

Prognostic factors for ARDS: clinical, physiological and atypical immunodeficiency

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Abstract

Background: The risk factors affecting the prognosis of acute respiratory distress syndrome (ARDS) in adults were investigated. The aim was to identify new predictors of prognosis in ARDS patients, including those with clinical, pathophysiological, and atypical immunodeficiency. **Methods:** ARDS patients were retrospectively included. Patients were grouped and analysed according to different oxygenation index grades and prognosis, and the factors influencing prognosis and survival were analysed. Adolescent patients, patients with typical immunodeficiency and patients who died within 24 hours after being diagnosed with ARDS were excluded. The predictive value for mortality was determined by Cox proportional hazard analysis. **Results:** In total, 201 patients who fulfilled the Berlin definition of ARDS were included. The severity of critical illness on the day of enrolment, as measured by the Acute Physiology and Chronic Health Evaluation (APACHE) II score ($P < 0.05$), Sequential Organ Failure Assessment (SOFA) score ($P < 0.05$), and $\text{PaO}_2 / \text{FiO}_2$ ($P < 0.001$), worsened from mild to severe ARDS. Compared with survivors, non-survivors had significantly older age and higher APACHE II and SOFA scores. Moreover, significantly lower lymphocyte/neutrophil ratios and leukocyte counts were found in non-survivors than in survivors ($P < 0.01$, $P < 0.05$). Furthermore, there was a moderate positive correlation between the lymphocyte/neutrophil and $\text{PaO}_2 / \text{FiO}_2$ ratios ($P < 0.05$). The area under the curve (AUC) for the lymphocyte/neutrophil ratio was significantly greater than those for the body mass index (BMI) alone, $\text{PaO}_2 / \text{FiO}_2$ ratio alone, and lymphocyte/neutrophil ratio alone for predicting 100-day survival in patients with ARDS ($P = 0.0062$, 0.0001 , and 0.0154). Age (per log 10 years), $\text{BMI} < 24$, the SOFA score, leukocyte count, and the lymphocyte/neutrophil ratio were independent predictors of 28-day mortality in ARDS patients. Moreover, ARDS patients with a lymphocyte/neutrophil ratio < 0.0537 had elevated 28-day mortality ($P < 0.05$). Old age affected both 28-day and 100-day mortality ($P < 0.05$). **Conclusions:** Age (per log 10 years), $\text{BMI} < 24$, SOFA score, lymphocytes, and the lymphocyte/neutrophil ratio were independent predictors of 100-day mortality in patients with ARDS. The lymphocyte/neutrophil ratio may represent a potential molecular marker to evaluate atypical immunosuppression or impairment in patients with ARDS.

Background

Acute respiratory distress syndrome (ARDS) is a life-threatening respiratory disease with a high mortality rate in critically ill patients [1,2]. Although many in-depth studies on ARDS have been conducted, the specific pathogenesis and prognostic factors of ARDS are not very clear. Despite improvements in ventilatory techniques and the extensive research undertaken, ARDS continues to be associated with high mortality [3,4].

However, clinical and animal studies have shown that the activation of multiple inflammatory cells and the release of inflammatory mediators play important roles in the development and outcome of ARDS [5]. The involvement of immune cells, including neutrophils [6,7] and lymphocytes [8], has become an active topic of research in the pathogenesis of ARDS. Bronchoalveolar lavage fluid (BALF) from patients with ARDS is highly chemotactic for human neutrophils [7]. Neutrophil numbers in BALF correlated with acute

lung injury (ALI) severity have been shown to be predictive of mortality in human and animal studies [8,9]. Some evidence shows that ARDS could be mediated by regulatory T-cell-mediated suppressive mechanisms [10].

Currently, few clinical studies on the immune status of ARDS patients have focused on the aetiology, treatment and prognosis [11]. Some studies have shown that ARDS occurs in patients with previous immunodeficiencies, such as haematologic malignancies, active solid tumours, solid organ transplantation, acquired immunodeficiency syndrome, patients taking long-term or high-dose corticosteroids or immunosuppressants, and those who use extra-corporeal membrane oxygenation (ECMO) may achieve a better prognosis [12]. However, atypical causes of immunosuppression may be relatively more common in patients with ARDS, and atypical immune impairment caused by viral infection may be an important factor affecting the prognosis in patients with ARDS. In particular, a clear reduction in the number of circulating lymphocytes was evident in this group when compared to the group of patients discharged from the intensive care unit (ICU). The reduction in lymphocytes involves all lymphocyte populations: T (CD3+), B (CD19+) and NK cells (CD3CD16/56+) [13]. A high neutrophil-to-lymphocyte ratio (NLR) was associated with poor outcomes in critically ill patients with ARDS [14,15]. Cumulative survival rates at the 28-day follow-up also differed significantly between ARDS patients with different Th17/Treg ratios, and the Th17/Treg imbalance was a potential novel risk indicator in patients with early ARDS [16]. ARDS patients with resolved inflammation who survived the syndrome had significantly higher levels of Tr1 cells than ARDS patients who died [17]. Tfr cells were strongly enriched in ARDS patients, especially in the lung infiltrates, and they may exert critical ameliorating effects in ARDS [18]. ARDS patients who survived presented significantly higher frequencies of TIM-1+ B cells, especially CD27+TIM-1+ B cells, than non-surviving patients [19]. ALI/ARDS patients showed a significant increase in CD4 and CD8 T-cell activation compared to the control group [20]. An increased T-regulatory cell ratio in the admission BAL of patients with ARDS is an important and independent risk factor for 30-day mortality [21].

The immune status of ARDS patients has long been the focus of researchers. The prognosis of ARDS patients may be affected by either typical immunosuppression or atypical immunosuppression or impaired patient status. Atypical immunosuppression or impaired status is very common in ARDS patients, but there is currently a lack of uniform molecular markers for patients with atypical immunosuppression or impaired status. We hope to find a convenient and easy-to-operate molecular biomarker to detect and evaluate the status of patients with atypical immunosuppression or ARDS deficiency to facilitate targeted interventions and achieve the goal of improving the prognosis of these patients.

Methods

Study design and patients

A retrospective observational cohort study was conducted with ARDS patients hospitalized in the critical care centre of a university-based tertiary care hospital (The Second Xiangya Hospital of Central South University) in Hunan, China, from January 2011 to August 2018. Institutional approval was provided by the Second Xiangya Hospital of Central South University Biomedical Research Ethics Committee (Hunan, China). Written informed consent was waived because of the retrospective observational design. All patient data were anonymously recorded to ensure confidentiality.

