admission (P = 0.838). The most common cause of ICU

TABLE 1 Demographic data, clinical characteristics, incidence of successful and failure weaning in both derivation and validation groups and total population.

Variables	Total Population (n = 1,175)	Derivation dataset (n = 208)	Validation dataset (n = 967)	P-value
Gender				
Male, N (%)	523 (44.5)	92 (44.2)	431 (44.6)	0.929
Female, N (%)	652 (55.5)	116 (55.8)	536 (55.4)	
The cause of admission				
Cancer, N (%)	97 (8.3)	14 (6.7)	83 (8.6)	0.838
ARDS, N (%)	216 (18.4)	39 (18.8)	177 (18.3)	
COPD, N (%)	273 (23.2)	44 (21.2)	229 (23.7)	
Multiple traumas, N (%)	223 (19)	38 (18.3)	185 (19.1)	
Abdominal surgery, N (%)	193 (16.4)	38 (18.3)	155 (16)	
Pneumonia, N (%)	119 (10.1)	23 (5.8)	96 (9.9)	
Sepsis, N (%)	54 (4.6)	12 (5.8)	42 (4.3)	
Weaning rate				
Successful, N (%)	939 (79.9)	169 (81.3)	770 (79.6)	0.569
Unsuccessful, N (%)	236 (20.1)	39 (18.8)	197 (20.4)	
Age, Year, Mean (SD)	58.36 (7.94)	56.19 (9.16)	58.82 (7.57)	<**0.001**
ICU-related variables				
ICU LOS, Day, Mean (SD)	23.73 (7.96)	19.71 (8.17)	24.59 (7.64)	<**0.001**
Hospital LOS, Day, Mean (SD)	13.56 (5.06)	12.89 (5.60)	13.71 (4.93)	**0.034**
APACHE II, Mean (SD)	26.43 (4.83)	26.35 (5.63)	26.44 (4.64)	0.804
SOFA, Mean (SD)	14.90 (3.86)	14.20 (3.01)	15.27 (3.92)	0.052
SAPS, Mean (SD)	49.11 (4.64)	48.56 (4.90)	49.22 (4.57)	0.060
Hemoglobin, g/dl, Mean (SD)	11.13 (1.54)	10.98 (1.50)	11.17 (1.55)	0.118
Pulmonary-related variables				
$C_{Dynamic}$, ml/cmH_2O, Mean (SD)	23.39 (3.63)	23.10 (3.60)	23.46 (3.64)	0.192
C_{Static}, ml/cmH_2O, Mean (SD)	36.16 (6.42)	36.94 (5.75)	35.99 (6.54)	0.053
VE, l/min, Mean (SD)	7.84 (1.43)	8.61 (1.75)	7.67 (1.30)	<**0.001**
VT, ml/min, Mean (SD)	391.31 (40.69)	422.31 (39.37)	384.64 (37.79)	<**0.001**
RR, breath/min, Mean (SD)	20.01 (2.82)	20.34 (3.36)	19.94 (2.69)	0.063
Gas exchange-related variables				
PaO_2, mmHg, Mean (SD)	88.76 (5.30)	84.31 (3.60)	89.72 (5.19)	<**0.001**
SaO_2,%, Mean (SD)	88.89 (1.47)	89.03 (1.56)	88.87 (1.45)	0.144
$PaCO_2$, mmHg, Mean (SD)	43.92 (2.73)	43.57 (2.88)	44 (2.69)	**0.035**
FiO_2,%, Mean (SD)	35.27 (2.27)	35.39 (2.28)	35.24 (2.26)	0.404
$P_{ALV}O_2$, mmHg, Mean (SD)	159.86 (14.30)	161.04 (1453)	159.61 (14.24)	0.191
$PaO_2/P_{ALV}O_2$, mmHg, Mean (SD)	0.56 (0.06)	0.53 (0.05)	0.57 (0.06)	<0.001
P(ALV-a) O_2, mmHg, Mean (SD)	71.10 (15.10)	76.73 (14.57)	69.89 (14.95)	<0.001
Conventional weaning indices				
RSBI, breath/l/min, Mean (SD)	90.39 (10.62)	92.74 (10.89)	89.89 (10.50)	<**0.001**
NIF, cmH_2O, Mean (SD)	23.72 (2.97)	24.63 (2.90)	23.53 (2.96)	<**0.001**
P.01, milli/second, Mean (SD)	5.72 (1.91)	6.49 (2.34)	5.55 (1.76)	<**0.001**
New integrated weaning indices				
1. (PPR/RSBI*FiO_2), Mean (SD)	180.28 (40.34)	164.80 (33.66)	183.62 (40.89)	<**0.001**
2. (PPR/RSBI*FiO_2*P.01), Mean (SD)	350.64 (133.86)	290.46 (117.33)	363.59 (133.71)	<**0.001**
3. (PPR*NIF/RSBI*FiO_2), Mean (SD)	426.29 (104.02)	405.25 (93.04)	430.82 (105.73)	<**0.001**
4. (PPR*NIF/RSBI*FiO_2*P.01), Mean (SD)	821.33 (305.06)	712.75 (289.14)	844.68 (303.48)	<**0.001**
5. (NIF/P.01), Mean (SD)	454.74 (132.84)	435.52 (162.32)	458.87 (125.32)	**0.021**
6. (SaO_2/P(ALV-a) O_2*RSBI*FiO_2), Mean (SD)	430.59 (166.75)	380.26 (120.34)	441.42 (173.28)	<**0.001**
7. (SaO_2/P(ALV-a) O_2*RSBI*FiO_2*P.01), Mean (SD)	838.89 (424.58)	669.65 (315.43)	875.29 (436.20)	<**0.001**
8. (SaO_2*NIF/P(ALV-a) O_2*RSBI*FiO_2), Mean (SD)	1016.78 (402.91)	933.82 (306.88)	1034.63 (418.70)	<**0.001**
9. (SaO_2*NIF/P(ALV-a) O_2*RSBI*FiO_2*P.01), Mean (SD)	196.23 (96.93)	164.10 (77.03)	203.14 (99.39)	<**0.001**
10. (SaO_2/P(ALV-a) O_2*P.01), Mean (SD)	256.81 (105.24)	214.01 (92.11)	266.01 (105.65)	<**0.001**

