

CD71⁺erythroid cells expansion in adult sepsis: potential causes and role in prognosis and nosocomial infection prediction

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Research

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Abstract

Background

Immune suppression contributes to nosocomial infections (NI) and poor prognosis in sepsis. Recent studies revealed CD71⁺ erythroid cells had unappreciated immunosuppressive functions. This study aimed to investigate the values of CD71⁺ erythroid cells in predicting NIs and prognosis among adult septic patients. The potential factors associated with the expansion of CECs were also explored.

Methods

In total, 112 septic patients and 32 critically ill controls were enrolled. The frequencies of CD71⁺ cells, CD71⁺CD235a⁺ cells (CECs) and CD45⁺ CECs were measured by flow cytometry. The associations between CECs and NIs and 30-day mortality were assessed by ROC curve analysis, Cox and competing-risk regression model. Factors associated with the frequency of CECs were identified by linear regression analysis.

Results

The percentage of CD71⁺ cells, CECs, CD45⁺ CECs were higher in septic patients than critically ill controls. In septic patients, the percentage of CD71⁺ cells, CECs and CD45⁺ CECs were associated with NI development, while CD71⁺ cells and CECs were independently associated with 30-day mortality. Linear regression analysis showed that the levels of interleukin (IL)-6 and interferon (IFN)- γ were positively associated with the frequencies of CD71⁺ cells, CECs and CD45⁺ CECs, while IL-10 was negatively associated with them. Additionally, the levels of red blood cells (RBCs) were negatively associated with the percentage of CD45⁺ CECs.

Conclusions

CECs were expanded in sepsis and can serve as independent predictors of the development of NI and 30-day mortality. Low levels of RBCs and high levels of IL-6 and IFN- γ may contribute to the expansion of CECs in sepsis.

Trial registration: ChiCTR, ChiCTR1900024887. Registered 2 August 2019, <http://www.chictr.org.cn/showproj.aspx?proj=38645>

Introduction

Sepsis and septic shock are major health-care problems worldwide and mortality and morbidity are still high[1]. Sepsis is often accompanied by immune suppression, which is associated with increased occurrence of nosocomial infection (NI) and adverse outcomes[2, 3]. The immunosuppression of sepsis is characterized by insufficient response of immune cells, decreased number of immune cells, increased

level of anti-inflammatory cytokines and expansion of immunosuppressive cells[4, 5]. It is generally acknowledged that immunosuppression mainly occurs in the late stage of sepsis, but recent evidence shows that this phenomenon also exists in the early stage of the disease and is closely related to the poor prognosis of patients[5–7].

Erythropoiesis is a highly regulated, multistep process that generates mature RBCs from hematopoietic stem cells (HSCs) in bone marrow. During anemia, pregnancy or infections, extramedullary erythropoiesis is induced in the spleen and a large number of immature erythrocytes with immunosuppressive function were produced[8, 9]. Although, immunophenotype pattern for these erythroid cells are not totally identified, transferrin receptor I (CD71) combined with glycophorin A (CD235a) are widely used to characterize them in humans[8, 9, 10]. Recently, two subtypes of CD71⁺ erythroid cells (CECs) with different immunosuppressive abilities were found, they are CD45⁺CECs and CD45⁻ CECs. Compared to CD45⁺ones, some studies showed that CD45⁻CECs have relatively poor immunosuppressive properties. However, in animal models, transfer of CD45⁻ CECs also promotes tumor growth indicating they may also have immunomodulatory roles[10–12].

In neonates, the relationship between the expansion of CECs and numerous pathogens infections has been well described. CECs from newborns suppressed cytokine production by myeloid cells and T cells [13]. Additionally, CECs deficient neonatal mice are more resistant to pathogens such as *Listeria monocytogenes*, *Escherichia coli* and *Bordetella pertussis* [14, 15]. However, the frequency of CECs in adult critical illness patients with sepsis and its clinical significance remains unclear. In this study, we evaluated whether CECs can be used to evaluate the risk of nosocomial infection and prognosis of adult septic patients. Furthermore, the potential factors associated with the expansion of CECs in sepsis were also explored.

Methods

Patients

The study was conducted at an 18-bed emergency intensive care unit (ICU) of the First Affiliated Hospital of Wenzhou Medical University, which is a tertiary hospital of City University, with about 300,000 emergency admissions every year. The study was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China (2019040) and was registered on the website of China Registered Clinical Trial Registration Center with No.ChiCTR1900024887. Informed consent was obtained from all participants prior to enrollment.

Between July 15, 2019 to August 15, 2020, patients with sepsis and septic shock were prospectively enrolled in our study. Sepsis was defined as a documented infection and an acute increase of 2 SOFA points according to the diagnostic criteria of Sepsis-3[16]. Patients with age<18 years old or acute bleeding were excluded. Patients were excluded from the study if they received erythropoietin or stored red blood cells (RBCs) during hospitalization or within 3 months before admission.

Data collection and definitions

Baseline data collection included demographics (age and gender), height, weight, comorbidities, source of sepsis and nosocomial infections. Septic patients were screened daily for nosocomial infection which was defined as a new infection acquired more than 48 hours after admission. The diagnosis of nosocomial infection in the present study was made based on the criteria of Centers for Disease Control and Prevention (CDC, 2008), as we previously described[3,17,18]. Clinical scores, including acute physiology and chronic health evaluation (APACHE) II and Sepsis Related Organ Failure Assessment (SOFA) score, also recorded at the first 24 hours after admission. Length of stay in hospital and ICU and the outcome after 30 days (death or survival) were collected.

Laboratory Examinations

Sysmex XE2100 automatic blood Cell analyzer was used to detect the levels of white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) and lymphocytes. The levels of serum creatinine (Scr) were tested using Vitros 5.1 FS Chemistry System (Ortho Clinical Diagnostics, Johnson & Johnson, USA). Cytokines including interleukin (IL) -2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)- α and interferon (IFN)- γ in serum were detected by a microsphere-based immunofluorescence assaying kit (Saiji Biotechnology, Jiangxi, China).

