

# Predictive Value of the Baseline and Early Changes in Blood Eosinophils for Short-Term Mortality in Patients with Acute Respiratory Distress Syndrome

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**Background:** Eosinophils play an essential role in the pathogenesis of acute respiratory distress syndrome (ARDS). We aimed to assess the association between the baseline blood eosinophils, eosinophil changes during the first week in the intensive care unit (ICU) and short-term patient outcomes.

**Methods:** All patients meeting the Berlin definition of ARDS from the Medical Information Mart for Intensive Care IV database were retrospectively analyzed. We used logistic regression, Kaplan–Meier survival and random forest analysis to determine the association between the baseline eosinophils and short-term mortality. Then the trends in eosinophils over time were compared between the survivors and non-survivors using a generalized additive mixed model (GAMM), which is a common approach used for analysis of repeated measurement data. Area under the receiver-operating characteristic curve (AUC) was used to evaluate the predictive value.

**Results:** A total of 1685 patients were included, and the 30-day mortality was 25.1%. Kaplan–Meier analysis showed that patients with high baseline eosinophils (>0.3%) had lower 30-day mortality ( $p < 0.001$ ). Random forest model selected the baseline eosinophils as an important factor associated with 30-day mortality. Multivariable logistic regression analysis identified high baseline eosinophils as an independent factor for 30-day mortality (OR 0.743, 95% CI 0.568–0.970). The GAMM result showed that the levels of eosinophils were increased in both survival and non-survival groups, and the between-group differences increased over time, with an average of 0.154 daily after adjusting for confounders. The AUC of changes in eosinophils within the first week was significantly higher than that of baseline eosinophils.

**Conclusion:** There is a negative association between the baseline eosinophils and short-term mortality in ARDS patients, and the differences in eosinophils increased over time between the survivors and non-survivors. Higher increase in eosinophils is associated with decreased short-term mortality, and dynamic monitoring of eosinophils could better predict the survival of ARDS patients.

**Keywords:** ARDS, eosinophils, 30-day mortality, generalized additive mixed model, change

## Introduction

Acute respiratory distress syndrome (ARDS) is a major contributing cause of hospitalization to the intensive care unit (ICU), with a high mortality rate of 30–40%.<sup>1</sup> It is characterized mainly by massive inflammation, increased permeability of alveolar-capillary barrier, and subsequent non-cardiogenic pulmonary edema and refractory hypoxia.<sup>2</sup> For decades, researchers and clinicians have dedicated significant efforts to treat ARDS, but most of them have met disappointing results, largely as a consequence of the marked heterogeneity of ARDS.<sup>3</sup> Therefore, accelerating precision medicine in

ARDS has become a top priority, and biomarkers are suggested to play an essential role in this field, due to their potential implications in molecular phenotype.<sup>4</sup>

Inflammation is critically involved in the development of ARDS.<sup>5</sup> Innate immune cells play crucial roles in the modulation of inflammatory responses.<sup>6</sup> Neutrophils are the classical effector cells of the innate immune response during ARDS, which is associated with type-1 inflammation. In contrast, eosinophils, an effector cell of type-2 inflammation, have recently attracted much interest, as their potential diagnostic and prognostic abilities in ARDS.<sup>7–13</sup> About 40 years ago, eosinopenia among patients with ARDS was first described and may be a predictor of impending ARDS.<sup>7–9</sup> Subsequently, the accumulation of eosinophils in lung was found to be a favorable prognostic indicator for patients with ARDS.<sup>10</sup> Moreover, recent studies have found that eosinophils could ameliorate neutrophilic inflammation and exert a protective effect against ARDS.<sup>11–13</sup> It is therefore plausible to speculate that “eosinophilic” ARDS is a distinct phenotype, and may present a better clinical prognosis. Unfortunately, the collection of biopsies and bronchoalveolar lavage requires invasive and laborious process, which is often infeasible in clinics. Thus, the measurement of peripheral blood eosinophils seems to be a more simple, efficient and secure option. Actually, blood eosinophils have been proposed as a surrogate biomarker to identify the eosinophil-mediated phenotype in various respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD).<sup>14,15</sup> In our previous study, we have demonstrated that blood eosinophils have predictive utility for clinical outcomes in patients with COPD.<sup>16</sup>

To date, the predictive value of eosinophils, especially considering longitudinal monitoring data, for patients with ARDS has not been fully elucidated. Therefore, we aimed to evaluate the association between the baseline eosinophils, early changes (the first week of ICU admission) in its level and adverse patient outcomes.

## Method

### Study Design

We performed this retrospective study using a sizeable US-based critical care database called the Medical Information Mart for Intensive Care (MIMIC)-IV. The MIMIC-IV database is an updated version of MIMIC-III and currently contains 76,540 patients in the ICU of the Beth Israel Deaconess Medical Center in Boston, Massachusetts between 2008 and 2019.<sup>17</sup> These data were collected before the coronavirus disease 2019 (COVID-19) outbreak. This database was approved by the Institutional Review Boards of the Massachusetts Institute of Technology. We have completed the National Institutes of Health Web-based training course and the Protecting Human Research Participants examination (no. 9555299) to gain access to the database. The approval of our institution and informed consent were exempted given the retrospective nature of the study on an anonymized database. Our study was conducted and reported according to the Reporting of Studies Conducted using Observational Routinely Collected Health Data (RECORD) statement.<sup>18</sup>

### Selection of Patients

We reviewed and retrieved the patient clinical data from the database. Patients who were  $\geq 16$  years of age, stayed more than 72 hours in the ICU, and met the Berlin definition of ARDS at the time of ICU admission were considered eligible for study inclusion. We excluded the patients who lacked eosinophil values within 24 hours after ICU admission. Additionally, if a patient had multiple ICU admissions, we used the data from his first admission.

Berlin definitions are as follows:<sup>19</sup> (1) acute onset of respiratory symptoms; (2) presence of bilateral infiltrates on chest radiograph; (3) arterial oxygen partial pressure ( $\text{PaO}_2$ )/fraction of inspired oxygen ( $\text{FiO}_2$ )  $< 300$  mmHg with a minimum requirement for positive end-expiratory pressure (PEEP)  $\geq 5$  cmH<sub>2</sub>O; (4) absence of heart failure. The severity of ARDS was classified into mild ( $200 \text{ mmHg} < \text{PaO}_2/\text{FiO}_2 \text{ ratio} \leq 300 \text{ mmHg}$ ), moderate ( $100 \text{ mmHg} < \text{PaO}_2/\text{FiO}_2 \text{ ratio} \leq 200 \text{ mmHg}$ ), or severe ( $\text{PaO}_2/\text{FiO}_2 \text{ ratio} \leq 100 \text{ mmHg}$ ) according to the Berlin criteria.

