

Early alterations of circulating immune cell counts in severe trauma patients are related to later occurrence of nosocomial infection, sepsis and mortality

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Abstract

Background: Severe trauma leads to extensive disturbances of the innate and adaptive arms of the immune system, which in turn may affect the prognosis. The main objective of this study was to investigate the relationship between the alterations of circulating immune cell counts in the early stage of severe trauma and the later occurrence of nosocomial infection, sepsis and mortality.

Methods: This was a retrospective study of 876 patients with an Injury Severity Score (ISS) ≥ 16 . Demographic data, the absolute counts of neutrophil, lymphocyte and monocyte (ANC, ALC and AMC) on days 1, 3, and 7 (D1, D3, and D7) after trauma, and whether nosocomial infection, sepsis or death occurred within 60 days were recorded. Ratios were calculated between immune cell counts of each two time points, namely day 3/day 1 (D3/D1) and day 7/day 3 (D7/D3). Patients were grouped based on ISS and the occurrence of nosocomial infection, sepsis or death. Comparative studies were conducted between each two groups. Univariate and multivariate logistic regression analysis were used to identify variables related to the risk of nosocomial infection, sepsis, and mortality. Receiver operating characteristic (ROC) curve was plotted to assess the predictive value of various risk factors.

Results: More severe trauma leads to more pronounced increase in ANC and more slowly recovery of ALC. In patients with subsequent nosocomial infection and sepsis, ANC was higher and ALC recovery was slower than those without nosocomial infection and sepsis within 7 days. In non-survivors, ALC had not recovered and AMC (D3) and AMC (D7) was lower than survivors within 7 days. ALC (D3) and ALC (D3/D1) are independent risk factors for nosocomial infection and sepsis. ALC (D3), ALC (D3/D1), ALC (D7) and AMC (D7) are independent risk factors for death. The combination of ALC (D3/D1) and ALC (D3) had a good predictive value for the occurrence of nosocomial infection, sepsis, and mortality.

Conclusions: More severe trauma causes more intense interference to circulating immunocyte counts. Worse alterations in circulating immunocyte counts within 7 days may increase the risk of subsequent nosocomial infection, sepsis and mortality.

Background

Trauma is a disease with high morbidity and mortality. More than five million people die of trauma

every year. It is also the primary cause of death in individuals under 40 years old [1, 2]. The main cause of early death at the scene or after admission is mainly severe brain damage or massive bleeding due to heart or large blood vessel injury. With the advancement of resuscitation strategies, damage control surgery and supportive care, many inpatients can survive the early stage. However, for these early survivors, delayed death due to sepsis and multiple organ dysfunction syndrome (MODS) is also frequent [3-6].

Extensive tissue injury and ischemia-induced release of damage-related molecular models (DAMP) after severe trauma leads to a tremendous inflammatory response (systemic inflammatory response syndrome, SIRS) and an extremely important anti-inflammatory response (compensatory anti-inflammatory response syndrome, CARS) occurs almost simultaneously [4, 7-10]. These early events are generally believed to disrupt the homeostasis of the immune system and are important causes of nosocomial infection, sepsis and MODS in the later stage [11-14]. And this disruption of homeostasis is quite complex, affecting the innate and adaptive arms of the immune system, leading to significant changes in the count, function and phenotype of the immune cells [4, 15, 16]. Nevertheless, if we continue to systematically deepen the study of these immune cell changes, we may also have the opportunity to find out general rules in the complexity. This in turn may help us to predict that some patients may have adverse complications or poor prognosis in advance, for which we can conduct early intervention. Moreover, the changes of these immune cells may also be the cause of adverse complications or poor prognosis, which may also help us to expand the idea of immunotherapy. Many studies have previously reported some changes in the post-traumatic immune system and the impact of these changes on prognosis. However, the following points may be worth further consideration: Simultaneously studying the response of innate and adaptive immune cells in the same group of patients to post-traumatic immune system status is more comprehensive than studying one type of immune cells alone; Considering that the immune system is dynamic, it is more persuasive to study the law of change in a period of time than to investigate the state of a time point; Although some of the predictive test indicators are very meaningful, they are not routinely used clinically, more easily available and repeatable indicators need to be introduced.

In this study, we collected a large number of target patients and used a common clinical test item, blood routine, to comprehensively study the alterations in the absolute counts of neutrophil, lymphocyte and monocyte (ANC, ALC and AMC) of severe trauma patients within 7 days. They cover most of the innate and adaptive immune cells. In addition, we evaluated the predictive value of these immune cell counts at certain time points and their changes over time for the risk of nosocomial infections, sepsis and death within 60 days.

Methods

Study design, setting and patients

This retrospective study was performed at a 7,000-bed university-affiliated hospital. Approval was obtained from the Medical Ethics Committee of the hospital with a waiver of informed consent. Patients admitted within 24 hours after trauma with an Injury Severity Score (ISS) ≥ 16 were enrolled in this study. The exclusion criteria were patients less than 18 years old; those with autoimmune or hypersensitivity diseases; neoplastic or proliferative hematologic diseases; severe systemic inflammation caused by other diseases (e.g. liver cirrhosis, myocardial infarction); concurrent chronic disease requiring treatment with any form of immunomodulation therapy. Besides, patients were also excluded if they had non-survivable injuries (ISS = 75) or died within 7 days. This is because our purpose was to investigate the relationship between the alterations of immunocyte counts within 7 days in the early survivors and the later emergence of nosocomial infections, sepsis and death. These patients are not suitable. Furthermore, to minimize the impact of surgery on the immune cell count results, we excluded patients who underwent surgery from after blood collection on day 1 to before blood collection on day 7.

Data collection

Demographic data, Acute Physiology and Chronic Health Evaluation (APACHE) II score, ISS, Glasgow coma scale (GCS), the mechanism of injury, days of ventilator use, co-morbidities, smoking or not, whether nosocomial infection, sepsis or death occurred within 60 days, and blood routine results (ANC, ALC and AMC) on days 1, 3, and 7 (D1, D3, and D7) were obtained from the clinical information systems. Patients with incomplete information were also withdrawn from this study.

In our study, nosocomial infection refers to bacteremia, respiratory system, urinary system, surgical site, or skin and soft tissue infection occurring after 3 days from admission. Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection; organ dysfunction can be identified as an acute change in total SOFA score ≥ 2 due to infection [17].

Statistical analysis

Continuous and categorical variables are reported as mean (SD), median (interquartile range [IQR]) and number (proportion) as appropriate. The Kolmogorov-Smirnov test was used to verify the normality of continuous data. Comparisons of three correlated samples were performed using Friedman`s rank test (for non-normally distributed data) followed by the Wilcoxon signed rank test. Comparisons of two independent samples were performed using Wilcoxon rank sum test (for non-normally distributed data), Mann-Whitney U test (for ordered categorical data) or chi-square tests (for unordered categorical data). Pearson correlation coefficient was used to measure the multicollinearity of continuous variables. Univariate and multivariate logistic regression analysis were used to identify variables related to the risk of nosocomial infection, sepsis, and mortality. Receiver operating characteristic (ROC) curve was plotted to calculate the area under the ROC curve and determine the cut-off value. Binary logistic regression model was used for the combination analysis of two indicators. Statistical analyses and graphics were developed using SPSS v20.0 (IBM, Armonk, NY), MedCalc v19.0.7 (MedCalc Software, Ostend, Belgium) and GraphPad Prism v8.3.0 (GraphPad Software, San Diego, CA). A p value less than 0.05 was considered significant.

Results

Patients

A total of 876 patients with an ISS of 24.0 (19.0, 29.0) were screened for inclusion into the present study. Table 1 shows baseline characteristics for the entire patient cohort. The incidence of nosocomial infection and sepsis was 54.3% (476 patients) and 27.2% (238 patients), respectively. The overall mortality rate was 18.4% (161 patients).