P-values of 0.05 are shown in bold and are considered significant.

admission for both groups was COPD (validation: 23.7% vs. derivation: 21.2%, $P = 0.745$). Illness severity as measured by the Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA), and Simplified Acute Physiology Score (SAPS) scores, which the mean ± SD scores of all participants was 26.43 ± 4.83, 14.90 ± 3.86, and 49.11 ± 4.64, respectively. No significant differences were observed between groups according to APACHE II (26.35 ± 5.63 vs. 26.44 ± 4.64, $P = 804$), SOFA (14.20 ± 3.01 vs. 15.27 ± 3.92, $P = 0.052$), and SPAS (48.56 ± 49.22, $P = 0.060$) scores. The prevalence of extubation failure in all patients was 20.1% and no significant difference was observed between groups in terms

TABLE 2 Univariate and multivariate logistic regression analysis to determine the effect of demographic characteristics and clinical data on weaning outcome.

Variables	Univariate		Multivariate	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	1.002 (0.984–1.02)	0.817	1.003 (0.985–1.022)	0.749
Gender (Female vs. male)	0.957 (0.718–1.276)	0.764	0.96 (0.718–1.282)	0.78
APACHE II	1.003 (0.973–1.033)	0.861	1.003 (0.973–1.033)	0.867
SOFA	0.991 (0.955–1.028)	0.627	0.994 (0.957–1.033)	0.769
SAPS	1.006 (0.975–1.037)	0.705	1.008 (0.977–1.04)	0.601
ICU LOS	0.986 (0.968–1.004)	0.13	0.986 (0.968–1.005)	0.154
Hospital LOS	0.979 (0.952–1.006)	0.132	0.98 (0.953–1.008)	0.152
Cause of ICU admission	1.021 (0.934–1.116)	0.648	1.024 (0.937–1.121)	0.597
Groups (derivation vs. validation)	1.109 (0.757–1.624)	0.596	1.02 (0.68–1.53)	0.925

OR, odds ratio; CI, confidence interval.

of weaning rate (18.8% vs. 20.4%, $P = 0.569$). However, the mean ± SD of ICU ($P < 0.001$) and hospital (P-0.034) length of stay (LOS) were significantly higher in the validation group than that the derivation group. Pulmonary-related variables, gas exchange-related variables, conventional weaning indices, and the mean ± SD of 10 new integrated weaning indices were observed in Table 1 can be seen in detail in both derivation and validation data sets groups.

Findings of logistic regression analysis

Univariate and multivariate Binary logistic regression analysis to determine the effect of demographic characteristics and clinical data on outcome of weaning are presented in Table 2. However, according to the results, we not found any statistical significance between the variables and the weaning outcome.

