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CLINICAL TRIAL REPORT

Dynamic Changes in Serum Choline Acetyltransferase and Acetylcholinesterase in Septic versus Non-Septic ICU Patients: A Prospective Exploratory Study

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Objective: To compare serum choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) levels between septic and non-septic critically ill patients, and to evaluate their dynamic changes, associations with inflammatory markers, disease severity, and prognosis in sepsis.

Methods: In this prospective observational study, 87 patients with sepsis and 93 non-septic critically ill controls were enrolled. Blood samples were collected from septic patients within 12 hours (T1), 24 hours (T2), 48 hours (T3), and 7 days (T4) after intensive care unit (ICU) admission, while the non-septic group underwent sampling only at T1. Serum levels of ChAT, AChE, and inflammatory cytokines (IL-6, IL-10, TNF-α) were measured. A linear mixed model was used to analyze dynamic changes, repeated measures correlation (rmcorr) to assess associations between variables, receiver operating characteristic curves to evaluate discriminative ability, and multivariable logistic regression to identify independent influencing factors.

Results: Septic patients had significantly lower ChAT at T1 than non-septic controls ($P<0.001$), whereas AChE levels did not differ. Both ChAT and AChE were lower in septic shock than in non-shock septic patients (both $P<0.05$). Linear mixed-model analysis revealed a significant overall group effect for AChE, with lower levels in non-survivors (group effect, $P = 0.013$); however, no significant intergroup difference was observed at T1 ($P = 0.098$). ChAT showed moderate discriminative ability for sepsis, with an area under the ROC curve of 0.70, and was identified as an independent factor associated with sepsis in this exploratory cohort (OR = 0.85, 95% CI: 0.76–0.94, $P = 0.001$). The inverse association between ChAT and sepsis was stronger in patients aged ≥65 years (interaction $P=0.023$).

Conclusion: This exploratory study demonstrates that serum ChAT levels are significantly decreased in septic patients, correlate with disease severity, and show moderate discriminative ability for sepsis. Linear mixed-model analysis revealed that AChE levels were overall lower in non-survivors than in survivors, although the prognostic value at individual time points was limited. Age modifies the ChAT–sepsis association, which should be considered in clinical evaluation.

Keywords: sepsis, cholinergic anti-inflammatory pathway, choline acetyltransferase, acetylcholinesterase, prognosis

Introduction

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. It represents a common and critical clinical syndrome in the field of critical care medicine worldwide and is a major cause of death among patients in intensive care units (ICUs).[1,2] In recent years, despite significant advances in early identification,[3] fluid resuscitation,[4,5] anti-infective therapy,[6] and organ support,[1,7] the mortality rate of sepsis, particularly septic shock, remains persistently high,[8] imposing a substantial medical burden on families and society.[9] The pathogenesis of sepsis

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is complex and involves multiple interconnected mechanisms, including dysregulated inflammatory responses, immunosuppression, mitochondrial dysfunction, and coagulation disorders,[1,7,10] among which uncontrolled inflammation is considered the core pathophysiological basis leading to tissue damage and multiple organ dysfunction.

The cholinergic anti-inflammatory pathway (CAP) is a key component of the neuro-immune regulatory network and plays an important role in modulating the systemic inflammatory response in sepsis.[11,12] This pathway involves the release of acetylcholine (ACh) by the vagus nerve, which binds to the α7 nicotinic acetylcholine receptor (α7nAChR) on the surface of splenic macrophages, thereby inhibiting the synthesis and release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), thus exerting a negative regulatory role in the inflammatory response.[13] In the context of sepsis, CAP often exhibits functional impairment or suppression, leading to amplification of the inflammatory response, immune dysfunction, and multiple organ injury.[14]

Choline acetyltransferase (ChAT) is a key enzyme in the synthesis of acetylcholine within the cholinergic anti-inflammatory pathway, and its activity directly influences the efficiency of acetylcholine production.[15] Acetylcholinesterase (AChE) is the key enzyme responsible for hydrolyzing acetylcholine, reflecting the metabolic status of cholinergic neurotransmitters.[16] The α7nAChR expressed by macrophages and the ACh generated thereby are components of the non-neuronal cholinergic system, and have become a research focus due to their potential to regulate inflammatory and immune responses;[17] they also represent the final effector target of CAP-mediated neuroimmune regulation. The present study focuses on changes in ChAT and AChE within the CAP and their clinical significance.

Previous studies have shown that peripheral blood cholinesterase activity is often significantly decreased in patients with sepsis, and its decline is associated with disease severity, organ failure, and short-term mortality risk.[18–20] However, only one small-sample study (n = 11) has reported changes in serum ChAT activity in septic patients, with results suggesting elevated ChAT levels;[15] due to the limited sample size, this conclusion requires validation in larger cohorts. Therefore, prospective observational studies are needed to further clarify the pattern of ChAT changes in sepsis and to examine the dynamic trends of both enzymes and their prognostic implications.

We hypothesized that ChAT and AChE may exhibit distinct dynamic patterns during the course of sepsis. Accordingly, this prospective observational study was designed to compare serum ChAT and AChE levels between septic and non-septic critically ill patients, to dynamically track the trajectories of these two enzymes over a 7-day period in septic patients, and to analyze their associations with inflammatory cytokines, disease severity, and prognosis. The aim was to preliminarily explore the potential value of these two enzymes in the clinical evaluation of sepsis.

Materials and Methods

Study Design and Patient Characteristics

This study was a single-center, prospective, observational cohort study. Patients consecutively admitted to the Intensive Care Unit (ICU) of the Fourth Hospital of Hebei Medical University from December 2024 to May 2025 were enrolled and divided into a sepsis group and a non-sepsis group according to the Sepsis-3 criteria. Patients in the sepsis group were diagnosed with sepsis upon ICU admission. Inclusion criteria were: (1) age ≥ 18 years; (2) for the sepsis group, fulfillment of the Sepsis-3 diagnostic criteria, defined as infection plus an increase in the Sequential Organ Failure Assessment (SOFA) score of ≥ 2 points; (3) newly diagnosed sepsis, with the time interval from diagnosis to enrollment ≤ 2 hours. Exclusion criteria were: (1) a history of neuropsychiatric disorders such as schizophrenia or Alzheimer's disease, or long-term use of medications affecting mental status; (2) a history of splenectomy; (3) for patients in the sepsis group, fewer than two time points with valid serum ChAT or AChE measurements; (4) ICU length of stay < 24 hours; and (5) pregnant or breastfeeding patients.

Blood samples were collected from patients in the sepsis group within 12 hours (T1), at 24 hours (T2), 48 hours (T3), and 7 days (T4) after ICU admission. In the non-sepsis group, a single blood sample was collected within 12 hours of admission. Patients with sepsis were further divided into survivor and non-survivor groups based on their 28-day survival status. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (Approval No. 2024KS159). Written informed consent was obtained from all patients or their legal proxies. This study was registered in the Chinese Clinical Trial Registry (registration number: ChiCTR2500106909).

Sample Measurement

Venous blood samples were centrifuged at 3000 rpm for 10 min at 4°C. Serum was separated and stored at −80°C until analysis. Serum choline acetyltransferase (ChAT) activity was measured using a commercial colorimetric assay kit (Nanjing Jiancheng Bioengineering Institute, China; Cat. No. A079-2-1) following the manufacturer's instructions. Absorbance was read with a microplate reader. Serum acetylcholinesterase (AChE) activity was determined using a commercial activity assay kit (Shanghai Zhuocai Biotechnology Co., Ltd., China; Cat. No. ZC-S0384) according to the manufacturer's protocol. Concentrations of inflammatory cytokines (IL-6, IL-10, TNF-α) were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Wuhan Boster Biological Technology Co., Ltd., China).