Table 1
Overall characteristics of trauma patients

Parameter	Patient (n = 876)
Age, mean (SD), year	49.8 (14.7)
Male, n (%)	194 (22.1)
APACHE II score, median (IQR)	11.0 (7.0, 15.0)
ISS, median (IQR)	24.0 (19.0, 29.0)
GCS, n (%)	
3-8	166 (18.9)
9-12	120 (13.7)
13-15	590 (67.4)
Mechanism of injury, n (%)	
Fall	336 (38.4)
Road traffic collision	328 (37.4)
Tumble	151 (17.2)
Assault	20 (2.3)
Other/unknown	41 (4.7)
Operated on admission, n (%)	187 (21.3)
Ventilator days, median (IQR)	0 (0, 4.0)
Co-morbidities, n (%)	
Hypertension	111 (12.7)
Coronary artery disease	17 (1.9)
Cerebrovascular disease	18 (2.1)
Diabetes mellitus	48 (5.5)
COPD	18 (2.1)
Smoking, n (%)	289 (33.0)
Nosocomial infection, n (%)	476 (54.3)
Sepsis, n (%)	238 (27.2)
60-day mortality, n (%)	161 (18.4)

Table shows the demographic and clinical data of the severe trauma patients involved in this study. APACHE II score = Acute Physiology and Chronic Health Evaluation II score; ISS = Injury Severity Score; GCS = Glasgow coma scale; ICU = intensive care unit; COPD = chronic obstructive pulmonary diseases.

Severity of trauma

According to ISS, we divided the patients into two groups: $16 \leq \text{ISS} \leq 24$ and $25 \leq \text{ISS} \leq 74$. The latter has higher APACHE II score, lower GCS and longer ventilator use time (Additional file 1: Table S1). ANC and AMC rise and ALC decline were common in severe trauma patients. The proportions of patients with immune cell counts above the upper limit of the normal range of ANC and AMC or below the lower limit of the normal range of ALC are shown on the x-axis (Fig. 1). Patients with higher ISS had higher ANC within 7 days. Although ANC of both groups showed a decrease on the 3rd day, it rebounded in patients with higher ISS on the 7th day (Fig. 1A). On the 1st day, there was no significant difference in ALC between the two groups. Then, ALC of the lower ISS group gradually increased from the 3rd day, while ALC of the higher ISS group began to rise on the 7th day. And ALC of the latter was lower than the former on the 3rd and 7th day (Fig. 1B). Besides, AMC increased in the higher ISS group on the 7th day, but there was no significant difference between the two groups (Fig. 1C). These results show that patients suffering from more severe trauma have worse alterations

in immune cell counts, especially ANC and ALC.

Nosocomial infection

According to whether nosocomial infection occurred, patients were divided into two groups: non-nosocomial infection and nosocomial infection. A total of 476 patients had nosocomial infection, with the lungs being the most common site, and Gram-negative bacteria being the most common pathogen (data not shown). Nosocomial infection group had higher ISS and APACHE II score, lower GCS and longer ventilator use time (Additional file 1: Table S1). The trends of differences in ANC and ALC between the non-nosocomial and nosocomial infection groups were similar to those between the lower and the higher ISS groups (Fig. 1A, 1B, 2A and 2B). The AMC of the nosocomial infection group increased on the 7th day, and was higher than the non-nosocomial infection group (Fig. 2C). Besides, given the wide fluctuation of the immune cell counts, ratios were calculated between their counts of each two time points, namely day 3/day 1 (D3/D1) and day 7/day 3 (D7/D3). ANC (D3/D1), ALC (D3/D1), ANC (D7/D3) and AMC (D7/D3) were different between the two groups (Fig. 2D). These results suggest that subsequent nosocomial infection may be associated with worse alterations of immune cell counts in the early stage.

Sepsis

According to whether sepsis occurred, we divided the patients into two groups: non-sepsis and sepsis. A total of 238 patients developed sepsis, with the lungs being the most common site of infection, and Gram-negative bacteria being the most common pathogen (data not shown). Sepsis patients were older, with higher ISS and APACHE II score, lower GCS and longer ventilator use time (Additional file 1: Table S1). The trends of differences in ANC and ALC between the non-sepsis and sepsis groups were similar to those between the lower and higher ISS groups or the non-nosocomial infection and nosocomial infection groups (Fig. 1A, 1B, 2A, 2B, 3A and 3B). On day 7, AMC of sepsis group increased and was higher than non-sepsis group (Fig. 3C). Besides, ANC (D3/D1), ALC (D3/D1) and ANC (D7/D3) was different between the two groups (Fig. 3D). These results indicate that worse alterations of immune cell counts may increase the risk of sepsis.

Mortality

According to whether death occurred within 60 days, patients were divided into two groups: alive and death. A total of 161 patients were included in the latter group, and they were older, with higher ISS and APACHE II score, lower GCS, and longer ventilator use time compared with the former group (Additional file 1: Table S1). ANC of both groups reduced on the 3rd day and rebounded on the 7th day, but the latter group was different from the former group in that the ANC increased to the same level as the 1st day on day 7. And there was a difference in ANC between the two groups on day 7 (Fig. 4A). There was no difference in ALC between the two groups on day 1, it gradually increased in the alive group but no significant change in the death group. As a result, ALC differed between the two groups on the 3rd and 7th days (Fig. 4B). Besides, AMC increased on the 7th day in the alive group and was higher than the death group on the 3rd and 7th days (Fig. 4C). Moreover, ALC (D3/D1), AMC (D3/D1) and AMC (D7/D3) were different between the two groups (Fig. 4D). These results suggest that worse alterations in immune cell populations may increase the risk of mortality.

We further divided the non-survivors into two groups according to the cause of death: sepsis and trauma. There were 96 (59.6%) patients died of sepsis and 65 (40.4%) patients died of trauma. There were no significant differences in age, gender, ISS, APACHE II score, and GCS between the two groups, as well as ANC, ALC, and AMC (Fig. 4E, 4F, 4G and Additional file 1: Table S1). These results imply that regardless of whether the patients eventually died of sepsis or trauma, they may suffer a similar degree and potentially fatal immune system insult in the early stage.

Risk factor analysis

To further determine the relationship between the immune cell counts and the occurrence of nosocomial infection, sepsis and mortality, we performed risk factor analysis. Since the median time of nosocomial infection was day 6 (data not shown) in our study, we did not analyze the effect of ANC (D7), ALC (D7) and AMC (D7) on nosocomial infection. The results of univariate and multivariate analysis for immune cell counts at fixed points in time and their slopes of each two time points are showed in Tables 2 and 3, respectively. This separate evaluation was to prevent collinearity. After adjustment for other confounding factors, ISS, APACHE II score, ALC (D3) and ALC (D3/D1) were significantly associated with nosocomial infection; ISS, APACHE II score, ANC (D7), ALC (D3), ANC

(D7/D3) and ALC (D3/D1) were significantly associated with sepsis; ISS, APACHE II score, GCS, ALC (D3), ALC (D7), AMC (D7) and ALC (D3/D1) were significantly associated with mortality.

