Nucleated red blood cell and its specific fluctuations are risk factors of 28-day and 90-day all-cause mortality in ICU patients: an observational cohort study

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Research

Keywords: ICU, Nucleated red blood cell, CCI; APACHE II score, SOFA score, Dynamic change, All-cause mortality

Posted Date: January 15th, 2020

DOI: https://doi.org/10.21203/rs.2.20960/v1

Abstract

Background Little was known about the first occurrence time of Nucleated red blood cell (NRBC)and the persistent duration of the positive results in ICU patients, nor the dynamic changes of NRBC count and quantified predictive value of NRBC for mortality in ICU patients. We hypothesized that the persistent presence of NRBC in ICU patients would be associated with all-cause mortality and death patients would have specific NRBC fluctuations compared to survival ones.

Methods A total of 224 newly admitted ICU patients in our hospital were collected and were followed up to 90 days after admission to ICU. Biological information, clinical characteristics and laboratory indicators and dynamic changes of NRBC count was compared between survival group and death group. Factors plausibly interact with both NRBC and outcome were included in logistic regression analysis to explore the risk factors of 28-day and 90-day mortality,ROC curve were drawn to determine the predictive value of NRBC for 28-day and 90-day all-cause mortality for ICU patients.

Results NRBC was positively correlated with NRBC-positive duration days, APACHE II score, SOFA score, CCI, CRP, PCT and RDW. The 28-day mortality and 90-day were 15.0%,31.3%, 41.2%, 56.7% and 32.7%,52.9%, 59.4%, 80.0% in patients with 0/μL, 1–100/μL, 101–200/μL and more than 200/μL NRBC,respectively.NRBC in the death group had a rapid upward trend before death, while those in the survival group were stable at low levels.NRBC was a robust predictor of 28-day and 90-day all-cause mortality following multivariable adjustment. The adjusted odds of 28-day and 90-day all-cause mortality in patients with more than 200/μL NRBC were 5.087(95% CI, 1.960-13.202) and 4.922(95% CI, 1.369-17.703), relative to patients without NRBC. Area under the curve(AUC) of predicting 28-day all-cause mortality and 90-day all-cause mortality by NRBC were 0.685 and 0.670, and NRBC had high predictive values for sepsis patients and non-respiratory failure patients.

Conclusion In ICU patients especially sepsis patients and non-respiratory failure patients, the presence of NRBC is a robust predictor of 28-day all-cause mortality and 90-day all-cause mortality. Specific NRBC fluctuations were found in death patients compared to survival ones.

Background

The essence of the Nucleated red blood cell (NRBC) is the non-denucleated red blood cells(RBCs), i.e. Immature RBCs[1]. NRBC also has a certain capacity of carrying oxygen, but it is significantly lower than mature RBC[2]. In addition, because NRBC is larger than mature RBC, it is easy to rupture when passing through narrow capillaries[3]. Under normal condition, NRBC can only exist in a small amount in the peripheral blood of newborn within one week of birth, but not in adult peripheral blood[4]. But in some pathological conditions, such as excessive erythropoiesis or the abnormal function of spleen,NRBC can not be removed from the blood circulation and appear in the peripheral blood. The common diseases or pathological processes leading to the increase of erythropoiesis mainly include massive hemorrhage, the increase of destruction of erythrocytes caused by various reasons, anemia, systemic inflammation or the

existence of hypoxia, etc[5]. High portal vein pressure can lead to splenomegaly or damage of spleen immune function caused by autoimmune antibody[6, 7]. Therefore, once the NRBC appears in the peripheral blood, it indicates that there is a disorder of physiological balance in the body.

It was reported that NRBC was related to the prognosis of various diseases, including pregnancy complications, neonatal-related diseases, severe infectious diseases, hematopathy, malignant tumors and immunological diseases[8–10].Many scholars have found that NRBC was related to the mortality of intensive care unit (ICU)patients, Stachon A,et al. [11]found that the NRBC positive rate in ICU patients was 20.0%, while the mortality rate of NRBC-positive patients was 21.1%, which was significantly higher than that of NRBC-negative ones, and the mortality rate increased with the increase of NRBC content. Menk M's study[12] on the patients with severe acute respiratory distress syndrome found that the positive rate of NRBC was 75.5%, while the mortality rate of NRBC-positive patients was 50.8%, and the NRBC content at the time of admission to ICU was an independent risk factor for mortality. A large sample studies showed that the 30-day mortality rate of NRBC-positive ICU patients was 9.0%, and the 90-day mortality rate was 14.0%, and mortality increased with the increase of NRBC,90-day mortality rate of the patients with more than 300 / μ L NRBC is over 20.0%[13].

However, most of the above studies were limited to comparing the mortality of NRBC-positive and NRBC-negative groups or different NRBC content groups, but did not discuss the time of the first occurrence of NRBC and the persistent presence days of NRBC,nor the dynamic changes of NRBC count and quantified predictive value of NRBC for mortality in ICU patients. We sought to determine whether the persistent presence of NRBC in ICU patients would be associated with 28-day and 90-day all-cause mortality.We hypothesized that death patients would have specific NRBC fluctuations compared to survival ones.

Methods

Source population

We abstracted 358 cases of newly admitted ICU patients from August 2018 to April 2019 by the electronic medical records of taizhou hospital in zhejiang province, China.A total of 109 patients did not have NRBCs measured,12 patients did not have blood pressure measured , 5 patients died within 24 hours,6 patients did not take blood test after admission to the ICU were excluded.Thus,224 patients finally constituted the cohort.

Data sources

Biological and clinical datas on all patients were collected retrospectively from Laboratory information system(LIS)and Hospital information system(HIS).

Specimens and laboratory test

About 10 mL fasting venous blood samples of ICU patients within 24 hours after admitted were collected. 2 ml of them were placed in the vacuum tube containing EDTA-K2 anticoagulant (BD company), and

automatically detected in the assembly line of Mindray BC-6800plus automated Hematology System.The remaining 8 mL were placed in two tubes with coagulant (BD company), and centrifuged at 3500rpm for 5 minutes after keeping at room temperature for 30 minutes for serum. The serum samples were tested for liver and kidney function, electrolyte, C-reactive protein (CRP), serum amyloid A (SAA) and procalcitonin (PCT) by Abbott C16000 automatic biochemical analyzer and Robas E601 automatic electrochemiluminescence analyzer,respectively.At the same time, 2ml arterial blood was drawn and placed in a special tube for blood gas analysis and detection by Robas b121 automatic blood gas analyzer.All samples should be mixed immediately after extraction.

Exposure of interest and score systems

The exposure of interest was the highest absolute NRBC count occurring after ICU initiation. The absolute NRBC count was determined via fluorescent flow cytometry using the Mindray BC-6800plus Automated Hematology System. The absolute NRBC count was reported as the number of NRBCs per microliter of blood, and was stratified as 0/μL,1–100/μL, 101–200/μL and more than 200/μL.

We utilized the charlson comorbidity index(CCI) to assess the burden of chronic illness, which is well studied and validated. CCI was put forward by Charlson in 1987. It refers to the injury and abnormality of other organs or tissues except the basic diseases, and carries out integral evaluation on the patients' complications, such as congestive heart failure, myocardial infarction, cerebrovascular disease, dementia, peripheral vascular disease, chronic obstructive pulmonary disease, peptic ulcer disease, connective tissue disease, diabetes, moderate Severe chronic kidney disease, hemiplegia, leukemia, lymphoma, solid tumor, liver disease, etc[14]. APACHE was used for evaluation of the severity of ICU patients.APACHE II score is the sum of acute physiology score, age score and chronic health score. Acute physiology score includes body temperature, heart rate, respiration and blood pressure, arterial oxygen pressure and pH, serum potassium (K), sodium (Na), creatinine (Cr), white blood cell (WBC) and Hematocrit (HCT), record the worst value of each index within 24 hours after entering ICU[15].We used SOFA score to determine the number of organs with failure. Index of respiratory, blood, circulation, nerve and kidney are involved in the SOFA score. The worst value of daily evaluation was recorded[16].

Respiratory failure was defined as arterial partial pressure of oxygen (PaO2) < 60mmHg. Sepsis was defined as the positive result of blood culture within 24 hours after admitting in ICU.