Inclusion and exclusion criteria

Patients admitted to the critical care centre with a diagnosis of ARDS based on the 2012 Berlin definition [22] were included in our study if they met the inclusion criteria and none of the exclusion criteria. All eligible patients had to be over 18 years old and had neutrophil and lymphocyte count results within 24 hours after ICU admission. Patients who were repeatedly admitted to the ICU, lacked the neutrophil and lymphocyte records, had chronic haematological disorders, were under the age of 18 years, or died within 24 h of receiving a diagnosis of ARDS were excluded. In addition, we excluded patients who were defined as having an immunodeficiency with the following aetiologies: (1) haematological malignancies, (2) active solid tumours or specific anti-tumour treatment within a year, (3) solid organ transplant, (4) acquired immunodeficiency syndrome (AIDS), or (5) long-term or high-dose corticosteroid (CS) or immunosuppressant (IS) therapy. Long-term CS therapy was defined as >7.5 mg of prednisone/day for >3 months, and a high dose was defined as >1 mg/kg for >1 week within the last 3 months. According to the arterial blood oxygen/oxygen fraction ($\text{PaO}_2/\text{FiO}_2$) ratio, patients were divided into mild ($200 \text{ mmHg} < \text{PaO}_2/\text{FiO}_2 \leq 300 \text{ mmHg}$, $n=31$), moderate ($100 \text{ mmHg} < \text{PaO}_2/\text{FiO}_2 \leq 200 \text{ mmHg}$, $n=61$), and severe ($\text{PaO}_2/\text{FiO}_2 \leq 100 \text{ mmHg}$, $n=109$) groups. Meanwhile, 201 patients were included and divided into a survivor group ($n=80$) and a non-survivor group ($n=121$) according to the final clinical results.

Data extraction and outcome

We retrospectively reviewed all case data from ARDS patients and selected the patients who met the criteria. Therefore, demographic and baseline characteristics such as age, gender, body mass index (BMI), ARDS risk factors, severity of illness at admission to the ICU (Acute Physiology and Chronic Health Evaluation (APACHE) II score) [23], and the Sequential Organ Failure Assessment (SOFA) score [24] were recorded and analysed. We recorded routine blood examination results within 24 hours and within three days after ICU admission. Two independent authors completed the data collection. The primary outcome was mortality. The secondary outcomes were ICU mortality and hospital mortality. We also calculated the 28-day mortality and 100-day mortality rates.

Blood measurements and flow cytometric analysis

The white blood cell count, C-reactive protein (CRP) level and procalcitonin (PCT) level were immediately measured in the clinical chemistry laboratory of the Second Xiangya Hospital. The serum levels of haemoglobin, albumin, immunoglobulins (IgG, IgA, IgM, IgE), and complement components (C3, C4) (R&D Systems, USA) were determined by enzyme-linked immunosorbent assays (ELISA) following the

instructions. To analyse T-lymphocytes, a cell staining kit (BD ingen™, USA) was used to detect CD4+ CD8+ CD3+ cells in accordance with the manufacturer's protocol. Briefly, peripheral blood mononuclear cells (PBMCs) were incubated with a mixture of luciferin isothiocyanate anti-CD4 and apc anti-CD8 at 4 ° C for 30 min. A facscalibur flow cytometer (BD Biosciences, USA) equipped with CellQuest software (BD Biosciences, USA) was used for flow cytometry analysis. A homotype control was used to ensure antibody specificity [25,26].

Statistical analysis

All analyses were performed using SPSS, version 22.0 (IBM Corp, Armonk, NY, USA) and MedCalc version 11.0 (MedCalc Software, Inc, Mariakerke, Belgium). After the Kolmogorov-Smirnov test was used to assess the normality of the distribution, continuous variables were reported as the mean±SD or median (IQR). An independent samples t-test was used to evaluate normally distributed data, and the Mann-Whitney test was used to evaluate non-normally distributed data when comparing the two groups. In multi-group comparisons, one-way ANOVA and the Kruskal-Wallis test were used to analyse normally and non-normally distributed data, respectively, and *P*-values adjusted by Bonferroni correction were used for multi-group comparison. Classified data were aggregated using numbers (percentages) and compared using the chi-square or Fisher's exact test. Spearman's rank correlation was adopted to determine correlations among variables. The area under the operating characteristic (ROC) curve was used to assess the characteristics of the subjects and to evaluate the diagnostic and prognostic value of the test parameters. The cut-off point was obtained by determining the optimal den index (sensitivity+specificity-1). Survival rates were compared between groups by Kaplan-Meier plots and log-rank tests. To calculate independent predictors of 100-day mortality, binary logistic regression was used with stepwise regression for variables with a value of *P*<0.05 (one variable was entered when *P*<0.05, and one was deleted when *P*>0.10). The odds ratio (OR), *P*-value and 95% CI were used to represent the results. All tests were double-tailed, and *P*<0.05 was considered statistically significant.

Results

Baseline characteristics and patient outcome

A total of 201 patients meeting the Berlin definition of ARDS were included in this study from January 2011 to August 2018. The characteristics at enrolment and outcomes of the study population are shown in Table 1 and Table 2. No statistically significant differences were found in age, gender or BMI among the mild, moderate and severe ARDS groups. Pneumonia, sepsis and pancreatitis were the most common aetiologies of ARDS. As measured by the APACHE II score (*P*<0.05), SOFA score (*P*<0.05), and PaO₂/FiO₂ (*P*<0.001), the severity of critical illness on the day of enrolment worsened from mild to severe ARDS, as shown in Tables 1 and 2. The 100-day mortality rate was 60.2% (121/201) in patients with ARDS. Compared with survivors, non-survivors had significantly older age and higher APACHE II and SOFA scores. Additionally, survivors had higher BMIs and PaO₂/FiO₂ ratios than non-survivors.

Correlations of the lymphocyte/neutrophil ratio with disease severity and outcome

As shown in Table 1, the frequencies of lymphocyte cells were evidently decreased in the peripheral blood of severe ARDS patients compared with those in the mild group ($P < 0.05$). Moreover, the lymphocyte/neutrophil ratio progressively decreased with increasing ARDS severity from mild to moderate and severe ARDS ($P < 0.01$). Moreover, in non-survivors, a significantly lower lymphocyte/neutrophil ratio was found compared with that of the survivors ($P < 0.01$) (Table 2), and the frequencies of leukocyte cells in non-survivors were lower than those in survivors ($P < 0.05$) (Table 2). However, although the frequencies of leukocytes and neutrophil cells in non-survivors were higher than those in survivors ($P < 0.05$) (Table 2), there were no significant differences among the three groups in terms of the frequencies of leukocytes and neutrophil cells (Table 1).