Flow cytometry

Samples of peripheral blood were collected from patients within 2 days after admission and transported to the laboratory at 4 °C immediately. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood through Ficoll centrifugation. Specifically, the following antibodies were used: FITC labelled anti-CD71 and its isotype control antibody (Ab) (IgG2a, κ), APC labelled anti-CD235a and its isotype control Ab (IgG2b, κ), PE labelled anti-CD45 and its isotype control Ab (IgG1, κ). All antibodies were purchased from BD Bioscience Company. The frequency of cells was analyzed by flow cytometry on a BD FACSCanto II flow cytometer (BD Bioscience). Fluorescence minus one (FMO) isotype control was used to confirm the specificity of staining.

Data analysis and statistics

Continuous variables in the present study were described as a median with interquartile range, and categorical variables were reported as frequencies. Univariate analyses were conducted using Mann-Whitney U test or Kruskal-Wallis H test for continuous variables, Chi-square test for categorical data. Cox regression model was used for estimating the relationships between CECs and outcomes (NI and mortality) adjusting for confounding variables selected based on the results of univariate analysis. To determine the discriminative power of the variables for NI and mortality, we constructed ROC curves and calculated areas under the curve (AUC) with 95% CI. The best predictive cutoff values maximizing the sum of sensitivity and specificity were defined. Further, cumulative incidence curves were used to compare the incidence of NI and 30-day mortality among septic patients according to these cutoff

values. Competing risk regression model were used to assess the risk of NI and 30-day mortality. Multiple linear regression was performed to detect independent variables associated with the frequency of CD71⁺ cells and CECs. In all test, two-tailed $P < 0.05$ was considered significant. The calculations were performed with IBM SPSS Statistics version 22.0 (IBM, Armonk, NY, USA), GraphPad Prism (GraphPad Software) and R 3.6.2 software for windows.

Results

Characteristics of study subjects

Initially, 112 septic patients were admitted to the ICU of emergency department. However, five septic patients having a delayed measurement. Five patients were discharged automatically and losing follow-up. One patient with gastrointestinal bleeding was excluded. Thus, 101 septic patients were included in survival analysis. 32 critically ill patients without sepsis were enrolled as control. Flow diagram of selecting study subjects was shown in Figure 1. The demographic and clinical characteristics of patients were shown in Table S1. There were no differences in baseline characteristics between the septic patients and critically ill patients without sepsis on admission (Table S1).

Nosocomial Infection Characteristics

For NIs analysis, 8 septic patients who died within 48 hours after admission were excluded, and 93 patients were enrolled finally (Figure 1). Twenty-nine percent (27/93) of patients developed NIs. Of these, 23 patients had a NI at one site, 5 patients had NIs at two sites. The median time of the first diagnosis of NIs was 8 days [interquartile ranges (IQR):4-15]. Among 33 NIs, pulmonary infection (PI) was the most frequent NI (60.6%), followed by urinary tract infection (UTI) (21.2%), bloodstream infection (BSI) (9.1%) and catheter-related infections (CRI) (9.1%). Of all NIs, 39 microorganisms were isolated. PIs were mostly caused by *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*, followed by *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Corynebacterium*. *Candida* was the most common pathogen responsible for UTIs and CRIs. *Staphylococcus* accounted for 66.7% of all isolates from BSIs. Details regarding the frequency of isolated microorganisms are given in Table S2.

The baseline characteristics of patients with and without NI were shown in Table 1. There were no significant differences in age, gender, and co-morbidities between patients that will develop NI or not. Septic patients who developed an NI were more severely ill on admission than those who did not, as indicated by higher APACHE II score and SOFA score. Moreover, these patients were characterized by greater exposure to intubation and central venous catheterization, and longer duration of mechanical ventilation, and urinary tract and central venous catheterization. 30-day mortality was significantly higher in patients with NI ($P=0.034$) (Table 1).

Table 1
Baseline characteristics of septic patients with and without NIs

Septic patients				
Variables	Total (n=93)	Without NI (n=66)	With NI (n=27)	P value
Age (year)	65.0(55.5, 70.0)	65.0(54.0, 69.3)	65.0(60.0, 70.0)	0.744
Gender (male), n(%)	50(53.8%)	34(51.5%)	16(59.3%)	0.327
BMI, kg/m ²	22.0(20.0, 25.0)	22.0(21.0, 26.3)	21.0(20.0, 24.0)	0.125
Percentage of CD71 ⁺ cells	2.9(1.2, 6.4)	2.5(1.1, 5.7)	4.0(1.4, 12.6)	0.113
Percentage of CD71 ⁺ CD235a ⁺ cells	0.9(0.2, 1.9)	0.7(0.2, 1.3)	1.2(0.5, 4.1)	0.021
Percentage of CD45 ⁺ CD71 ⁺ CD235a ⁺ cells	0.10(0.04,0.19)	0.08(0.04,0.17)	0.15(0.07,0.36)	0.016
Circulating cytokines (pg/ml)				
IL-2	0.7(0.5, 1.2)	0.7(0.5, 1.2)	0.6(0.5, 1.4)	0.879
IL-4	0.4(0.1, 0.6)	0.4(0.1, 0.5)	0.3(0.2, 0.7)	0.549
IL-6	267.1(72.1, 2971.3)	181.1(66.4, 2971.3)	922.9(105.3, 3000.1)	0.139
IL-10	44.1(12.2, 398.1)	43.2(13.2, 407.7)	50.7(7.6, 313.2)	0.997
TNF- α	1.8(0.7, 5.6)	1.8(0.7, 5.6)	1.5(0.5,5.6)	0.836
IFN- γ	1.6(0.5, 3.2)	2(0.6, 3.2)	1(0.1, 4.8)	0.381
Co-morbidities, n(%)				
Diabetes	20(21.5%)	13(19.7%)	7(25.9%)	0.343
Chronic heart disease	9(9.7%)	5(7.6%)	4(14.8%)	0.240
Hypertension	50(53.8%)	37(56.1%)	13(48.1%)	0.320

Abbreviations: NI, nosocomial infection; IQR: interquartile ranges; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment.