End points

The outcomes were 28-day and 90-day mortality.The end point of our research was September 1, 2019.All the cohort had vital status present at 28-day and 90-day after ICU initiation.

Research methods

Biological,clinical characteristics and laboratory indexes of ICU patients were compared between survival group and death group at 28-day and 90-day to explore the risk factors of 28-day and 90-day all-cause mortality.

Highest absolute NRBC count of ICU patients during hospitalization and days in ICU when NRBC initially appeared in peripheral blood were recorded.Correlation between absolute NRBC count and NRBC positive duration were analyzed.And correlations between absolute NRBC count and other laboratory indexes were discussed to speculate the possible causes of NRBC in ICU patients.

28-day and 90-day all-cause mortality compared among different NRBC levels to explore the relationship between absolute NRBC count and all-cause mortality of ICU patients.

Multiple results of NRBC in NRBC-positive patients during hospitalization were divided into 8 time periods according to the length of stay in ICU: 1-3 days, 4-6 days, 7-10 days, 11-15 days, 16-20 days, 21-30 days, 31-60 days and 61-90 days. The highest NRBC value was recorded in each time period.

Dynamic changes of absolute NRBC count of ICU patients in survival group and death group were compared in order to explore the similarities and differences of NRBC fluctuations in the two groups.

Multivariate logistic regression was used to explore the risk factors of 28-day and 90-day all-cause mortality and ROC curve was utilized to determine the predictive value of NRBC for 28-day and 90-day all-cause mortality .

Statistical analysis

SPSS19.0 statistical software was used for data processing and analysis, and Graphpad prism 8 was used for mapping. Datas with the normal distribution were represented by mean ± SD, comparison between two groups was represented by t-test, and the comparison among multiple groups was represented by one-way ANOVA.Non-normal distribution datas were represented by Median (P25-P75), comparison between two groups was represented by Mann -Whitney U test, and comparison among multiple groups was represented by Kruskal-Wallis H test.Categorical covariates were described by n (%), comparison between two groups or among multiple groups using contingency tables and chi-square testing. Spearman correlation analysis was used to analyze the correlation between non-normal distribution datas.Multivariate logistic regression was used to screen the risk factors of mortality, and ROC curve was used to analyze the predictive value of NRBC for 28-day and 90-day all-cause mortality in ICU patients. P <0.05 was considered with statistically differences.

Results

Biological and clinical characteristics of ICU patients

A total of 224 patients, 145 males and 79 females, aged 74 (60–82) years, were included. According to the 28-day and 90-day survival status, patients were divided into survival group and death group.Statistical differences were found in proportion of chronic kidney disease, APACH□score, SOFA score, PLT count and absolute NRBC count between 28-day death group and 28-day survival

group,indexes in 28-day death group were higher than 28-day survival group except for PLT count($P < 0.05$).

Proportion of acute respiratory failure,chronic kidney disease and malignant tumor, values of CCI, APACHE score, SOFA score, PCT and absolute NRBC count of 90-day death group were higher than 90-day survival group ($P < 0.05$)(Table 1)

Table 1

Comparison of biological and clinical characteristics between the survival group and the death group of ICU patients

	28-day			**90-day**		
	Survival group	Death group	P	Survival group	Death group	P
N	163	61		117	107	
Male gender	106(65.0%)	39(63.9%)	1.000	72(61.5)	73(68.2)	0.296
Age (years)	71(60–81)	78(56–85)	0.230	71(60–81)	76(58–84)	0.350
Surgical patient	25(15.3%)	7(11.5%)	0.602	17(14.5)	15(14.0)	0.913
CCI	7(5–8)	7(6–9)	0.065	6(4–8)	7(6–10)	0.000
CCI < 7	81(49.7%)	25(41.0%)	0.312	65(55.6)	41(38.3)	0.010
CCI ≥ 7	82(50.3%)	36(59.0%)		52(44.4)	66(61.7)	
Sepsis	45(27.6%)	19(31.1%)	0.722	27(23.1)	37(34.6)	0.057
Pneumonia	130(79.8%)	55(90.2%)	0.103	93(79.5)	92(86.0)	0.200
Acute respiratory failure	130(79.8%)	54(88.5%)	0.184	88(73.9)	96(89.7)	0.002
Hematopathy	8(4.9%)	4(6.6%)	0.739	4(3.4)	8(7.5)	0.178
Chronic kidney disease	26(16.0%)	18(29.5%)	0.037	15(12.8)	29(27.1)	0.007
Malignant tumor	22(13.5%)	14(23.0%)	0.131	11(9.4)	25(23.4)	0.004
APACHE□score	19(14–26)	23(19–29)	0.000	16(12–22)	23(20–29)	0.000
APACHE□<25	116(71.2%)	40(65.6%)	0.518	98(83.8)	58(54.2)	0.000
APACHE□≥25	47(28.8%)	21(34.4%)		19(16.2)	49(45.8)	
SOFA score	8(6–11)	12(11–16)	0.000	7(6–9)	12(10–16)	0.000
SOFA < 7	49(30.1%)	0(0.0%)	0.000	48(41.0)	1(0.9)	0.000

CCI: Charlson complication index, APACHE□: Acute physiological and chronic health score □, SOFA: Sequential organ failure assessment,WBC:White blood cell count,RBC:Red blood cell count,Hb: Hemoglobin,Hct:Hematocrit,RDW:Red blood cell distribution width,PLT:Platelet count, CRP:C-reactive protein,SAA:Serum amyloid A,PCT:Procalcitonin,NRBC:Nucleated red blood cells.

Data are expressed in n (%), Mean ± SD or Median (P25-P75)

P value is obtained by chi square test or Mann Whitney test

	28-day			90-day		
SOFA ≥ 7	114(69.9%)	61(100.0%)		69(59.0)	106(91.9)	
WBC($\times10^9$/L)	11.1(8.2–15.3)	9.9(6.9–15.0)	0.153	11.2(8.6–15.2)	10.2(7.3–15.1)	0.300
RBC($\times10^{12}$/L)	3.45(2.98–3.99)	3.40(2.75–3.97)	0.352	3.47(3.05–4.03)	3.40(2.79–3.90)	0.195
Hb(g/L)	102(86–121)	95(79–121)	0.151	103(88–121)	98(80–121)	0.146
Hct	31.5(26.6–37.1)	29.9(25.3–37.1)	0.144	32.3(27.2–37.1)	30.0(25.5–36.5)	0.126
RDW(%)	13.4(12.6–14.5)	13.8(13.0-15.4)	0.124	13.2(12.5–14.5)	13.8(13.0-14.7)	0.025
PLT($\times10^9$/L)	169(103–230)	123(68–199)	0.008	176(113–233)	131(76–201)	0.006
CRP(mg/L)	74.6(24.8–153.0)	85.3(35.9-169.3)	0.323	63.4(26.1-148.2)	92.7(25.8-166.5)	0.273
SAA(mg/L)	320.0(101.2-645.2)	257.7(86.8–455.0)	0.410	320.0(93.3-792.1)	320.0(93.9-499.2)	0.554
PCT(ng/ml)	1.14(0.21–5.43)	1.35(0.34–13.58)	0.102	0.68(0.18–2.96)	2.41(0.39–13.58)	0.002
NRBC(/μL)	0(0–48)	44(0-237)	0.000	0(0–39)	38(0-168)	0.000
CCI: Charlson complication index, APACHE□: Acute physiological and chronic health score □, SOFA: Sequential organ failure assessment,WBC:White blood cell count,RBC:Red blood cell count,Hb: Hemoglobin,Hct:Hematocrit,RDW:Red blood cell distribution width,PLT:Platelet count, CRP:C-reactive protein,SAA:Serum amyloid A,PCT:Procalcitonin,NRBC:Nucleated red blood cells.						
Data are expressed in n (%), Mean ± SD or Median (P25-P75)						
P value is obtained by chi square test or Mann Whitney test						

Distribution of highest absolute NRBC count in ICU patients during hospitalization

111 patients among the selected cases were NRBC-positive, with a positive rate of 49.6%, of which absolute NRBC count with 1-100/μL accounted for 28.6%, 101–200/μL accounted for 7.6%, and > 200/μL accounted for 13.4%.Highest absolute NRBC count of 0 /μL was more seen in survival group,and that of > 200 /μL was more appeared in the death group (Table 2).