Data Collection

Demographic data (age, sex, body mass index [BMI]), clinical characteristics (type of infection, underlying diseases, surgical history), laboratory parameters (routine blood tests, biochemical profiles, coagulation function, blood gas analysis, etc), disease severity scores (Acute Physiology and Chronic Health Evaluation [APACHE] II score, SOFA score), and prognostic information (28-day survival status, ICU length of stay, sepsis-associated acute kidney injury, delirium, etc.) were collected for all patients.

Statistical Analysis

This study was an exploratory analysis, and the sample size was determined based on clinical feasibility and preliminary experimental data; no formal power calculation was performed. All statistical analyses were conducted using R version 4.3.0, Zstats version 1.0, and SPSS version 27.0. Data visualization was performed using GraphPad Prism 10 and R software.

For continuous variables, those following a normal distribution were expressed as mean ± standard deviation (SD), and comparisons between groups were performed using the independent samples t-test. Non-normally distributed continuous variables were described as median (interquartile range), and comparisons between two groups were performed using the Mann-Whitney *U*-test. Categorical variables were described as frequencies and percentages, and comparisons between groups were performed using the chi-square (χ^2) test. A two-tailed significance level of $\alpha = 0.05$ was used for all tests. A linear mixed model was used to analyze the dynamic changes in serum ChAT and AChE levels in patients with sepsis, with patient ID as a random intercept and time point (T1, T2, T3, T4), group (non-survivor vs survivor), and their interaction as fixed effects. An unstructured covariance matrix was assumed. Post-hoc pairwise comparisons were adjusted using Bonferroni correction. Repeated measures correlation (rmcorr) was used to assess the within-individual correlations of ChAT and AChE with IL-6, IL-10, and TNF-α, accounting for the non-independence of repeated measurements from the same patient. Results are reported as r_rm, 95% CI, degrees of freedom, and p-values. Receiver operating characteristic (ROC) curves were constructed to assess the discriminative ability of serum ChAT and AChE for sepsis, and the area under the curve (AUC), sensitivity, specificity, and Youden index were calculated. Multivariate logistic regression with forward stepwise selection was used to identify independent factors associated with sepsis, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test. Bootstrap internal validation (500 resamples) was performed to assess the stability of the final model, including the variables selected in the final model, and the corrected AUC was calculated. Subgroup analyses were performed using multivariate logistic regression, and interaction effects were tested. A P value < 0.05 was considered statistically significant. Missing data were handled using complete-case analysis, with a low proportion of missing values (<5%) across key variables.

Results

Baseline Characteristics and Clinical Features

Patient Enrollment Process

A total of 226 critically ill patients admitted to the ICU of the Fourth Hospital of Hebei Medical University from December 2024 to May 2025 were screened. According to the inclusion and exclusion criteria, 180 patients were finally

enrolled, including 87 patients in the sepsis group and 93 patients in the non-sepsis group. Among the sepsis group, 27 patients (31.03%) died within 28 days, and 60 patients (68.97%) survived. The patient enrollment process is illustrated in Figure 1.

Comparison of Baseline Characteristics Between the Sepsis and Non-sepsis Groups

Comparison of baseline characteristics between the two groups is shown in Table 1. There were no statistically significant differences between the sepsis group and the non-sepsis group in terms of age, sex, or BMI (P>0.05). Statistically significant differences were observed between the two groups in albumin, lymphocyte count, fibrinogen, hemoglobin, body temperature, heart rate, platelet count, creatinine, oxygenation index, lactate, total bilirubin, ChAT level, proportion of patients with coronary heart disease, and proportion of patients receiving immunosuppressive therapy (P<0.05). Compared with the control group, the sepsis group had higher body temperature, heart rate, creatinine, fibrinogen, lactate, total bilirubin levels, and a higher proportion of patients receiving immunosuppressive therapy, whereas albumin, lymphocyte count, hemoglobin, platelet count, oxygenation index, ChAT levels, and the proportion of patients with coronary heart disease were lower.

Comparison of Baseline Characteristics Between Different Prognostic Subgroups in Patients with Sepsis

Among the 87 patients with sepsis, 27 were classified into the non-survivor group and 60 into the survivor group based on 28-day survival status. Comparison of baseline characteristics between the two subgroups is shown in Table 2. Statistically significant differences were observed between the survivor and non-survivor groups in body temperature, lactate level, SOFA score, postoperative admission status, and proportion of patients with septic shock (all P<0.05). Compared with the survivor group, the non-survivor group had higher lactate levels and SOFA scores, a higher proportion of non-surgical admissions, a higher proportion of patients with septic shock, and lower body temperature at ICU admission.

Comparison of Serum ChAT and AChE Levels

Comparison Between the Sepsis and Non-sepsis Groups

Serum ChAT levels at T1 were significantly lower in the sepsis group than in the non-sepsis group [15.50 (12.25, 20.70) U/mL vs 21.21 (17.00, 23.57) U/mL, P<0.001], whereas no statistically significant difference was observed in serum AChE levels between the two groups (P>0.05) (Table 3 and Figure 2).

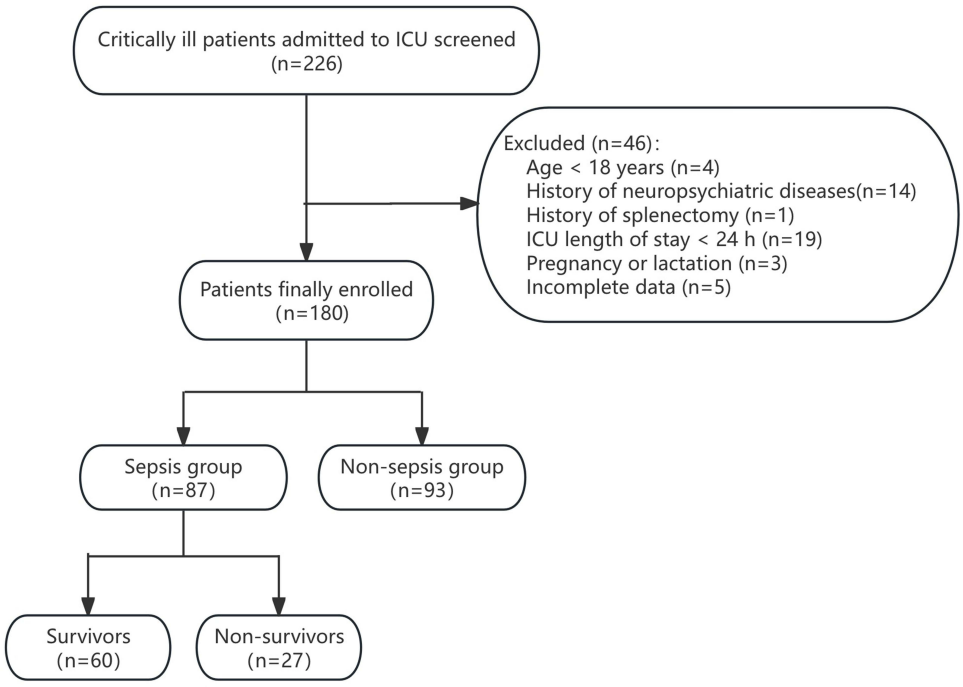

Figure 1 Patient enrollment flowchart.