### Data Extraction

We used the Structured Query Language with Navicat Premium (version 15) to extract the data. The following variables were extracted or calculated: baseline characteristics (age, gender, body mass index, ethnicity, smoking history, admission type), comorbidities (hypertension, atherosclerosis, diabetes, COPD, acute kidney failure, tumor), the risk factors of

ARDS (pneumonia, sepsis, trauma, shock, pancreatitis and others, like transfusion, pulmonary contusion or inhalation of gastric contents), the severity of organ dysfunction (Acute Physiology Score III, APS III; Oxford Acute Severity of Illness Score, OASIS; Logistic Organ Dysfunction System, LODS), the vital signs during the first 24 hours in the ICU (heart rate, mean arterial pressure, respiratory rate, temperature, SpO<sub>2</sub>), PaO<sub>2</sub>/FiO<sub>2</sub> at diagnosis, PEEP at diagnosis, the laboratory findings during the first 24 hours in the ICU (bicarbonate, blood urea nitrogen, creatinine, lactate, platelet, glucose, white blood cell), and treatment received (mechanical ventilation, antibiotic therapy, vasopressor therapy, renal replacement therapy, corticosteroids therapy). If a variable was recorded more than once in the first 24 hours, we used the value at the first measurement. All comorbidities were diagnosed based on ICD-9 or ICD-10 codes in the MIMIC-IV database. The organ dysfunction scores were estimated during the first 24 hours in the ICU for all patients. The baseline level of eosinophils was obtained based on the first laboratory value within 24 hours after ICU admission. The repeated measurements of eosinophils were performed within the first 7 days after ICU admission, and were irregularly spaced over time.

## Missing Data

Details of missing data can be found in [Supplementary Table S1](#). To avoid loss of statistical power, all missing data were assumed to be missing at random and imputed using a random Forest-based imputation method.<sup>20</sup> Specifically, we used the R package MissForest, which handles missing data by iteratively using Random Forests. The superiority of the MissForest method over other methods for imputation of missing values has been reported, especially when faced with heavy missingness.<sup>21</sup>

## Outcomes

The primary outcome in the present study was 30-day mortality, which was defined as the occurrence of death at 30 days. Secondary outcomes included in-hospital mortality, length of ICU and hospital stay, which were defined as the occurrence of death during the hospital, and the duration of patients' ICU and hospital stays measured in days, respectively.

## Statistical Analysis

We used R statistical software (version 3.6.1), GraphPad Prism (version 8.0, San Diego, CA) or SPSS software (version 26.0, IBM, USA) to perform statistical analysis and create pictures. We first analyzed the normality of continuous data using the Kolmogorov–Smirnov test. We described continuous variables by mean with standard deviation (SD) if they are normally distributed or median with interquartile range (IQR) if they are not, and categorical variables by count with percentage (%). We estimated the significance of variations between groups using Student's *t*-test and Mann–Whitney *U*-test for normally and non-normally distributed continuous variables, respectively, and chi-squared test for categorical variables when appropriate.

Then, we assessed the relationship between the baseline eosinophils and adverse clinical outcomes in patients with ARDS. We arbitrarily divided the patients into two groups according to the median instead of the mean value of the baseline eosinophils, due to its skewed distribution. We used Kaplan–Meier survival analysis to compare the overall survival between the two groups. We further performed multivariable logistic regression analysis and smooth curve fitting to test the independent effects of the baseline eosinophils and 30-day and in-hospital mortality with crude and adjusted models. We purposefully selected potential confounders for this study based on their possible relationships with the outcome or a *p*-value less than 0.1 in the univariate analysis. Variables for inclusion were carefully chosen, given the number of events available, to ensure parsimony of the final model. As vital signs and laboratory parameters are usually included in the disease severity scores, we did not enter them into the multivariate model except for PaO<sub>2</sub>/FiO<sub>2</sub> and PEEP. The following variables were included: age, gender, BMI, ethnicity, tobacco history, admission type, hypertension, diabetes, asthma, acute kidney failure, tumor, sepsis, trauma, shock, APS III, OASIS, LODS, PaO<sub>2</sub>/FiO<sub>2</sub>, PEEP, mechanical ventilation, antibiotics, vasopressor, CRRT, and glucocorticoid. Besides, we built a random forest Random model to determine the feature importance of baseline eosinophils. Because comorbidities and certain medications may influence the baseline value of eosinophils, we therefore conducted a subgroup analysis for hypertension, coronary

atherosclerosis, diabetes, COPD, asthma, acute kidney failure, antibiotic and corticosteroids therapy on the first day of ICU admission.

Lastly, we analyzed the relationship between the early changes of eosinophils and 30-day mortality. We compared the difference of eosinophils between survivors and non-survivors during the first 7 days in ICU. Receiver operating characteristic (ROC) curves with area under the curve (AUC) values were used to evaluate the predictive value. We used a generalized additive mixed model (GAMM) to examine the temporal variation of early changes in eosinophils and mortality with crude and adjusted models. The GAMM is often applied to analyze the results of repeated measurements, especially when the interval between repeated measurements is irregular and some data is missing.<sup>22</sup>

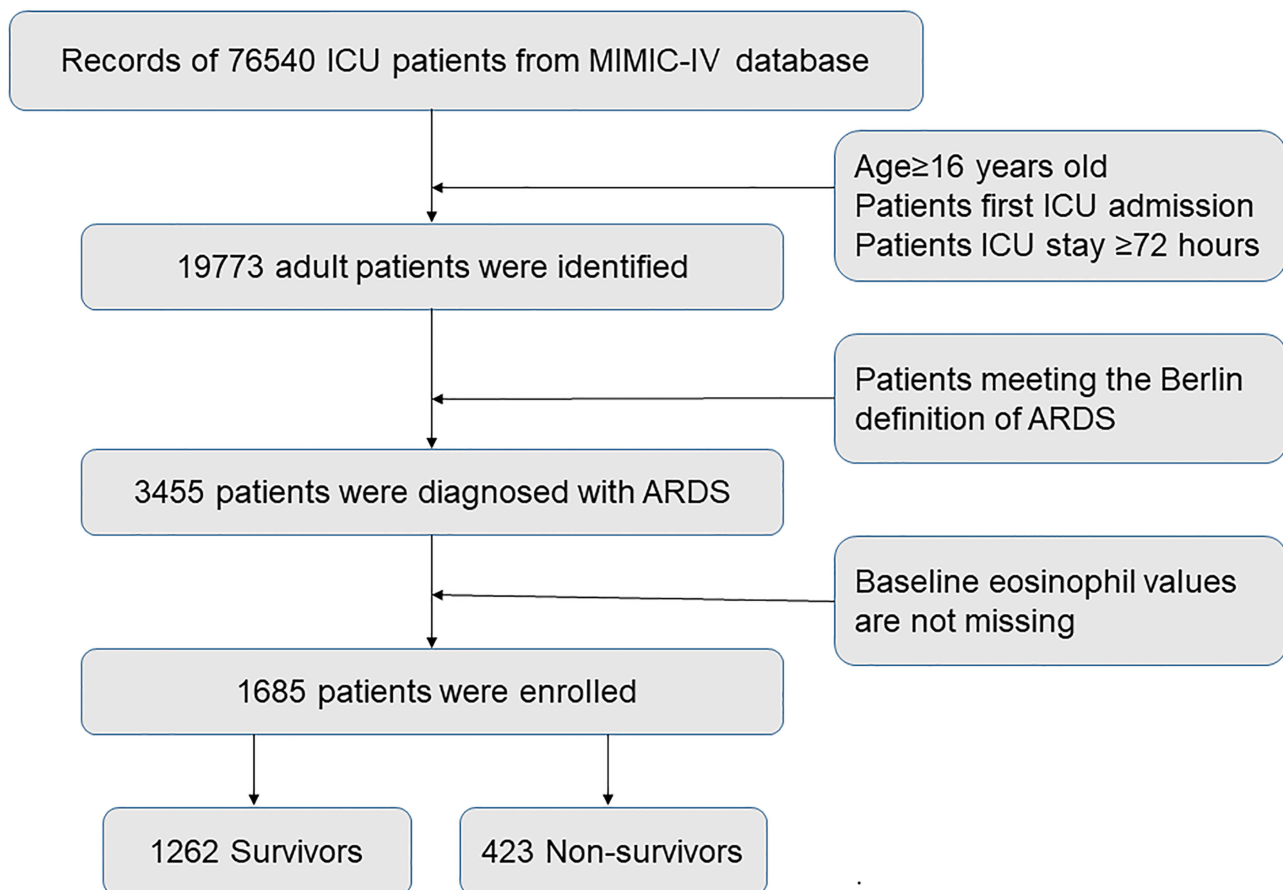
All tests were two-tailed. A  $p$ -value  $<0.05$  was considered significant.