Table 2

Comparison of highest absolute NRBC count distribution in survival group and death group

NRBC count	All patients	28-day			90-day		
		Survival group	Death group	P	Survival group	Death group	P
N	224	163	61		117	107	
0	113(50.4%)	96(58.9%)	17(27.9%)	0.000	76(65.0%)	37(34.6%)	0.000
1-100	64(28.6%)	44(27.0%)	20(32.8%)	0.491	26(22.2%)	38(35.5%)	0.040
101–200	17(7.6%)	10(6.1%)	7(11.5%)	0.254	8(6.8%)	9(8.4%)	0.848
> 200	30(13.4%)	13(8.0%)	17(27.8%)	0.000	7(6.0%)	23(21.5%)	0.001

Distribution of days in ICU when NRBC first appeared

Among all NRBC-positive patients, 34.2% showed positive results for the first time on the second day after admission to ICU, and 18.0% first appeared over 10 days after admission to ICU. Proportion of more than 10 days after admission with NRBC first appeared of the 28-day death group was higher than those in 28-day survival group(29.5% vs. 10.4%, P = 0.021),and there was no statistical difference between 90-day death group and 90-day survival group (P > 0.05).(Table 3)

Table 3

Comparison of days in ICU when NRBC first appeared in survival group and death group

Days in ICU when NRBC first appeared	All	28-day			90-day		
		survival group	death group	P	survival group	death group	P
N	111	67	44		41	70	
1 day	10(9.0%)	5(7.5%)	5(11.4%)	0.514	1(2.4%)	9(12.9%)	0.088
2 day	38(34.2%)	27(40.3%)	11(25.0%)	0.145	17(41.5%)	21(30.0%)	0.307
3 day	11(9.9%)	8(11.9%)	3(6.8%)	0.522	4(9.8%)	7(10.0%)	1.000
4 day	7(6.3%)	5(7.5%)	2(4.5%)	0.701	4(9.8%)	3(4.3%)	0.420
5 day	7(6.3%)	5(7.5%)	2(4.5%)	0.701	4(9.8%)	3(4.3%)	0.420
6 day	8(7.2%)	4(6.0%)	4(9.1%)	0.710	3(7.3%)	5(7.1%)	1.000
7 day	4(3.6%)	3(4.5%)	1(2.3%)	1.000	2(4.9%)	2(2.9%)	0.625
8 day	4(3.6%)	1(1.5%)	3(6.8%)	0.299	1(2.4%)	3(4.3%)	1.000
9 day	2(1.8%)	2(3.0%)	0(0.0%)	0.517	1(2.4%)	1(1.4%)	1.000
≥ 10 day	20(18.0%)	7(10.4%)	13(29.5%)	0.021	4(9.8%)	16(22.9%)	0.140

NRBC: Nucleated red blood cells. The data were expressed in n (%). P value was obtained by chi-square test.

Correlation between highest absolute NRBC count and NRBC continuous positive days in NRBC-positive group

M (P25-P75) of the highest absolute NRBC count in NRBC-positive patients was 79 (38–227) / μ L, with the minimum 11 / μ L and the maximum 11814 / μ L. M (P25-P75) of Lg (NRBC) was 1.58 (1.90–2.36), with the minimum value 1.04 and the maximum value 4.07.M (p25-p75) of NRBC positive duration days was 4 (2–11) day, with minimum 1 day, and maximum 90 day. The correlation between Lg (NRBC) and NRBC continuous positive days was shown in Fig. 1. There was a positive correlation between the highest absolute NRBC count and NRBC continuous positive days ($r = 0.292$, $P < 0.05$).

Correlation of absolute NRBC count with clinical scores and laboratory indicators

The absolute NRBC count was positively correlated with APACHE Ⅱ score, SOFA score, CCI, CRP, PCT and RDW ($r = 0.189$, $P = 0.005$; $r = 0.305$, $P = 0.000$; $r = 0.281$, $P = 0.000$; $r = 0.205$, $P = 0.002$; $r = 0.332$, $P = 0.000$; $r = 0.289$, $P = 0.000$), and was negatively correlated with RBC, Hb and HCT ($r = -0.174$, $P = 0.009$; $r = -0.205$, $P = 0.002$; $r = -0.196$, $P = 0.003$), but no correlation with SAA, WBC and PLT, as shown in Table 4.

Table 4
Correlation of the highest absolute NRBC count with clinical scores and laboratory indicators in NRBC-positive group

	r	P
APACHE Ⅱ score	0.189	0.005
SOFA score	0.305	0.000
CCI	0.281	0.000
CRP(mg/L)	0.205	0.002
SAA(mg/L)	− 0.014	0.873
PCT(ng/ml)	0.332	0.000
WBC($\times 10^9$/L)	0.056	0.406
RBC($\times 10^{12}$/L)	− 0.174	0.009
Hb(g/L)	− 0.205	0.002
Hct	− 0.196	0.003
RDW(%)	0.289	0.000
PLT($\times 10^9$/L)	− 0.128	0.055
APACHE Ⅱ: Acute physiological and chronic health score Ⅱ, SOFA: Sequential organ failure assessment, CCI: Charlson complication index, CRP:C-reactive protein,SAA:Serum amyloid A,PCT:Procalcitonin,WBC:White blood cell count,RBC:Red blood cell count,Hb: Hemoglobin,Hct:Hematocrit,RDW:Red blood cell distribution width,PLT:Platelet count.		
r and P value were obtained by Spearman correlation analysis.		

Biological and clinical characteristics of ICU patients with different NRBC counts

According to the NRBC count, the participants were divided into four groups: 0 / μ L, 1-100 / μ L, 101–200 / μ L and > 200 / μ L. Patients with high NRBC count had relatively high proportion of sepsis, pneumonia and malignant tumor, and had higher CCI,APACHE Ⅱ score, SOFA score and RDW. RBC, Hb and Hct decreased first and then increased with the increase of NRBC count($P < 0.05$).There were no statistical differences in sex, age, surgery, respiratory failure, proportion of blood disease and chronic kidney disease, WBC, PLT, CRP, SAA and PCT ($P > 0.05$), as shown in Table 5.

Table 5

Biological and clinical characteristics of ICU patients with different NRBC contents

	0/μL	1-100/μL	101–200/μL	> 200/μL	P
N	113	64	17	30	
Male gender	40(35.4)	21(32.8)	7(41.2)	11(36.7)	0.929
Age (years)	72(60–82)	78(60–84)	73(58–82)	69(56–81)	0.427
Surgical patient	14(12.4)	12(18.8)	3(17.6)	3(10.0)	0.576
CCI	6(4–8)	8(6–9)	7(6–9)	8(6–11)	0.000
CCI < 7	67(59.3)	22(34.4)	7(41.2)	10(33.3)	0.004
CCI ≥ 7	46(40.7)	42(65.6)	10(58.8)	20(66.7)	
Sepsis	19(16.8)	25(39.1)	4(23.5)	16(53.3)	0.000
Pneumonia	16(14.2)	55(85.9)	13(76.5)	24(80.0)	0.000
Acute respiratory failure	94(83.2)	51(79.7)	13(76.5)	26(86.7)	0.768
Hematopathy	2(1.8)	7(10.4)	1(5.9)	2(6.7)	0.075
Chronic kidney disease	15(13.3)	15(23.4)	5(29.4)	9(30.0)	0.088
Malignant tumor	10(8.8)	14(21.9)	5(29.4)	7(23.3)	0.025
APACHE Ⅱ score	19(14–25)	21(15–27)	23(19–27)	24(19–29)	0.035
APACHE Ⅱ<25	83(73.5)	45(70.3)	11(64.7)	17(56.7)	0.337
APACHE Ⅱ≥25	30(26.5)	19(29.7)	6(35.3)	13(43.3)	
SOFA score	8(6–11)	11(7–13)	13(8–16)	13(8–19)	0.000
SOFA < 7	32(28.3)	11(17.2)	2(11.8)	4(13.3)	0.118
SOFA ≥ 7	81(71.7)	53(82.8)	15(88.2)	26(86.7)	
WBC($\times 10^9$/L)	10.2(7.8–14.6)	10.6(7.8–15.2)	10.7(8.5–16.3)	12.5(7.6–17.0)	0.596

CCI: Charlson complication index, APACHE Ⅱ: Acute physiological and chronic health score Ⅱ, SOFA: Sequential organ failure assessment,WBC:White blood cell count,RBC:Red blood cell count,Hb: Hemoglobin,Hct:Hematocrit,RDW:Red blood cell distribution width,PLT:Platelet count, CRP:C-reactive protein,SAA:Serum amyloid A,PCT:Procalcitonin,NRBC:Nucleated red blood cells.