Table 2

Univariate and multivariate logistic regression analysis including immune cell counts at fixed time points

Variable	Nosocomial infection				Sepsis				Mortality			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age	1.02 (1.00, 1.03)	0.027	1.01 (0.99, 1.03)	0.348	1.02 (1.00, 1.03)	0.023	1.00 (0.98, 1.02)	0.768	1.03 (1.01, 1.05)	0.001	0.99 (0.97, 1.02)	0.580
ISS	1.10 (1.06, 1.13)	< 0.001	1.04 (1.01, 1.09)	0.028	1.10 (1.07, 1.14)	< 0.001	1.05 (1.01, 1.09)	0.015	1.08 (1.05, 1.11)	< 0.001	1.05 (1.03, 1.08)	0.010
APACHE II	1.14 (1.10, 1.19)	< 0.001	1.10 (1.04, 1.17)	0.001	1.18 (1.13, 1.23)	< 0.001	1.18 (1.10, 1.26)	< 0.001	1.24 (1.18, 1.30)	< 0.001	1.28 (1.18, 1.38)	< 0.001
GCS	0.89 (0.83, 0.94)	< 0.001	1.05 (0.97, 1.13)	0.225	0.84 (0.79, 0.89)	< 0.001	1.07 (0.98, 1.16)	0.138	0.82 (0.77, 0.88)	< 0.001	1.09 (1.00, 1.18)	0.043
ANC (D1)	1.06 (0.01, 1.11)	0.021	1.01 (0.95, 1.07)	0.802	1.05 (1.01, 1.10)	0.029	0.99 (0.93, 1.05)	0.638	1.05 (1.00, 1.11)	0.157		
ANC (D3)	1.14 (1.08, 1.22)	< 0.001	1.02 (0.94, 1.11)	0.702	1.16 (1.09, 1.23)	< 0.001	1.08 (0.99, 1.18)	0.094	1.06 (1.01, 1.12)	0.034	1.05 (0.95, 1.17)	0.369
ANC (D7)					1.23 (1.16, 1.30)	< 0.001	1.17 (1.08, 1.26)	< 0.001	1.07 (1.03, 1.12)	0.003	1.07 (0.98, 1.16)	0.114
ALC (D1)	1.04 (0.65, 1.67)	0.879			0.95 (0.57, 1.59)	0.851			1.08 (0.64, 1.91)	0.806		
ALC (D3)	0.55 (0.37, 0.83)	0.004	0.48 (0.29, 0.79)	0.003	0.55 (0.34, 0.89)	0.015	0.47 (0.24, 0.91)	0.026	0.34 (0.18, 0.65)	0.001	0.38 (0.16, 0.89)	0.025
ALC (D7)					0.54 (0.35, 0.83)	0.005	0.60 (0.33, 1.08)	0.088	0.15 (0.08, 0.30)	< 0.001	0.27 (0.11, 0.63)	0.003
AMC (D1)	1.47 (0.86, 2.52)	0.258			1.36 (0.79, 2.33)	0.270			1.38 (0.75, 2.54)	0.308		
AMC (D3)	1.48 (0.84, 2.58)	0.272			1.29 (0.73, 2.26)	0.379			0.47 (0.22, 1.00)	0.049	1.32 (0.42, 4.13)	0.637
AMC (D7)					2.23 (1.28, 3.53)	0.003	1.73 (0.76, 3.94)	0.190	0.24 (0.11, 0.52)	< 0.001	0.21 (0.06, 0.68)	0.023

Table shows the results of variables related to nosocomial infections, sepsis, and mortality determined by univariate and multivariate logistic regression analysis. OR = odds ratio; CI = confidence interval; ISS = Injury Severity Score; APACHE II = Acute Physiology and Chronic Health Evaluation II score; GCS = Glasgow coma scale; ANC = absolute neutrophil count; ALC = absolute lymphocyte count; AMC = absolute monocyte count; D1 = day 1; D3 = day 3; D7 = day 7.

Table 3

Univariate and multivariate logistic regression analyses including slopes of immune cell counts over time

Variables	Nosocomial infection				Sepsis				Mortality			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age	1.02 (1.00, 1.03)	0.027	1.01 (0.99, 1.02)	0.460	1.02 (1.00, 1.03)	0.023	1.01 (0.99, 1.03)	0.344	1.03 (1.01, 1.05)	0.001	1.02 (1.00, 1.04)	0.122
ISS	1.10 (1.06, 1.13)	< 0.001	1.07 (1.03, 1.11)	< 0.001	1.10 (1.07, 1.14)	< 0.001	1.06 (1.03, 1.10)	0.001	1.08 (1.05, 1.11)	< 0.001	1.07 (1.04, 1.10)	0.013
APACHE II	1.14 (1.10, 1.19)	< 0.001	1.10 (1.05, 1.17)	< 0.001	1.18 (1.13, 1.23)	< 0.001	1.16 (1.09, 1.24)	< 0.001	1.24 (1.18, 1.30)	< 0.001	1.30 (1.20, 1.41)	< 0.001
GCS	0.89 (0.83, 0.94)	< 0.001	1.02 (0.95, 1.09)	0.610	0.84 (0.79, 0.89)	< 0.001	1.04 (0.96, 1.13)	0.377	0.82 (0.77, 0.88)	< 0.001	1.08 (1.00, 1.18)	0.044
ANC (D3/D1)	1.48 (0.99, 2.21)	0.158			1.52 (1.05, 2.19)	0.027	1.49 (0.94, 2.37)	0.089	1.18 (0.79, 1.76)	0.413		
ANC (D7/D3)					1.71 (1.24, 2.35)	0.001	1.63 (1.13, 2.33)	0.008	1.13 (0.80, 1.59)	0.479		
ALC (D3/D1)	0.70 (0.56, 0.89)	0.004	0.72 (0.56, 0.93)	0.010	0.70 (0.53, 0.94)	0.017	0.71 (0.55, 0.96)	0.046	0.69 (0.48, 0.97)	0.035	0.72 (0.51, 0.99)	0.049
ALC (D7/D3)					1.33 (1.06, 1.66)	0.014	1.16 (0.86, 1.55)	0.338	0.86 (0.66, 1.18)	0.404		
AMC (D3/D1)	1.03 (0.93, 1.13)	0.616			1.05 (0.95, 1.17)	0.316			1.06 (0.96, 1.16)	0.239		
AMC (D7/D3)					1.21 (1.02, 1.44)	0.029	1.09 (0.89, 1.34)	0.389	0.91 (0.72, 1.16)	0.447		

Table shows the results of variables related to nosocomial infections, sepsis, and mortality determined by univariate and multivariate logistic regression analysis. OR = odds ratio; CI = confidence interval; ISS = Injury Severity Score; APACHE II = Acute Physiology and Chronic Health Evaluation II score; GCS = Glasgow coma scale; ANC = absolute neutrophil count; ALC = absolute lymphocyte count; AMC = absolute monocyte count; D3/D1 = day 3/day 1; D7/D3 = day 7/day 3.

Predictive value

The neutrophil-lymphocyte ratio (NLR) has been widely proved to be a simple and reliable marker for

assessing systemic inflammation and prognosis in non-traumatic and traumatic patients [1, 18-23]. In our study, we validated the predicted value of NLR (D3) and NLR (D7) using ROC curve given their differences between each two groups (Fig. 5). Since the median time of nosocomial infection was day 6 (data not shown) in our study, which may be the cause of the rise of ANC (D7), we did not analyze the effect of NLR (D7) on nosocomial infection. The results show that NLR (D7) has a larger area under the ROC curve (AUC) in predicting the risk of sepsis and mortality than NLR (D3) (Table 4). Then, we selected NLR which has a larger AUC and the independent risk factors of nosocomial infection, sepsis and mortality (Tables 2 and 3) for comparison. As depicted in Fig. 6A, 6B, 6C and Table 5, APACHE II score has the best predicted value for the risk of nosocomial infection, sepsis and mortality (AUC: 0.71, 0.80 and 0.87, respectively). ISS for nosocomial infection and NLR (D7) for sepsis and mortality were the next best (AUC: 0.69, 0.76 and 0.77, respectively).