## Results

### Basic Characteristics

After reviewing the data of 76,540 ICU patients in the MIMIC-IV database, we finally identified a total of 1685 patients in our study. Among them, 423 (25.1%) patients died within 30 days after admission to ICU. The flow chart of patient selections is presented in Figure 1.

Baseline characteristics of 30-day survivors and non-survivors are presented in Table 1. In general, survivors were more likely to be younger, male, non-white, and had lower BMI than non-survivors. A higher proportion of survivors were diagnosed with hypertension and diabetes, while a higher proportion of non-survivors were diagnosed with acute kidney failure and tumor. Concerning risk factors of ARDS, survivors were more likely to have trauma and less likely to have sepsis and shock than non-survivors. The severity scores, included APS III, OASIS, LODS, were all lower in the



**Figure 1** Flow chart of this study.

**Abbreviations:** ARDS, acute respiratory distress syndrome; ICU, intensive care unit; MIMIC, Medical Information Mart for Intensive Care.

**Table 1** Baseline Characteristics of Survivors and Non-Survivors at Day 30

Variables	Survivors (n=1262)	Non-Survivors (n=423)	p-value
Age (years)	62.47±15.63	64.70±15.08	0.011
Gender (males, %)	767(60.8)	234(55.3)	0.048
BMI (kg/m <sup>2</sup> )	30.75±8.12	29.24±7.78	0.001
Ethnicity			0.015
White	439(34.8)	175(41.4)	
Non-white	823(65.2)	248(58.6)	
Tobacco use (yes, %)	247(19.6)	79(18.7)	0.686
Admission type			0.1
Emergency	652(51.7)	199(47.0)	
Non-emergency	610(48.3)	224(53.0)	
<b>Comorbidity, n (%)</b>			
Hypertension	569(45.1)	167(39.5)	0.044
Coronary atherosclerosis	146(11.6)	44(10.4)	0.511
Diabetes	178(14.1)	41(9.7)	0.02
COPD	124(9.8)	36(8.5)	0.425
Asthma	121(9.6)	21(5.0)	0.003
Acute kidney failure	580(46.0)	298(70.4)	<0.001
Tumor	215(17.0)	131(31.0)	<0.001
<b>Risk factor, n (%)</b>			
Pneumonia	286(22.7)	85(20.1)	0.27
Sepsis	352(27.9)	224(53.0)	<0.001
Trauma	151(12)	30(7.1)	0.005
Shock	396(31.4)	224(53)	<0.001
Pancreatitis	70(5.5)	17(4.0)	0.219
Others	18(1.4)	3(0.7)	0.37
<b>Severity scale</b>			
APS III	62.61±26.63	91.24±29.91	<0.001
OASIS	39.54±8.50	45.59±8.15	<0.001
LODS	7.54±3.28	10.50±3.23	<0.001
<b>Baseline vital data</b>			
Heart rate (beats/min)	93.55±21.41	98.89±21.73	<0.001
MAP (mmHg)	82.83±19.64	82.28±19.67	0.677
RR (times/min)	20.81±6.85	23.13±7.26	<0.001
Temperature (°C)	36.83±1.00	36.79±1.09	0.482
SpO <sub>2</sub> (%)	96.27±4.45	95.24±5.30	<0.001
PaO <sub>2</sub> /FiO <sub>2</sub> (mmHg)	202.49±62.70	188.04±68.68	<0.001
Berlin classification, n (%)			<0.001
Mild	688(54.5)	189(44.7)	
Moderate	481(38.1)	181(42.8)	
Severe	93(7.4)	53(12.5)	
PEEP (cmH <sub>2</sub> O)	7.09±3.24	7.91±3.86	<0.001
Bicarbonate (mEq/L)	22.62±4.92	21.76±5.68	0.005
BUN (mg/dL)	26.93±20.74	35.46±24.84	<0.001
Creatinine (mg/dL)	1(0.7–1.5)	1.3(0.8–2)	<0.001
Lactate (mmol/L)	2.29±1.67	2.93±2.37	<0.001
Platelet (k/uL)	208.92±130.53	193.57±136.52	0.042
Glucose (mg/dL)	155.18±75.97	161.41±92.52	0.212
WBC (k/uL)	12.5(8.5–17.9)	13.15(7.7–20.2)	0.274
Eosinophils at day 1 (%)	0.4 (0–1.1)	0.1(0–0.6)	<0.001

(Continued)

**Table 1** (Continued).

Variables	Survivors (n=1262)	Non-Survivors (n=423)	p-value
<b>Treatment, n (%)</b>			
Mechanical ventilation	1154(91.4)	408(96.5)	0.001
Antibiotic	1210(95.9)	416(98.3)	0.017
Vasopressor	215(17.0)	173(40.9)	<0.001
CRRT	115(9.1)	105(24.8)	<0.001
Glucocorticoid	174(13.8)	125(29.6)	<0.001

**Notes:** Data were presented as mean±standard deviation or median (interquartile range) or numbers (percentages). p-values comparing groups were from Student's *t*-test or Mann–Whitney *U*-test for continuous variables and chi-squared test for categorical variables.