Data are expressed in n (%), Mean ± SD or Median (P25-P75)

P value is obtained by chi square test or Mann Whitney test

	0/μL	1-100/μL	101–200/μL	> 200/μL	P
RBC(×10^{12}/L)	3.62(3.18–4.17)	3.04(2.50–3.68)	3.45(2.85–4.06)	3.38(2.88–4.05)	0.000
Hb(g/L)	107(95–126)	88(75–104)	102(79–111)	102(80–121)	0.000
Hct	33.1(29.1–38.4)	27.4(23.8–32.7)	30.0(25.5–33.7)	31.1(25.7–38.2)	0.000
RDW(%)	13.1(12.5–14.1)	13.9(12.9–15.6)	13.7(12.8–16.2)	14.1(13.2–17.4)	0.000
PLT(×10^{9}/L)	166(113–229)	166(59–227)	121(83–202)	154(69–212)	0.295
CRP(mg/L)	51.7(14.3-117.8)	120.9(52.7-171.1)	84.8(32.5-187.5)	93.5(50.4-155.2)	0.537
SAA(mg/L)	320.0(75.3-553.8)	320.0(113.6-762.4)	320.0(230.5-362.8)	254.3(61.9-458.9)	0.395
PCT(ng/ml)	0.47(0.14–2.68)	2.41(0.46–9.65)	2.78(0.37–71.50)	3.56(0.65–21.43)	0.626
NRBC(/μL)	0(0–0)	42(25–71)	138(113–182)	813(316–1762)	0.000
CCI: Charlson complication index, APACHE□: Acute physiological and chronic health score □, SOFA: Sequential organ failure assessment,WBC:White blood cell count,RBC:Red blood cell count,Hb: Hemoglobin,Hct:Hematocrit,RDW:Red blood cell distribution width,PLT:Platelet count, CRP:C-reactive protein,SAA:Serum amyloid A,PCT:Procalcitonin,NRBC:Nucleated red blood cells.					
Data are expressed in n (%), Mean ± SD or Median (P25-P75)					
P value is obtained by chi square test or Mann Whitney test					

All-cause mortality in NRBC-negative and NRBC-positive groups

28-day all-cause mortality in all cases of NRBC-positive group was higher than NRBC-negative group (39.6% vs. 15.0%, $P < 0.05$). In patients with respiratory failure, sepsis, transfer from other departments and pneumonia, 28-day all-cause mortality of NRBC-positive group were higher than NRBC-negative group ($P < 0.05$). 90-day all-cause mortality in all cases of NRBC-positive group was higher than NRBC-negative group (63.1% vs. 32.7%, $P < 0.05$). In patients with respiratory failure, sepsis, transfer from other departments and pneumonia, 90-day all-cause mortality of NRBC positive group was significantly higher than that of NRBC-negative group ($P < 0.05$),as shown in Fig. 2.

Relationship between NRBC counts and all-cause mortality in ICU

The 28-day and 90-day all-cause mortality rate of all patients were 27.2% (61/224) and 47.8% (107/224). There were significant differences in 28-day and 90-day all-cause mortality rate among the groups of 0 /

μ L, 1-100 / μ L, 101–200 / μ L and > 200 / μ L NRBC(P < 0.05). The higher the NRBC count, the higher the mortality was(Fig. 3).

Dynamic changes of NRBC in survival group and death group on 28 days and 90 days of NRBC-positive patients

The mean NRBC count of the 28-day survival group were 89.2 / μ L, 56.5 / μ L, 56.5 / μ L, 90.6 / μ L, 30.8 / μ L, 188.0 / μ L, 14.8 / μ L, 22.7 / μ L and 9.5 / μ L on day 1–3, 4–6, 7–10, 11–15, 11–15, 16–20, 21–30, 21–30, 31–60 and 61–90 after admitted to ICU, respectively,while in 28-day death group were 242.3 / μ L, 223.6 / μ L, 135.2 / μ L, 289.9/ μ L, 55.1/μ L, 177.1/μ L. The mean value of NRBC in the 28-day death group was higher than that in the 28-day survival group. NRBC in the 28-day death group had a downward trend at 16–20 days, but recovered again at 21–30 days (one week before death),while in survival group it was stable within 100 / μ L, except for that it quickly fell back and stabilized at a lower value at 21–30 days after rising at 16–20 days.

The mean NRBC count of 90-day survival group were 63.9/μL□61.1/μL□42.6/μL□39.0 /μL□21.4/μL□12.2/μL□30.9 /μL and 9.5/μL, respectively,while it was 200.3/μL□162.3/μL□148.7 /μL□225.1/μL□174.6/μL□144.4/μL□1059.9 /μL and 15.5/μL of 90-day death group.NRBC in the 90-day death group was higher than that in the 90-day survival group in each time period. NRBC in the 90-day death group increased to over 1000 / μ L in 31–60 days, and NRBC in the 90-day survival group was kept at a low level, and gradually decreased.(Fig. 4)

Risk factors for 28-day all-cause mortality in ICU patients

Blood transfusion, NRBC and PLT were statistically significant through univariate logistic regression(P < 0.05) and included in multivariate logistic regression. NRBC was an independent risk factor of 28-day all-cause mortality (OR = 5.087, P = 0.001)(Table 6).

Table 6

Risk factors of 28-day all-cause mortality by univariate and multivariate logistic regression

	Univariate logistic regression			Multivariate logistic regression		
	OR	CI(95%)	P	OR	CI(95%)	P
Biological indicators						
Gender						
Male	1.000					
Female	1.049	0.568–1.938	0.879			
Age	1.007	0.988–1.026	0.485			
Clinical indicators						
APACHE Ⅱ score						
APACHE Ⅱ<25	1.000					
APACHE Ⅱ≥25	1.296	0.692–2.427	0.418			
SOFA score						
SOFA < 7	1.000					
SOFA ≥ 7	8.644	0.000	0.997			
CCI						
CCI < 7	1.000					
CCI ≥ 7	1.422	0.784–2.580	0.246			
Sepsis						
No	1.000					
Yes	1.186	0.625–2.253	0.602			
Surgical patient						
No	1.000					
Yes	0.716	0.292–1.752	0.464			
Acute respiratory failure						

OR: odds ratio,APACHE Ⅱ: Acute physiological and chronic health score Ⅱ,SOFA:Sequential organ failure assessment.CCI: Charlson complication index, NRBC: Nucleated red blood cells,CRP: C-reactive protein,SAA: serum amyloid A, PCT: procalcitonin, WBC: White blood cell count,RBC: Red blood cell count, Hb: Hemoglobin,HCT: Hematocrit,RDW:Red blood cell distribution width,PLT: Platelet count.