Results on derivation sample

Comparison of the AUCs of ten new integrated weaning indices for predicting successful weaning are presented in Figure 2A. Best performing predictive value for successful weaning were related to the first and third formulas with (AUC: 0.788, 95% CI: 0.727–0.842, $P < 0.001$), and (AUC: 0.783, 95% CI: 0.721–0.837, $P < 0.001$), respectively. However, no significant difference was observed between AUCs of first and third formula (0.788 vs. 0.783, $P = 0.779$). Poor predictive value for successful weaning was related to the fifth formula with (AUC: 0.610, 95% CI: 0.541–0.677, $P = 0.035$). Predictive value of tenth formula was not significant (AUC: 0.602, 95% CI: 0.532–0.669, $P = 0.067$). The results according to DeLong test indicated a significant difference of AUCs among fifth and tenth formulas with the others ($P < 0.05$). The ROC area for ten, first 5, and second five new integrated weaning indices are presented in Figure 2B. The results indicated AUC of about 0.98, 0.81, and 0.94 respectively, for ten, first five, and second five new integrated weaning indices simultaneously on predicting successful weaning after fitting a multiple logistic regression in derivation dataset.

The cumulative effect of all new integrated weaning indices in derivation dataset

To have a cumulative effect of all indices, we conducted a logistic regression, and then using the probability of successful weaning in this model, we computed the diagnostic indices. Diagnostic indices for this model considering 0.5 and optimal cutoffs for predicted probability is shown in Table 3. Diagnostic indices in derivation dataset indicated that the model by new integrated weaning indices had higher accuracy, SN, SP, LR + , PPV, and NPV and lower values of LR− in both 0.5 and optimal cutoff values as compared to two other sets of formulae. Additionally, model by second 5 formulae had higher accuracy, SN, SP, LR + , PPV, and NPV and lower values of LR- in both 0.5 and optimal cutoff values as compared to the model by first five formulae. The optimal cutoff values were estimated based on sensitivity and specificity tradeoff in Figure 3. The Figure 2C showed a tradeoff of sensitivity and specificity in the cutoff of around 0.7, 0.8, and 0.7 for predicted probability in the model by ten, first five and 2nd five new integrated weaning indices, respectively, based on the multivariate logistic regression.

Results on the validation sample

Good performing predictive value for successful weaning in the validation group were related to the first and third