**Abbreviations:** APS III, Acute Physiology Score III; BMI, body mass index; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; CRRT, continuous renal replacement therapy; LODS, Logistic Organ Dysfunction System; MAP, mean arterial pressure; OASIS, Oxford Acute Severity of Illness Score; PaO<sub>2</sub>/FiO<sub>2</sub>, arterial oxygen partial pressure/fraction of inspired oxygen; PEEP, positive end-expiratory pressure; RR, respiratory rate; WBC, white blood cells.

group of survivors, which indicated that non-survivors were more critically ill than the survivors upon ICU admission. On day 1, the median value of eosinophils was 0.3% (IQR, 0–1.0%) in all patients, and survivors had higher value of eosinophils than non-survivors [0.4% (IQR, 0–1.1%) vs 0.1% (IQR, 0–0.6%),  $p<0.001$ ]. Moreover, we found that there were significant differences in terms of vital signs (heart rate, respiratory rate, SpO<sub>2</sub>, PaO<sub>2</sub>/FiO<sub>2</sub>), ventilator and laboratory parameters (PEEP, bicarbonate, BUN, creatinine, lactate), and treatment (mechanical ventilation, antibiotics, vasopressor, CRRT, and glucocorticoid) between the two groups.

## Association Between Baseline Eosinophils and Mortality

We divided all the patients into two groups based on the median of baseline eosinophils. As shown in [Table 2](#), patients with high eosinophils (>0.3%) had shorter length of ICU and hospital stay, and lower 30-day and in-hospital mortality. The Kaplan–Meier curve also revealed significant differences in the 30-day and in-hospital mortality among patients stratified according to baseline eosinophils ([Figure 2A](#) and [B](#)). Besides, the random forest model selected the baseline eosinophils as the 8th important factor in predicting mortality ([Figure 3](#)). The subgroup analyses suggested that the significant inverse relationship between baseline eosinophils and the risk of mortality still existed except for patients with COPD, asthma and corticosteroids therapy on the first day of ICU admission ([Supplementary Table S2](#)).

We performed a smooth curve fitting to illustrate the association between baseline eosinophils and the risk of in-hospital and 30-day mortality. Taking eosinophils on admission as a continuous variable, we observed a significant negative correlation between eosinophils and short-term mortality in the adjusted model ([Figure 4A](#) and [B](#)).

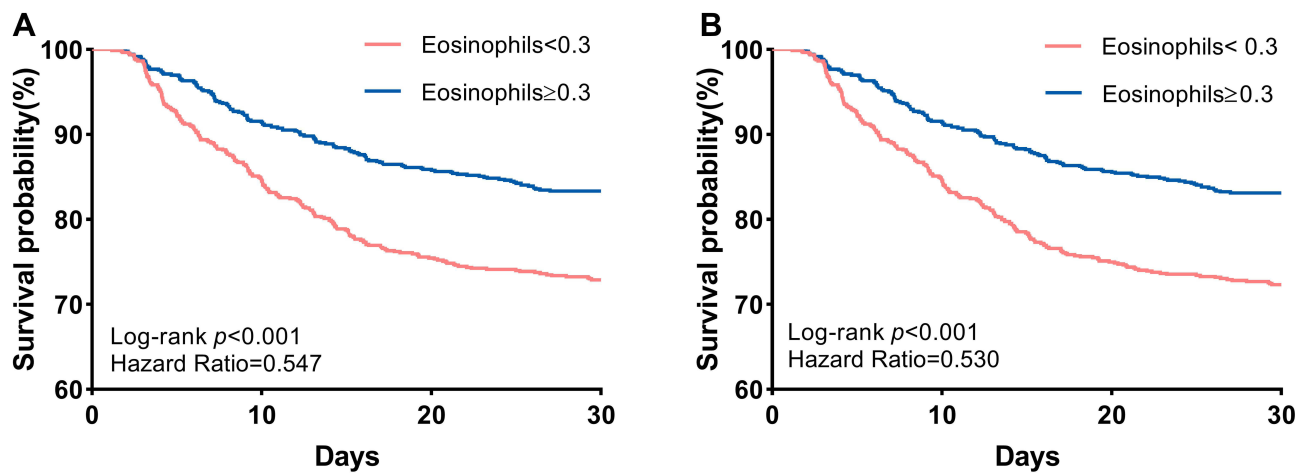
We also conducted the multivariable logistic regression analysis to further test our findings, which was shown in [Table 3](#). When we treated eosinophils as a categorical variable, patients with high eosinophils (>0.3%) had a significantly lower risk of 30-day mortality (OR 0.743, 95% CI 0.568–0.970,  $p=0.029$ ), as well as a lower but not significant risk of in-hospital mortality

**Table 2** Relationship Between Baseline Eosinophils and Clinical Outcomes

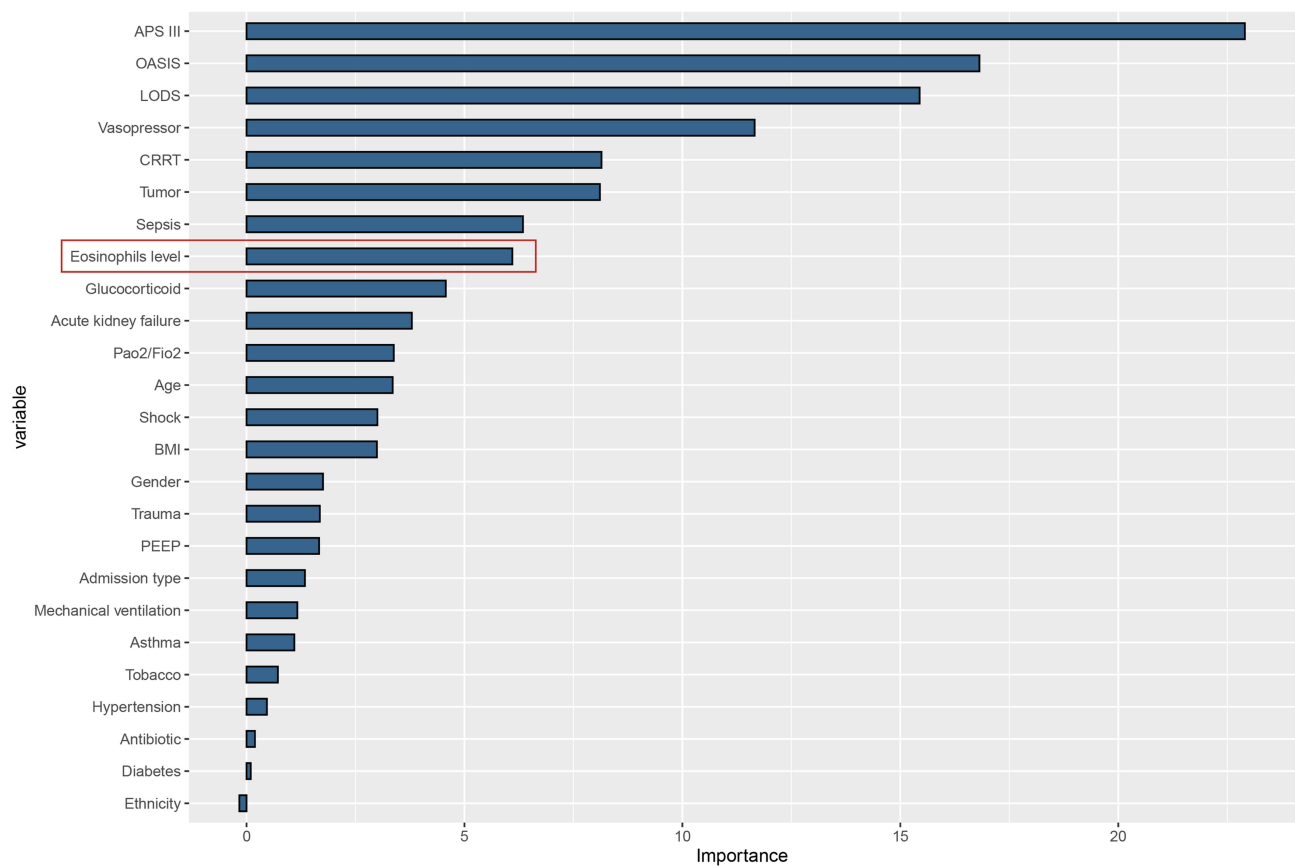
Variables	All Patients	Eosinophils (%)		p-value
		≤0.3	>0.3	
n	1685	820	865	
LOS ICU (days)	7.03(4.31–12.08)	7.59(4.74–13.25)	6.39(4.14–10.99)	<0.001
LOS hospital (days)	13.70(8.61–21.91)	14.44(9.11–23.51)	13.04(8.06–20.38)	0.001
In-hospital mortality, n (%)	410 (24.3)	252(30.7)	158(18.3)	<0.001
30-day mortality, n (%)	423 (25.1)	263(32.1)	160(18.5)	<0.001