*P < 0.05

	Univariate logistic regression			Multivariate logistic regression		
No	1.000					
Yes	1.958	0.816–4.698	0.132			
Blood transfusion						
No	1.000					
Yes	3.439	1.813–6.521	0.000	2.014	0.960–4.224	0.064
Laboratory indicators						
NRBC						
0/μL	1.000					
1-100/μL	2.567	1.227–5.372	0.012	1.816	0.806–4.089	0.150
101–200/μL	3.953	1.322–11.815	0.014	2.602	0.817–8.292	0.106
> 200/μL	7.385	3.040-17.935	0.000	5.087	1.960-13.202	0.001
CRP(mg/L)	1.001	0.998–1.003	0.540			
SAA(mg/L)	1.000	0.999–1.001	0.969			
PCT(ng/mL)	1.002	0.997–1.008	0.441			
WBC($\times 10^9$/L)	0.972	0.920–1.026	0.306			
RBC($\times 10^{12}$/L)	0.844	0.579–1.230	0.377			
Hb(g/L)	0.991	0.978–1.003	0.138			
Hct	0.969	0.929–1.011	0.143			
RDW(%)	1.041	0.933–1.160	0.472			
PLT($\times 10^9$/L)	0.997	0.993-1.000	0.043	0.998	0.994–1.001	0.188
OR: odds ratio,APACHE Ⅱ: Acute physiological and chronic health score Ⅱ,SOFA:Sequential organ failure assessment.CCI: Charlson complication index, NRBC: Nucleated red blood cells,CRP: C-reactive protein,SAA: serum amyloid A, PCT: procalcitonin, WBC: White blood cell count,RBC: Red blood cell count, Hb: Hemoglobin,HCT: Hematocrit,RDW:Red blood cell distribution width,PLT: Platelet count.						
*P < 0.05						

Risk factors for 90 day all-cause mortality in ICU patients

Univariate logistic regression showed that APACHE Ⅱ score, SOFA score, CCI, respiratory failure, blood transfusion, NRBC and PLT may be potential risk factors of 90-day all-cause mortality, and were included in multivariate logistic regression for further screening. APACHE Ⅱ score, SOFA score, respiratory failure,

blood transfusion and NRBC were the risk factors of all-cause mortality Risk factors (OR = 2.210, P = 0.033;OR = 61.156, P = 0.000; OR = 3.061, P = 0.030;OR = 2.649, P = 0.016; OR = 4.922, P = 0.015). (Table 7).

Table 7

Univariate and multivariate regression analysis of predictors of 90-day all-cause mortality in ICU patients

	Univariate logistic regression			Multivariate logistic regression		
	OR	CI(95%)	P	OR	CI(95%)	P
Biological indicators						
Gender						
Male	1.00					
Female	0.745	0.429–1.294	0.296			
Age	1.008	0.991–1.024	0.349			
Clinical indicators						
APACHE Ⅱ score						
APACHE Ⅱ<25	1.00					
APACHE Ⅱ≥25	4.358	2.341–8.111	0.000*	2.210	1.067–4.577	0.033*
SOFA score						
SOFA < 7	1.00					
SOFA ≥ 7	73.739	9.946–546.690	0.000*	61.156	7.609-491.531	0.000*
CCI						
CCI < 7	1.00					
CCI ≥ 7	2.012	1.180–3.431	0.010*	1.401	0.705–2.781	0.336
Sepsis						
No	1.00					
Yes	1.762	0.980–3.166	0.058			
Surgical patient						
No	1.00					
Yes	0.959	0.453–2.030	0.913			
Acute respiratory failure						

OR: odds ratio,APACHE Ⅱ: Acute physiological and chronic health score Ⅱ,SOFA:Sequential organ failure assessment.CCI: Charlson complication index, NRBC: Nucleated red blood cells,CRP: C-reactive protein,SAA: serum amyloid A, PCT: procalcitonin, WBC: White blood cell count,RBC: Red blood cell count, Hb: Hemoglobin,HCT: Hematocrit,RDW:Red blood cell distribution width,PLT: Platelet count.

*P < 0.05

	Univariate logistic regression			Multivariate logistic regression		
No	1.00					
Yes	2.876	1.356-6.100	0.006*	3.061	1.113–8.417	0.030
Blood transfusion						
No	1.00					
Yes	3.139	1.817–5.421	0.000*	2.649	1.195–5.870	0.016
Laboratory indicators						
NRBC						
0/μL	1.00					
1-100/μL	3.002	1.591–5.665	0.001*	2.206	0.941–5.170	0.069
101–200/μL	2.311	0.825–6.474	0.111	1.088	0.305–3.879	0.897
> 200/μL	6.749	2.655–17.154	0.000*	4.922	1.369–17.703	0.015
CRP(mg/L)	1.002	0.999–1.005	0.120			
SAA(mg/L)	1.000	0.999-1.000	0.483			
PCT(ng/mL)	1.004	0.998–1.011	0.214			
WBC($\times 10^9$/L)	0.994	0.948–1.042	0.799			
RBC($\times 10^{12}$/L)	0.783	0.562–1.102	0.163			
Hb(g/L)	0.991	0.980–1.002	0.115			
Hct	0.969	0.934–1.005	0.093			
RDW(%)	1.084	0.973–1.208	0.145			
PLT($\times 10^9$/L)	0.997	0.994-1.000	0.022	0.998	0.995–1.002	0.357
OR: odds ratio,APACHE□: Acute physiological and chronic health score □,SOFA:Sequential organ failure assessment.CCI: Charlson complication index, NRBC: Nucleated red blood cells,CRP: C-reactive protein,SAA: serum amyloid A, PCT: procalcitonin, WBC: White blood cell count,RBC: Red blood cell count, Hb: Hemoglobin,HCT: Hematocrit,RDW:Red blood cell distribution width,PLT: Platelet count.						
*P < 0.05						

Disscussion

About 750,000 cases of severe infection in the United States every year, more than the total number of AIDS, breast cancer, congestive heart failure and colon cancer patients, with a mortality rate of about 20%

– 63%. In the world, the incidence, mortality and related medical expenses of patients with severe infection increase year by year. There are more than 18 million cases of severe infection every year in the world, among which more than 1,400 cases die every day[17]. Systemic infection is the leading cause of death in ICU. China's research showed that the aging population and the increase of patients with chronic diseases, as well as the use of various immunosuppressants after some medical operations, such as tumor chemotherapy and organ transplantation, could increase the incidence of severe infection. The prevalence of severe systemic infection in surgical ICU and general ICU were 8.7% and 37.3% respectively, similar to that in European countries[18].

Most patients in ICU were with severe diseases and poor prognosis, and the probability of death in a short time was relatively high. At present, 28-day mortality or 90-day mortality are mostly used as the end point in the study of prognosis of ICU patients at home and abroad[19]. Lilly,et al [20]reported that the average length of stay in ICU was about 12.6 days, and chant C,et al[21] found that was 49 days, which were not the same. In this study, the average length of stay was 20.5 days, and 28-day mortality and 90-day mortality were set to explore the risk factors of ICU patients. Although both of them were short-term mortality, the 28-day mortality rate was closely related to patients' treatment, various medical operations and hospital infections[22, 23], while the 90-day mortality rate was not only related to the influencing factors of 28-day mortality, but also related to patients' age, family care after discharge, nutrition and natural progress of the disease itself[19].

A meta-analysis in 2008 showed that the total in-hospital mortality rate of ICU patients was 11–45%, among which 6.3–37% died in ICU[24]. Rui,et al. believed that the mortality rate of ICU patients after discharge was between 6.4% and 40%, depending on the severity of patients[25]. Braber,et al. found that the in-hospital mortality rate of ICU patients was 16.3%, and the two-year mortality rate was 26.6%[26]. In this study, 224 inpatients in ICU were included in the retrospective study, of which 61 died at 28-day after admission, with an all-cause mortality rate of 27.2%.107 died at 90-day after admission, with an all-cause mortality rate of 47.8%, similar to the above studies. In addition, because our hospital was a tertiary general hospital with large flow of patients, ICU patients had certain representativeness.

We found that risk factors of 28-day and 90-day mortality of ICU patients were different. CCI, the proportion of acute respiratory failure and malignant tumor were the risk factors of 90-day mortality, but they did not affect the 28-day mortality, which may be related to the natural progress and outcome of various complications, including tumors. However, patients with acute respiratory failure usually use ventilator to assist in maintaining respiration after tracheotomy in hospital. However, due to the limitation of conditions after discharge, most families were unable to assist in breathing, which affects the respiratory circulation of patients and increases the risk of hypoxia[27]. However, the proportion of chronic kidney disease, APACHE Ⅱ score, SOFA score and NRBC count were not only the risk factors of 28-day mortality, but also the risk factors of 90-day mortality. Kidney is one of the important excretory organs of human body,and creatinine and blood potassium are excreted through kidney. Most patients with severe chronic kidney disease need hemodialysis instead of renal function to discharge waste[28]. Because of the high cost of hemodialysis, some patients are unable to bear, and there are complications

such as infection and thrombus[29], so it has certain impact on the 28-day and 90-day mortality of ICU patients.APACHE Ⅱ score and SOFA score cover respiratory, circulatory, urinary, digestive system, etc., which are generally recognized as the scoring standards for evaluating the severity of patients' condition[30]. There were many studies proved that APACHE Ⅱ score and SOFA score were related to the mortality of ICU patients[31–33], which were consistent with this study.