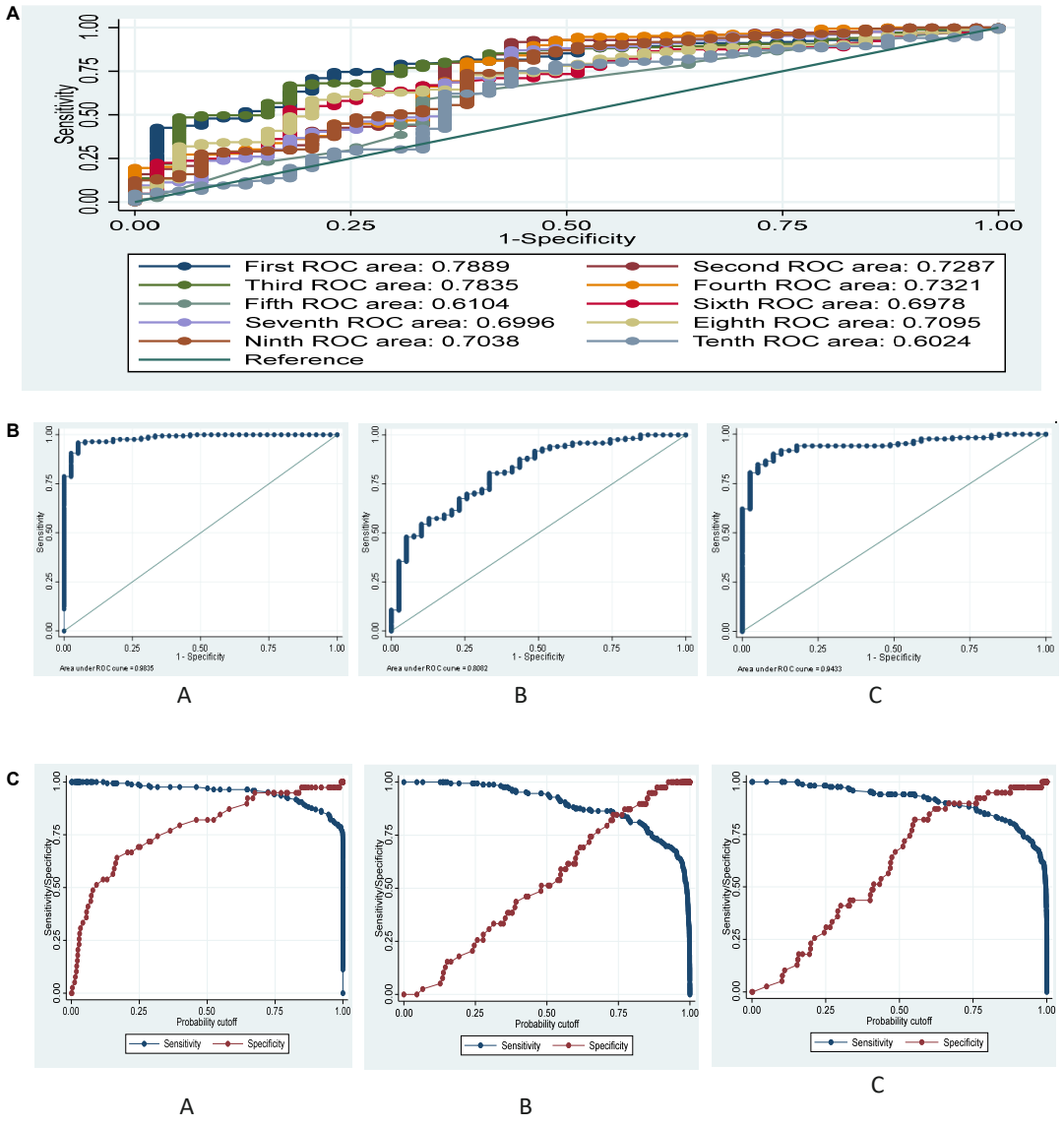


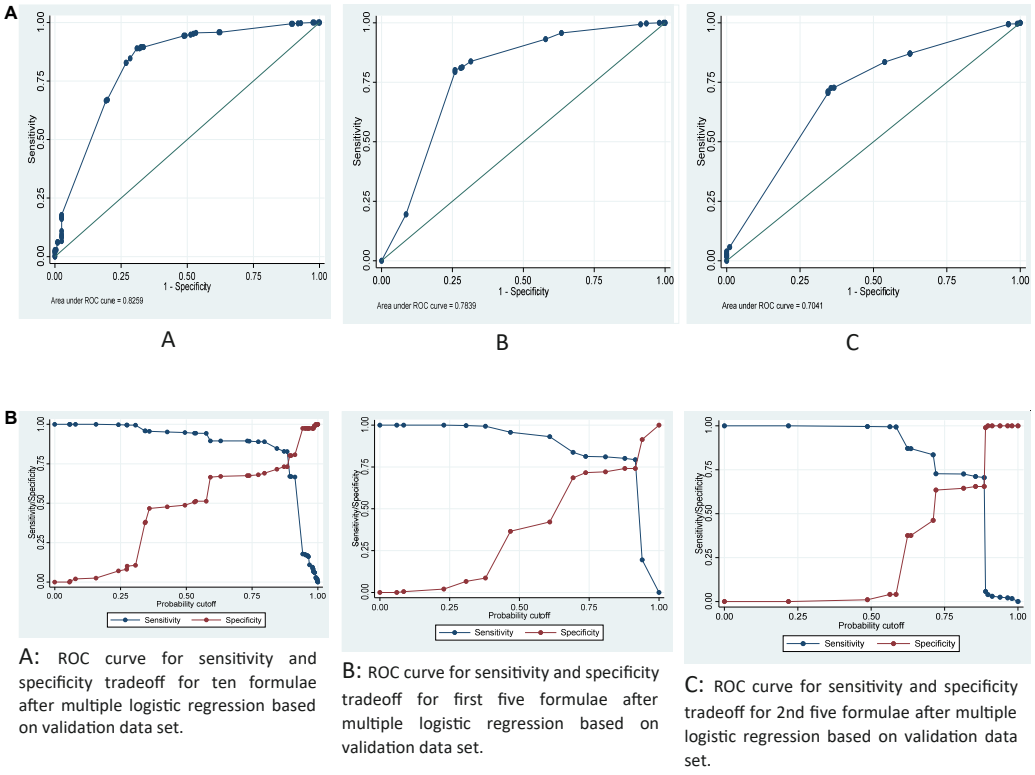
FIGURE 2
(A) ROC curve for ten formulae based on derivation data set. Ho: area (First) = area (Second) = area (Third) = area (Fourth) = area (Fifth) = area (Sixth) = area (Seventh) = area (Eighth) = area (Ninth) = area (Tenth), chi^2 (9) = 205.6, $P < 0.001$, **(B)** ROC curve for **(A)** ten **(A)**, first five **(B)**, and second five **(C)** new integrated weaning indices after multiple logistic regression based on the derivation data set. **(C)** ROC curve for sensitivity and specificity tradeoff for ten **(A)**, first five **(B)**, and 2nd five **(C)** new integrated weaning indices after multiple logistic regression based on derivation data set.