**Notes:** Data were presented as median (interquartile range) or numbers (percentages). p-values comparing groups were from Mann–Whitney *U*-test for continuous variables and chi-squared test for categorical variables.

**Abbreviations:** LOS hospital, length of hospital stay; ICU LOS, length of intensive care unit stay.



**Figure 2** Survival curves for the relationship between baseline eosinophils and risk of 30-day mortality (A)/in-hospital mortality (B).

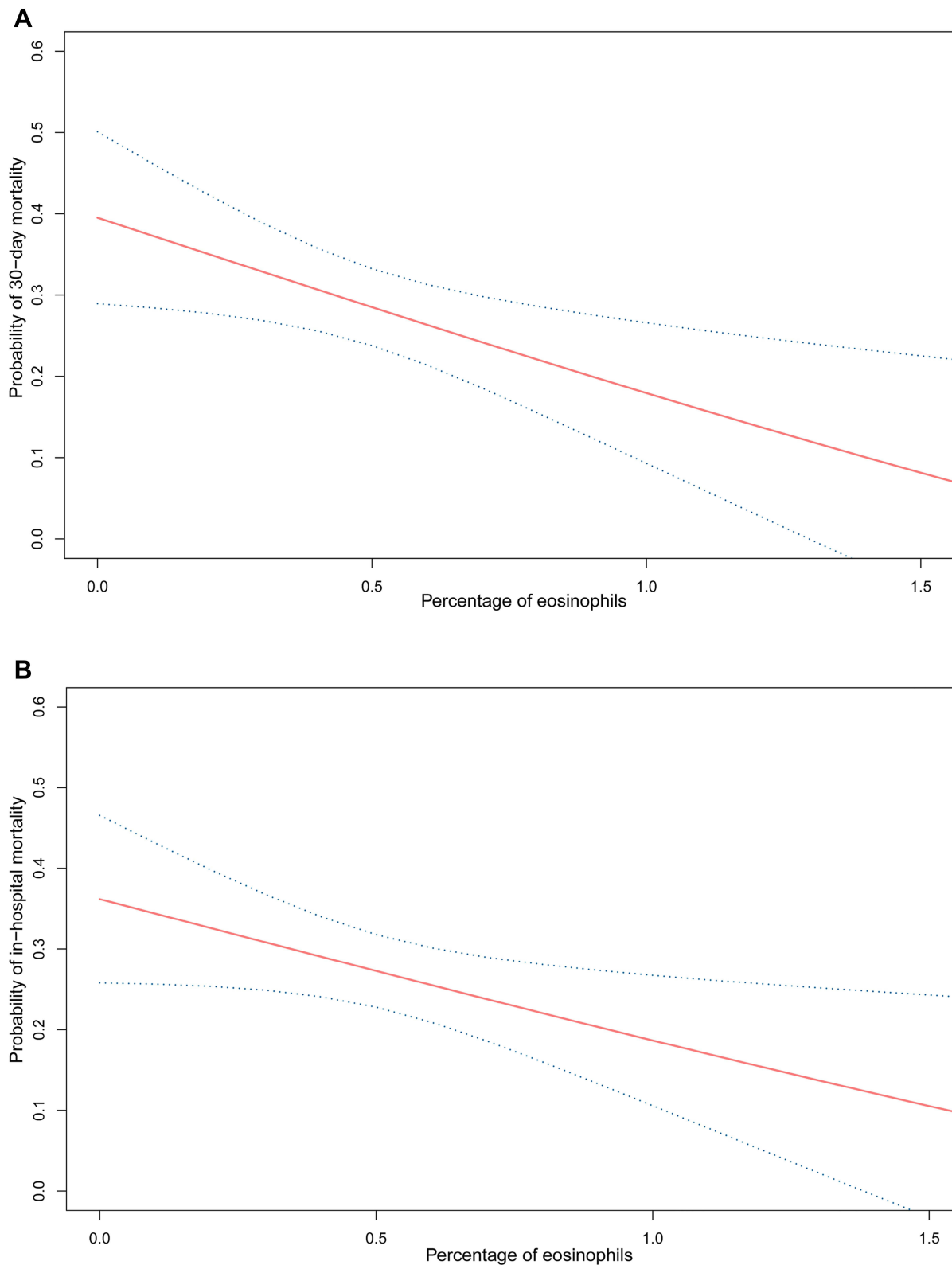


**Figure 3** Random forest feature importance with 30-day mortality.

**Note:** The red box indicates the importance of the baseline eosinophils level in the random forest model.

**Abbreviations:** APS III, Acute Physiology Score III; BMI, body mass index; CRRT, continuous renal replacement therapy; LODS, Logistic Organ Dysfunction System; OASIS, Oxford Acute Severity of Illness Score; PaO<sub>2</sub>/FIO<sub>2</sub>, arterial oxygen partial pressure/fraction of inspired oxygen; PEEP, positive end-expiratory pressure.

(OR 0.794, 95% CI 0.607–1.040,  $p=0.094$ ) than patients with low eosinophils ( $\leq 0.3\%$ ) in the full adjusted model. However, when we treated eosinophils as a continuous variable, this association was no longer present (all  $p>0.05$ ).



**Figure 4** A smooth curve fitting for the relationship between the baseline eosinophils and the risk of 30-day mortality (**A**)/in-hospital mortality (**B**).