Table 1 Comparison of Baseline Characteristics Between Sepsis and Non-Sepsis Groups

Variables	Total (n = 180)	Non-Sepsis Group (n = 93)	Sepsis Group (n = 87)	*P*
Age (years)	69.00 (61.00, 74.00)	68.00 (60.00, 74.00)	70.00 (61.00, 74.00)	0.858
BMI (kg/m^2)	24.12 ± 3.87	24.62 ± 4.07	23.57 ± 3.58	0.069
Gender (%)				0.264
Female	59 (32.78)	34 (36.56)	25 (28.74)	
Male	121 (67.22)	59 (63.44)	62 (71.26)	
Laboratory tests				
Temperature (°C)	36.70 (36.30, 37.30)	36.50 (36.20, 37.00)	36.90 (36.50, 37.80)	<0.001
Heart rate (bpm)	84.00 (69.00, 102.00)	73.00 (64.00, 89.00)	96.00 (82.50, 113.50)	<0.001
WBC (×10^9/L)	10.04 (7.34, 13.94)	10.07 (7.67, 12.47)	9.86 (6.66, 15.39)	0.621
Neutrophil (×10^9/L)	8.63 (6.00, 12.74)	8.30 (6.04, 10.45)	8.83 (5.85, 14.18)	0.133
Lymphocyte (×10^9/L)	0.71 (0.42, 1.10)	0.96 (0.64, 1.32)	0.48 (0.30, 0.73)	<0.001
Hemoglobin (g/L)	114.00 (94.00, 129.00)	123.00 (108.00, 133.00)	97.00 (87.00, 120.00)	<0.001
Platelet (×10^9/L)	181.50 (127.75, 240.25)	197.00 (152.00, 236.00)	159.00 (90.00, 244.00)	0.015
Creatinine (umol/L)	66.00 (52.00, 91.25)	60.00 (49.00, 71.00)	86.00 (62.00, 130.65)	<0.001
Albumin (g/L)	31.05 ± 5.33	33.43 ± 4.46	28.52 ± 5.02	<0.001
Fibrinogen (g/L)	3.60 (2.92, 4.67)	3.17 (2.73, 4.03)	4.36 (3.47, 5.26)	<0.001
Oxygenation Index (mmHg)	282.00 (201.62, 376.02)	351.50 (266.60, 423.00)	211.00 (163.00, 307.90)	<0.001
Lactic acid (mmol/L)	1.62 (1.10, 2.30)	1.50 (1.00, 2.00)	1.80 (1.20, 2.72)	0.005
Total Bilirubin (umol/L)	11.75 (8.28, 16.96)	11.00 (8.30, 13.50)	13.70 (8.20, 22.45)	0.013
Comorbidity				
Hypertension (%)				0.866
No	94 (52.22)	48 (51.61)	46 (52.87)	
Yes	86 (47.78)	45 (48.39)	41 (47.13)	
Diabetes (%)				0.926
No	136 (75.56)	70 (75.27)	66 (75.86)	
Yes	44 (24.44)	23 (24.73)	21 (24.14)	
Coronary heart disease (%)				0.026
No	145 (80.56)	69 (74.19)	76 (87.36)	
Yes	35 (19.44)	24 (25.81)	11 (12.64)	
Cerebral infarction (%)				0.983
No	153 (85.00)	79 (84.95)	74 (85.06)	
Yes	27 (15.00)	14 (15.05)	13 (14.94)	
Immunosuppressant use (%)				0.018
No	156 (86.67)	86 (92.47)	70 (80.46)	
Yes	24 (13.33)	7 (7.53)	17 (19.54)	
Smoke (%)				0.080
No	101 (56.11)	58 (62.37)	43 (49.43)	
Yes	79 (43.89)	35 (37.63)	44 (50.57)	

Notes: Mean±SD for continuous variables, (%) for categorical variables.
Abbreviations: BMI, Body Mass Index; WBC, White Blood Cell.

Table 2 Comparison of Baseline Characteristics Between Survivors and Non-Survivors In Septic Patients

Variables	Total (n = 87)	Survivors (n = 60)	Non-Survivors (n = 27)	*P*
Age (years)	70.00 (61.00, 74.00)	68.50 (58.00, 74.00)	71.00 (68.50, 74.50)	0.083
BMI (kg/m^2)	23.57 ± 3.58	23.62 ± 3.23	23.47 ± 4.32	0.863
Gender (%)				0.368
Female	25 (28.74)	19 (31.67)	6 (22.22)	
Male	62 (71.26)	41 (68.33)	21 (77.78)	

(*Continued*)

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Table 2 (Continued).

Variables	Total (n = 87)	Survivors (n = 60)	Non-Survivors (n = 27)	*P*
Postoperative ICU admission (%)				0.012
No	54 (62.07)	32 (53.33)	22 (81.48)	
Yes	33 (37.93)	28 (46.67)	5 (18.52)	
Infection type (%)				0.291
Community acquisition	41 (47.13)	26 (43.33)	15 (55.56)	
Hospital acquisition	46 (52.87)	34 (56.67)	12 (44.44)	
Laboratory tests				
Temperature (°C)	36.90 (36.50, 37.80)	37.05 (36.70, 38.00)	36.70 (36.45, 37.25)	0.019
Heart rate (bpm)	99.31 ± 22.99	99.57 ± 21.96	98.74 ± 25.56	0.878
WBC ($\times 10^9$/L)	9.86 (6.66, 15.39)	9.41 (5.91, 14.85)	11.54 (8.06, 15.97)	0.156
Neutrophil ($\times 10^9$/L)	8.83 (5.85, 14.18)	8.62 (5.50, 13.72)	10.74 (6.83, 15.24)	0.289
Lymphocyte ($\times 10^9$/L)	0.48 (0.30, 0.73)	0.48 (0.31, 0.71)	0.46 (0.29, 0.84)	0.989
Hemoglobin (g/L)	97.00 (87.00, 120.00)	96.50 (87.00, 119.25)	99.00 (88.50, 120.50)	0.829
Platelet ($\times 10^9$/L)	159.00 (90.00, 244.00)	173.50 (114.75, 253.75)	124.00 (75.50, 233.50)	0.138
Creatinine (umol/l)	86.00 (62.00, 130.65)	82.95 (62.75, 113.50)	103.80 (61.30, 180.00)	0.315
Albumin (g/L)	28.52 ± 5.02	28.99 ± 5.60	27.46 ± 3.27	0.114
Fibrinogen (g/L)	4.36 (3.47, 5.26)	4.46 (3.53, 5.22)	3.82 (3.21, 5.67)	0.801
Oxygenation Index (mmHg)	211.00 (163.00, 307.90)	222.80 (168.00, 306.95)	202.50 (135.40, 291.15)	0.539
Lactic acid (mmol/L)	1.80 (1.20, 2.72)	1.70 (1.10, 2.36)	2.23 (1.40, 3.60)	0.018
Total Bilirubin (umol/L)	13.70 (8.20, 22.45)	12.80 (8.15, 19.68)	16.00 (9.30, 32.19)	0.172
IL-6 (pg/mL)	99.18 (29.01, 383.16)	101.80 (36.61, 381.50)	76.41 (21.37, 479.93)	0.748
IL-10 (pg/mL)	7.00 (4.54, 22.65)	7.23 (4.48, 15.02)	6.48 (4.64, 25.94)	0.494
TNF-a (pg/mL)	21.07 (10.19, 51.31)	19.95 (11.50, 50.82)	25.10 (7.85, 54.56)	0.912
Comorbidity				
Hypertension (%)				0.291
No	46 (52.87)	34 (56.67)	12 (44.44)	
Yes	41 (47.13)	26 (43.33)	15 (55.56)	
Diabetes (%)				0.173
No	66 (75.86)	43 (71.67)	23 (85.19)	
Yes	21 (24.14)	17 (28.33)	4 (14.81)	
Coronary heart disease (%)				0.146
No	76 (87.36)	55 (91.67)	21 (77.78)	
Yes	11 (12.64)	5 (8.33)	6 (22.22)	
Malignant tumor (%)				0.935
No	36 (41.38)	25 (41.67)	11 (40.74)	
Yes	51 (58.62)	35 (58.33)	16 (59.26)	
Cerebral infarction (%)				0.341
No	74 (85.06)	53 (88.33)	21 (77.78)	
Yes	13 (14.94)	7 (11.67)	6 (22.22)	
Immunosuppressant use (%)				0.314
No	70 (80.46)	50 (83.33)	20 (74.07)	
Yes	17 (19.54)	10 (16.67)	7 (25.93)	
Smoke (%)				0.533
No	43 (49.43)	31 (51.67)	12 (44.44)	
Yes	44 (50.57)	29 (48.33)	15 (55.56)	
Severity score				
APACHE II	16.57 ± 5.63	15.87 ± 5.49	18.15 ± 5.72	0.080
SOFA	7.00 (5.00, 11.00)	6.50 (4.75, 10.00)	10.00 (7.00, 11.50)	0.014