Alterations in inflammatory biomarkers, immunoglobulins, complement components, circulating T-lymphocyte cells, B-lymphocyte cells and NK cells in ARDS

The changes in the levels of inflammatory biomarkers, immunoglobulins, complement components, circulating T-lymphocyte cells, B-lymphocyte cells and NK cells in each group are shown in Tables 1 and 2. The CPR, PCT, and albumin levels were higher in patients with severe ARDS than in patients with mild ARDS, and patients with severe ARDS also had higher leukocyte and neutrophil counts compared to patients with mild ARDS ($P < 0.05$) (Table 1). Interestingly, the lymphocyte count decreased as the severity of ARDS increased from mild to severe ($P < 0.05$) (Table 1). Furthermore, compared with survivors, non-survivors had older age, higher leukocyte counts, neutrophil counts and lymphocyte/neutrophil ratios, and lower BMI and lymphocyte counts ($P < 0.05$) (Table 1). The CRP and PCT levels were similar in the two groups (Table 2). As shown in Table 1, the peripheral blood immunoglobulin IgE and complement C3 levels in patients with mild ARDS were significantly lower than those in patients with severe ARDS ($P < 0.05$). Moreover, non-survivors had lower immunoglobulin IgE and complement C3 levels than did survivors ($P < 0.05$) (Table 2). However, peripheral blood complement C4 levels in patients with mild ARDS were significantly higher than those in patients with severe ARDS ($P < 0.05$) (Table 1), but the complement C4 levels were similar between the survivors and non-survivors (Table 2).

As shown in Table 2, the level of peripheral blood B-lymphocyte cells were significantly lower in non-survivors than in survivors ($P < 0.01$), and the level of peripheral blood CD8+ cells was significantly lower in non-survivors than survivors ($P < 0.05$). However, the levels of both peripheral blood B-lymphocyte cells and CD8+ cells were similar in the three groups (Table 1). In addition, the proportions of CD3+ cells, CD4+ cells, and NK cells and the CD4+/CD8+ ratio in the peripheral blood showed no significant differences in three groups of ARDS patients stratified by oxygenation index or in the survivor and non-survivor groups.

Correlations of lymphocytes, the lymphocyte/neutrophil ratio, immunoglobulin IgE levels, complement C3 levels, T-CD8+ lymphocyte levels and B-lymphocyte levels with disease severity and outcome

The Spearman correlation analyses of the relationships between the lymphocyte/neutrophil ratio and age, APACHE II score, SOFA score, and PaO₂/FiO₂ in ARDS patients are displayed in Figure 1. In all ARDS patients, significant moderate negative correlations were found between the lymphocyte/neutrophil ratio and age ($r=-0.153$, $P<0.05$), the SOFA score ($r=-0.140$, $P<0.05$), and the APACHE II score ($r=-0.177$, $P=0.012$). In addition, we noted a moderate positive correlation between the lymphocyte/neutrophil ratio and PaO₂/FiO₂ ratio ($r=0.143$, $P<0.05$). However, the lymphocyte to neutrophil ratio was negatively correlated with viral infection status ($r=-0.091$, $P=0.557$). Moreover, significant mild positive correlations were found between the lymphocyte count and BMI ($r=0.145$, $P=0.041$) and the lymphocyte count and the PaO₂/FiO₂ ratio ($r=0.110$, $P=0.121$). However, the lymphocyte count was negatively correlated with age ($r=-0.045$, $P=0.523$), the APACHE II score ($r=-0.060$, $P=0.395$), the SOFA score ($r=-0.012$, $P=0.864$) and the viral infection status ($r=-0.042$, $P=0.557$) although not significantly.

In all ARDS patients, moderate negative correlations were found between the immunoglobulin IgE level and age ($r=-0.033$, $P=0.817$), the SOFA score ($r=-0.0140$, $P=0.918$), the APACHE II score ($r=-0.289$, $P=0.036$), and the viral infection status ($r=-0.118$, $P=0.399$); however, we noticed moderate positive correlations between the immunoglobulin IgE level and BMI ($r=0.261$, $P=0.059$) and the immunoglobulin IgE level and the PaO₂/FiO₂ ratio ($r=0.288$, $P=0.036$).

In all ARDS patients,, moderate negative correlations were found between the level of C3 and age ($r=-0.103$, $P=0.515$), the SOFA score ($r=-0.021$, $P=0.896$), the APACHE II score ($r=-0.360$, $P=0.813$), and the viral infection status ($r=-0.072$, $P=0.648$); however, we noticed moderate positive correlations between the C3 level and BMI ($r=0.342$, $P=0.026$) and the C3 level and the PaO₂/FiO₂ ratio ($r=0.038$, $P=0.811$).

In all ARDS patients,, moderate negative correlations were found between T-CD8+ lymphocyte cell counts and age ($r=-0.162$, $P=0.520$), the SOFA score ($r=-0.101$, $P=0.690$), the APACHE II score ($r=-0.206$, $P=0.690$), and the viral infection status ($r=-0.409$, $P=0.092$). However, we noticed moderate positive correlations between the T-CD8+ lymphocyte counts and BMI ($r=0.248$, $P=0.534$), the PaO₂/FiO₂ ratio ($r=0.121$, $P=0.633$), and lymphocyte counts ($r=0.755$, $P=0.001$).

In all ARDS patients, moderate negative correlations were found between B-lymphocyte cell counts and age ($r=-0.198$, $P=0.447$), the SOFA score ($r=-0.020$, $P=0.940$), the APACHE II score ($r=-0.071$, $P=0.787$), and viral infection status ($r=-0.059$, $P=0.823$). However, we noticed moderate positive correlations between B-lymphocyte cell counts and BMI ($r=0.588$, $P=0.013$), the PaO₂/FiO₂ ratio ($r=0.240$, $P=0.353$), and lymphocyte counts ($r=0.582$, $P=0.014$).

The ROC curves for the lymphocyte/neutrophil ratio, lymphocyte count, BMI, PaO₂/FiO₂ ratio, lymphocyte/neutrophil ratio in combination with the PaO₂/FiO₂ ratio, and lymphocyte/neutrophil ratio in combination with the PaO₂/FiO₂ ratio for predicting 100-day survival in patients with ARDS are shown in

Figure 2. The area under the ROC curve (AUC) for the lymphocyte/neutrophil ratio in combination with the lymphocyte count for the prediction of 100-day survival in ARDS patients was 0.723 (95% CI 0.656 to 0.784), which was higher than that for the lymphocyte/neutrophil ratio (0.721, 95% CI 0.653 to 0.782) and that for the lymphocyte/neutrophil ratio in combination with the PaO₂/FiO₂ ratio (0.719, 95% CI 0.651 to 0.780), although the differences were not statistically significant (P=0.8601 and 0.7734). The AUC for the PaO₂/FiO₂ was 0.625 (95% CI 0.554 to 0.692), the AUC for BMI was 0.593 (95% CI 0.521 to 0.661) and the AUC for the lymphocyte count was 0.592 (95% CI 0.520 to 0.660), all of which were significantly lower than the AUC for the lymphocyte/neutrophil ratio (P=0.0062, 0.0001, and 0.0154, respectively). The AUC for the lymphocyte/neutrophil ratio in combination with the lymphocyte count was 0.723 (95% CI 0.656 to 0.784), which was significantly higher than those for the PaO₂/FiO₂ ratio alone (P=0.0060), BMI alone (P=0.0001), and lymphocyte count alone (P=0.0067) for predicting survival in patients with ARDS. The AUC for the lymphocyte/neutrophil ratio in combination with the PaO₂/FiO₂ ratio was 0.719 (95% CI 0.651 to 0.780), which was significantly higher than those for the PaO₂/FiO₂ ratio alone (P=0.0014), BMI alone (P=0.0001), and lymphocytes alone (P=0.0162) for predicting survival in patients with ARDS.