^a Other pathogens are rickettsia, leptospira and plasmodium falciparum.

^b Scores were calculated within the first 24 h after the ICU admission using the value associated with the greatest severity of illness.

Septic patients				
Chronic liver disease	8(8.6%)	4(6.1%)	4(14.8%)	0.167
Chronic kidney disease	12(12.9%)	9(13.6%)	3(11.1%)	0.519
Cancer	17(18.3%)	11(16.7%)	6(22.2%)	0.361
Infection sites, n(%)				0.262
Digestive tract	30(32.3%)	19(28.8%)	11(40.7%)	
Urinary tract	25(26.9%)	21(31.8%)	4(14.8%)	
Skin and soft tissue	15(16.1%)	9(13.6%)	6(22.2%)	
Respiratory tract	7(7.5%)	4(6.1%)	3(11.1%)	
Others	16(17.2%)	13(19.7%)	3(11.1%)	
Pathogen isolates, n(%)				0.036
Gram-negative bacteria	19(20.4%)	15(22.7%)	4(14.8%)	
Gram-positive bacteria	9(9.7%)	5(7.6%)	4(14.8%)	
Fungus	2(2.2%)	1(1.5%)	1(3.7%)	
Virus	1(1.1%)	0(0%)	1(3.7%)	
^a Other pathogens	3(3.2%)	3(4.5%)	0(0%)	
Mixed	3(3.2%)	0(0%)	3(11.1%)	
No	56(60.2%)	42(63.6%)	14(51.9%)	
^b SOFA score, median (IQR)	7(4, 9)	6(4, 9)	9(7, 12)	0.002
^b APACHE II score				
Overall, median (IQR)	13(9, 17)	12(8, 15)	17(13, 20)	0.001
<8	21(22.6%)	17(25.8%)	4(14.8%)	0.005
9-13	26(28%)	23(34.8%)	3(11.1%)	
14-17	25(26.9%)	17(25.8%)	8(29.6%)	

Abbreviations: NI, nosocomial infection; IQR: interquartile ranges; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment.

^a Other pathogens are rickettsia, leptospira and plasmodium falciparum.

^b Scores were calculated within the first 24 h after the ICU admission using the value associated with the greatest severity of illness.

Septic patients				
>17	21(22.6%)	9(13.6%)	12(44.4%)	
Hemoglobin (g/L)	103.0(86.5, 116.0)	105.0(90.8, 118.0)	97.0(71.0, 112.0)	0.069
Red blood cells ($\times 10^{12}/L$)	3.5(2.9, 3.9)	3.5(3, 3.9)	3.5(2.6, 3.8)	0.345
White blood cells ($\times 10^9/L$)	13.9(8.2, 20.3)	14.5(8.3, 19.9)	12.4(7.6, 21.2)	0.651
Lymphocytes ($\times 10^9/L$)	0.7(0.5, 1.0)	0.7(0.5, 1)	0.7(0.5, 1)	0.343
Serum creatinine ($\mu\text{mol}/L$)	136.0(83.5, 266.5)	111.5(76.8, 219.0)	214.0(102.0, 296.0)	0.048
Interventions, n(%)				
Intubation	22(23%)	6(9.1%)	16(59.3%)	<0.001
Duration of Mechanical ventilation	0(0, 0)	0(0, 0)	3(0, 11)	<0.001
Central venous catheterization	65(69.9%)	43(65.2%)	22(81.5%)	0.093
Duration of Central venous catheterization	4.0(0, 8.0)	3(0, 6)	9(3, 15)	<0.001
Urinary tract catheterization	87(93.5%)	61(92.4%)	26(96.3%)	0.436
Duration of urinary tract catheterization	6.0(4.0, 11.5)	5(3, 7)	12(7, 26)	<0.001
30-day mortality, n(%)	13(14.0%)	6(9.1%)	7(25.9%)	0.034
Abbreviations: NI, nosocomial infection; IQR: interquartile ranges; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment.				
^a Other pathogens are rickettsia, leptospira and plasmodium falciparum.				
^b Scores were calculated within the first 24 h after the ICU admission using the value associated with the greatest severity of illness.				

CD71⁺cells and CECs in different groups

Representative flow dot plots of gating strategies of CD71⁺cells, CD71⁺CD235a⁺ cells and CD45⁺ subset of CD71⁺CD235a⁺cells were shown in Figure 2. Their frequency varies greatly among adult critically ill patients; The proportion of CD71⁺CD235⁺ cells in PBMC ranged from under detectable level to 38.80%. Compared with critically ill controls, the percentages of CD71⁺cells, CD71⁺CD235a⁺cells as well as CD71⁺CD235a⁺CD45⁺cells in PMBCs were significantly higher in septic patients (all $P < 0.01$) (Figure 3 A-C). Septic patients who developed an NI had significantly higher percentages of CD71⁺CD235a⁺cells ($P = 0.021$) and CD71⁺CD235a⁺CD45⁺cells ($P = 0.016$), but not CD71⁺cells ($P = 0.113$), than those who did

no (Figure 3 D-F). The percentage of CD71⁺CD235a⁺ cells was higher in non-survivors when compared with survivors [1.3(0.4,6.6) vs 0.7(0.2, 1.3); P=0.037] (Figure 3 G-I).