formulas with (AUC: 0.846, 95% CI: 0.822–0.869, $P < 0.001$), and (AUC: 0.828, 95% CI: 0.803–0.851, $P < 0.001$), respectively. Fair performing predictive were related to the sixth and eight formulas with (AUC: 0.737, 95% CI: 0.708–0.764, $P < 0.001$), and (AUC: 0.730, 95% CI: 0.708–0.765, $P < 0.001$), respectively. Poor performing predictive were related to the second, four, seven and nine formulas with (AUC: 0.681, 95% CI: 0.651–0.710, $P < 0.001$), (AUC: 0.686, 95% CI: 0.656–0.716, $P < 0.001$), (AUC: 0.649, 95% CI: 0.618–0.679, $P < 0.001$), and (AUC: 0.657, 95% CI: 0.626–0.687, $P < 0.001$), respectively. Predictive value of fifth and tenth formulas were not significant (AUC: 0.507, 95% CI: 0.475–0.539, $P = 0.751$) and (AUC: 0.502, 95% CI: 0.470–0.534, $P = 0.933$), respectively. The results according to DeLong test indicated a significant difference of AUCs among the second, four, seven and nine formulas with first, third, sixth and eight formulas ($P < 0.05$). Diagnostic indices for each proposed integrated weaning indices in predicting successful weaning is presented in Table 4. Diagnostic indices in the validation dataset indicated that the first, second, third, fourth, and ninth formulae had higher accuracy which was more elaborated in continue.

TABLE 3 Diagnostic indices after multiple logistic regression model based on derivation data set.

Model	SN (95% CI)	SP (95% CI)	LR + (95% CI)	LR- (95% CI)	PPV (95% CI)	NPV (95% CI)	Youden Index	Accuracy
LR on 10 formulae, cut point = 0.5	97 (93–99)	82 (67–93)	5.4 (2.8–10.6)	0.04 (0.02–0.09)	96 (92–98)	87 (71–96)	0.79	94.2
LR on 10 formulae, Optimal cut point = 0.7	95 (91–98)	95 (83–99)	18.6 (4.8–71.7)	0.05 (0.03–0.1)	99 (96–100)	82 (68–92)	0.90	95.2
LR on 1–5 formulae, cut point = 0.5	96 (92–98)	31 (17–48)	1.4 (1.1–1.7)	0.14 (0.06–0.30)	86 (80–90)	63 (38–84)	0.27	83.7
LR on 1–5 formulae, Optimal cut point = 0.8	78 (71–84)	67 (50–81)	2.3 (1.5–3.7)	0.34 (0.24–0.48)	91 (85–95)	41 (29–54)	0.45	75.5
LR on 6–10 formulae, cut point = 0.5	94 (89–97)	69 (52–83)	3.1 (1.9–4.9)	0.09 (0.05–0.16)	93 (88–96)	73 (56–86)	0.63	89.4
LR on 6–10 formulae, Optimal cut point = 0.7	89 (83–93)	90 (76–97)	8.7 (3.4–21.9)	0.13 (0.08–0.19)	97 (94–99)	65 (51–77)	0.79	88.9

LR10, Logistic regression on 10 formulae; LR 1_5, Logistic regression on first 5 formulae; LR 6_10, Logistic regression on second 5 formulae; Cut, cut point; Optimal cut point, optimal cut point based on sensitivity and specificity in the ROC curve after logistic; CI, Confidence interval; SN, Sensitivity; SP, Specificity; LR + , Positive Likelihood Ratio; LR-, Negative Likelihood Ratio; PPV, Positive Predictive value; NPV, Negative Predictive value.



A: ROC curve for sensitivity and specificity tradeoff for ten formulae after multiple logistic regression based on validation data set.

B: ROC curve for sensitivity and specificity tradeoff for first five formulae after multiple logistic regression based on validation data set.

C: ROC curve for sensitivity and specificity tradeoff for 2nd five formulae after multiple logistic regression based on validation data set.

FIGURE 3
(A) ROC curve for ten **(A)**, first five **(B)** and 2nd five **(C)** new integrated weaning indices after multiple logistic regression based on validation data set. **(B)** ROC curve for sensitivity and specificity tradeoff for ten **(A)**, first five **(B)**, and 2nd five **(C)** new integrated weaning indices after multiple logistic regression based on validation data set.