A cut-off value of the lymphocyte/neutrocyte ratio of >0.0537 was used to predict the survival of ARDS patients, with a sensitivity of 83.8%, specificity of 80.2%, positive likelihood ratio of 4.23 and negative likelihood ratio of 0.20. Moreover, using a leukocyte count cut-off of >0.415 (10⁹/L) for predicting survival in patients with ARDS, the sensitivity and specificity were 87.5% and 81.0%, respectively, and the positive and negative likelihood ratios were 4.61 and 0.15, respectively.

Predictors of 28-day and 100-day mortality in patients with ARDS

Table 3 shows that the age (per log₁₀ years) (OR=1.269, P=0.019), BMI<24 (OR=1.665, P=0.015), SOFA score (OR=1.287, P=0.002), leukocyte count<0.415 (10⁹/L) (OR=1.671, P=0.042), and lymphocyte/neutrophil ratio (OR=2.132, P=0.009) were the independent predictors of 100-day mortality in patients with ARDS. Moreover, ARDS patients with a lymphocyte/neutrophil ratio <0.0537 had a higher 28-day mortality rate than those with a lymphocyte/neutrophil ratio >0.0537 (P=0.0283, Figure 3A). The 28-day and 100-day mortality rates were significantly lower in the under-40 years old and 40-60 years old age groups than in the over-60 years old age group (P=0.0064, 0.0057, Figure 3B, C). The 100-day mortality rate was significantly higher in the over-80 years old age group than in the under-40 years old age group, the 40-60 years old age group and the 60-80 years old age group (P=0.0029, Figure 3D).

Discussion

In this study, we found associations between age, BMI, the SOFA score, and the lymphocyte/neutrophil ratio at ICU admission and clinical outcomes in patients with ARDS. Age (per log₁₀ years), BMI<24, SOFA score (per point) and lymphocyte/neutrophil ratio were independent risk factors for predicting 100-day mortality in ARDS patients. The secondary outcome was the lymphocyte/neutrophil ratio, and age was related to ICU mortality and hospital mortality. In addition, we also found associations between baseline

lymphocyte/neutrophil ratio and age, the SOFA score, the APACHE II score, the PaO₂/FiO₂ ratio, and the severity of ARDS according to the Berlin classification.

During the past decade, there have been a few investigations addressing the potential function of the lymphocyte/neutrophil ratio, and the lymphocyte/neutrophil ratio remains a useful test for the diagnosis of tuberculous pleuritis [27]. The lymphocyte to neutrophil ratio can be used as an early biomarker for predicting acute rejection after heart transplantation [28]. The lymphocyte/neutrophil ratio may help predict ARDS patients with a high immunologic risk. To the best of our knowledge, the current study is the first longitudinal clinical outcome study in ARDS patients demonstrating the predictive significance of the lymphocyte/neutrophil ratio.

Previous studies focused on early T-cell lymphopenia, which has been widely attributed to viral infection, and patients with a poor prognosis had severe lymphopenia from the first day of ICU admission [29]. In our study, it was found that peripheral blood lymphopenia was very common in ARDS patients without typical underlying diseases causing immunosuppression. The frequencies of lymphocyte cells were evidently decreased in the peripheral blood of severe ARDS patients compared with those in the mild group, and they were lower in non-survivors than in survivors. However, there was no significant difference in the probability of viral infection between the survivors and non-survivors in ARDS patients. Neither the frequencies of leukocytes nor the frequencies of lymphocytes were associated with mortality in patients with ARDS. Moreover, the lymphocyte/neutrophil ratio progressively decreased with increasing ARDS severity from mild to moderate and severe ARDS, and a significantly lower lymphocyte/neutrophil ratio was found in non-survivors than in survivors. The lymphocyte/neutrophil ratio reveals the balance between lymphocyte and neutrophil counts. This may mean that the combination of lymphopenia and neutrophilia contributes to the outcome. Multivariate analysis showed that a decrease in the lymphocyte count was associated with a 2.32-3.76-fold increase in the risk of death in patients with or without septic shock [30]. Patients develop T-lymphocyte dysfunctions leading to T-cell exhaustion, which is associated with an increased risk of death in septic shock patients [31]. The surviving ARDS patients had significantly higher frequencies of TIM-1+ B cells than did non-survivors, especially CD27+TIM-1+B cells [32]. ARDS patients with resolved inflammation who survived the syndrome had significantly higher levels of Tr1 cells than did ARDS patients who died [33]. In our study, both peripheral blood B-lymphocyte and CD8+T-lymphocyte counts in survivors were significantly higher than those in non-survivors among ARDS patients, and B-lymphocyte and CD8+T-lymphocyte counts were also found to be positively correlated with peripheral blood lymphocyte counts. In addition, when the lymphocyte count was below a certain value, the risk of death in ARDS patients was increased. Lymphocytes are important immune cells in the response to the ARDS process and the prognosis. Neutrophils are another type of immune cells. During sepsis, the inappropriate activation and positioning of neutrophils within the microvasculature contributes to the pathological manifestations of multiple organ failure in sepsis [34]. Neutrophils are involved in the process of sepsis, and the relative increase in the total number of circulating neutrophils and the percentage increase in the neutrophils with immature morphology are also closely related to sepsis [35]. Higher neutrophil counts were found in patients who eventually died as a result of sepsis-

induced ARDS compared with the counts in survivors, and the excessive accumulation of neutrophils in patients with ARDS may therefore contribute to disease progression [36,37]. This may explain why the lymphocyte/neutrophil ratio was a strong independent predictor of prognosis in ARDS patients.