CD71⁺cells and CECs for predicting NIs in septic patients

Cox proportional hazard model was used to analyze the relationship between CECs and NIs (Table 2). The results show that the percentage of CD71⁺cells (HR 1.06; 95% CI 1.02-1.11; P=0.006), CD71⁺CD235a⁺cells (HR 1.08; 95% CI 1.02-1.14; P=0.006) and CD71⁺CD235a⁺CD45⁺cells (HR 9.63; 95% CI 2.31-40.27; P =0.002) were associated with increased incidence of NIs after adjusting for BMI, APACHE II score, pathogen isolates, IL-6, hemoglobin, Serum creatinine, intubation, central venous catheterization, and durations of mechanical ventilation, central venous catheterization and urinary tract catheterization. From ROC curve analysis, 2.96 was the best cutoff value of CD71⁺cells to predict NI development [area under the curve (AUC) 0.605, p = 0.067, sensitivity 66.7%, specificity 57.6%]. The best cutoff values of CD71⁺CD235a⁺cells and CD71⁺CD235a⁺CD45⁺cells to predict NI development were 2.08 (AUC 0.653, p=0.021, sensitivity 40.7%, specificity 86.4%) and 0.25 (AUC 0.660, p=0.016, sensitivity 37.0%, specificity 90.9%).

In competing-risk regression analyses, the percentages of CD71⁺cells (HR 1.06; 95% CI 1.02-1.09; P<0.001), CD71⁺CD235a⁺cells (HR 1.08; 95% CI 1.04-1.12; P=0.001) and CD71⁺CD235a⁺CD45⁺cells (HR 6.77; 95% CI 1.39-32.93; P=0.018) were associated with NI development after adjusting for possible confounders (Table 2). According to cumulative incidence curves, for patients with high levels of CD71⁺cells (>2.96%), CD71⁺CD235a⁺cells (>2.08%) and CD71⁺CD235a⁺CD45⁺cells (>0.25%), the probabilities of developing an NI within the next 30 days were 52.8%, 66.6% and 71.3%, whereas for patients with low percentage of them (CD71⁺cells≤2.96%∩CD71⁺CD235a⁺cells≤2.08%; CD71⁺CD235a⁺CD45⁺cells≤0.25%), the probabilities were 33.8%, 32.4% and 32.9%, respectively (all P<0.05) (Figure 4 A-C).

CD71⁺cells and CECs for predicting mortality in septic patients

In Cox regression analysis, the percentages of CD71⁺CD235a⁺CD45⁺cells were not significantly associated with 30-day mortality after adjusting for confounders (HR 2.20; 95% CI 0.51-9.45; P=0.288), while the percentage of CD71⁺cells (HR 1.05; 95% CI 1.00-1.10; P=0.041) and CD71⁺CD235a⁺cells (HR 1.06; 95% CI 1.00-1.12; P=0.031) can predict 30-day mortality outcome (Table 2, Table S3). From ROC curve analysis, 3.48% was the best cutoff value of CD71⁺CD235a⁺cells to predict 30-day mortality (AUC 0.640, p=0.046, sensitivity 42.9%, specificity 90.0%), while 6.05% was the best cutoff value of CD71⁺cells (AUC 0.62, p=0.068, sensitivity 47.6%, specificity 77.5%). In competing-risk regression analysis, the percentage of CD71⁺cells (HR 1.04; 95% CI 1.01-1.07; P=0.015) and CD71⁺CD235a⁺cells (HR 1.06; 95% CI 1.01–1.10; P=0.011) were associated with 30-day mortality after adjusting for possible confounding factors (Table 2). According to cumulative incidence curves, for patients with the percentage of

CD71⁺CD235a⁺ cells more than 3.48%, the probability of mortality in 30 days was 14.3%, whereas for patients with low percentage of the cells ($\leq 3.48\%$), the probability was 52.9% ($P < 0.001$).

Table 2. Cox regression analysis and competing-risk regression analysis of CD71⁺ cells and CECs for predicting nosocomial infection and mortality in septic patients

Variables	Unadjusted model			Adjusted model		
	HR	CI ₉₅	P value	HR	CI ₉₅	P value
^a Nosocomial infection						
Cox regression analysis						
Percentage of CD71 ⁺ cells	1.05	1.01~1.09	0.010	1.06	1.02~1.11	0.006
Percentage of CD71 ⁺ CD235a ⁺ cells	1.09	1.05~1.15	<0.001	1.08	1.02~1.14	0.006
Percentage of CD45 ⁺ CD71 ⁺ CD235a ⁺ cells	6.34	2.15~18.69	0.002	9.63	2.31~40.27	0.002
Competing-risk regression analysis						
Percentage of CD71 ⁺ cells	1.05	1.01~1.09	0.011	1.06	1.02~1.09	<0.001
Percentage of CD71 ⁺ CD235a ⁺ cells	1.09	1.06~1.13	0.001	1.08	1.04~1.12	0.001
Percentage of CD45 ⁺ CD71 ⁺ CD235a ⁺ cells	5.81	1.87~18.70	0.003	6.77	1.39~32.93	0.018
^b 30-day mortality						
Cox regression analysis						
Percentage of CD71 ⁺ cells	1.04	1.00~1.08	0.035	1.05	1.00~1.10	0.041
Percentage of CD71 ⁺ CD235a ⁺ cells	1.07	1.02~1.11	0.002	1.06	1.00~1.12	0.031
Percentage of CD45 ⁺ CD71 ⁺ CD235a ⁺ cells	3.14	0.93~10.63	0.065	2.20	0.51~9.45	0.288
Competing-risk regression analysis						
Percentage of CD71 ⁺ cells	1.04	1.01~1.07	0.009	1.04	1.01~1.07	0.015
Percentage of CD71 ⁺ CD235a ⁺ cells	1.07	1.05~1.09	<0.001	1.06	1.01~1.10	0.011
Percentage of CD45 ⁺ CD71 ⁺ CD235a ⁺ cells	2.91	1.10~7.74	0.032	2.06	0.90~4.75	0.089

Abbreviations: CECs, CD71⁺ erythroid cells; HR, HR hazard ratio; CI, confidence interval.