Table 4
Predictive value of neutrophil-lymphocyte ratio for nosocomial infection, sepsis and mortality

	AUC	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	p
Nosocomial infection							
NLR (D3)	0.66 (0.61, 0.71)	> 6.74	72.44 (66.1, 78.2)	52.98 (44.7, 61.1)	69.7 (65.6, 73.5)	56.3 (49.9, 62.6)	< 0.001
Sepsis							
NLR (D3)	0.72 (0.67, 0.76)	> 8.57	78.45 (69.9, 85.5)	59.92 (53.7, 65.9)	46.4 (42.1, 50.8)	86.3 (81.4, 90.0)	< 0.001
NLR (D7)	0.76 (0.71, 0.80)	> 7.34	81.03 (72.7, 87.7)	61.30 (55.1, 67.2)	48.2 (43.8, 52.6)	87.9 (83.1, 91.5)	< 0.001
Mortality							
NLR (D3)	0.69 (0.64, 0.73)	> 8.01	83.33 (72.7, 91.1)	50.98 (45.2, 56.7)	28.6 (25.5, 31.8)	92.9 (88.5, 95.7)	< 0.001
NLR (D7)	0.77 (0.72, 0.81)	> 14.23	56.94 (44.7, 68.6)	86.23 (81.8, 89.9)	49.4 (40.9, 58.0)	89.5 (86.6, 91.7)	< 0.001

AUC = area under the ROC; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value; NLR = neutrophil-lymphocyte ratio; D3 = day 3; D7 = day 7.

Table 5

Predictive value of various indexes for nosocomial infection, sepsis and mortality

	AUC	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	p
Nosocomial infection							
ISS	0.69 (0.65, 0.73)	> 22	67.65 (61.3, 73.5)	63.00 (55.9, 69.7)	68.5 (64.0, 72.7)	62.1 (57.0, 66.9)	< 0.001
APACHE II	0.71 (0.67, 0.75)	> 11	61.76 (55.3, 68.0)	73.00 (66.3, 79.0)	73.1 (68.0, 77.7)	61.6 (57.2, 65.8)	< 0.001
ALC (D3)	0.62 (0.57, 0.68)	≤ 0.83	67.76 (59.7, 75.1)	53.54 (46.8, 60.2)	49.5 (45.1, 54.0)	71.2 (65.6, 76.2)	< 0.001
ALC (D3/D1)	0.68 (0.64, 0.73)	≤ 1.36	79.65 (73.8, 84.7)	46.05 (37.9, 54.3)	68.7 (65.1, 72.1)	60.3 (52.7, 67.5)	< 0.001
NLR (D3)	0.66 (0.61, 0.71)	> 6.74	72.44 (66.1, 78.2)	52.98 (44.7, 61.1)	69.7 (65.6, 73.5)	56.3 (49.9, 62.6)	< 0.001
Sepsis							
ISS	0.71 (0.67, 0.75)	> 27	55.46 (46.1, 64.60)	77.12 (72.1, 81.6)	47.5 (41.1, 53.9)	82.3 (79.0, 85.10)	< 0.001
APACHE II	0.80 (0.76, 0.84)	> 11	81.90 (73.7, 88.4)	65.31 (59.8, 70.5)	46.1 (41.9, 50.4)	90.9 (87.0, 93.7)	< 0.001
ANC (D7)	0.75 (0.70, 0.79)	> 11.27	58.62 (49.1, 67.7)	83.59 (78.5, 87.9)	61.3 (53.6, 68.4)	82.0 (78.5, 85.1)	< 0.001
ALC (D3)	0.64 (0.59, 0.69)	≤ 1.02	80.17 (71.7, 87.0)	46.18 (40.0, 52.4)	39.7 (36.3, 43.2)	84.0 (78.1, 88.6)	< 0.001
ANC (D7/D3)	0.71 (0.57, 0.67)	> 1.13	92.24 (85.8, 96.4)	44.66 (38.5, 50.9)	42.5 (39.5, 45.4)	92.9 (87.2, 96.1)	< 0.001
ALC (D3/D1)	0.75 (0.70, 0.79)	≤ 0.97	74.14 (65.2, 81.8)	64.5 (58.4, 70.3)	48.0 (43.2, 52.9)	84.9 (80.3, 88.6)	< 0.001
NLR (D7)	0.76 (0.71, 0.80)	> 7.34	81.03 (72.7, 87.7)	61.30 (55.1, 67.2)	48.2 (43.8, 52.6)	87.9 (83.1, 91.5)	< 0.001
Mortality							
ISS	0.70 (0.63, 0.76)	> 23	80.77 (70.3, 88.8)	52.50 (47.2, 57.8)	26.9 (24.0, 30.0)	92.6 (88.8, 95.3)	< 0.001
APACHE II	0.87 (0.83, 0.91)	> 14	80.52 (69.9, 88.7)	83.39 (78.7, 87.4)	55.4 (48.5, 62.0)	94.4 (91.4, 96.4)	< 0.001
GCS	0.70 (0.65, 0.75)	≤ 13	67.57 (55.7, 78.0)	63.7 (58.0, 69.1)	31.3 (26.8, 36.1)	88.9 (85.1, 91.9)	< 0.001
ALC (D3)	0.68 (0.63, 0.75)	≤ 0.82	70.83 (58.9, 81.0)	61.11 (55.4, 66.6)	30.0 (25.9, 34.5)	89.9 (86.0, 92.8)	< 0.001
ALC (D7)	0.75 (0.71, 0.80)	≤ 0.93	75.00 (63.4, 84.5)	70.59 (65.1, 75.6)	37.5 (32.5, 42.8)	92.3 (88.9, 94.7)	< 0.001
AMC (D7)	0.65 (0.60, 0.70)	≤ 0.59	43.06 (31.4, 55.3)	81.70 (76.9, 85.9)	35.6 (27.9, 44.1)	85.9 (83.2, 88.2)	< 0.001
ALC (D3/D1)	0.76 (0.71, 0.80)	≤ 0.91	79.17 (68.0, 87.8)	61.11 (55.4, 66.6)	32.4 (28.5, 36.5)	92.6 (88.7, 95.2)	< 0.001
NLR (D7)	0.77 (0.72, 0.81)	> 14.23	56.94 (44.7, 68.6)	86.23 (81.8, 89.9)	49.4 (40.9, 58.0)	89.5 (86.6, 91.7)	< 0.001

AUC = area under the ROC; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value; ISS = Injury Severity Score; APACHE II = Acute Physiology and Chronic Health Evaluation II score; GCS = Glasgow coma scale; ANC = absolute neutrophil count; ALC = absolute lymphocyte count; AMC = absolute monocyte count; NLR = neutrophil-lymphocyte ratio; D3 = day 3; D7 = day 7; D3/D1 = day 3/day 1; D7/D3 = day 7/day 3.

Considering that the independent assessment of immune cell counts at fixed time points or their slopes of two time points is one-sided, the combination of them was assessed using ROC curve. The results are shown in Fig. 6 and Table 6. For nosocomial infection, the combination of ALC (D3/D1) and ALC (D3) had better predicted value than APACHE II score (AUC: 0.73 VS 0.71). For sepsis, the predictive value of the combination of ALC (D3/D1) and ALC (D3) was slightly better than combination of ANC (D7/D3) and ANC (D7) (AUC: 0.81 VS 0.80), and also slightly better than APACHE II score (AUC:

0.81 VS 0.80). For mortality, the combination of ALC (D3/D1) and ALC (D3) provided a better predicted value than the other two combinations (AUC: 0.84 VS 0.65 and 0.60). Although its predicted value was lower than APACHE II score (AUC: 0.84 VS 0.87), it was higher than all other predictors.