(*Continued*)

Table 2 (Continued).

Variables	Total (n = 87)	Survivors (n = 60)	Non-Survivors (n = 27)	*P*
Treatment measures				
Vasopressor use on day 1 (%)				0.581
No	36 (41.38)	26 (43.33)	10 (37.04)	
Yes	51 (58.62)	34 (56.67)	17 (62.96)	
MV on day 1 (%)				0.588
No	26 (29.89)	19 (31.67)	7 (25.93)	
Yes	61 (70.11)	41 (68.33)	20 (74.07)	
Sedative use on day 1 (%)				0.942
No	23 (26.44)	16 (26.67)	7 (25.93)	
Yes	64 (73.56)	44 (73.33)	20 (74.07)	
Dexmedetomidine (%)				0.262
No	47 (54.02)	30 (50.00)	17 (62.96)	
Yes	40 (45.98)	30 (50.00)	10 (37.04)	
Esmolol (%)				0.410
No	57 (65.52)	41 (68.33)	16 (59.26)	
Yes	30 (34.48)	19 (31.67)	11 (40.74)	
Event				
Length of ICU stay (day)	10.00 (6.00, 21.00)	9.00 (6.00, 24.00)	11.00 (5.50, 17.50)	0.620
Delirium (%)				0.741
No	81 (93.10)	55 (91.67)	26 (96.30)	
Yes	6 (6.90)	5 (8.33)	1 (3.70)	
SAKI (%)				0.051
No	52 (59.77)	40 (66.67)	12 (44.44)	
Yes	35 (40.23)	20 (33.33)	15 (55.56)	
Septic Shock (%)				<0.001
No	41 (47.13)	36 (60.00)	5 (18.52)	
Yes	46 (52.87)	24 (40.00)	22 (81.48)	

Notes: Mean±SD for continuous variables, (%) for categorical variables.
Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, Body Mass Index; ICU, Intensive Care Unit; IL-6, Interleukin-6; IL-10, Interleukin-10; MV, Mechanical Ventilation; SAKI, Sepsis-Associated Acute Kidney Injury; SOFA, Sequential Organ Failure Assessment; TNF-α, Tumor Necrosis Factor-alpha; WBC, White Blood Cell.

Table 3 Comparison of Serum ChAT and AChE Levels Between Sepsis and Non-Sepsis Groups at T1

Variables	Non-Sepsis Group (n = 93)	Sepsis Group (n = 87)	*P*
ChAT (U/mL)	21.21 (17.00, 23.57)	15.50 (12.25, 20.70)	<0.001
AChE (nmol/min/mL)	12.25 (7.84, 17.15)	11.76 (7.11, 15.19)	0.405

Notes: Data are presented as median (interquartile range).
Abbreviations: AChE, acetylcholinesterase; ChAT, choline acetyltransferase.

Comparison Between Survivor and Non-Survivor Subgroups in Sepsis

Among the 87 patients with sepsis, 27 (31.03%) died within 28 days and 60 (68.97%) survived. No statistically significant differences were observed in serum ChAT or AChE levels at T1 between the non-survivor and survivor groups (P>0.05) (Table 4).

Subgroup Analysis

According to the Sepsis-3 diagnostic criteria, the 87 patients with sepsis were further divided into a septic shock group (n = 46) and a non-shock septic group (n = 41). Serum ChAT levels were significantly lower in the septic shock group than in the non-shock septic group (15.10 ± 5.78 U/mL vs 17.96 ± 7.07 U/mL, P=0.041). Serum AChE levels were also significantly

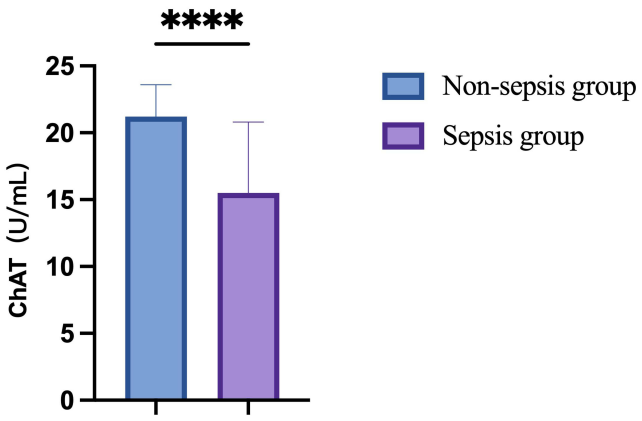

Figure 2 Comparison of serum ChAT levels between non-sepsis and sepsis groups. Data are presented as median with interquartile range (Q1-Q3). ChAT levels were significantly lower in the sepsis group (15.5 U/mL, IQR: 12.25–20.70) compared to the non-sepsis group (21.21 U/mL, IQR: 17.00–23.57).****P < 0.001 (Mann–Whitney *U*-test).

lower in the septic shock group than in the non-shock septic group [9.07 (6.00, 13.23) nmol/min/mL vs 12.74 (8.33, 20.09) nmol/min/mL, P=0.005] (Table 5, Figure 3A and B).

Dynamic Trends of Serum ChAT and AChE in Patients with Sepsis

A linear mixed model was used to analyze the dynamic changes in serum ChAT and AChE levels at four time points (T1, T2, T3, and T4) in 87 patients with sepsis. The results are shown in Figure 4.

Serum AChE: The group effect was statistically significant (F=6.497, P=0.013), indicating that overall AChE levels were significantly lower in the non-survivor group than in the survivor group. Neither the time effect (F = 0.570, P = 0.637) nor the time-by-group interaction effect (F = 0.032, P = 0.992) reached statistical significance. Detailed results of intergroup comparisons at each time point are presented in Table 6. Among the four time points, only at T3 did non-survivors have significantly lower AChE levels than survivors [8.33 (nmol/min/mL) vs 11.26 (nmol/min/mL), P = 0.048]; no significant differences were observed at T1, T2, or T4. Both groups showed a descriptive trend of an initial decline followed by a partial recovery (Figure 4A).

Table 4 Comparison of Serum ChAT and AChE Levels Between Survivors and Non-Survivors at T1

Variables	Survivors (n = 60)	Non-Survivors (n = 27)	*P*
ChAT (U/mL)	16.81 ± 7.10	15.65 ± 5.14	0.448
AChE (nmol/min/mL)	12.25 (7.72, 17.89)	8.33 (6.86, 13.23)	0.098

Notes: Data are presented as mean ± SD for normally distributed variables or median (interquartile range) for non-normally distributed variables.
Abbreviations: AChE, Acetylcholinesterase; ChAT, Choline Acetyltransferase.