There have been many clinical studies on ARDS patients with typical immunodeficiency [38,39]. The immunodeficiency in ARDS patients is usually atypical. Although not it does not reach the level found in typical immunodeficiency, there is already a degree of immune impairment. However, there are few studies on atypical immune deficiency or impaired immunity in ARDS patients, and there is no uniform scale or biomarker to measure immune impairment in ARDS patients and its relationship with the prognosis of ARDS patients. A consensus has not been reached regarding whether viral infection causes immune deficiency, but it is commonly thought to cause immune impairment in infected people but not to the extent of immune deficiency. In our study, there was no significant difference in viral infection status between the survivor and non-survivor groups. However, we observed that the number of lymphocytes and the ratio of lymphocytes to neutrophils in ARDS patients in the survivor group were higher than those in the non-survivor group. Moreover, significantly moderate negative correlations were found between the lymphocyte/neutrophil ratio and age, the SOFA score, and the APACHE II score. Furthermore, we noticed a moderate positive correlation between the lymphocyte/neutrophil ratio and the PaO₂/FiO₂ ratio. These results suggest that the lymphocyte/neutrophil ratio can comprehensively and plausibly reflect the patient's physiological, pathophysiological and respiratory oxygenation index status.

We hypothesized that atypical immunodeficiency in ARDS patients may affect their prognosis and that a low lymphocyte count and lymphocyte/neutrophil ratio may be markers of this atypical immunodeficiency in ARDS patients. Until now, there have been no recognized biomarkers that could be used to identify the immune status of ARDS patients. In this study, therefore, we propose a new biomarker to use for the identification of atypical immune status in patients with ARDS. This status may be due to abnormal inhibitors that have been activated. We hope that by observing the association of the early lymphocyte/neutrophil ratio with the prognosis of disease may results in better detection and more timely treatment of abnormal immune status.

Our findings must be understood in view of the following limitations. First, this study had a relatively small sample size, although it was the first study to explore the prognostic value of the lymphocyte/neutrophil ratio for prognosis in ARDS patients. Second, because only patients in whom the absolute lymphocyte and neutrophil counts were measured soon after being diagnosed with ARDS were included, there may be a selection bias. Third, the current study was not pre-specified but is a post hoc analysis from a prospective, randomized controlled trial that was not intended to assess the impact of the lymphocyte/neutrophil ratio on the prognosis in ARDS patients. Thus, further prospective studies are needed.

Conclusions

In the present study, we found that age (per \log_{10} years), a BMI<24, the SOFA score, the lymphocyte count, and the lymphocyte/neutrophil ratio were independent predictors of 100-day mortality in patients with ARDS. We also found significant moderate negative correlations between the lymphocyte/neutrophil ratio and age, the SOFA score, and the APACHE II score, and a significant mild positive correlation was found between the lymphocyte count and BMI. However, a moderate positive correlation was found between the lymphocyte/neutrophil ratio and the PaO₂/FiO₂ ratio in all patients with ARDS. The AUC was greatest for the lymphocyte/neutrophil ratio combined with the lymphocyte count for the prediction of 100-day survival in ARDS patients. Moreover, ARDS patients with a lymphocyte/neutrophil ratio <0.0537 had a higher 28-day mortality rate than those with a lymphocyte/neutrophil ratio >0.0537. The lymphocyte/neutrophil ratio was also revealed to be a strong and independent predictor of prognosis in ARDS patients, especially those with atypical immunodeficiency.

Abbreviations

ARDS: Acute respiratory distress syndrome; APACHE: Acute Physiology and Chronic Health Evaluation; AUC: Area under the curve; BALF: Bronchoalveolar lavage fluid; BMI: Body mass index; CI: Confidence interval; CRP: C-reactive protein; ICU: Intensive care unit; LDH: Lactate dehydrogenase; MOF: Multiple organ failure; OR: Odds ratio; PCT: Procalcitonin; ROC: Receiver operating characteristic; SAPS: Simplified Acute Physiology Score; SD: Standard deviation; SOFA: Sequential Organ Failure Assessment.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of the Second Xiangya Hospital, Central South University, and written informed consent was waived because of the retrospective design.

Consent for publication

Not applicable

Availability of data and material

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors participated in the interpretation of the study results and review of the manuscript. MS designed and conducted all experiments, drafted the manuscript, and performed the statistical analyses. HL planned the study and drafted the manuscript. YJ L and ZW L participated in the data collection. HP and PC supervised the project. All authors read and approved the final manuscript.

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Tables

Table 1 Baseline characteristics of the enrolled study population

Variables	Acute respiratory distress syndrome					Δ p-value
	Total	Mild	Moderate	Severe		
Number		201	31	61	109	
Age, years	54.24±16.35	55.38±15.60	52.43±	54.93±16.03		0.579
Sex, male/female, n	130/71	19:12	40/21	71/38		0.415
BMI, kg/m ²	23.90±3.82	24.22±3.93	23.83±4.05	23.90±3.82		0.896
Cause of ARDS						
Pneumonia	125	19	37	69		
Non-pulmonary sepsis	38	6	15	17		
Pancreatitis	15	4	7	4		
Trauma	7	0	0	7		
Aspiration	2	0	0	2		
Others	14	2	2	10		
APACHE II score	14.19(7.69 to 31.00)	12.74(7.69 to 24.37)	14.52 (9.86 to 27.69)	14.34 (9.68 to 31.00)*		0.016
SOFA score	4.98[4.65 to 5.30]	4.59(3.97 to 5.20)	5.11 (4.71 to 5.53)	5.26 (4.89 to 5.63)		0.027
PaO ₂ /FiO ₂ , mmHg	123 (112 to 134)	280 (267 to 294)	140 (132 to 146)*	69 (65 to 72)*#		0.000
CRP, mg/L	129.82 [129.82 to 169.91)	110.09 (76.59 to 143.59)	163.90 (138.67 to 189.14)*	203.33 (115.97 to 290.68)*		0.011
PCT, ng/mL	12.20 (8.12 to 16.29)	9.37 (5.45 to 13.30)	10.15 (1.51 to 18.80)	17.05 (7.58 to 27.73)*#		0.175
Albumin, g/L	27.67±5.82	27.13±3.17	27.07±6.05	28.10±6.05*		0.759
Haemoglobin, g/L	107.67±28.32	104.04±23.76	107.29±28.05	108.82±29.73		0.759
Leukocytes, 10 ⁹ /L	11.33(10.12 to 12.54)	10.98 [8.67 to 13.28)	10.91 (9.62 to 12.22)	13.65 (8.65 to 18.64)		0.298
Lymphocytes, 10 ⁹ /L	1.10 (0.95 to 1.26)	1.28 (0.86 to 1.75)	1.10 (8.67 to 13.28)	1.07 (0.87 to 1.27)*		0.025
Neutrophils, 10 ⁹ /L	10.78 (8.38 to 13.18)	8.35 (6.91 to 9.78)	11.66 (7.59 to 15.74)	13.03 (7.31 to 18.76)		0.371
Lymphocyte/neutrophil ratio	0.19±0.03	0.35±0.23	0.17±0.03*	0.15±0.02*		0.001
Virus infection, n,%	23(11.44%)	1(3.23%)	10(16.39%)	12(11.0%)		
28-day mortality, n,%	103(51.24%)	13(41.94%)	31(50.82%)	59(54.13%)		
100-day mortality, n,%	121(60.20%)	15(48.39%)	37(60.66%)	69(63.30%)		
Immunoglobulin						
IgG, g/L	10.87±1.86	11.71±4.96	10.82±1.94	10.59±5.36		0.095
IgA, g/L	1.98±0.24	1.92±1.09	1.85±0.72	2.11±1.40		0.270
IgM, g/L	1.05±0.86	1.32±1.18	1.02±0.92	1.00±0.72		0.598
IgE, ng/mL	620.36±145.11	1426.20±119.89	640.55±32.45	356.87±78.73*		0.025
Complement components						
C3	5.62±1.78	12.27±1.83	7.13±3.14	0.84±0.33*		0.186
C4	1.33±0.58	0.25±0.13	0.24±0.11	2.25±0.95*		0.663
T-lymphocyte subsets						
CD3+ cells, %	62.75±15.29	71.00±13.78	63.35±12.44	60.62±17.11		0.229
CD4+ cells, %	35.15±15.48	36.40±13.69	34.10±11.14	35.66±8.47		0.951
CD4+ cell count (PCS/ μ L)	383.20±67.18	176.50±67.18	383.67±94.79	434.08±78.66		0.708
CD8+ cells, %	27.33±13.46	34.33±6.37	25.60±11.59	26.92±14.01		0.275
CD8+ cell count (PCS/ μ L)	212.33±64.30	134.00±30.63	286.67±51.72	178.91±70.96		0.409
CD4+/CD8+ ratio	1.95±0.26	1.29±0.23	1.91±0.93	2.14±0.48		0.623
B-lymphocytes						
B-lymphocytes, %	30.25±17.28	18.00±3.24	26.20±7.81	33.36±7.81		0.598