The cumulative effect of all new integrated weaning indices in validation dataset

Diagnostic indices in validation dataset indicated that the model by ten formulae had higher accuracy, and relatively higher values of SN, SP, LR + , PPV, and NPV and lower values of LR- in both 0.5 and optimal cutoff values as compared to two other sets of formulae. Additionally, model by second 5 formulae had higher accuracy, SN, SP, LR + , PPV, and NPV and lower values of LR- in both 0.5 and optimal cutoff values as compared to the model by first five formulae. The

TABLE 4 Diagnostic indices for each proposed new integrated weaning indices based on validation data set.

Formula	Cut point	SN (95% CI)	SP (95% CI)	LR + (95% CI)	LR-(95% CI)	PPV (95% CI)	NPV (95% CI)	Youden Index	Accuracy
First	145.67	92 (90–94)	45 (38–52)	1.7 (1.5–1.9)	0.18 (0.14–0.24)	87 (84–89)	59 (50–67)	0.37	82.3
Second	161.68	99 (98–100)	9 (5–14)	1.09 (1.04–1.14)	0.08 (0.03–0.21)	81 (78–83)	77 (55–92)	0.08	80.9
Third	369.44	81 (78–83)	70 (63–76)	2.7 (2.2–3.3)	0.28 (0.23–0.33)	91 (89–93)	48 (42–54)	0.51	78.5
Fourth	379.04	98 (97–99)	5 (2–9)	1.04 (1.01–1.08)	0.26 (0.11–0.60)	80 (78–83)	50 (27–73)	0.03	79.6
Fifth	333.33	74 (71–77)	24 (18–30)	0.98 (0.89–1.07)	0.9 (0.6–1.3)	79 (76–82)	19 (14–25)	0.02	63.9
Sixth	338.51	78 (75–81)	49 (42–56)	1.5 (1.3–1.8)	0.44 (0.36–0.54)	86 (83–88)	37 (31–43)	0.27	72.4
Seventh	376.12	94 (92–96)	12 (8–17)	1.07 (1.01–1.13)	0.51 (0.31–0.81)	81 (80–83)	33 (22–46)	0.06	77.3
Eighth	839.87	70 (67–74)	65 (57–71)	1.9 (1.6–2.4)	0.46 (0.40–0.54)	89 (86–91)	36 (31–41)	0.35	69.1
Ninth	93.08	95 (93–96)	13 (8–18)	1.09 (1.03–1.15)	0.42 (0.26–0.67)	81 (78–83)	38 (26–51)	0.08	78.0
Tenth	138.22	90 (88–92)	11 (6–16)	1.01 (0.96–1.07)	0.93 (0.59–1.45)	80 (77–82)	21 (14–31)	0.01	73.8

CI, Confidence interval; SN, Sensitivity; SP, Specificity; LR + , Positive Likelihood Ratio; LR-, Negative Likelihood Ratio; PPV, Positive Predictive value; NPV, Negative Predictive value.

TABLE 5 Diagnostic indices after multiple logistic regression model based on validation data set.

Model	SN (95% CI)	SP (95% CI)	LR + (95% CI)	LR- (95% CI)	PPV (95% CI)	NPV (95% CI)	Youden Index	Accuracy
LR on 10 formulae, cut point = 0.5	94 (93–96)	51 (44–58)	1.9 (1.7–2.2)	0.11 (0.08–0.15)	88 (86–90)	70 (62–77)	0.45	85.5
LR on 10 formulae, Optimal cut point = 0.8	85 (82–87)	72 (65–78)	3.0 (2.4–3.7)	0.21 (0.18–0.26)	92 (90–94)	54 (48–61)	0.57	82.0
LR on 1–5 formulae, cut point = 0.5	93 (91–95)	42 (35–49)	1.6 (1.4–1.8)	0.16 (0.12–0.22)	86 (84–89)	61 (52–69)	0.35	82.7
LR on 1–5 formulae, Optimal cut point = 0.9	79 (76–82)	74 (67–80)	3.1 (2.4–3.9)	0.28 (0.23–0.33)	92 (90–94)	48 (42.54)	0.53	78.3
LR on 6–10 formulae, cut point = 0.5	99 (98–100)	4 (2–8)	1.04 (1.01–1.07)	0.13 (0.04–0.42)	80 (78–83)	67 (35–90)	0.03	80.0
LR on 6–10 formulae, Optimal cut point = 0.8	73 (69–76)	65 (57–71)	2.0 (1.7–2.5)	0.43 (0.37–0.50)	89 (86–91)	38 (32–43)	0.38	70.9