Table 5 Comparison of Serum ChAT and AChE Levels Between Septic Shock and Sepsis without shock groups

Variables	Septic Shock Group (n = 46)	Sepsis Without Shock Group (n=41)	*P*
ChAT (U/mL)	15.10 ± 5.78	17.96 ± 7.07	0.041
AChE (nmol/min/mL)	9.07 (6.00, 13.23)	12.74 (8.33, 20.09)	0.005

Notes: Data are presented as mean ± SD for normally distributed variables or median (interquartile range) for non-normally distributed variables.
Abbreviations: AChE, Acetylcholinesterase; ChAT, Choline Acetyltransferase.

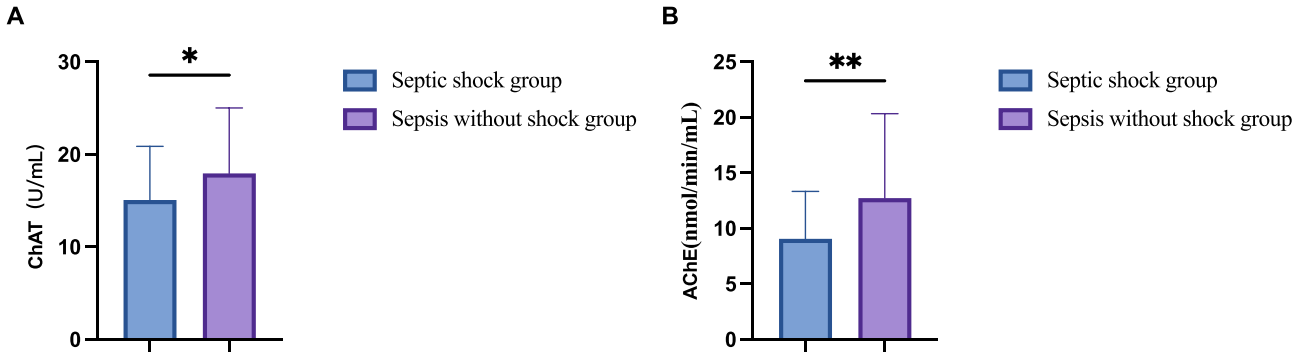

Figure 3 Comparison of serum ChAT and AChE levels between septic shock group and sepsis without shock group. (**A**) Serum ChAT levels. Data are presented as mean ± SD. Patients in the septic shock group (n=46) had significantly lower ChAT levels than those in the sepsis without shock group (n=41) (P = 0.041, independent samples *t*-test). *P < 0.05. (**B**) Serum AChE levels. Data are presented as median with interquartile range. AChE levels were significantly lower in the septic shock group (P = 0.005, Mann–Whitney *U*-test). **P < 0.01.

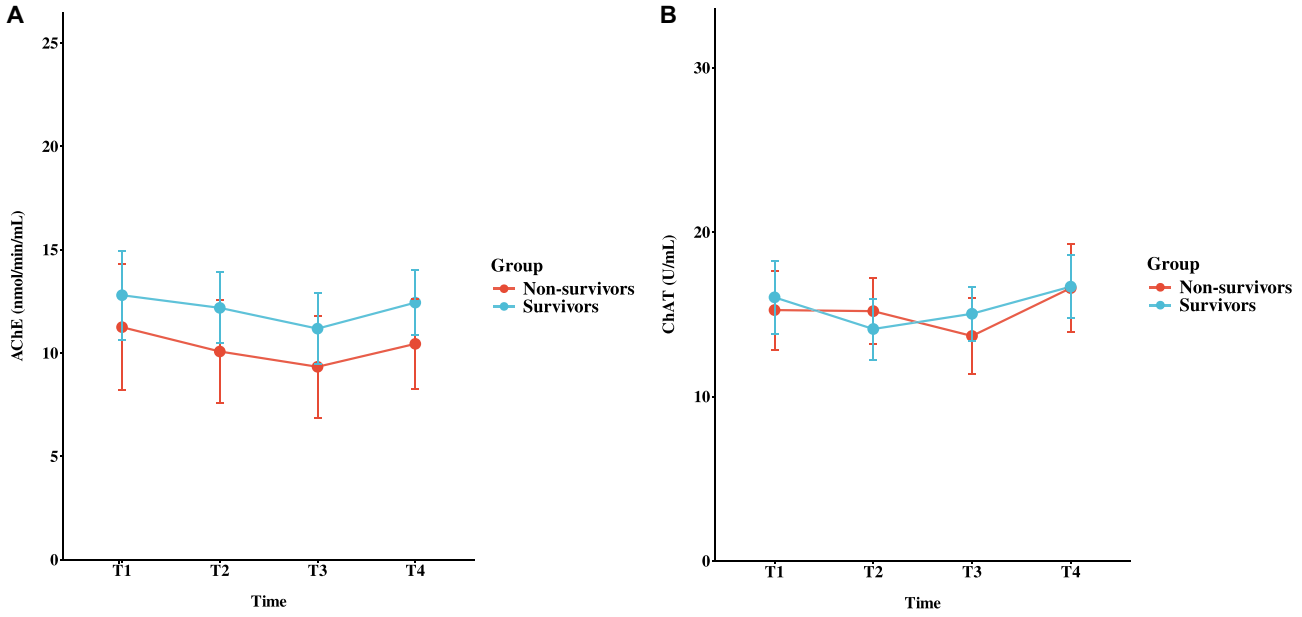

Figure 4 Dynamic changes of serum AChE (**A**) and ChAT (**B**) in sepsis patients with different outcomes. (**A**) Serum AChE levels in survivors (n=60) and non-survivors (n=27). Data are presented as mean (95% CI). Non-survivors had significantly lower AChE levels overall (group effect, P=0.013), with no significant time effect (P=0.637) or interaction (P=0.992). Both groups showed initial decline followed by partial recovery. (**B**) Serum ChAT levels. No significant group (P=0.539), time (P=0.359), or interaction effects (P=0.925) were observed.

Serum ChAT: The group effect (F=0.380, P=0.539), time effect (F=1.090, P=0.359), and interaction effect (F=0.157, P=0.925) were not statistically significant, indicating that ChAT levels did not differ significantly between the two groups and showed no significant change over time (Figure 4B).

Table 6 Comparison of Serum AChE Levels Between Survivors and Non-Survivors at Each Time Point

Variables	Survivors (n = 60)	Non-Survivors (n = 27)	*P*
T1	12.25 (7.72, 17.89)	8.33 (6.86, 13.23)	0.098
T2	11.27 (8.33, 14.70)	9.31 (5.63, 12.74)	0.082
T3	11.26 (8.21, 14.46)	8.33 (5.14, 12.00)	0.048
T4	12.64 ± 5.27	10.45 ± 4.59	0.140

Notes: Data are presented as mean ± SD for normally distributed variables or median (interquartile range) for non-normally distributed variables.

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Discriminative Ability of Serum ChAT and AChE for Sepsis

Receiver operating characteristic (ROC) curves were used to evaluate the discriminative ability of serum ChAT and AChE measured within 12 hours of ICU admission (T1) for sepsis, with 87 patients with sepsis as the case group and 93 non-septic critically ill patients as the control group. The results are shown in Figure 5.

The area under the curve (AUC) of ChAT for discriminating sepsis was 0.70 (95% CI: 0.62–0.78, P<0.001). Whereas the AUC of AChE was 0.54 (95% CI: 0.46–0.62, P=0.404), indicating no significant discriminative ability. The AUC for the combination of ChAT and AChE was 0.70 (95% CI: 0.62–0.78), which was identical to that of ChAT alone, suggesting that the addition of AChE did not improve discriminative performance.