^a Admission variables included in this model were BMI, APACHE II score, SOFA score, pathogen isolates, IL-6, hemoglobin, serum creatinine, intubation, central venous catheterization, and durations of mechanical ventilation, central venous catheterization and urinary tract catheterization.

^b Admission variables included in this model were gender, chronic heart disease, APACHE II score, SOFA score, IL-6, hemoglobin, serum creatinine, intubation, central venous catheterization, and durations of mechanical ventilation.

Factors associated with the expansion of CD71⁺ cells and CECs in sepsis

A multiple linear regression was performed to detect independent factors associated with the expansion of CD71⁺ cells and CECs (Table S4). Potential variables that included in the regression analysis were age, gender, co-morbidities, the severity scores (SOFA and APACHE II), WBC, RBC, Hb, Scr and cytokines (IL-2, IL-4, IL-10, TNF- α and IFN- γ). The results showed that the levels of IL-6 and IFN- γ were positively associated with percentage of CD71⁺ cells, CD71⁺CD235a⁺ cells and CD45⁺ subset of CD71⁺CD235a⁺ cells in PBMCs, while IL-10 was negatively associated with them. Additionally, the levels of RBC were negatively associated with the percentage of CD71⁺CD235a⁺CD45⁺ cells (Table S4).

Discussion

Immune paralysis is one of the main pathophysiological characteristics of sepsis. Due to the decreased ability to kill the invading harmful microorganisms, septic patients with immune paralysis are not only difficult to recover from the primary infection, but also have an increased susceptibility to nosocomial infections [2–4, 19, 20]. In light of these findings, immunomodulation has been considered as a promising therapeutic strategy for sepsis [5, 21, 22]. Consequently, biomarkers for selecting patients with immunosuppression and the potential targets of immunotherapy urgent to be exploring.

CECs, a population of erythroid progenitors and precursors, exhibit a wide range of immunosuppressive properties [8, 9]. Through produce reactive oxygen species (ROS), CD71⁺CECs suppress T-cells activation and production of pro-inflammatory which contribute to cancer development and human immunodeficiency virus (HIV) infection in animal or cell models [8, 9, 12]. Programmed death-ligand 1 (PD-L1) and arginase I/II also play essential roles in CECs-mediated T cells suppression [23, 24]. CECs promote regulatory T-cells expansion by the secretion of transforming growth factor β (TGF- β) [25]. Additionally, the increase levels of CECs have been found in patients with cancer, anemia, and HIV-infected patients [10, 12, 26]. Recently, in patients with COVID-19, a robust expansion of CECs was observed [27]. Nevertheless, no study has been specifically designed to investigate the frequency of CECs in adult septic patients, and the potential clinical significance of them.

The present study found that the frequency of CECs in septic patients was higher than control critically ill patients. Additionally, human leukocyte antigen-DR (HLA-DR), an immune maker, was lower in septic

patients when compared with other ICU patients[28]. Therefore, immune defects may more common and serious in critically ill patients with sepsis compared with those without sepsis. Immune dysfunctions are associated with a number of significant complications. Consequently, sepsis has been proved as an independent risk factor of NI, an important clinical sign of immunosuppression, in critically ill patients [29]. Nevertheless, for septic patients, the immune markers that can be used to predict NI, are still lacking. In the present study, septic patients who developed an NI had higher frequency of CD71⁺cells, CD71⁺CD235a⁺cells and CD71⁺CD235a⁺CD45⁺cells compared with who did no. The results of cox proportional hazard regression and competing-risk regression analyses showed that all these cells were independent risk factors of NIs in sepsis, and CD71⁺CD235a⁺cells can used to predict 30-day mortality. In addition to be a biomarker, in tumor-bearing mice, prevention of CD71⁺cells accumulation decreased tumor growth [12]. Recent studies also found CD71⁺cells depletion decreased bacterial load in mice model of polymicrobial sepsis and mice with various bacterial infection [15, 26]. Therefore, CD71⁺cells maybe a promising therapeutic target for sepsis.

Anemia is a common feature during sepsis [29]. Our study found that the levels of RBC were negatively associated with the frequency of CD45⁺CECs which indicates that anemia may contribute to the expansion of CECs. Erythropoietin (EPO) may be a mediator of CECs expansion caused by anemia in sepsis. Studies have illustrated that EPO can induce the expansion of highly proliferative early-stage CECs (CD45⁺ CECs), and neutralization of EPO prevents infection-related CECs accumulation[30, 31]. In addition to anemia, many pro-inflammatory cytokines play crucial roles in the expansion of CECs[8, 10]. IFN- γ stimulates CECs expansion by reducing RBC life span and increasing macrophage erythrophagocytosis [32]. Other cytokines, including TNF- α , IL-1, and IL-6, leading to the expansion of CECs and enrichment of early-stage CECs by impairing erythropoiesis and aggravating anemia[8, 10]. In the present study, IL-6 and IFN- γ were positively associated with the frequency of CECs indicates these two cytokines may contribute to CECs expansion in sepsis. Interestingly, IL-10, an important anti-inflammatory cytokine in sepsis, was negatively associated with the frequency of CECs. Previous in vitro study found that IL-10 increased the proliferation of BFU-E[33], the role of it in CECs expansion need further investigated.

Our study has some limitations. Firstly, the dynamic changes of the proportions of CECs in sepsis was not studied. Sustained high proportions of CECs may have high predictive value for the adverse outcomes of sepsis. Secondly, there were 21 non-survivors in the present cohort and the 30-day mortality was 20.8%. The frequencies of CD71⁺cells and CD71⁺CD235a⁺CD45⁺cells in non-survivors appeared to be higher than in survivors, although there was no statistical difference in some models. Relatively small number of non-survivors may lead to an inaccurately estimation of the prognostic value of these cells. Finally, this study is a single center study. The results should be validated prospectively in a multicenter trial.