LR10, Logistic regression on 10 formulae; LR 1_5, Logistic regression on first 5 formulae; LR 6_10, Logistic regression on second 5 formulae; Cut, cut point; Optimal cut point, optimal cut point based on sensitivity and specificity in the ROC curve after logistic; CI, Confidence interval; SN, Sensitivity; SP, Specificity; LR + , Positive Likelihood Ratio; LR-, Negative Likelihood Ratio; PPV, Positive Predictive value; NPV, Negative Predictive value.

results indicated AUC of about, 0.83, 0.78, and 0.70 respectively, for ten, first five, and second five formulas simultaneously on predicting successful weaning after fitting a multiple logistic regression in the validation dataset (Figure 3A). The optimal cutoff values were estimated based on sensitivity and specificity trade-off in Figure 3B, showed a tradeoff of sensitivity and specificity in the cutoff of around 0.8, 0.9 and 0.8 for predicted probability in the model by ten (A), first five (B) and 2nd five (C) integrated weaning indices, respectively, in the multivariate logistic regression (Table 5).

Discussion

The aim of weaning indices is to find patients who can be successfully weaned as clinical judgment is not accurate

enough to predict weaning outcome in most critically ill patients (26). In this study, we introduced ten new and integrated weaning indices (index 1 to index 10). Our results showed that patients that present poor prognosis for weaning according to a high f/Vt ratio, can present better prognosis according to new integrative indices, if variables of respiratory system dynamics, the respiratory drive and the oxygenation/ventilation are appropriate. These to ten integrated indices had compared favorably to previous indices such as RSBI, NIF, and P0.1, etc. A large spectrum of weaning predictors has been studied either simple weaning indices, others that measures load and capacity e.g., negative inspiratory force (NIP), maximum inspiratory pressure (P_{Imax}), tidal volume (V_T), and breathing frequency (f) or integrative weaning indices requiring special equipment e.g., minute ventilation ($\dot{V}_E$), the ratio of breathing frequency to tidal volume (f/VT), P0.1, and compliance, rate, oxygenation, pressure system (CROP) index (27–30). However, Conti and colleagues (28, 31), showed that vital capacity, V_T, P0.1, $\dot{V}_E$, respiratory frequency (RR), maximum inspiratory pressure (P_{Imax}), RSBI and f/V_T are poor predictors of weaning outcome in an ICU population.

This fact emphasizes the hypothesis that not only the clinical evaluation, but also the evaluation of weaning indexes should be considered in the weaning process of critically ill patients. Weaning indices are based one single function/parameter have usually presented poor accuracy and for this reason, an integrative index that can evaluate multiple essential functions and may represent better outcome (32). It was reported that from 66 predictors of weaning were reviewed and analyzed by McMaster university, only eight predictors were recognized as more valuable than others (33–35), the most frequently used was the RSBI that was assessed by at least 25 studies (36). Although other variables as CROP, RSBI, P0.1, and P_{Imax} are integrative variables, but they are affected not only by the respiratory system mechanics but also could be affected by other factors such as chest and abdominal wall compliance e.g., CROP index, and by the neurological drive e.g., (P0.1) which are variable from one moment to another (37). The current indices are rather heterogeneous variables that reflect the capacity and the integrative function of the respiratory system as a whole i.e., assess both ventilator pump and also oxygenation capacity of the lungs and the ability to maintain their function and endurance effectively for a certain time interval.

To our knowledge, there are three additional integrated weaning indices (WI) reported in the literature including Huaringa et al. (38), Jabour et al. (31), and De Souza et al. (35). The first weaning index (WI1) was proposed by Huaringa et al. (38), who added two corrective factors to RSBI including the elastance index (EI = peak pressure/NIF) and the ventilator demand index (VDI = minute ventilation/10). The sensitivity of the WI1 index was 98%, specificity was 89%, PPV was 95%, NPV was 94%, and the area under the ROC curve was 0.95. Although characterizations of Huaringa's index are excellent, further critique is required. For example, Huaringa's study consisted of a single group with a modest number of only 59 patients. In this situation, the pilot data set did not include WI1 consisting of two added variables EI and VDI. This omission nullifies the selection of data thresholds that were derived solely from the literature. The second weaning index (WI2) was proposed by Jabour et al. (31), who combined ventilator endurance and the efficiency of gas exchange to their index. However, the interpretation of WI2 is very difficult because of the scant weaning research employing this index. Indeed, we failed at finding a single study using WI2. The current weaning indices were derived from a large patient data set with high variability of disease types aiming for the introduction of reliable, reproducible and robust weaning predictor. Nevertheless, until additional studies can validate the performance of our weaning variables, the explicit and implied limitations of our study must be taken seriously. Thus, present interpretations of our ten variables must be done with caution. Ebrahimabadi et al. (39), in their study on 105 mechanically ventilated patients showed that the integrative weaning index (IWI) as a more objective indicator has acceptable accuracy and power for predicting the 2-h SBT result. Therefore, in addition to the reliable prediction of the final weaning outcome, it has favorable power to predict if the patient is ready to breathe spontaneously as the first step to weaning which is in accordance to our results.