These findings indicate that serum ChAT has moderate discriminative ability for sepsis, whereas AChE is not suitable as an independent discriminative marker.

Correlation Analysis of Serum ChAT and AChE with Inflammatory Cytokines in Patients with Sepsis

To account for the within-patient correlation inherent in repeated measurements, we used repeated measures correlation (rmcorr) to assess the associations of serum AChE and ChAT with inflammatory cytokines.

The rmcorr analysis showed no significant correlations between AChE and IL-6 ($r_{rm} = -0.04$, 95% CI: −0.168 to 0.089, df = 231, p = 0.543), IL-10 ($r_{rm} = -0.005$, 95% CI: −0.133 to 0.124, df = 231, p = 0.94), or TNF-α ($r_{rm} = -0.067$, 95% CI: −0.194 to 0.062, df = 231, p = 0.308). ChAT also showed no statistically significant correlations with any of the inflammatory cytokines (all $p > 0.05$).

In addition, the rmcorr analysis revealed a significant positive correlation between ChAT and AChE ($r_{rm} = 0.14$, 95% CI: 0.011 to 0.264, df = 231, p = 0.033).

Multivariable Logistic Regression Analysis of Factors Associated with Sepsis and Incremental Value Assessment

Using sepsis status as the dependent variable, forward stepwise selection (LR) was used to identify independent influencing factors. Five independent factors were ultimately identified (Table 7 and Figure 6). Model evaluation showed good calibration, as assessed by the Hosmer-Lemeshow test ($\chi^2 = 14.906$, P = 0.061), with a Nagelkerke R^2 of 0.726. The full model (including ChAT) yielded an area under the ROC curve of 0.94 (95% CI: 0.91–0.98) for predicting sepsis (Figure 7).

To assess the incremental value of ChAT, we compared the AUC of a baseline clinical model (total bilirubin, oxygenation index, heart rate, hemoglobin) with that of a model additionally including ChAT. In the whole cohort, the AUC increased from 0.929 to 0.943, with overlapping confidence intervals indicating no significant improvement.

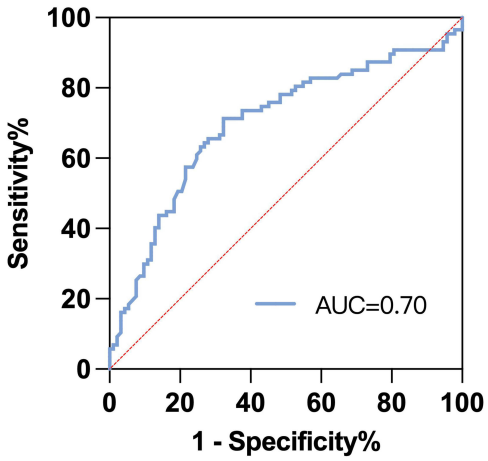

Figure 5 ROC curve of serum ChAT for discriminating sepsis. The area under the curve (AUC) for ChAT was 0.70 (95% CI: 0.62–0.78, P < 0.001).

Table 7 Multivariable Logistic Regression Analysis of Factors Associated with Sepsis

Variables	OR (95% CI)	*P*
ChAT (U/mL)	0.85 (0.76 ~ 0.94)	0.001
Total Bilirubin (umol/L)	1.10 (1.04 ~ 1.17)	0.002
Oxygenation Index (mmHg)	0.99 (0.98 ~ 0.99)	<0.001
Heart rate (bpm)	1.07 (1.04 ~ 1.11)	<0.001
Hemoglobin	0.94 (0.91 ~ 0.96)	<0.001

Notes: Variables were selected using the forward stepwise selection method (LR). Hosmer–Lemeshow test: χ^2 = 14.906, P = 0.061; Nagelkerke R^2 = 0.726.
Abbreviations: ChAT, Choline Acetyltransferase; CI, Confidence Interval; OR, Odds Ratio.

Bootstrap internal validation (500 resamples) was performed to assess the stability of the model, yielding a corrected AUC of 0.933, with a decrease of only 0.01 from the apparent AUC (0.943), suggesting a low risk of overfitting.

Subgroup Analysis

Multivariate logistic regression models (adjusted for total bilirubin, heart rate, hemoglobin, and oxygenation index) were used to evaluate the association between ChAT and sepsis risk across different subgroups. The results are shown in Figure 8. In the overall population, ChAT was significantly negatively associated with sepsis (OR = 0.85, 95% CI: 0.76–

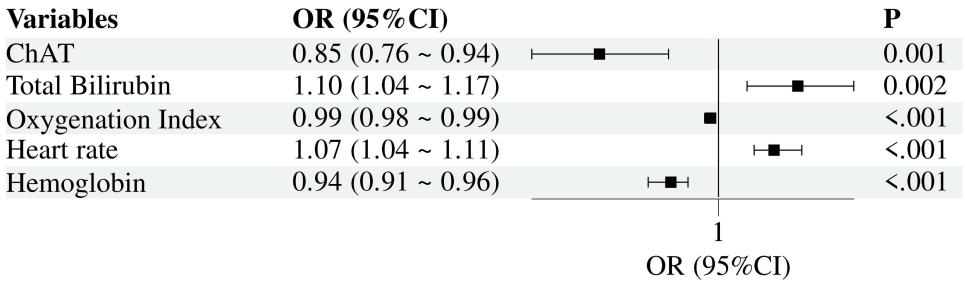

Figure 6 Forest plot of multivariable logistic regression analysis for independent factors associated with sepsis. ChAT, total bilirubin, oxygenation index, heart rate, and hemoglobin were identified as independent factors for sepsis. Decreased levels of ChAT, hemoglobin, and oxygenation index (OR < 1) were associated with increased sepsis risk, while increased levels of total bilirubin and heart rate (OR > 1) were associated with higher sepsis risk.

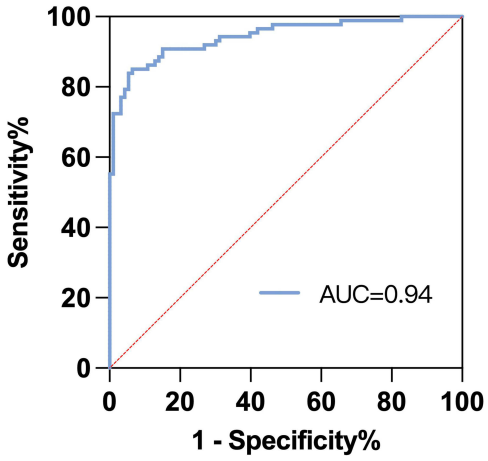

Figure 7 ROC curve of the multivariable logistic regression model for predicting sepsis. The ROC curve was based on the multivariable model including ChAT, total bilirubin, oxygenation index, heart rate, and hemoglobin. The area under the curve (AUC) was 0.94 (95% CI: 0.91–0.98).