B-lymphocyte cell count (PCS/ μ L)	301.05 \pm 73.77	69.01 \pm 8.89	413.20 \pm 41.42	271.18 \pm 78.37	0.665
NK cells					
NK cells, %	8.76 \pm 5.91	17.00 \pm 4.41	9.40 \pm 0.81	7.73 \pm 4.58	0.331
NK cell count (PCS/ μ L)	68.77 \pm 46.46	68.20 \pm 7.66	82.60 \pm 14.82	62.55 \pm 15.15	0.751

Normally distributed quantitative data are expressed as means \pm standard deviation. Non-normally distributed quantitative data are expressed as medians (IQR). Qualitative data are presented as numbers (%). ^ΔP-value for the three groups (mild, moderate, and severe ARDS groups); *P<0.01 versus mild ARDS; #P<0.01 versus moderate ARDS. BMI, body mass index; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; CRP, C-reactive protein; PCT, procalcitonin.

Table 2 Comparison of clinical characteristics of ARDS patients according to survival status

Variables	Non-survivors (n=121)	Survivors (n=80)	P-value
Age, years	56.96±17.09	50.16±14.33	0.004
Sex, male/female, n	76/45	54/26	0.496
BMI, kg/m ²	23.41±3.88	24.69±3.93	0.027
APACHE II score	14.96(14 to 16)	13.51 (13 to 15)	0.036
SOFA score	5.42 (5 to 6)	4.15 (4 to 5)	0.000
PaO ₂ /FiO ₂ ,mmHg	115 (102 to 188)	135 (115 to 154)	0.042
CRP, mg/L	152.20 (126.50 to 177.89)	146.32 (113.27 to 179.37)	0.778
PCT, ng/mL	13.67 (5.49 to 21.89)	11.30 (6.90 to 15.70)	0.577
Haemoglobin, g/L	105.63±27.68	110.41±30.74	0.298
Albumin, g/L	27.24±5.68	28.33±6.02	0.223
Leukocytes, 10 ⁹ /L	12.19 (10.41 to 13.97)	10.80 (9.47 to 12.14)	0.012
Lymphocytes, 10 ⁹ /L	1.03 (0.86 to 1.20	1.21 (0.92 to 1.50)	0.025
Neutrophils, 10 ⁹ /L	9.80 (8.29 to 11.31)	8.80 (7.64 to 9.95)	0.016
Lymphocyte/neutrophil ratio	0.15±0.05	0.20±0.28	0.008
Virus infection	12(9.91%)	11(13.75%)	
Immunoglobulin			
IgG, g/L	9.95±2.37	12.42±3.88	0.169
IgA, g/L	1.80±0.53	2.29±1.04	0.156
IgM, g/L	0.90±0.25	1.22±0.33	0.163
IgE, ng/mL	351.97±80.27	1030.29±220.94	0.009
Complement component			
C3	3.27±0.91	10.31±2.06	0.018
C4	1.84±0.46	0.25±0.10	0.170
T-lymphocyte subsets			
CD3+ cells, %	61.90±15.49	63.83±15.29	0.647
CD4+ cells, %	33.93±16.06	36.67±14.93	0.521
CD4+ cell count (PCS/μL)	328.85±72.23	487.86±65.04	0.512
CD8+ cells, %	29.47±5.42	24.38±9.75	0.190
CD8+ cell count (PCS/μL)	152.75±22.66	331.50±95.71	0.024
CD4+/CD8+ ratio	1.79±0.09	1.78±0.12	0.428
B-lymphocytes			
B-lymphocyte cells, %	29.81±8.89	31.33±5.49	0.169
B-lymphocyte cell count (PCS/μL)	113.27±29.45	601.33±52.26	0.009
NK cells			
NK cells, %	9.19±3.28	8.00±1.67	0.708
NK cell count (PCS/μL)	56.55±12.87	91.17±19.79	0.134

Normally distributed quantitative data are expressed as means±standard deviation. Non-normally distributed quantitative data are expressed as medians (IQR). BMI, body mass index; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; CRP, C-reactive protein; PCT, procalcitonin.

Table 3 Logistic regression analysis of the prediction of mortality for patients with acute respiratory distress syndrome (ARDS)

Variables	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Age, per log ₁₀ (years)	1.269(1.040,1.548)	0.019	2.982(2.073,4.654)	0.007
BMI, <24	1.665(0.883,3.137)	0.015		
APACHE II score, per point	1.016(0.940,1.098)	0.059		
SOFA score, per point	1.287(1.098,1.509)	0.002	2.560(1.457,5.430)	0.005
PaO ₂ /FiO ₂ , per log ₁₀ (mmHg)	0.652(0.280,1.004)	0.067		
PCT, per log ₁₀ (ng/mL)	1.028(0.797,1.810)	0.063		
CRP, >150 (mg/L)	1.256(0.618,2.553)	0.059		
Lymphocytes, <0.415 × 10 ⁹ /L	1.671(1.252,1.787)	0.042		
Lymphocyte/neutrophil ratio, <0.0537	4.137(1.452,6.832)	0.002	3.726(2.754,5.195)	0.003

BMI, body mass index; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; PCT, procalcitonin; CRP, C-reactive protein.