There were limited data in the literature regarding whether the use of IWI affected the success rate of weaning from mechanical ventilation. In our study, IWI had better predictive value for weaning patients from mechanical ventilation. Our results showed that the integration of important single functions into an index can be helpful to improve its weaning predictive value when compared with each single function component alone. Our integrative indices use essential parameters that are simple to measurement and are independent of the patient's cooperation. The scores, in a single equation, the respiratory system dynamics, the respiratory drive, the oxygenation/ventilation, and the respiratory pattern, through NIF, P0.01, PPR-P(A-a) O_2, SaO_2 and RSBI ratio respectively. Several reasons concurred to the choice of the parameters above: RSBI in most papers is considered as the best or one of the best indices to evaluate the weaning outcome; respiratory system indices (Resistance and static compliance) is associated with a shorter time to weaning when compliance is more than 20 ml/cmH_2O. Regarding oxygenation our indices use SaO_2 and P(A-a) O_2 which have fewer variation compared to other indices. Multiplying or dividing these indices, we can detect those patients who can or cannot maintain a good oxygenation, despite good or bad respiratory mechanics, patients who will or will not be able to maintain unassisted breathing. In that, they offer a more comprehensive perspective on pathophysiological conditions. It is proposed that the new indices maybe applied to a comprehensive continuum of hospitalized ICU patients presenting with a wide range of illnesses. Although these indices

assess oxygen saturation and respiratory mechanics/drive from different views, applying each index must be adjusted according to the clinician's goal while assessing these indices in each individual patient, i.e., the first to fifth indices are less complex than the sixth to tenth indices. Thus, the first to fifth indices are simpler and more applicable in patients with the more acceptable clinical situation while others (index six to index ten) are more suitable when confronting patients with the complex and elaborated situation since they incorporate more information reflecting the ventilator and oxygenation capacity of the respiratory pump and the lungs respectively.

Limitations of the study: Our weaning indices are more accurate than traditional and simple ones, however, it is considered that the indices aren't completely fit in simple weaning. Furthermore, before measurement of the indices, if patients had suitable RSBI, the patients didn't include in the measurements of new indices. Then, new indices were calculated for others (Specific populations, difficult and prolonged weaning). According to the last consensus conference, T tube or PSV 5-8 ± PEEP was recommended but in study settings, the SBT with CPAP 5 cmH2O is so common. Then, this may be an additional limitation to interpret the results. Moreover, the lack of subgroups based on ICU type and also not recorded the weaning outcome as prolong weaning can be considered as other limitations of this study. The routine clinical application of our findings should await further studies with larger samples. It should also be noted that our study population represents a heterogeneous collection and we think that it would be desirable to evaluate the validity of these indices in each one of population groups in the future. Despite these limitations, our results showed that the integration of important single functions/parameter can be helpful to improve the accuracy of successful weaning. Patients that present poor prognosis for weaning according conventional indices can present good prognosis according to the new weaning indices. Another significant point is that all previous studies were performed as comparative interventional studies whereas the current study has a cross-sectional design. It is obvious that the findings of a comparative study will be different from those of a descriptive study. According to our results, Integrative Weaning Indices compared to the physicians' selected indexes had higher sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and accuracy. This was consistent with Nemer's study in 2009 and could prove persistence of successful weaning in a 48-h period with an accuracy above 90% (37).

Conclusion

Our ten integrated weaning indices are reliable and reproducible indices that integrate ventilator pump efficiency, pulmonary gas exchange, the balance between respiratory demands and respiratory muscle reserve into more accurate predictors of weaning success. Although the comparison of these integrated weaning indices with others weaning indices revealed better predictive power of weaning outcome in intensive care patients and can apply for a comprehensive continuum of different hospitalized patients to predict the weaning outcome, interpretations of such variables must be done with caution until further validation.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. The clinical trial registered code is NCT01779297 and is available at: http://clinicaltrials.gov/c.

Author contributions

AV-A, AM, and AS designed the study. FR-B, KG-M, MK, SM, and LS contributed to acquisition of data, analysis and interpretation of data, and drafting the article. All authors contributed to the study, edited and revised manuscript, and approved final version of manuscript.

Conflict of interest

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

Publisher's note

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