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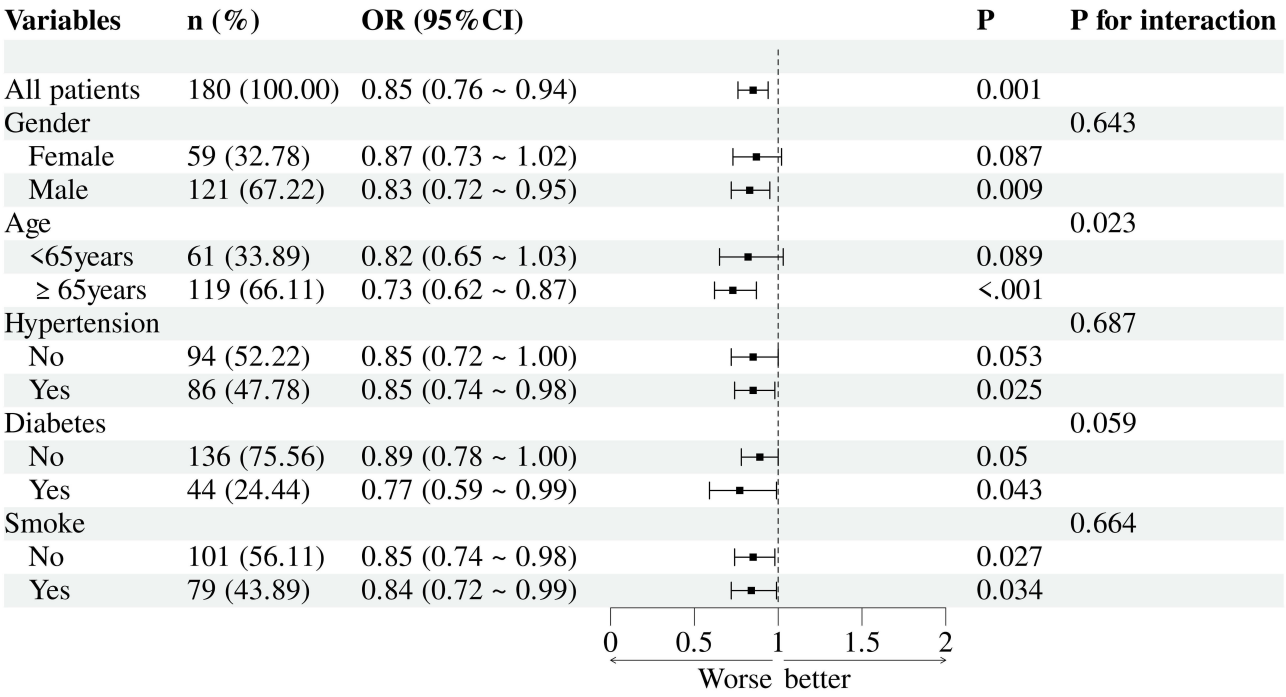

Figure 8 Forest plot of subgroup analysis for the association between ChAT and sepsis risk. ChAT was significantly inversely associated with sepsis risk in the overall population (OR=0.85, 95% CI: 0.76–0.94, P=0.001). A significant interaction was observed for age (P=0.023): the association was stronger in patients aged ≥65 years (OR=0.73, 95% CI: 0.62–0.87, P<0.001) but not significant in those <65 years (OR=0.82, 95% CI: 0.65–1.03, P=0.089). No significant interactions were found for sex, hypertension, diabetes, or smoking (all P>0.05).

0.94, P=0.001). Subgroup analyses revealed that the direction of the association between ChAT and sepsis was consistent across subgroups stratified by sex, hypertension, diabetes, and smoking status, with no significant interactions observed (all P for interaction > 0.05). Notably, a significant interaction was observed for age group (P for interaction = 0.023): among patients aged ≥65 years, the negative association between ChAT and sepsis was stronger (OR = 0.73, 95% CI: 0.62–0.87, P<0.001), whereas the association did not reach statistical significance in patients aged <65 years (OR = 0.82, 95% CI: 0.65–1.03, P=0.089), suggesting that age may modify the association between ChAT and sepsis.

Further stratified analysis by age was performed to evaluate the incremental discriminative ability of ChAT (Table 8). In patients aged ≥65 years, adding ChAT increased the AUC from 0.930 to 0.960; in patients aged <65 years, no notable change in AUC was observed (0.968 vs 0.973). Although the confidence intervals overlapped, the improvement in point estimates was consistent with the significant age interaction (P = 0.023), suggesting an age -modifying effect of ChAT.

Table 8 Incremental Discriminative Ability of ChAT by Age Subgroup

Age Subgroup	Model	AUC (95% CI)
≥65 years	Baseline clinical model	0.930 (0.887–0.974)
≥65 years	+ ChAT	0.960 (0.927–0.992)
<65 years	Baseline clinical model	0.968 (0.930–1.006)
<65 years	+ ChAT	0.973 (0.941–1.005)

Notes: Baseline clinical model: total bilirubin, oxygenation index, heart rate, hemoglobin. Age interaction P = 0.023.
Abbreviations: AUC, area under the curve; ChAT, choline acetyltransferase; CI, confidence interval.

Discussion

In this prospective observational study, we compared serum ChAT and AChE levels between septic and non-septic critically ill patients, dynamically tracked the changes in these two enzymes over a 7-day period in patients with sepsis, and analyzed their associations with inflammatory cytokines, disease severity, and prognosis. The main findings are as follows: (1) Serum ChAT levels at T1 were significantly lower in the sepsis group than in the non-sepsis group, and both ChAT and AChE levels were significantly lower in the septic shock group than in the non-shock septic group; (2) Serum AChE levels showed a descriptive trend of an initial decrease followed by a partial recovery within 7 days, with overall lower levels in non-survivors than in survivors, while ChAT levels showed no significant differences between the two groups; (3) ChAT was positively correlated with AChE, whereas neither ChAT nor AChE showed significant correlations with IL-6, IL-10, or TNF-α; (4) ChAT showed moderate discriminative ability for sepsis (AUC = 0.70); (5) Multivariable logistic regression analysis identified ChAT, total bilirubin, heart rate, hemoglobin, and oxygenation index as independent factors associated with sepsis; (6) Subgroup analysis revealed that the inverse association between ChAT and sepsis was more pronounced in elderly patients (≥65 years), with a significant interaction (P for interaction = 0.023).

CAP and Sepsis

CAP is a neuroimmune regulatory mechanism that has attracted extensive attention in recent years.[21,22] This pathway inhibits the synthesis and release of inflammatory cytokines through modulation of the vagus nerve. The role of the cholinergic anti-inflammatory pathway has been validated in the treatment of various diseases.[23–25] Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) are the key enzymes within this pathway.

In the present study, serum ChAT levels were significantly lower in patients with sepsis than in non-septic critically ill patients, which may be related to CAP dysfunction under septic conditions. In sepsis, excessive release of inflammatory cytokines to the central nervous system may impair central regulation of the peripheral immune system, leading to autonomic nervous system dysfunction and markedly attenuated anti-inflammatory effects of the cholinergic pathway.[14] Further analysis revealed that both ChAT and AChE levels were lower in patients with septic shock than in those with sepsis without shock, suggesting that the degree of cholinergic system impairment is closely associated with disease severity. This finding is also consistent with the potential role of the cholinergic anti-inflammatory pathway in sepsis.[12,26,27]

Dynamic Changes of AChE and Their Clinical Significance

In this study, serum AChE levels in patients with sepsis showed a descriptive downward trend within the first 48 hours after ICU admission, followed by a slight increase by day 7. This pattern may reflect initial consumption of the cholinergic system in response to excessive inflammation, with subsequent recovery of cholinergic function after effective anti-infective treatment and organ support.

Overall AChE levels were lower in non-survivors than in survivors, with a significant difference at T3 (48 hours). This finding is consistent with previous studies,[18–20] suggesting that lower overall AChE levels in non-survivors may reflect insufficient functional reserve of the cholinergic system. Of note, the above association between AChE and prognosis was derived from a univariable linear mixed model without adjustment for other confounding factors; therefore, its independent prognostic value remains uncertain.