Figures

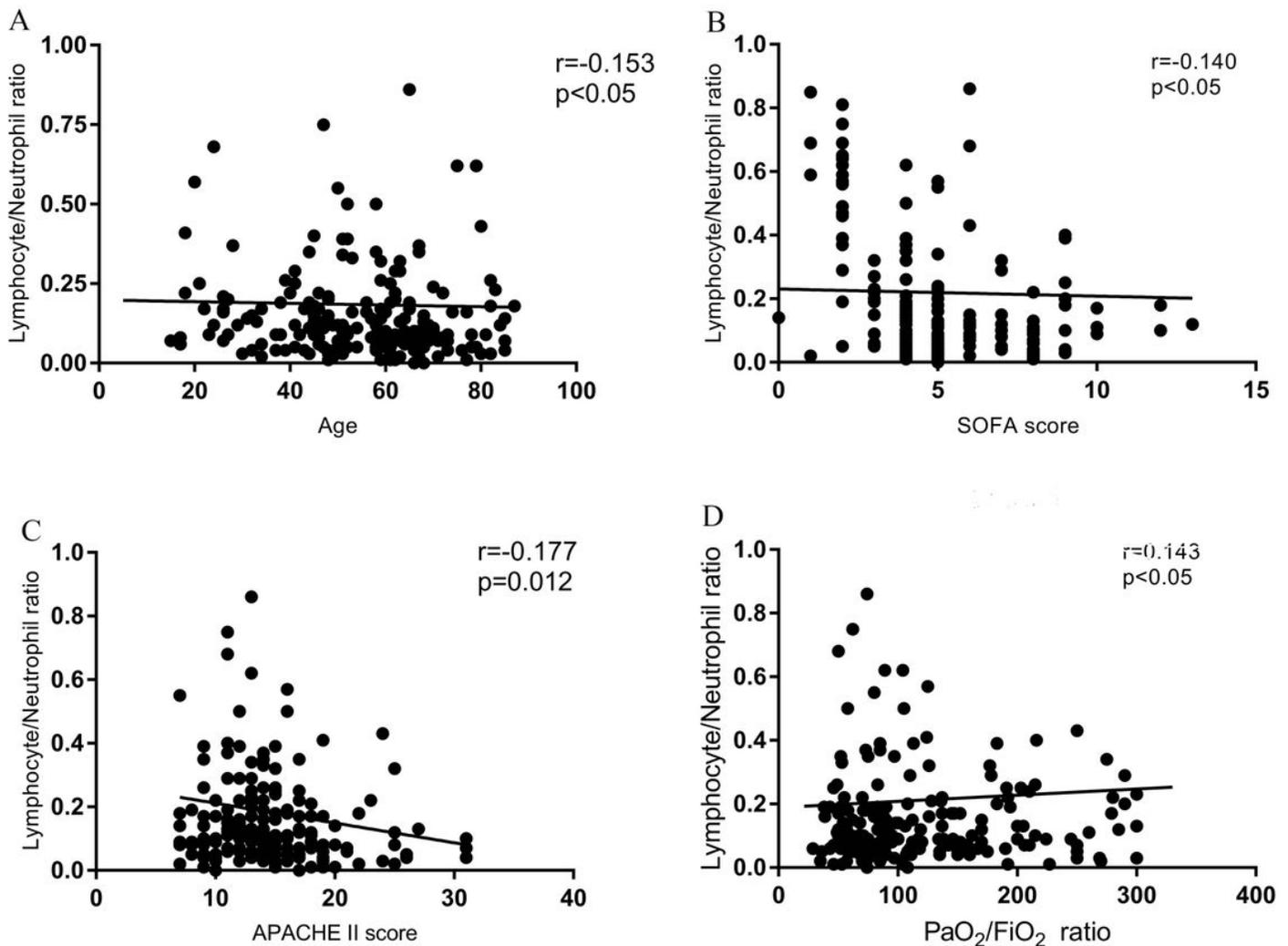


Figure 1

Relationships between the lymphocyte/neutrophil ratio and age, APACHE II score, SOFA score, and PaO₂/FiO₂ ratio in ARDS patients. Spearman rank correlation was used to assess the associations between variables. The lymphocyte/neutrophil ratio was negatively correlated with age (A), the SOFA score (B), and the APACHE II score (C), while it was positively correlated with the PaO₂/FiO₂ ratio (D) in ARDS patients.