Table 6

Combination of immune cell counts and their slope for predicting nosocomial infection, sepsis and mortality

	AUC (95% CI)	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	p
Nosocomial infection							
ALC (D3/D1) + ALC (D3)	0.73 (0.69, 0.78)	> 0.70	66.37 (59.8, 72.5)	68.42 (60.4, 75.7)	75.8 (70.8, 80.1)	57.8 (52.5, 62.9)	< 0.001
Sepsis							
ANC (D7/D3) + ANC (D7)	0.80 (0.76, 0.85)	> 0.70	78.24 (72.8, 83.1)	71.55 (62.4, 79.5)	86.1 (82.2, 89.3)	59.3 (53.0, 65.3)	< 0.001
ALC (D3/D1) + ALC (D3)	0.81 (0.75, 0.84)	> 0.66	72.9 (67.1, 78.2)	74.14 (65.2, 81.8)	86.4 (82.3, 89.7)	54.8 (49.1, 60.3)	< 0.001
Mortality							
ALC (D3/D1) + ALC (D3)	0.84 (0.75, 0.84)	> 0.24	76.39 (64.9, 85.6)	78.76 (73.7, 83.2)	45.8 (39.7, 52.1)	93.4 (90.3, 95.6)	< 0.001
AMC (D3/D1) + AMC (D3)	0.60 (0.54, 0.65)	> 0.23	29.17 (19.0, 41.1)	90.2 (86.3, 93.3)	41.2 (29.9, 53.5)	84.4 (82.3, 86.3)	0.022
AMC (D7/D3) + AMC (D7)	0.65 (0.60, 0.70)	> 0.24	47.89 (35.9, 60.1)	79.08 (74.1, 83.5)	34.7 (27.7, 42.4)	86.7 (83.9, 89.2)	< 0.001

Table shows the predictive values of the combination of immune cell counts at time point and their slope of each two time points for predicting the risk of nosocomial infection, sepsis and mortality. AUC = area under the ROC; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value; ANC = absolute neutrophil count; ALC = absolute lymphocyte count; AMC = absolute monocyte count; D3 = day 3; D7 = day 7; D3/D1 = day 3/day 1; D7/D3 = day 7/day 3.

Discussion

In this study, we comprehensively investigated the alterations of circulating ANC, ALC and AMC within 7 days in severe trauma patients and assessed the significance of these alterations. To our knowledge, this is the first time to analyze the relationship between the combination (immunocyte counts at time point and their changing trend with time) and prognosis in severe trauma patients. Based on the ISS, and whether nosocomial infection, sepsis or death occurred, patients were divided into $16 \leq \text{ISS} \leq 24$ and $25 \leq \text{ISS} \leq 74$ groups, non-nosocomial infection and nosocomial infection groups, non-sepsis and sepsis groups, and alive and death groups, respectively. Then, comparative studies were conducted between each two groups. Collectively, our data suggest that more severe damage can lead to more severe immune system interference, and the worsening alterations in circulating immune cell counts within 7 days are associated with the later occurrence of nosocomial infections, sepsis and death. In addition, the combination of immunocyte counts at time point and

their slope over time could provide good predictive value for the risk of nosocomial infection, sepsis and mortality.

Neutrophils are on the front line of the body's defense against pathogenic microorganisms. They have a strong phagocytosis of pathogens. However, they can also cause damage to blood vessels and surrounding tissues by releasing lysosomal enzymes. An uncontrolled innate immune system response may make sepsis and organ failure more likely to occur [1, 21, 24]. In our study, we found that patients with more severe trauma had higher ANC within 7 days. Patients with subsequent nosocomial infection and sepsis also had higher ANC within 7 days. According to the results of slope, ANC of patients with subsequent nosocomial infection and sepsis decreased less on the 3rd day, and rebounded significantly on the 7th day. Although the rise in ANC on day 7 may be attributed to the appearance of nosocomial infections on day 6, these results are sufficient to show that more severe trauma can lead to more severe deterioration of the innate immune system in addition to more severe damage to the body's organs. This in turn may exacerbate organ damage. The results of multivariate analysis showed that ANC (D7) and ANC (D7/D3) were independent risk factors for sepsis, however, this may be only the manifestation of nosocomial infection. Therefore, it cannot be concluded that higher ANC will increase the susceptibility to nosocomial infection and sepsis. Moreover, ANC (D7) of the death group was higher than the alive group, but multivariate analysis showed that it was not an independent risk factor for death. Nevertheless, it should be noted that the elevation of ANC is only one aspect of neutrophil abnormalities (e.g. neutrophil function is also impaired) [4, 25–27]. The overall abnormal response of neutrophils may still lead to immunosuppression, which increases the risk of adverse complications and poor prognosis [5]. Lymphocytes play a central role in the anti-infective immune response due to their ability to interact with cells of the innate immune system and other cells of adaptive immunity. They are not only passive bystanders, but also play a key role in the proper regulation of the inflammatory response [28, 29]. In contrast to the increase of ANC after trauma, ALC decreased, which has been widely reported [8, 30, 31]. Post-traumatic lymphopenia was also common in our study. More severe trauma did not initially lead to more severe lymphocyte depletion, but resulted in more slowly recovery of

ALC. So that patients with higher ISS on days 3 and 7 had lower ALC. This changing trend of ALC was also applicable to patients with subsequent nosocomial infection and sepsis. Moreover, for patients with subsequent nosocomial infections and sepsis, ALC showed a tendency to recover on the 7th day, but for patients with subsequent death, ALC had no tendency to recover at all within 7 days.

According to the results of slope, ALC did not recover or even decline on the 3rd day in patients with subsequent nosocomial infection, sepsis or death. In addition, multivariate analysis showed that ALC (D3) and ALC (D3/D1) were independent risk factors for nosocomial infection, sepsis and mortality, and ALC (D7) was also an independent risk factor for mortality. These results are similar to previous findings in critically ill patients such as trauma and sepsis, the extent and delayed recovery of lymphopenia have been widely reported to be related to nosocomial infection, sepsis and death [28, 30, 32–35]. Taking together, the importance of immediate recovery of ALC for prognosis is obvious.

Monocytes are the precursors of macrophages, which can phagocytize pathogens, mediate and promote inflammatory responses, process and present antigens, and participate in the regulation of adaptive immune responses. Monocyte dysfunctions, such as reduced HLA-DR expression and decreased ability to release inflammatory factors, have been widely investigated in critical diseases such as severe trauma and sepsis [4, 34, 36–41]. However, there are few reports of changes in their counts. In our study, the changes in AMC were not as extensive as those in ANC and ALC. They did not differ between the lower and higher ISS groups. In patients with subsequent nosocomial infection and sepsis, they rose on day 7 and were higher than those of non-nosocomial infection and non-sepsis patients. The overall rise of AMC after trauma may be due to endogenous stimulation (damaged tissue), but this scaling new heights on day 7 may be caused by new exogenous stimulation (microbial infection). That is to say, this scaling new heights may be just a sign of nosocomial infection on day 6. Interestingly, when comparing the alive group with the death group, we found that AMC was higher in survivors than those in non-survivors on days 3 and 7. This result is similar to the findings of two recent studies: survivors have significantly higher AMC than non-survivors in critically ill patients such as injured and septic shock patients [42, 43]. Furthermore, according to the results of slope, the change trend of AMC in the alive and death groups within 7 days is opposite, rising in the

alive group and falling in the death group. In addition, multivariate analysis showed that AMC (D7) is an independent risk factor for mortality. Considering the important role of monocytes and the fact that some of the existing monocytes may have functional impairment, the advantage of ensuring total number may mitigate the adverse outcome caused by dysfunction.