**Notes:** The resulting figures show the risk of mortality in the y-axis and the eosinophils (continuous variable) in the x-axis. The red line shows the dose-response curve between eosinophils and probability of short-term mortality, the two blue lines refer to 95% confidence intervals. A negative relationship between eosinophils and the risk of short-term mortality was observed after adjusting for age, gender, BMI, ethnicity, tobacco history, admission type, hypertension, diabetes, asthma, acute kidney failure, tumor, sepsis, trauma, shock, APS III, OASIS, LODS, PaO<sub>2</sub>/FiO<sub>2</sub>, PEEP, mechanical ventilation, antibiotic, vasopressor, CRRT, and glucocorticoid by spline smoothing plot.

**Abbreviations:** APS III, Acute Physiology Score III; BMI, body mass index; CRRT, continuous renal replacement therapy; LODS, Logistic Organ Dysfunction System; OASIS, Oxford Acute Severity of Illness Score; PaO<sub>2</sub>/FiO<sub>2</sub>, arterial oxygen partial pressure/fraction of inspired oxygen; PEEP, positive end-expiratory pressure.



**Table 3** Multivariate Logistic Regression Analysis of Baseline Eosinophils for Mortality

Exposure	Non-Adjusted			Adjusted Model I			Adjusted Model II		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
<b>30-day mortality</b>									
Eosinophils Per 1sd	0.939	(0.872–1.012)	0.1	0.952	(0.886–1.022)	0.173	0.973	(0.907–1.044)	0.451
<b>Eosinophil dichotomy</b>									
T1	1	(Reference)		1	(Reference)		1	(Reference)	
T2	0.481	(0.384–0.602)	<0.001	0.496	(0.395–0.624)	<0.001	0.743	(0.568–0.970)	0.029
<b>In-hospital mortality</b>									
Eosinophils Per 1sd	0.95	(0.884–1.021)	0.167	0.961	(0.896–1.029)	0.255	0.983	(0.916–1.056)	0.639
<b>Eosinophil dichotomy</b>									
T1	1	(Reference)		1	(Reference)		1	(Reference)	
T2	0.504	(0.401–0.632)	<0.001	0.518	(0.411–0.653)	<0.001	0.794	(0.607–1.040)	0.094

**Notes:** T1 means the percentage of eosinophils  $\leq 0.3\%$ ; T2 means the percentage of eosinophils  $> 0.3\%$ . Model I was adjusted for age, gender, ethnicity, tobacco, BMI, admission type. Model II was adjusted for age, gender, BMI, ethnicity, tobacco history, admission type, hypertension, diabetes, asthma, acute kidney failure, tumor, sepsis, trauma, shock, APS III, OASIS, LODS, PaO<sub>2</sub>/FiO<sub>2</sub>, PEEP, mechanical ventilation, antibiotic, vasopressor, CRRT, and glucocorticoid.

**Abbreviations:** OR, odds ratio; CI, confidence interval.

## Association Between Early Changes in Eosinophils and Mortality

We compared the changes in eosinophils at early time points between the 30-day survivors and non-survivors. In univariate analyses, we observed significant differences in eosinophils on admission, the 2–3rd, the 4–5th, and the 6–7th day between the two groups (Figure 5A–D, Table 4). Compared to the baseline eosinophils in the analysis of ROC curves, the change in eosinophils within the first week after ICU admission had a significantly higher AUC for 30-day mortality ( $p < 0.001$ ; Supplementary Figure S1).

We next applied GAMM to characterize the changes in eosinophils over time (the first week of ICU admission) between 30-day survivors and non-survivors after adjustment for the listed confounders. As shown in Figure 6A and B, we found that although eosinophils increased over time in both groups, the trend in survival group was much more apparent. Moreover, the between-group difference was gradually increased with time elapse.

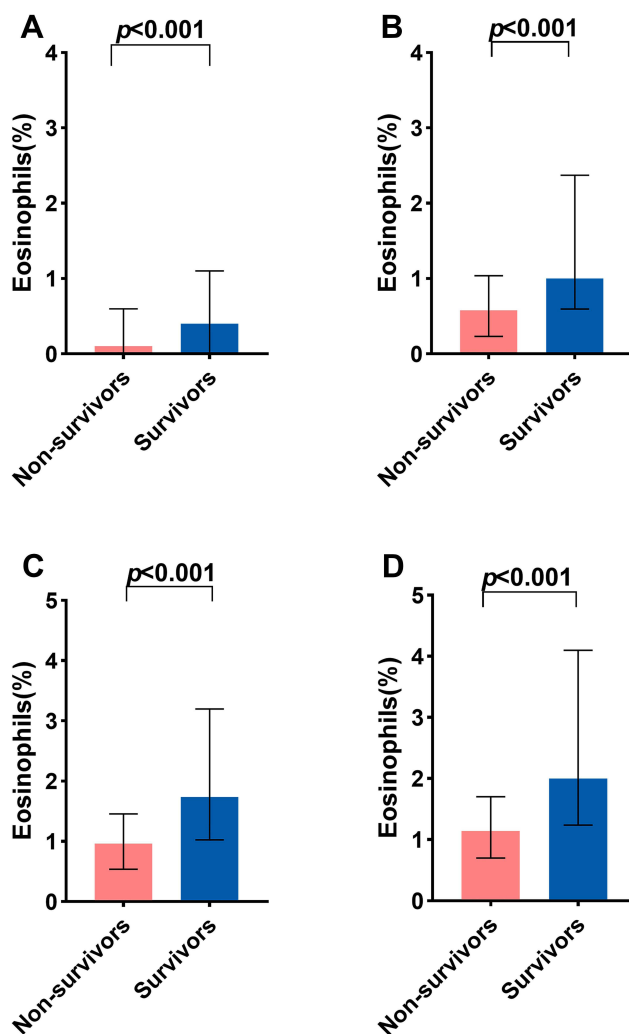
Furthermore, we explored the early longitudinal changes of eosinophils for prediction of 30-day mortality in patients with ARDS. As shown in Table 5, the level of eosinophils in the survival group was significantly higher than that in the non-survival group, and particularly the between-group difference presented an increasing trend during the first week of ICU admission, with an average of 0.154 per day. This association remained significant after adjusting for confounding factors ( $\beta = 0.154$ , 95% CI 0.138–0.169,  $p < 0.001$ ). The same results were observed for in-hospital mortality.

## Discussion

The present study is the first to explore the dynamic changes in eosinophils for prediction of adverse outcomes in ICU patients with ARDS. Our results show that high eosinophils at ICU admission was associated with lower 30-day and in-hospital mortality, as well as shorter ICU and hospital stays in patients with ARDS. Moreover, survivors had a persistently high eosinophil levels from ICU admission to day 7, and the between-group difference was gradually increased over time. This association remained materially unchanged after adjusting for potential confounders. Therefore, dynamic monitoring of eosinophils could help identify patients with better survival probabilities.

ARDS is a heterogeneous syndrome with a variety of risk factors.<sup>1</sup> Inflammation plays a pivotal role in the pathogenesis of ARDS,<sup>5</sup> which is associated with a concerted response of the innate immune system.<sup>6</sup> Eosinophil is an important innate immune cell. Bass et al<sup>23</sup> have shown that eosinophils can be activated by acute inflammation contributing from the infectious or non-infectious sources, instead of a specific type of pathogen. In ARDS, the level of eosinophil cationic protein (ECP), a specific granule protein of eosinophils, was increased,<sup>7</sup> and lavage ECP levels correlated strongly with the severity of lung damage.<sup>9</sup> Taken together, these findings indicate a pathophysiologic role for eosinophils in ARDS.