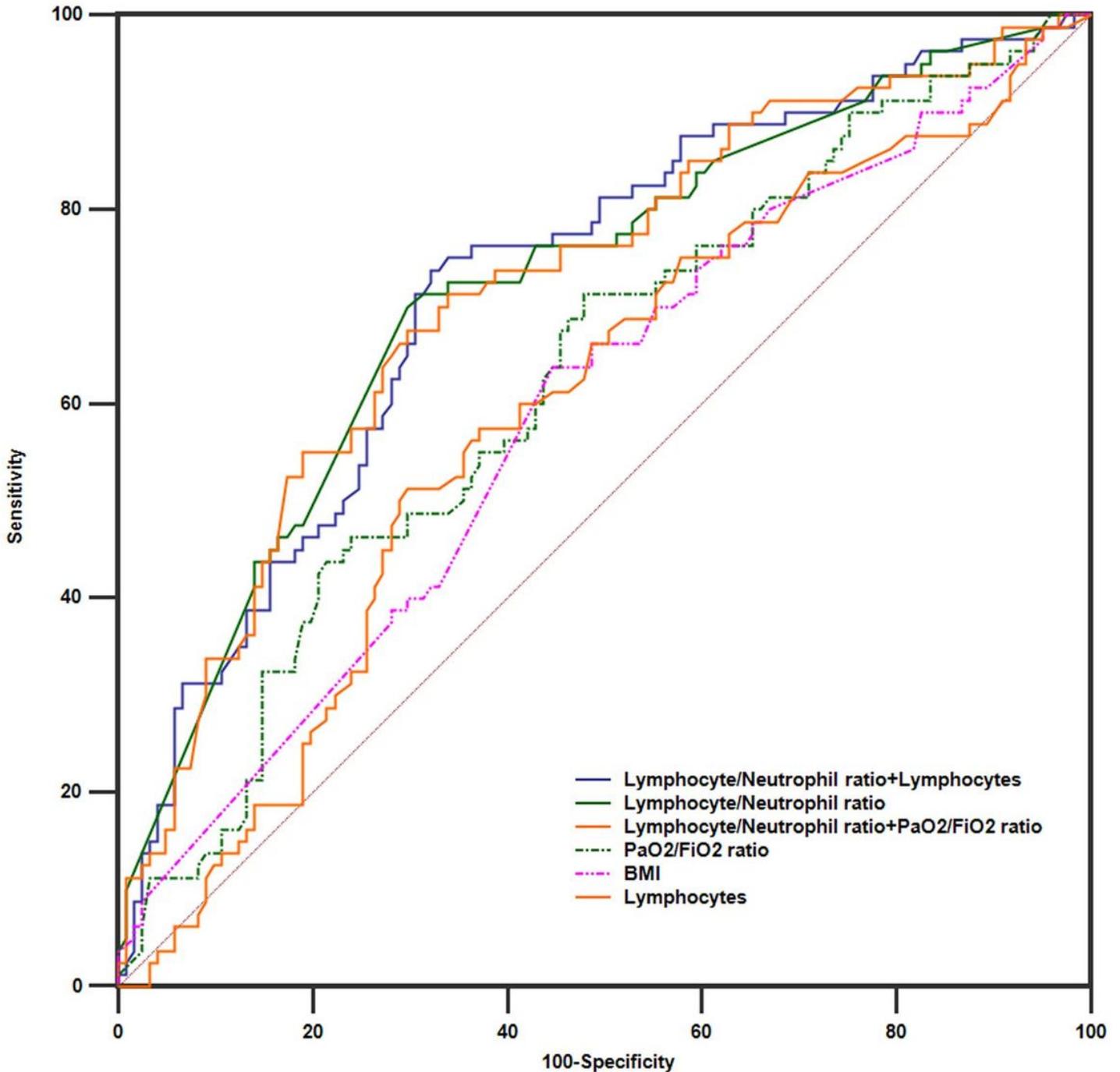


Figure 2

Receiver operating characteristic (ROC) curves for predicting 100-day survival in patients with acute respiratory distress syndrome (ARDS). The area under the curve (AUC) was 0.721 (95% CI 0.656 to 0.784) for the lymphocyte/neutrophil ratio, 0.625 (95% CI 0.554 to 0.692) for the PaO₂/FiO₂ ratio, 0.593 (95% CI 0.521 to 0.661) for the BMI, 0.592 (95% CI 0.520 to 0.660) for the lymphocyte count, 0.723 (95% CI 0.656 to 0.784) for the lymphocyte/neutrophil ratio combined with the lymphocyte count and 0.719 (95% CI 0.651 to 0.780) for the lymphocyte/neutrophil ratio in combined with the PaO₂/FiO₂ ratio. However, the AUC was 0.369 (95% CI 0.292 to 0.446) for age, 0.425 (95% CI 0.345 to 0.505) for the APACHE II score, and 0.355 (95% CI 0.278 to 0.433) for the SOFA score (not shown).

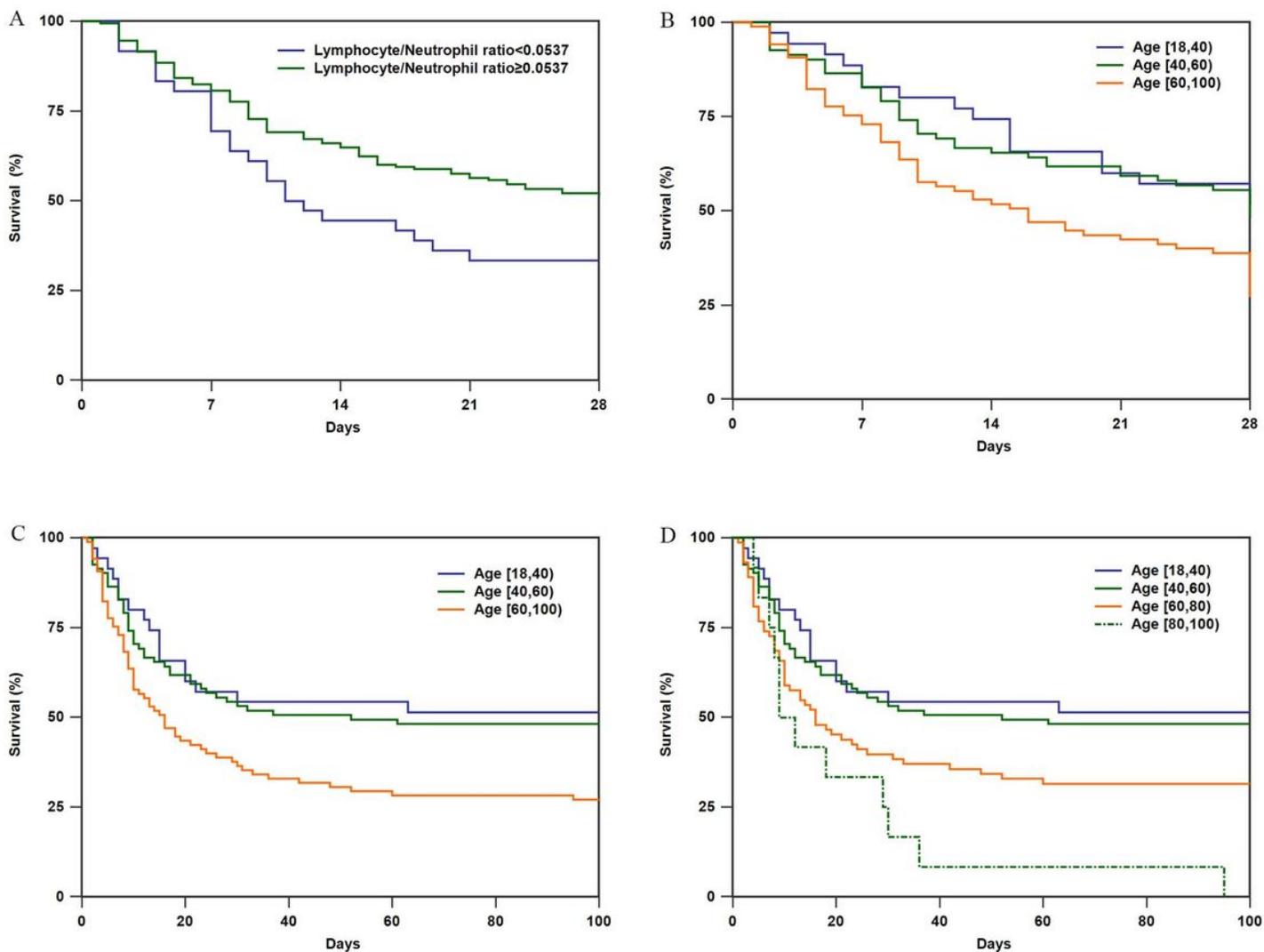


Figure 3

Kaplan-Meier survival curve for patients with ARDS using the cut-off values for the lymphocyte/neutrophil ratio and age obtained by ROC analysis. Log-rank test ($P=0.0283$) (A), ($P=0.0064$) (B), ($P=0.0057$) (C), and ($P=0.0029$) (D).

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