When analyzing the predictive value of various risk factors for nosocomial infection, sepsis and death, we innovatively combined immune cell counts at fixed time point with their slope over time. The results show that the combination provides better predictive value than alone. The combination of ALC (D3/D1) and ALC (D3) was the best predictor of nosocomial infection and sepsis, and APACHE II score was the best predictor of mortality. It is worth noting that although the predictive value of the combination of ALC (D3/D1) and ALC (D3) for mortality is not as good as APACHE II score, their predictive value is also quite high (AUC: 0.84). Therefore, the combination of ALC (D3/D1) and ALC (D3) can be regarded as an important predictive tool for post-traumatic adverse complications or poor prognosis.

This study has several limitations. Firstly, this is a single-center retrospective study. The nature of this study may lead to unintentional bias in patient selection. Besides, blood transfusion can affect the function of immune system [44–46], but the interference of blood transfusion is not excluded in our study. Finally, although we excluded patients who underwent surgery from after blood collection on day 1 to before blood collection on day 7, emergency surgery performed at admission and surgery performed 7 days after admission may also have an impact on the results. For example, emergency surgery on admission may cause additional interference to the immune system, and it is also uncertain whether the surgery 7 days after admission is related to nosocomial infection, sepsis and death. These limitations indicate that our conclusions need to be verified in the future by large prospective multi-center research with rigorous design.

Conclusions

First, more severe injuries result in more pronounced ANC elevations and more slowly ALC recovery within 7 days. The secondary injury caused by high ANC and immunosuppression caused by low ALC may be related to subsequent adverse events. Second, ALC (D3) and ALC (D3/D1) are independent

risk factors for nosocomial infection and sepsis, and ALC (D3), ALC (D3/D1), ALC (D7) and AMC (D7) are independent risk factors for death. Third, the combination of ALC (D3/D1) and ALC (D3) may be used as a valuable predictor of nosocomial infection, sepsis, and mortality in the later post-traumatic stage. Last and outlook, immunization interventions such as clearing excessive dysfunctional ANC, recovering ALC as soon as possible and supplementing AMC with normal function may help to improve the prognosis.

Abbreviations

ANC: absolute neutrophil count; ALC: absolute lymphocyte count; AMC: absolute monocyte count; D1: day 1; D3: day 3; D7: day 7; D3/D1: day 3/day 1; D7/D3: day 7/day 3; ROC curve: Receiver operating characteristic curve; AUC: area under the ROC curve; MODS: multiple organ dysfunction syndrome; DAMP: damage-related molecular models; SIRS: systemic inflammatory response syndrome; CARS: compensatory anti-inflammatory response syndrome; APACHE II score: Acute Physiology and Chronic Health Evaluation II score; ISS: Injury Severity Score; GCS: Glasgow coma scale; COPD: chronic obstructive pulmonary diseases; IQR: interquartile range; OR: odds ratio; CI: confidence interval; PPV = positive predictive value; NPV = negative predictive value.

Declarations

Ethics approval and consent to participate: Approval was obtained from the Medical Ethics Committee of the hospital with a waiver of informed consent.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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Authors' contributions: XJD and CTW were responsible for data acquisition, statistical assistance and data analysis. XHL were responsible for manuscript drafting and revision. XJD and ZFL were responsible for concept and design, data analysis, manuscript drafting and revision. XJD, CTW XHL, XJB and ZFL discussed the results. All authors read and approved the final manuscript.

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Figures

Figure 1

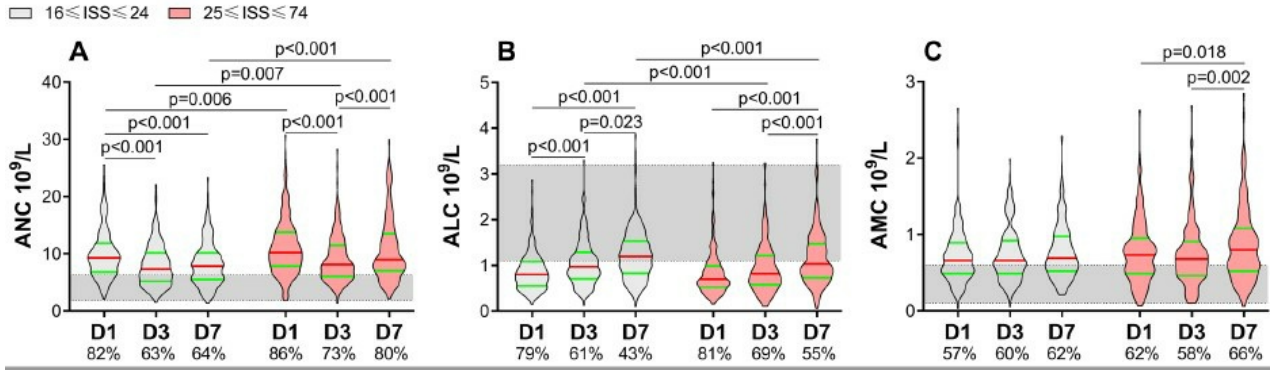


Figure 1

Patients suffering from more severe trauma have worse alterations in circulating immune cell counts. ANC (absolute neutrophil count) and AMC (absolute monocyte count) rise and ALC (absolute lymphocyte count) decline are common. The proportions of patients with ANC and AMC above the upper limit of the normal range (shaded area) are shown on the x-axis (A and C). The proportions of patients with ALC below the lower limit of the normal range (shaded area) are shown on the x-axis (B). ISS = Injury Severity Score; D1 = day 1; D3 = day 3; D7 = day 7.

Figure 2

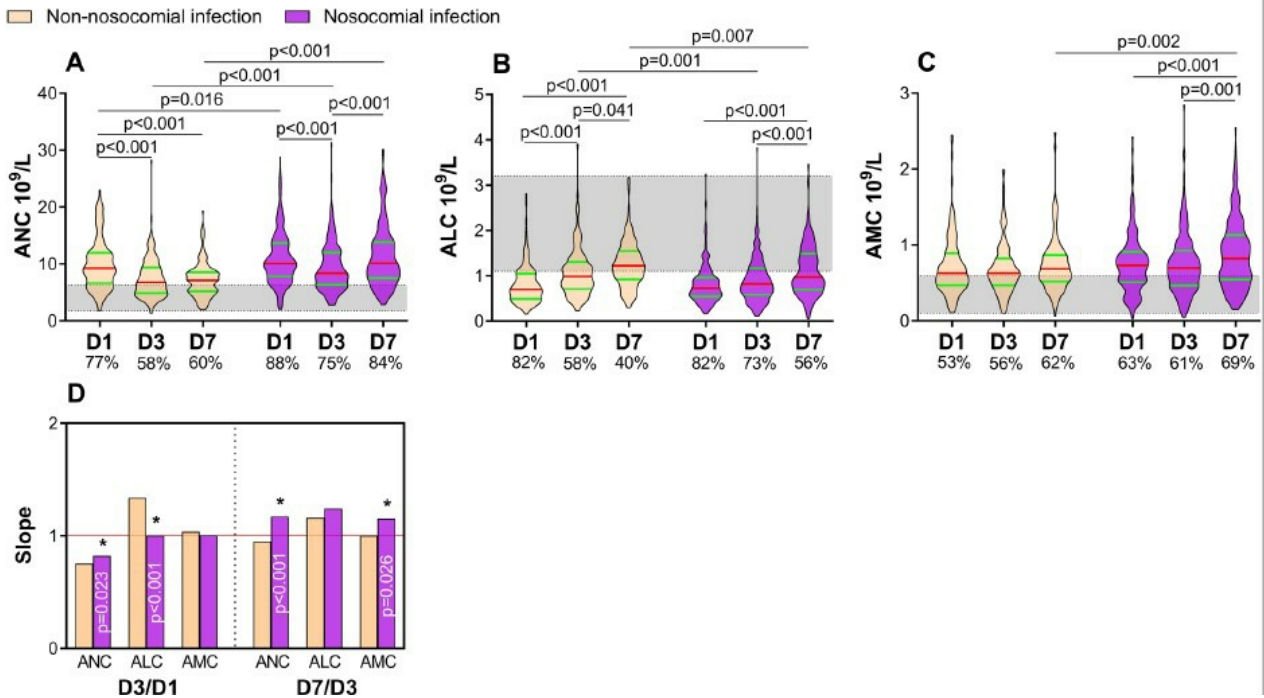


Figure 2

Patients who later developed nosocomial infection have worse alterations of circulating immune cell counts in the early stage. The proportions of patients with ANC (absolute neutrophil count) and AMC (absolute monocyte count) above the upper limit of the normal range (shaded area) are shown on the x-axis (A and C). The proportions of patients with ALC (absolute lymphocyte count) below the lower limit of the normal range (shaded area) are shown on the x-axis (B). A value of slope greater than 1 (red line) indicates an upward trend in immune cell counts, and a value less than 1 indicates a downward trend; * Represents a statistical difference between non-nosocomial infection and nosocomial infection groups, and the corresponding p-value is shown on the column (D). D1 = day 1; D3 = day 3; D7 = day 7.