Conclusions

In conclusion, the present study found that CD71⁺ erythroid cells were expanded in adult septic patients and can serve as independent predictors of the development of NI and 30-day mortality. Low levels of RBCs and high levels of IL-6 and IFN- γ may contribute to the expansion of CD71⁺ erythroid cells in sepsis.

Declarations

Ethics approval and Consent to participate

The study was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China (2019040) and was registered on the website of China Registered Clinical Trial Registration Center with No.ChiCTR1900024887. The informed consent was obtained from all participants prior to enrollment.

Consent for publication

Not applicable.

Availability of supporting data

The datasets used in the present study are available from the first author and corresponding authors on reasonable request.

Completing interests

The authors declare that they have no completing interests

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Authors' contributions

GJZ designed the study; collected and analyzed data; and contributed to writing this manuscript. DWJ and WCC collected and analyzed data; XYC, WD, LWC, GLH and BW helped with data analyzation. YMY and ZQL designed and supervised the study and drafted the manuscript. All authors have read and approved the final manuscript.

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Figures

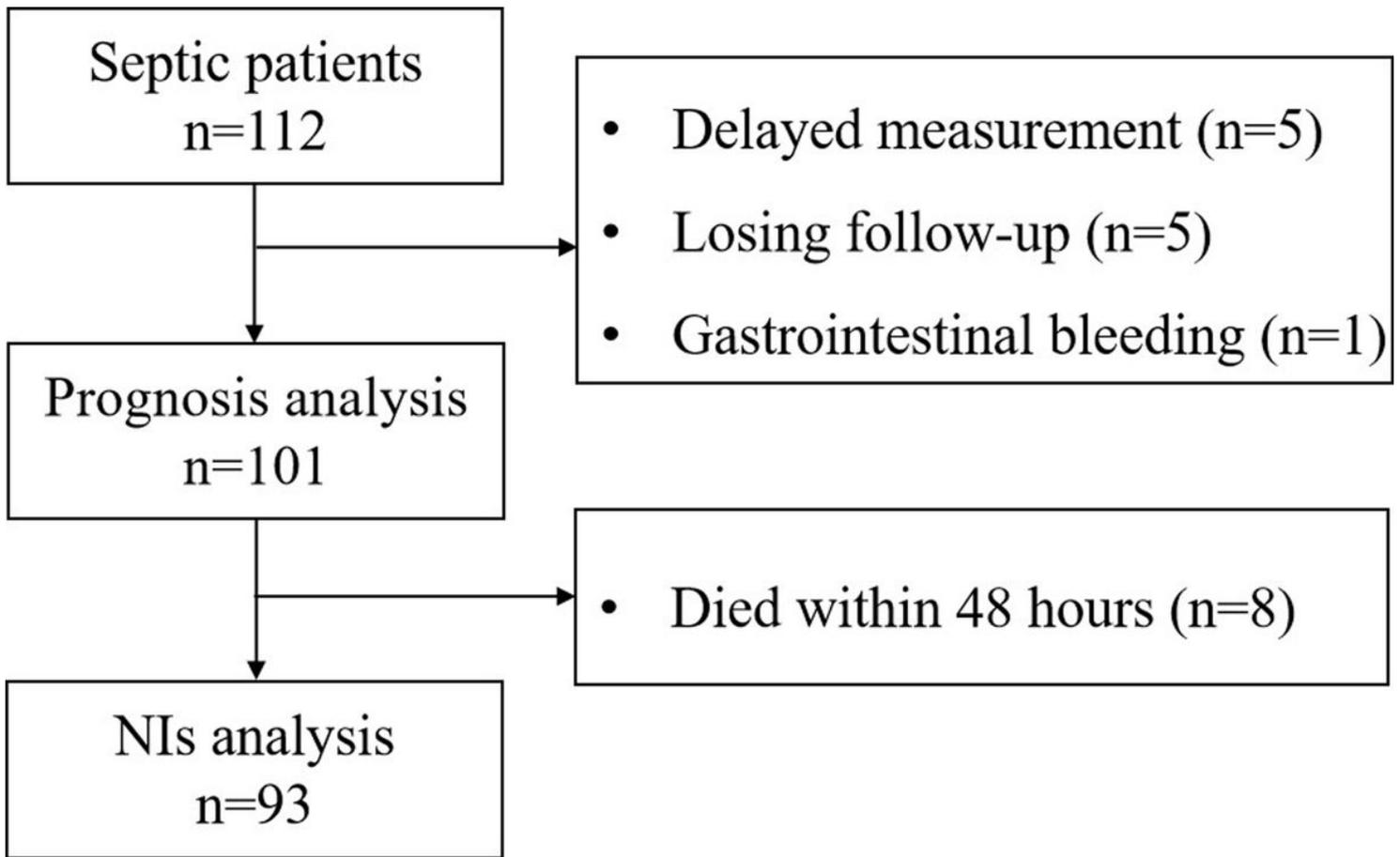


Figure 1

Flowchart of included patients

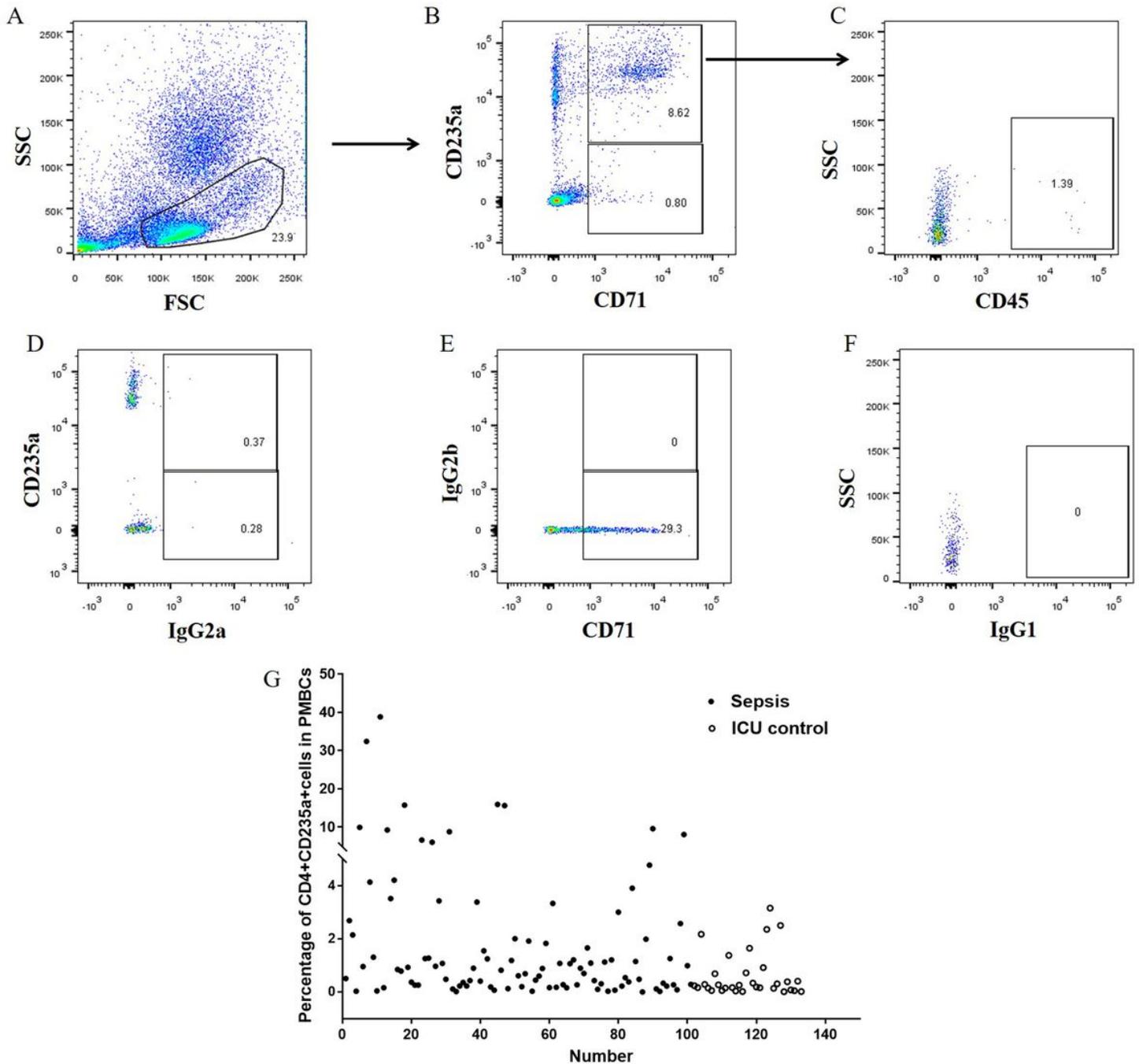


Figure 2

Gating strategy and representative dot plots for flow cytometry analysis (A-C). Fluorescence minus one (FMO) isotype control for proper identification of CD71+(D) , CD235a+(E) and CD45+cells (F). The percentages of CECs in PMBCs of 101 septic patients and 32 critically ill controls (ICU controls) (G).