Relationship between NRBC and diseases were mainly focused on fetal hypoxia[34], while the causes of NRBC in adults mainly involved infection[10], anemia[35] and hypoxia[36]. Some scholars thought that NRBC was closely related to severe infection and was associated with prognosis, such as Desai S, who found that the mortality rate increased with the elevated of NRBC count, and NRBC might be a biomarker for the prognosis of surgical septicemia patients[37]; Minior VK,et al. found the fetal NRBC count increased after rats exposed to hypoxia for more than 24 hours, with low weight and the slow organ growth, suggesting that the production of NRBC was related to hypoxia and had a negative effect for fetal development[38]. However, there were few comparative studies on the dynamic changes of NRBC in survival and death patients. Therefore, this study aimed to explore the relationship between 28-day mortality and 90-day mortality of NRBC and ICU patients by collecting all kinds of clinical and laboratory indicators of ICU patients for comprehensive analysis.

In this study, we found that the majority of NRBC in the survival group was negative, and that in death group was > 200 / μ L. Zhang Shan,et al. found that for patients with APACHE II score > 21, there was a certain correlation between admission time and mortality[39]. The mortality of patients admitted during non-statutory working hours was relatively higher than that admitted during statutory working hours. Therefore, we speculated that the time of NRBC presenting positive results for the first time can also affect the mortality of ICU patients. It was found that more than 1 / 3 of the NRBC-positive ICU patients showed positive results on the second day of admission, and the 28-day all-cause mortality rate of the patients who were positive for the first time more than 10 days in ICU was higher, while the number of days in ICU was not related to the 90-day all-cause mortality rate. Stachon a et al. believed that NRBC could show positive results for the first time on average 9 days (median 5 days) before death[40], while NRBC-positive death patients in this study showed positive results for the first time on average 14 days (median 6 days) before death, similar to the above study. NRBC-positive patients who died between 28-day and 90-day after admitting in ICU had relatively longer survival time than those who died within 28 days The influencing factors of mortality were complex, including the number and degree of complications, nursing and diet[41], so it was not difficult to understand that they have nothing to do with the number of days in ICU when the patient first presented positive.

In addition, we found that the higher the NRBC content, the longer the NRBC-positive duration. It might due to the clearance of NRBC in the spleen[42]. If the NRBC count was too high, the spleen couldn't clear it quickly led to prolong of the NRBC existence time in the peripheral blood.

Furthermore, correlation between NRBC count and laboratory indexes showed that NRBC was positively correlated with CRP, PCT and RDW, negatively correlated with RBC, Hb and Hct, but not significantly

correlated with SAA, WBC and PLT. CRP, PCT and RDW were commonly used inflammatory indexes[43], RBC, Hb and Hct were visual indexes to reflect whether anemia exists in the body[44], PLT could reflect the coagulation or thrombus state of patients[45], so it can be inferred that the production and release of NRBC in ICU patients were related to inflammation and anemia, but not to whether the body was in hypercoagulable state. Moreover, NRBC was positively correlated with APACHE Ⅱ score, SOFA score and CCI, and the above three clinical scores are generally recognized as indicators for disease severity assessment and prognosis[46]. Therefore, NRBC can also reflect disease severity and prognosis prediction.

This study concluded that the 28-day and 90-day all-cause mortality in NRBC-positive group were higher than that in NRBC-negative group, and were positive correlated with NRBC counts, inferred that NRBC can predict 28-day and 90-day all-cause mortality of ICU patients.

We also compared the NRBC count fluctuations between survival group and death group. Because of the irregular detection time of NRBC in ICU patients and the inconsistent positive time of NRBC, it was impossible to monitor the NRBC count of each patient every day. In our study,more than 50% of NRBC-positive patients showed positive results within 3 days after admission, 80% of them show positive results within 10 days. Therefore, by dividing the detection time o into 1–3 days, 4–6 days,7–10 days, 11–15 days, 6–20 days, 21–30 days, 31–60 days and 61–90 days after admitting in ICU. Recorded peak value of NRBC count in each time period. The study found that the NRBC count in the death group was higher than that in the survival group, and if the NRBC count fluctuated greatly or increased repeatedly, the mortality risk was higher.If the NRBC count kept at a low level or only after a temporary increase then quickly fell back and maintained at a lower level, the risk of mortality was low. It can be concluded that the repeated and large increase of NRBC indicated adverse outcomes.

We also found that NRBC was an independent risk factor of 28-day all-cause mortality of ICU patients,especially for sepsis and non respiratory failure patients, it has a high predictive value for mortality.Risk factors of 90-day all-cause mortality of ICU patients including APACHE Ⅱ score, SOFA score, respiratory failure, blood transfusion and NRBC. NRBC alone had the highest predictive value for 90-day all-cause mortality of non respiratory failure patients, while NRBC combined with APACHE Ⅱ score and SOFA score had the highest predictive value for 90-day all-cause mortality of sepsis patients. Sepsis refered to the systemic inflammatory response syndrome caused by infection[47], so it could be considered that the increased of NRBC count caused by infection could predict the mortality of sepsis patients.Moreover,it could be inferred that respiratory failure can independently lead to the increase of NRBC count, because the other cause of NRBC besides inflammation and anemia was hypoxia[48], and the increase of NRBC excluding respiratory failure had a higher predictive value for the mortality.

There are some limitations in this study. First of all, sample numbers in this study was small. Second, because the time of NRBC detection in ICU patients was irregular, and the positive time of NRBC was inconsistent, it was impossible to carry out the test for each patient daily.Thirdly, due to family and economic factors, each patient's nutrition and nursing reserves after discharge were different, which

could affect the survival time. Therefore,large samples of ICU patients should be collected for prospective study in the future.All patients should be tested for NRBC according to specific frequency, and analyzed in subgroups according to patients' basic state and disease type. In addition, samples of patients should be collected for cell culture and animal experiments to conduct basic research to explore the production of NRBC in patients in order to study the relationship between NRBC and all-cause mortality of ICU patients more comprehensively and deeply, and provide a better basis for disease diagnosis and treatment and prognosis improvement.

Conclusion

NRBC in ICU patients is mainly caused by inflammation, anemia and hypoxia, which is related to APACHE score, SOFA score and CCI, and can reflect the disease severity of patients. In addition, NRBC has different dynamic changes in survival group and death group. It has a high predictive value for 28 day all-cause mortality and 90 day all-cause mortality of ICU patients, especially sepsis patients and non-respiratory failure patients. Clinically, NRBC can be used as an indicator of disease treatment and dynamic monitoring of ICU patients, providing a new theoretical basis for improving the prognosis of ICU patients.

Declarations

Ethics approval and consent to participate

Approval for the study was granted by the Medical ethics committee of Taizhou Hospital of Zhejiang Province. Requirement for consent was waived because the data were analyzed anonymously.

Consent for publication

Not applicable.

Availability of data and materials

The dataset supporting the conclusions of this article is not available

Competing interests

None of the authors have any competing interests in the manuscript

Funding

This study was supported by grants from Taizhou Science and Technology Plan (1802ky18) (Zhejiang, China).

Authors' contributions

JW, GL,BS conceived, designed and coordinated the study, participated in acquisition and interpretation of data, and drafted the manuscript. X J participated in acquisition of data. Y Y participated in blood and urine determination levels and the interpretation of data.

Acknowledgements

We thank the intensive care unit of Taizhou Hospital of Zhejiang Province for their support to our research.

Authors' information (optional)

Not applicable.

Abbreviations

NRBC:Nucleated red blood cells;ICU:Intensive care unit;CRP:C-reactive protein;SAA:Serum amyloid A;PCT:Procalcitonin;CCI:Charlson Complication Index Score;GCS:Glasgow coma scale;APACHE:Acute Physiological and Chronic Health Score ;SOFA:Sequential Organ Failure Assessment;ROC:Receiver operating characteristic curve;AUC:Area Under Curve;WBC:White blood cell;RBC:Red blood cell;Hb:Hemoglobin;Hct:Hematocrit;RDW:Red blood cell distribution width;PLT:Platelet;OR:Odds ratio;95%CI:95% Confidence interval.