It has been recognized that the level of eosinophil was associated with the short-term prognosis in critical illness.<sup>24,25</sup> Abidi et al<sup>24</sup> found that eosinophils remained significantly lower in non-survivors from ICU admission to seventh day



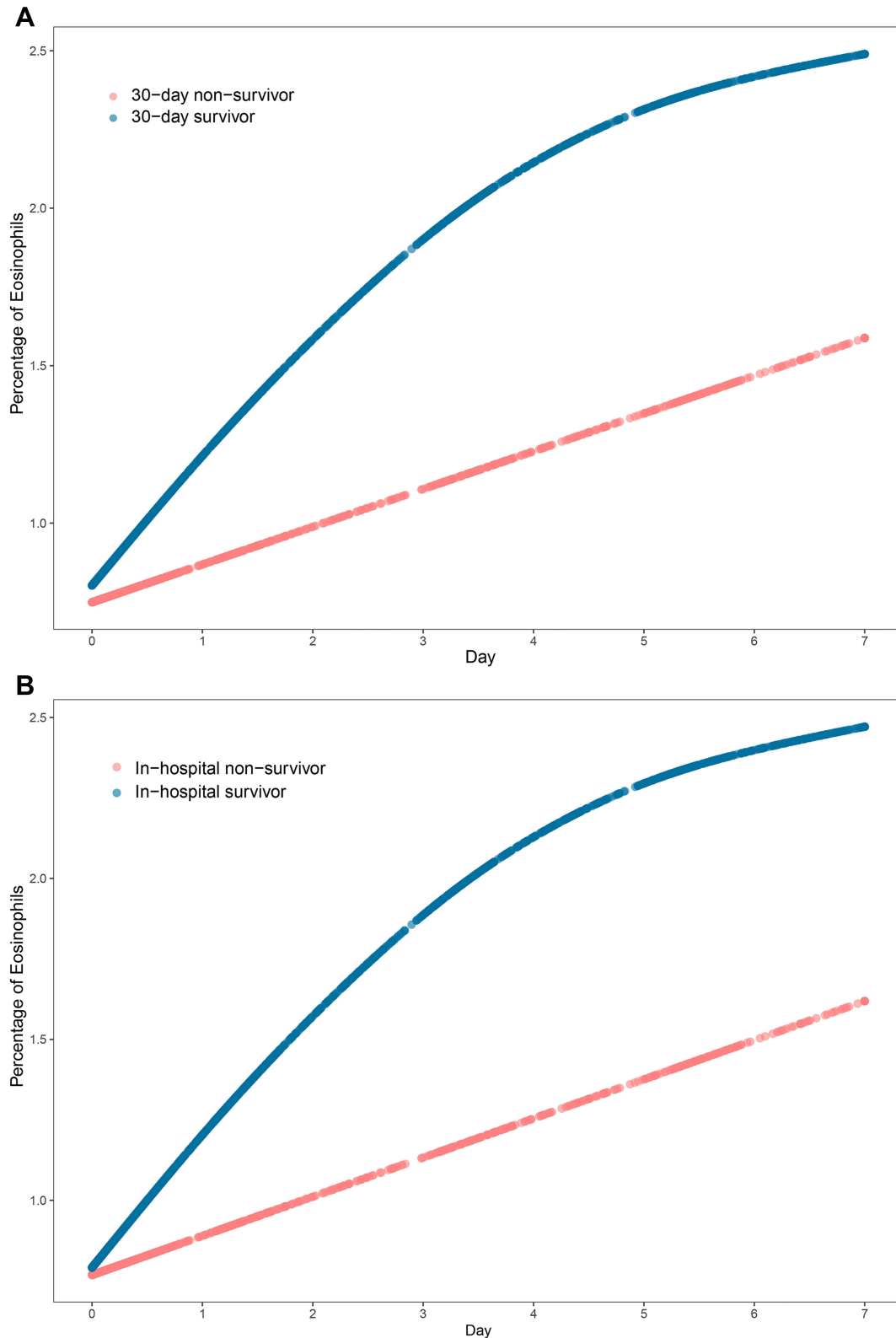
**Figure 5** Differences of eosinophil levels on the 1 day (A), 2–3 days (B), 4–5 days (C), 6–7 days (D) between the 30-day survivors and non-survivors. **Notes:** Data are presented as median (interquartile range). *p*-values are calculated using Mann–Whitney *U*-test.

than that in survivors, and lower eosinophil level was an independent predictor of 28-day all-cause mortality. Of note, the study population in their report was general hospitalized ICU patients and the number of included patients was relatively small ( $n=200$ ), limiting the ability to extrapolate their findings to a specific population. Up to date, there are only two published studies that have described the relationship between blood eosinophils and clinical outcomes in patients with ARDS.<sup>11,13</sup> Zhu et al<sup>11</sup> observed that following clinical treatment, patients with increased levels of blood eosinophils at discharge are associated with increased survival. Moreover, Chen et al<sup>13</sup> found that patients with higher baseline eosinophils had lower 28-day mortality. However, in the two studies, the blood eosinophils were only detected at

**Table 4** Comparison of the Early Changes (0–7 Days) in Eosinophils Between 30-Day Survivors and Non-Survivors

Time	Survivors	Non-Survivors	<i>p</i> -value
On admission	0.4 (0–1.1)	0.1(0–0.6)	<0.001
On 2–3rd day	1.0(0.6–2.37)	0.58(0.23–1.04)	<0.001
On 4–5th day	1.74(1.03–3.20)	0.96(0.54–1.46)	<0.001
On 6–7th day	2.00(1.24–4.1)	1.14(0.7–1.7)	<0.001

**Notes:** Data were presented as median (interquartile range). *p*-values comparing groups were from Mann–Whitney *U*-test.



**Figure 6** Association between the early changes (0–7 days) in eosinophils and 30-day mortality **(A)**/ in-hospital mortality **(B)**.

**Notes:** A linear association between changes in eosinophils and mortality was found in a generalized additive mix model (GAMM). Smooth curve fitting graph illustrated the eosinophils based on the days after admission to ICU. The red line represented the survivors. The blue line represented the non-survivors. All adjusted for age, gender, BMI, ethnicity, tobacco history, admission type, hypertension, diabetes, asthma, acute kidney failure, tumor, sepsis, trauma, shock, APS III, OASIS, LODS, PaO<sub>2</sub>/FiO<sub>2</sub>, PEEP, mechanical ventilation, antibiotic, vasopressor, CRRT, and glucocorticoid.

**Abbreviations:** APS III, Acute Physiology Score III; BMI, body mass index; CRRT, continuous renal replacement therapy; LODS, Logistic Organ Dysfunction System; OASIS, Oxford Acute Severity of Illness Score; PaO<sub>2</sub>/FiO<sub>2</sub>, arterial oxygen partial pressure/fraction of inspired oxygen; PEEP, positive end-expiratory pressure.