Correlation Between AChE and Inflammatory Cytokines

In this study, ChAT and AChE were found to be significantly positively correlated, which may reflect coordinated changes in the synthetic and degradative components of the cholinergic anti-inflammatory pathway (CAP) during sepsis, potentially serving as a neuroimmune compensatory mechanism in response to dysregulated inflammation. However, no significant correlations were observed between ChAT or AChE and the inflammatory cytokines (IL-6, IL-10, TNF-α). Possible explanations include: (1) AChE is predominantly synthesized and released by the liver, and its serum levels are influenced by multiple factors, including hepatic function and nutritional status;[28,29] (2) the pathogenesis of sepsis is complex, and inflammatory cytokine levels are regulated by various other physiological mechanisms; thus, the serum level of a single enzyme may be insufficient to fully reflect the overall inflammatory response.

Discriminative Ability, Incremental Value, and Age-Modifying Effect of ChAT

In this study, ChAT showed moderate discriminative ability for sepsis and was identified as an independent factor associated with sepsis. In contrast to the elevated ChAT levels reported by Gabalski et al[15] the present study found decreased ChAT levels, which may be attributed to differences in sample size, assay methods, and sampling timing. Incremental value analysis showed that adding ChAT did not significantly improve the discriminative ability of the baseline clinical model in the whole cohort. After age stratification, the AUC increased from 0.930 to 0.960 in patients aged ≥65 years, whereas no notable change was observed in those aged <65 years. Although the confidence intervals still overlapped, the improvement in point estimates was consistent with the significant age interaction, suggesting that the clinical significance of ChAT may be modified by age.

With advancing age, the cholinergic system undergoes a series of changes:[30] degeneration of basal forebrain nerve fibers leads to cholinergic neuron dysfunction and reduced ChAT expression.[31] These changes diminish the capacity of elderly individuals to buffer inflammatory insults, resulting in reduced cholinergic functional reserve. Consequently, when ChAT levels decrease, the cholinergic system in elderly individuals may be more vulnerable to impairment, leading to an increased risk of sepsis. In younger individuals, however, the cholinergic system maintains relatively adequate functional reserve, and a certain degree of ChAT decline may still be compensated through regulatory mechanisms, thereby accounting for the weaker association with sepsis risk. This finding suggests that age should be taken into consideration when evaluating the association between ChAT and sepsis.

It should be acknowledged that this study represents a post-hoc analysis in an already diagnosed cohort; therefore, the AUC of ChAT reflects only its discriminative ability in known groups, and its independent diagnostic value warrants further validation. In addition, the subgroup analysis was limited by a relatively small sample size, and the interaction effect should be interpreted as exploratory, requiring confirmation in larger cohorts.

Innovations and Clinical Significance

This prospective exploratory study on serum ChAT and AChE levels in patients with sepsis has the following novel aspects: (1) it is the first to simultaneously measure serum ChAT and AChE levels in septic patients, preliminarily observing changes in the cholinergic system during the pathophysiological process of sepsis; (2) it is the first to dynamically track the trajectories of these two enzymes over a 7-day period; and (3) it is the first to report a possible age-modifying effect on the association between ChAT and sepsis.

From a clinical perspective, the present study suggests that decreased ChAT levels may assist in identifying sepsis and assessing disease severity, with particular warning significance in elderly patients, while decreased AChE levels are associated with poor prognosis. In addition, reduced ChAT levels may indirectly suggest impaired function of the cholinergic anti-inflammatory pathway, and therapeutic strategies such as exogenous ChAT supplementation,[15] vagus nerve stimulation,[32,33] and α7nAChR agonists[34–36] warrant further investigation.

Limitations

This study has several limitations. First, the sample size was limited. As an exploratory study, the sample size was determined primarily based on clinical feasibility and preliminary experimental data, without formal power calculation. The sepsis group comprised only 87 patients, with 27 deaths (31.0%) within 28 days. According to the sample size requirement for multivariate regression analysis (events per variable [EPV] ≥ 10), the number of death events in this study would allow stable inclusion of only 2–3 variables; therefore, multivariate analysis with mortality as the outcome was not performed. The independent associations of ChAT and AChE with prognosis warrant validation in larger sample studies. Second, this was a single-center prospective study, which may introduce selection bias. Third, the exclusion of patients with ICU stay < 24 hours and those with fewer than two valid ChAT/AChE measurements may have systematically excluded the most severely ill patients who died early and were unable to complete follow-up sampling, potentially biasing the enrolled cohort toward a relatively stable intermediate group. Fourth, although we enrolled only patients with newly diagnosed sepsis (within 2 hours of diagnosis) and performed the first blood sampling within 12 hours of ICU admission, using ICU admission as the baseline (T1) cannot completely eliminate inter-individual

differences in pathophysiological onset, which may introduce bias when aligning dynamic trajectories. Fifth, serum ChAT and AChE levels may not fully reflect cholinergic functional status in tissues or synaptic clefts, where changes may be more pronounced. Sixth, the lack of longitudinal sampling in the non-septic group precludes comparison of dynamic trajectories between the two groups. Seventh, despite adjustment for multiple confounders in the multivariable analysis, residual confounding (eg, medication use, nutritional status) may still exist. Eighth, the discriminative ability of AChE for sepsis was limited; it is not recommended as an independent discriminative marker. However, its association with disease severity (shock vs non-shock) and dynamic trends may still provide certain clinical insights. Ninth, Bootstrap internal validation of the multivariable logistic regression model showed a decrease of only 0.01 in the corrected AUC, suggesting a low risk of overfitting. Nevertheless, the model remains exploratory, and its predictive performance requires external validation in an independent cohort.

Conclusion

This exploratory study suggests that serum ChAT levels are significantly decreased in patients with sepsis and correlate with disease severity, while AChE levels are overall lower in non-survivors than in survivors. These findings indicate that ChAT and AChE may be associated with sepsis status and certain clinical features; however, their clinical value remains to be confirmed through larger, multicenter studies with more rigorous methodological designs.

Abbreviations

ACh, acetylcholine; AChE, acetylcholinesterase; APACHE II, Acute Physiology and Chronic Health Evaluation II; AUC, area under the curve; BMI, body mass index; CAP, cholinergic anti-inflammatory pathway; ChAT, choline acetyltransferase; ChiCTR, Chinese Clinical Trial Registry; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; EPV, events per variable; ICU, intensive care unit; IL-6, interleukin-6; IL-10, interleukin-10; LR, likelihood ratio (stepwise forward selection method); MV, mechanical ventilation; OR, odds ratio; ROC, receiver operating characteristic; SAKI, sepsis-associated acute kidney injury; SD, standard deviation; SOFA, Sequential Organ Failure Assessment; TNF-α, tumor necrosis factor-α; WBC, white blood cell.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and ethical restrictions, but are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (Approval No. 2024KS159). Written informed consent was obtained from all patients or their legal proxies.

Consent for Publication

No individual person's data, images, videos, or recordings are presented in this paper. Therefore, consent for publication is not applicable. All patients or their legal proxies provided written informed consent for participation in the study, and were informed that the anonymized results would be submitted for publication.

Author Contributions

Ziyi Zhu: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review & editing. Haiping Wang: conceptualization, data curation, formal analysis, investigation, methodology, writing – review & editing. Yaxin Huo: investigation, data curation – review & editing. Bingqi Lu: investigation, data curation – review & editing. Yujun Zhang: investigation, data curation – review & editing. Lixia Liu: conceptualization, funding acquisition, project administration, supervision – review & editing. All authors met the ICMJE authorship criteria, agreed on the journal to which the article would be submitted, reviewed and agreed on all versions of the paper before

submission, during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage, and agree to take responsibility and be accountable for the contents of the article.

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Disclosure

The authors declare that they have no competing interests.

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