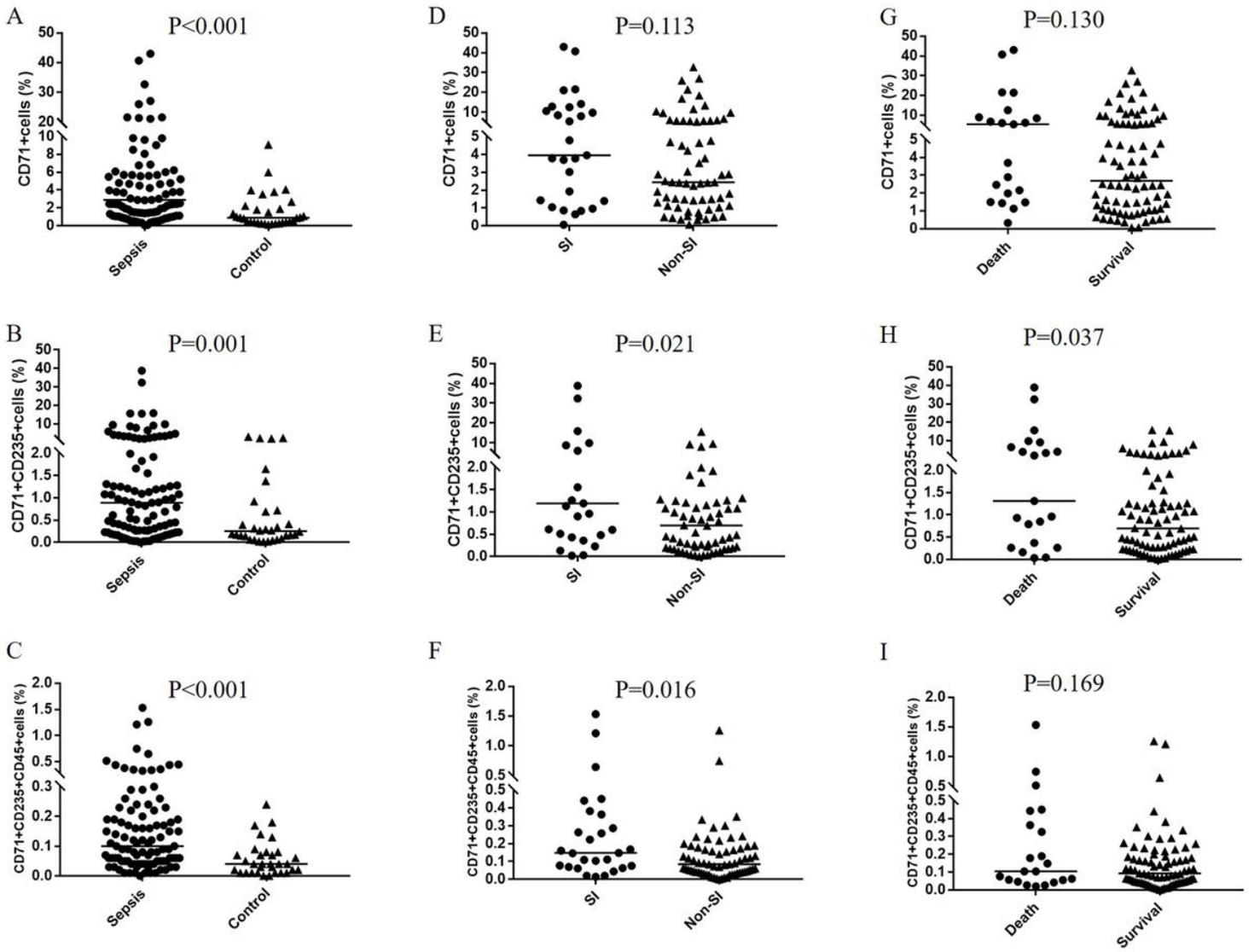


Figure 3

CD71+cells and CECs in different groups.

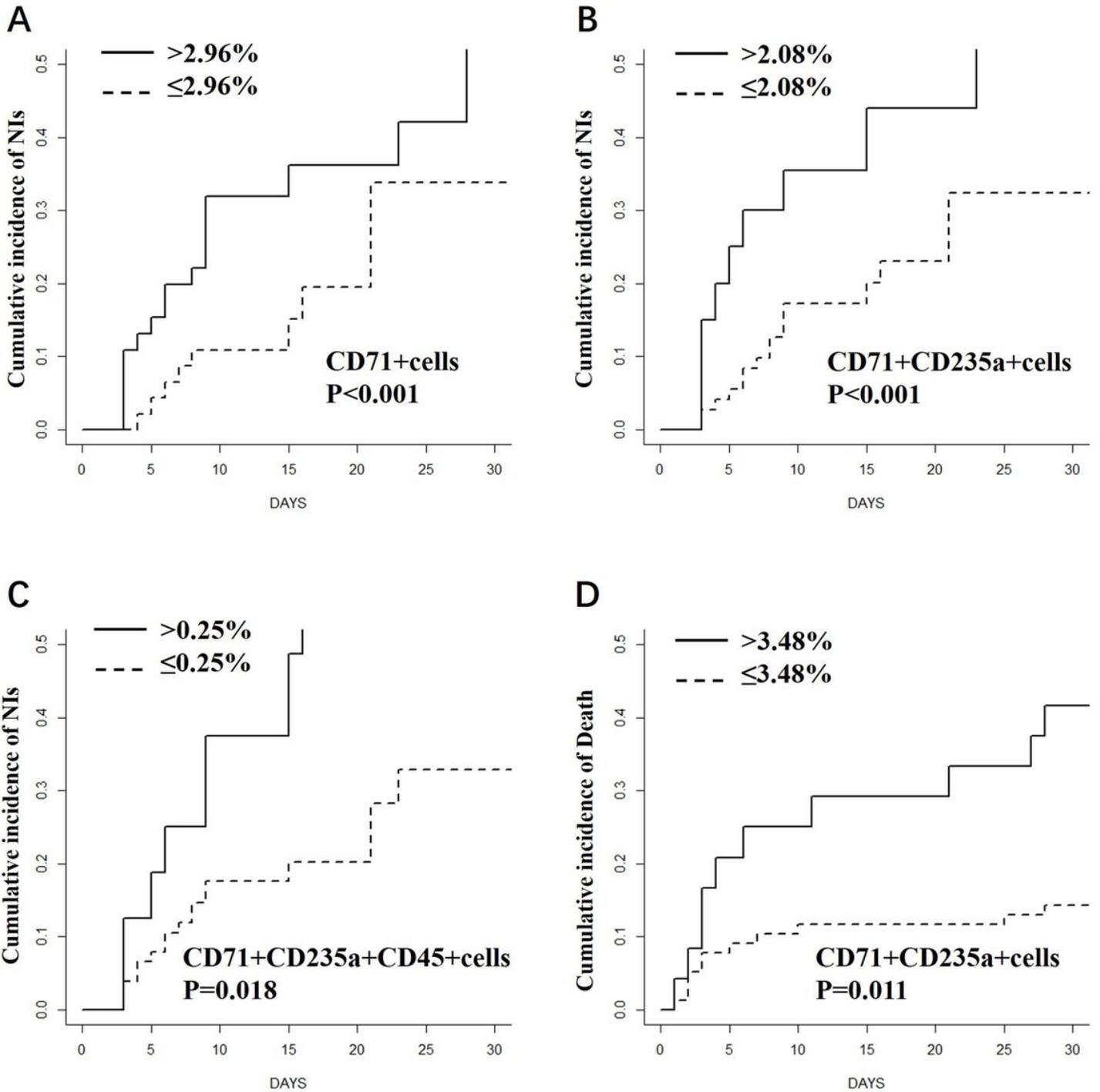


Figure 4

Cumulative incidence curves for nosocomial infections (A-C) and mortality (D) stratified based on the percentage of CD71+ cells and (or) CECs in septic patients.

Supplementary Files

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