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Figures

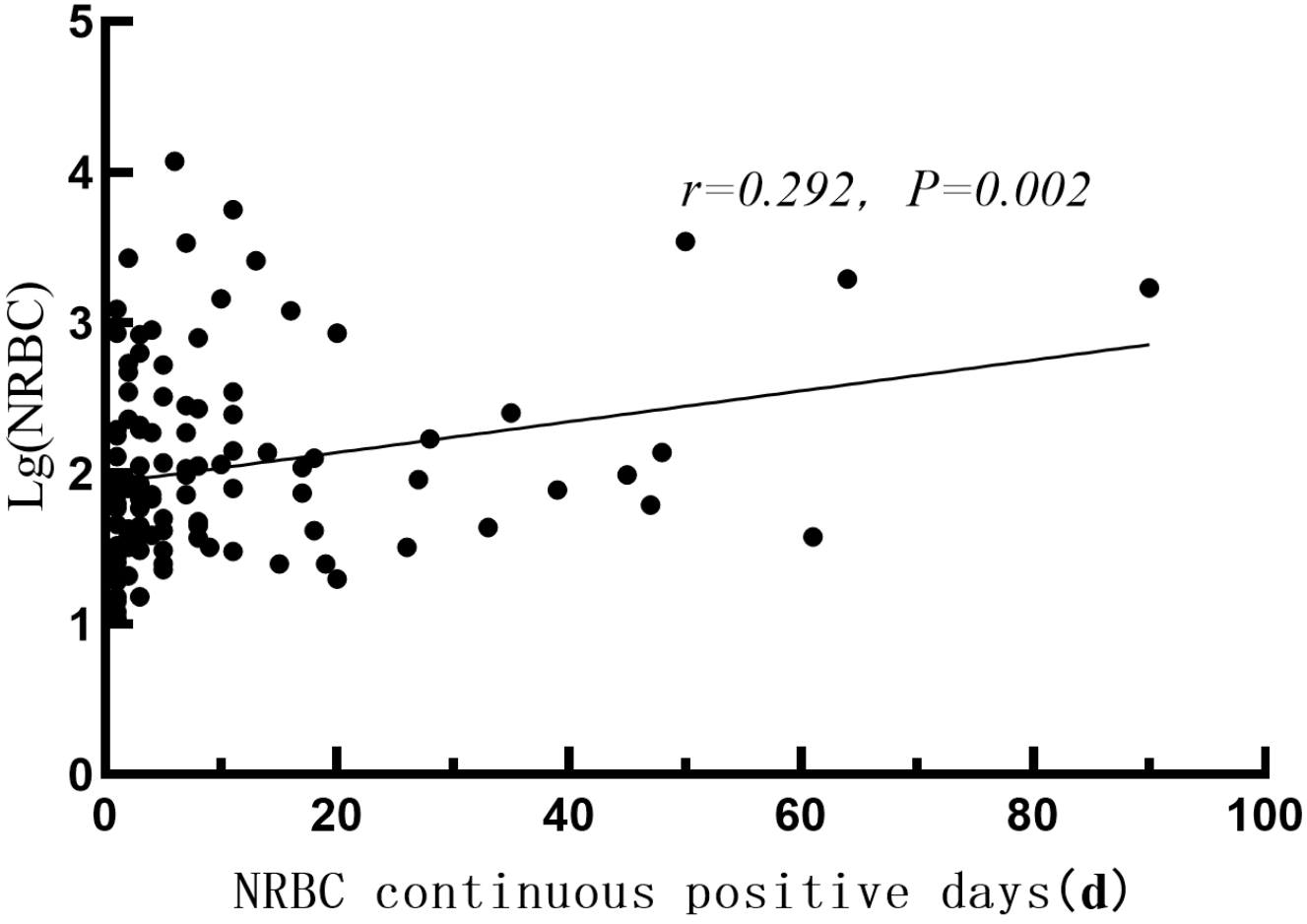

Figure 1

Correlation between highest absolute NRBC count and NRBC continuous positive days *P value is obtained by Spearman correlation analysis.

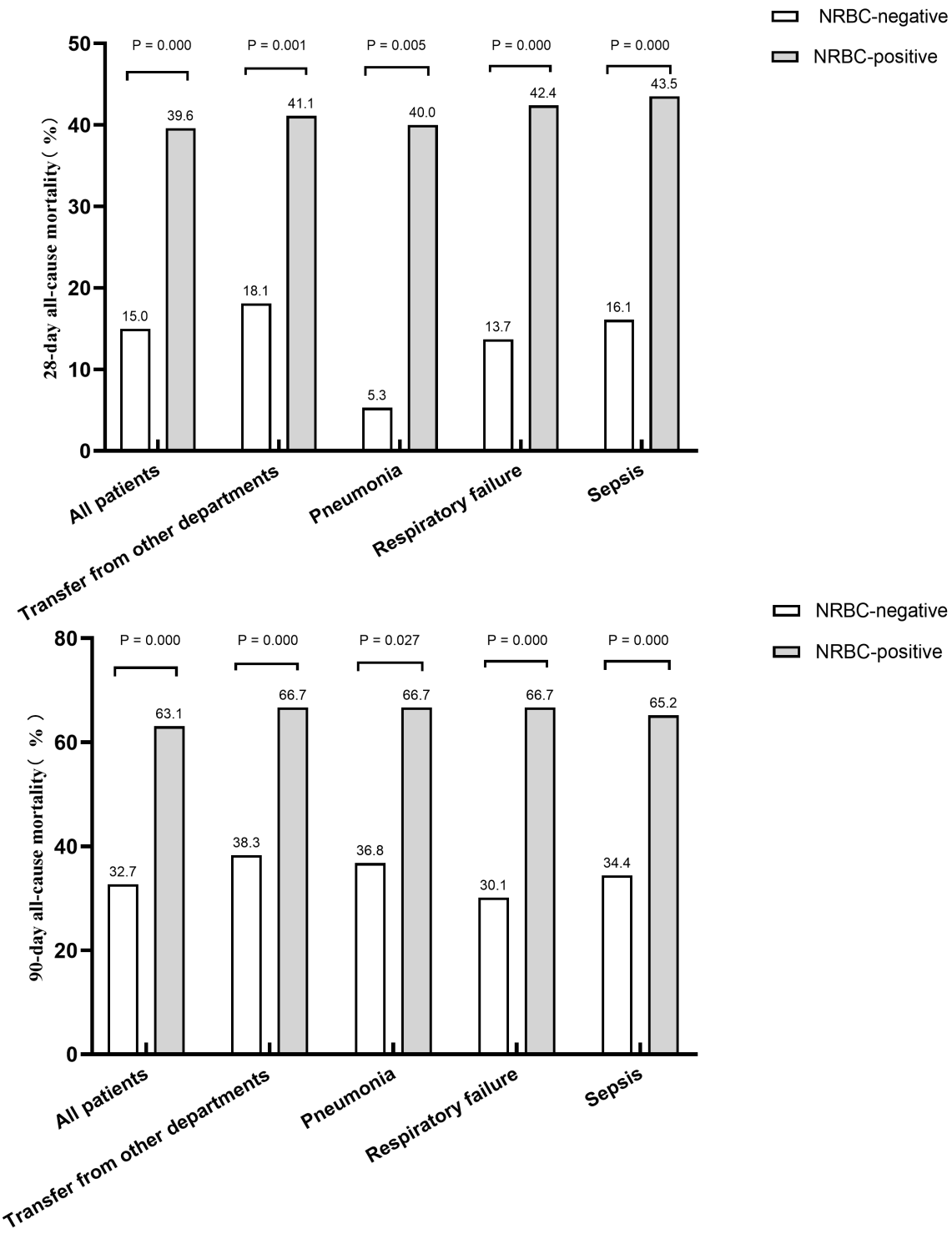

Figure 2

Comparison of 28-day and 90-day all-cause mortality in NRBC-negative group and NRBC-positive group