Figure 3

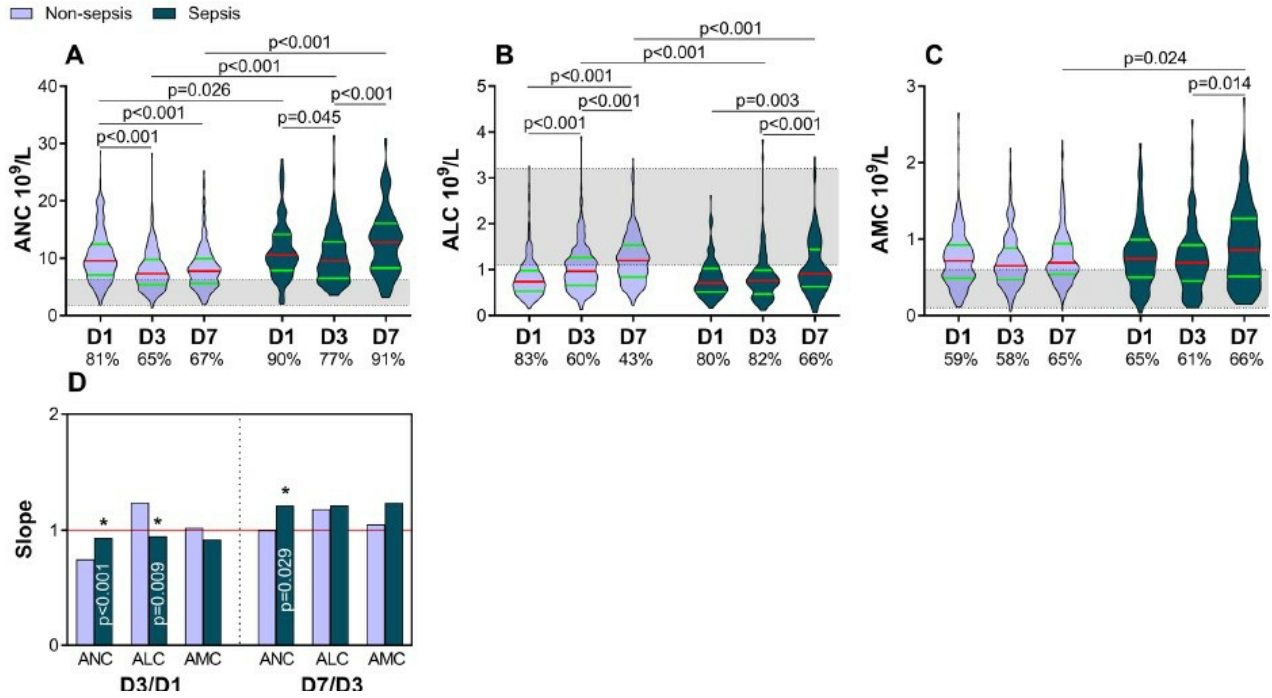


Figure 3

Patients who later developed sepsis have worse alterations of circulating immune cell counts in the early stage. The proportions of patients with ANC (absolute neutrophil count) and AMC (absolute monocyte count) above the upper limit of the normal range (shaded area) are shown on the x-axis (A and C). The proportions of patients with ALC (absolute lymphocyte count) below the lower limit of the normal range (shaded area) are shown on the x-axis (B). A value of slope greater than 1 (red line) indicates an upward trend in immune cell counts, and a value less than 1 indicates a downward trend; * Represents a statistical difference between non-sepsis and sepsis groups, and the corresponding p-value is shown on the column (D). D1 = day 1; D3 = day 3; D7 = day 7.