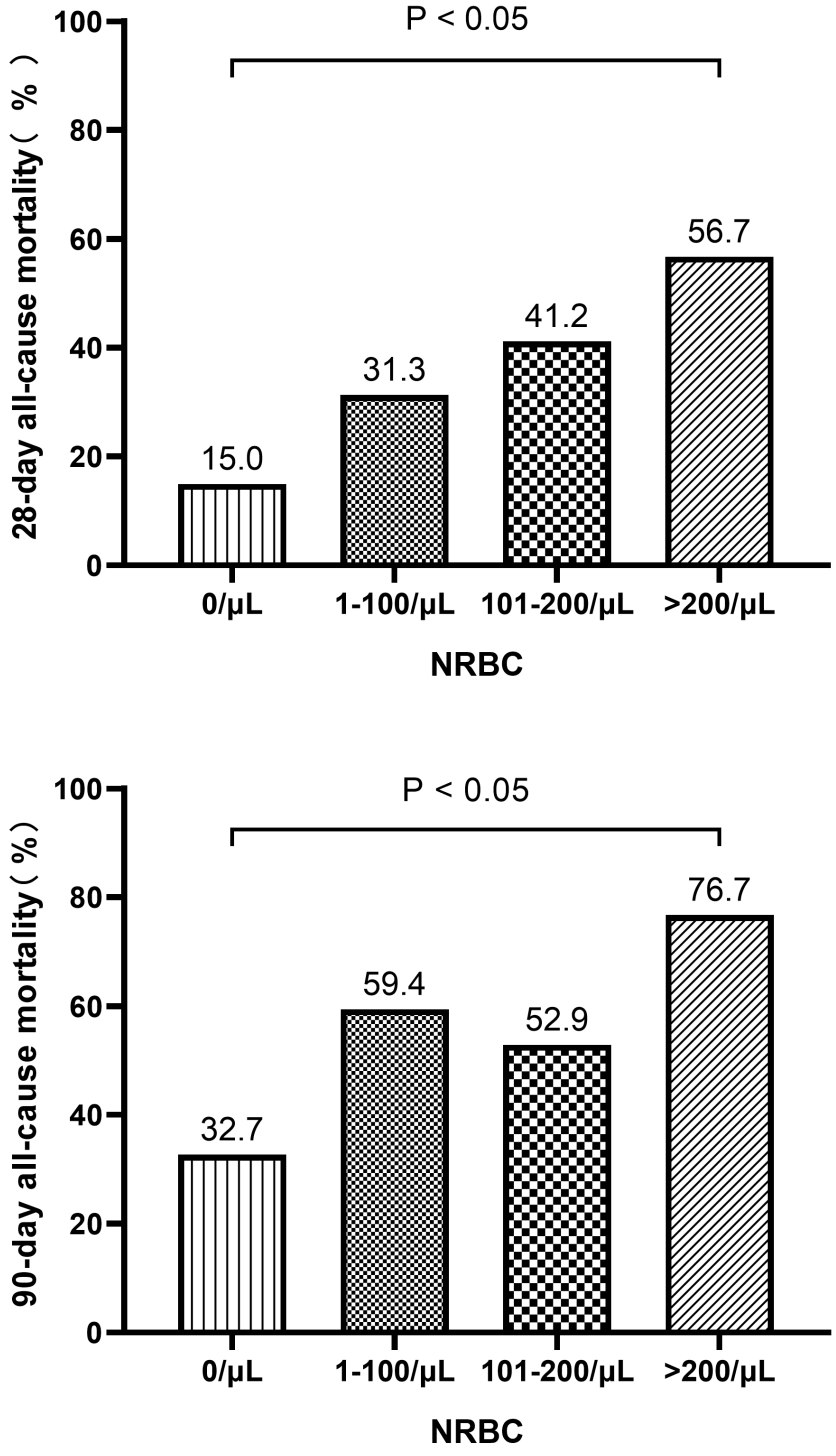

Figure 3

Relationship between NRBC content and all-cause mortality at 28 and 90 days in ICU

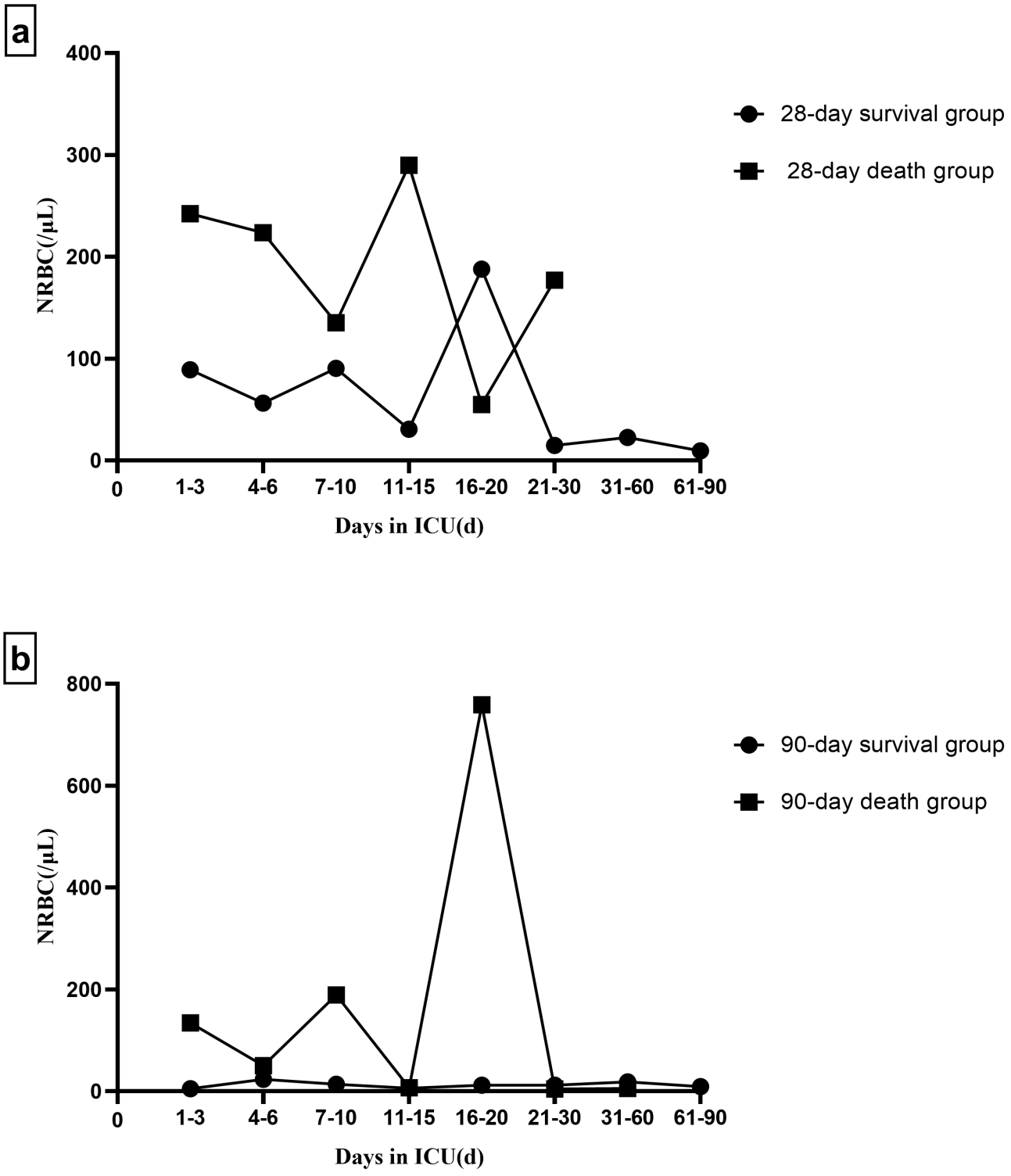

Figure 4

Dynamic changes of NRBC count in survival group and death group of NRBC positive patients a:Dynamic change of NRBC count in the 28-day survival group and the death group of NRBC-positive patients living in ICU b:Dynamic change of NRBC count in the 90-day survival group and death group of NRBC-positive patients in ICU