Figure 4

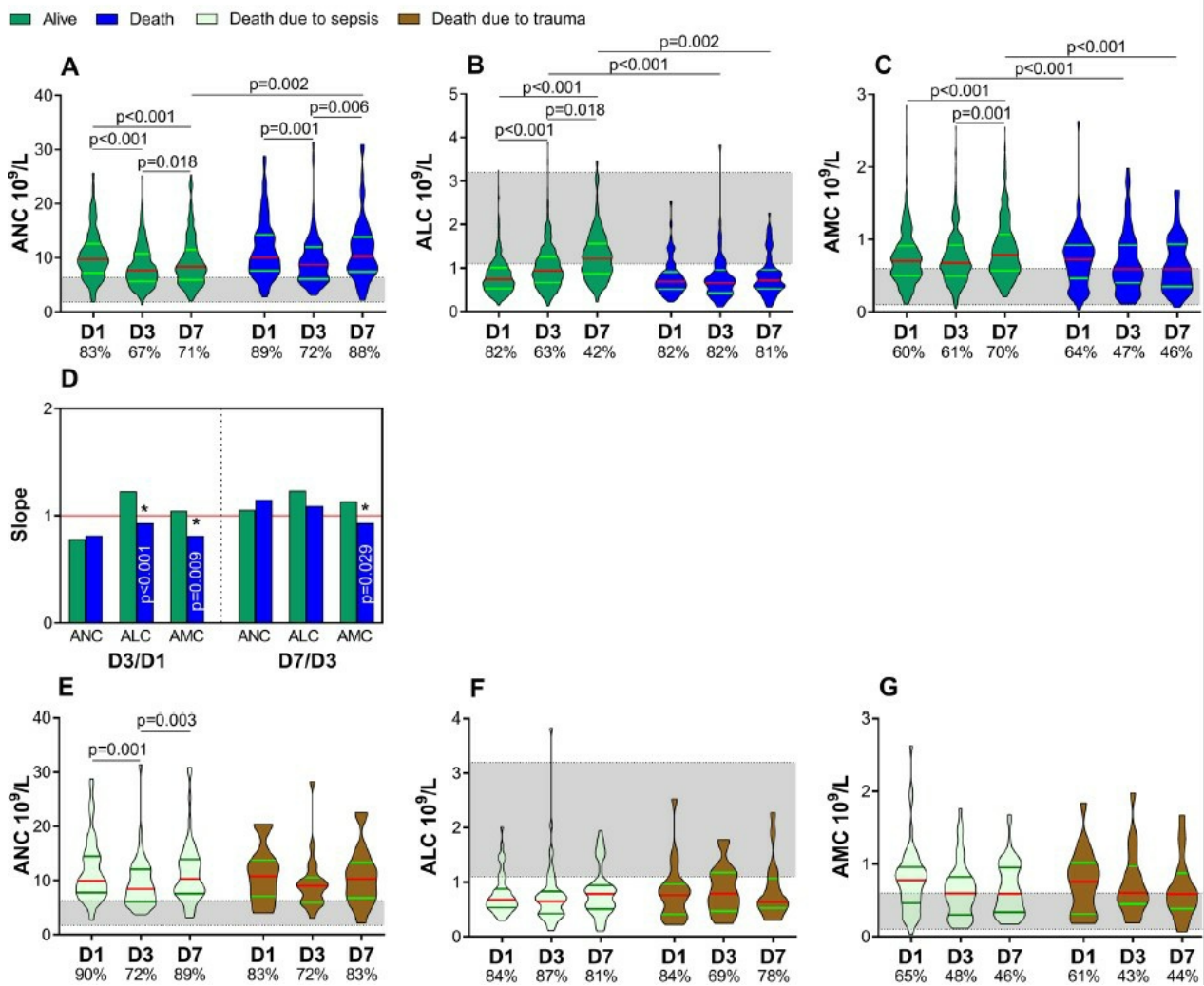


Figure 4

Early circulating immune cell counts show worse alterations in non-survivors, but there is no difference between non-survivors who died of sepsis and trauma. The proportions of patients with ANC (absolute neutrophil count) and AMC (absolute monocyte count) above the upper limit of the normal range (shaded area) are shown on the x-axis (A, C, E and G). The proportions of patients with ALC (absolute lymphocyte count) below the lower limit of the normal range (shaded area) are shown on the x-axis (B and F). A value of slope greater than 1 (red line) indicates an upward trend in immune cell counts, and a value less than 1 indicates a downward trend; * Represents a statistical difference between alive and death groups, and the corresponding p-value is shown on the column (D). D1 = day 1; D3 = day 3; D7 = day 7.

Figure 5

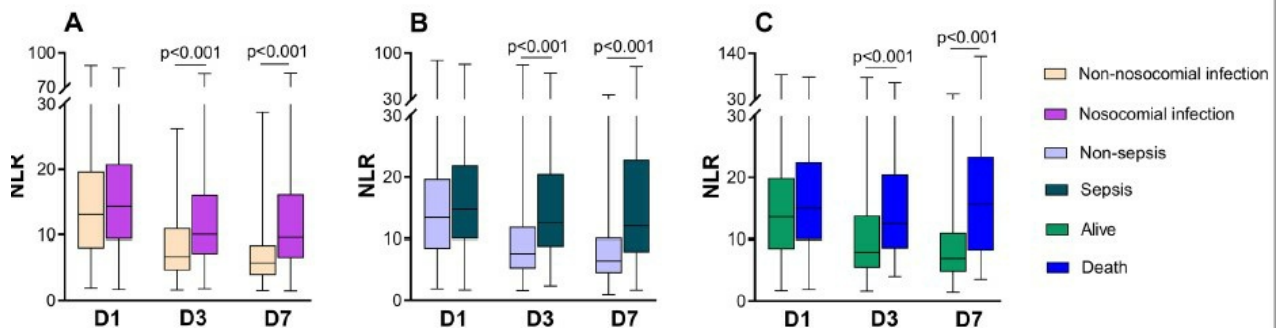


Figure 5

Differences in NLR (neutrophil-lymphocyte ratio) were compared between non-nosocomial infection and nosocomial infection groups (A), non-sepsis and sepsis groups (B), and alive and death groups (C) on day 1 (D1), day 3 (D3) and day 7 (D7).

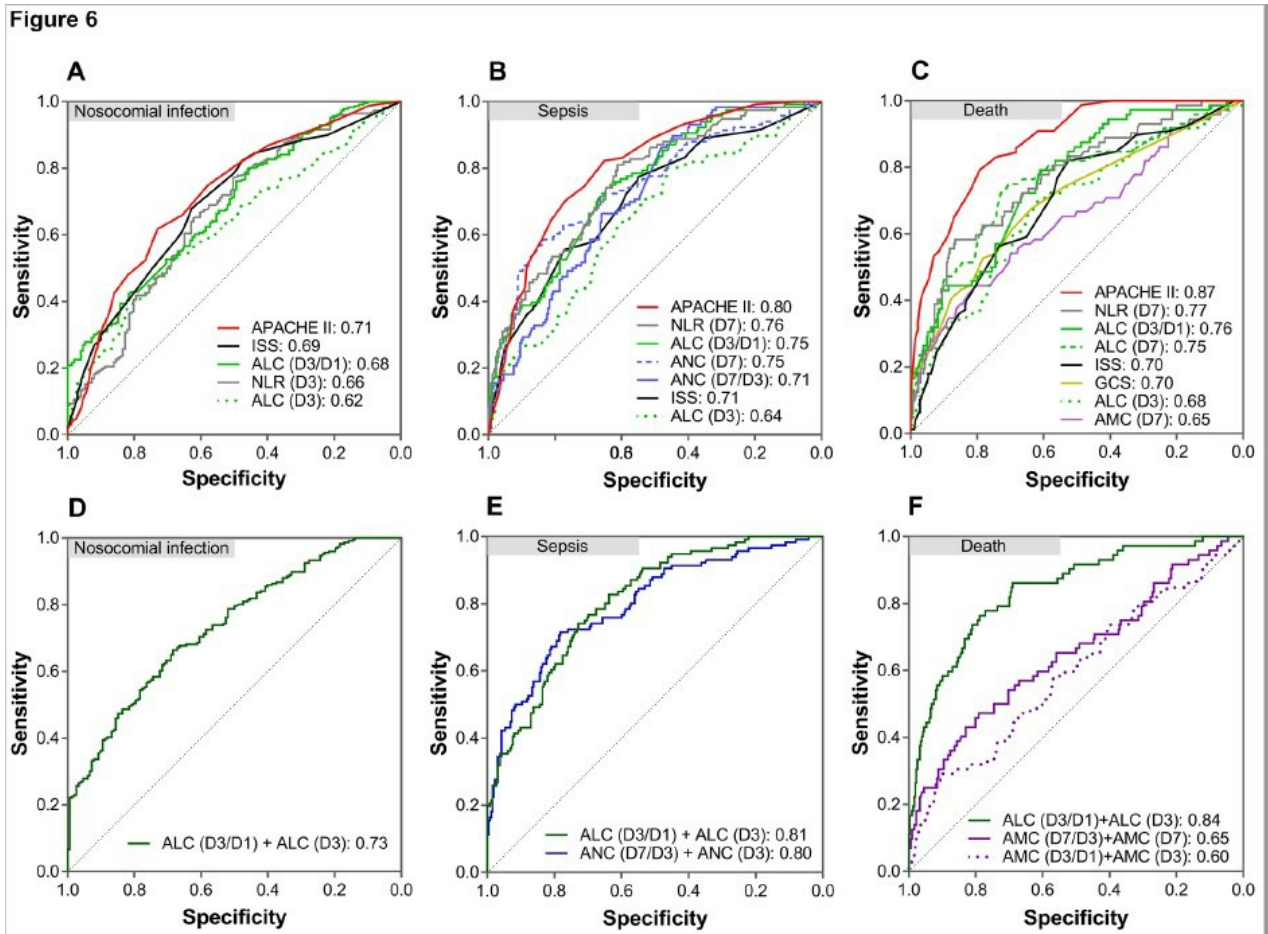


Figure 6

Receiver operating characteristic (ROC) curve analysis to compare the values of indicators in predicting the risk of nosocomial infection, sepsis and mortality. The area under the ROC curve of each indicator is presented in the figure. APACHE II = Acute Physiology and Chronic Health Evaluation II score; ISS = Injury Severity Score; GCS = Glasgow coma scale; ANC = absolute neutrophil count; ALC = absolute lymphocyte count; AMC = absolute monocyte count; D1 = day 1; D3 = day 3; D7 = day 7; D3/D1 = day 3/day 1; D7/D3 = day 7/day 3.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Additional file 1.xlsx