**Table 5** Relationship Between Early Changes (0–7 Days) in Eosinophils and Mortality in Patients with ARDS Derived from a Generalized Additive Mixed Model (GAMM)

30-Day Mortality	Unadjusted		Adjusted Model I		Adjusted Model II	
	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value
Death	-0.194 (-0.297, -0.092)	0.058	-0.140 (-0.242, -0.038)	0.172	0.180 (0.070, 0.290)	0.101
Day	0.275 (0.268, 0.283)	<0.001	0.275 (0.268, 0.283)	<0.001	0.274 (0.267, 0.282)	<0.001
Day*death	0.154 (0.139, 0.169)	<0.001	0.154 (0.139, 0.169)	<0.001	0.154 (0.138, 0.169)	<0.001
In-hospital mortality	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value	$\beta$ (95% CI)	p-value
Death	-0.171 (-0.275, -0.068)	0.099	-0.124 (-0.227, -0.020)	0.231	0.204 (0.093, 0.315)	0.067
Day	0.274 (0.266, 0.281)	<0.001	0.274 (0.266, 0.281)	<0.001	0.273 (0.266, 0.281)	<0.001
Day*death	0.152 (0.137, 0.167)	<0.001	0.152 (0.136, 0.167)	<0.001	0.151 (0.136, 0.167)	<0.001

**Notes:** Values indicate the estimated effect ( $\beta$ ) and 95% confidence interval (CI). Day\* death means the average daily increased differences in eosinophils between the survival and non-survival groups. Model I was adjusted for age, gender, ethnicity, tobacco, BMI, admission type. Model II was adjusted for age, gender, BMI, ethnicity, tobacco history, admission type, hypertension, diabetes, asthma, acute kidney failure, tumor, sepsis, trauma, shock, APS III, OASIS, LODS, PaO<sub>2</sub>/FiO<sub>2</sub>, PEEP, mechanical ventilation, antibiotic, vasopressor, CRRT, and glucocorticoid.

a single time point, and were not evaluated for their dynamic changes. Our study further provided evidence of the relationship between early changes in eosinophils and mortality in patients with ARDS, which may better reflect the dynamic process of the disease situation.

In present study, we found that patients with high level of baseline eosinophils (>0.3%) had significantly lower 30-day mortality than non-survivors in univariate and multivariate analyses, which is similar to previous studies.<sup>13,24</sup> The results from Kaplan–Meier curve and random forest model also supported the above findings. Of note, when we regarded blood eosinophils as a continuous variable, no significant association was found in the multivariate logistic regression analysis. The reason might be the distribution of baseline eosinophil values was skewed, with a high proportion of zero or extremely low data, and this was also evident in previous studies.<sup>11,13</sup> The statistical power for the logistic regression would be considerably influenced in the case of highly skewed data.<sup>26</sup> By using a categorical variable, the data processing is significantly simplified, which leads to improved robustness of the model. Moreover, in the subgroup analysis, we found that the protective effect of high baseline eosinophils on short-term mortality still existed, except for patients with COPD, asthma and corticosteroids therapy on the first day of ICU admission. However, after excluding patients with these conditions, the significant differences in baseline eosinophils between the survivors and non-survivors remained, indicating that these factors might be surrogate markers but not necessarily the main reason for the difference in baseline eosinophils between the two groups.

Subsequently, we used the GAMM to explore the temporal variation of eosinophils in ARDS patients and its relationship with short-term outcomes. Our result showed that the levels of eosinophils were increased over time in both survival and non-survival groups, and the between-group differences of eosinophils remained significant within the first week after ICU admission. Further, we found the between-group difference presented an increasing trend (an average of 0.154 per day) during the first week in ICU based on the GAMM analysis. The AUC of early changes in eosinophils was significantly higher than that of baseline eosinophils, suggesting a better predictive value for mortality. Taken together, these results indicate that dynamic monitoring of eosinophils can predict the survival probabilities of patients with ARDS. Moreover, several studies have also suggested that the longitudinal changes in eosinophils are helpful to predict prognosis in some diseases, such as COVID-19, COPD and septic shock.<sup>25,27,28</sup>

Our study suggests that eosinophils exert a possible protective role in ARDS, and some possible mechanisms might explain this protective effect. Zhu et al<sup>11</sup> found that CD101<sup>+</sup> eosinophils, a subtype of eosinophils, could attenuate neutrophil recruitment and accelerate clean-up of neutrophil debris through the production of protectin D1 in lipopolysaccharide (LPS)-induced ARDS murine model. Besides, Krishack et al<sup>12</sup> observed that IL-33–mediated eosinophilia protects against acute lung injury, by reducing neutrophil-associated proinflammatory cytokines and mobilizing tissue repair responses in the bacteria-induced pneumonia model. Eosinophils have been reported to participate in the process of tissue regeneration and repair by producing growth factors.<sup>29</sup> Moreover, Willetts et al<sup>10</sup> found that surviving patients with ARDS had higher lung eosinophil numbers and increased

eosinophil degranulation than non-surviving patients. Taken together, these findings indicate that the protective effect of eosinophils is mainly achieved by alleviating neutrophil-dependent inflammation and accelerating lung repair.

There are several limitations in our present study. First, we only used the blood eosinophil concentrations for analysis. The blood eosinophil counts were not discussed, as a large proportion of them are missed in the MIMIC-IV database. The study would be more complete if we take blood eosinophil counts into account together. Second, we did not analyze the outcomes stratified by different subtypes of eosinophils (eg CD101<sup>+</sup> and CD101<sup>-</sup>), because the blood specimens were unavailable in this study. Third, we did not have information about the treatment regimens (especially for corticosteroids) before hospitalization, which might alter the value of eosinophils. Fourth, due to the retrospective nature of the study, missing data and a possible selection bias could have affected the outcome. Our results should be considered exploratory rather than definitive. Therefore, more in-depth studies with prospective design are required to validate and further expand our findings.

## Conclusions

Our present study revealed that increased baseline eosinophils were associated with better clinical outcomes, such as decreased 30-day and in-hospital mortality as well as shortened ICU and hospital length of stay, in patients with ARDS. Moreover, a significant difference in the changes of eosinophils over time were observed between survivors and non-survivors. Further, we found a relationship between a higher increased trend of eosinophils and lower short-term mortality in patients with ARDS. Taken together, our results indicate the potential benefit of monitoring early changes in eosinophils for prediction of short-term mortality in patients with ARDS. As a peripheral biomarker, blood eosinophil concentration may reflect lung pathology in this cohort of ARDS patients. Further studies may be suggested to clarify the role of eosinophils as a protective factor.

## Abbreviations

APS III, Acute Physiology Score III; ARDS, acute respiratory distress syndrome; AUC, area under the curve; BMI, body mass index; BUN, blood urea nitrogen; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CRRT, continuous renal replacement therapy; ECP, eosinophil cationic protein; GAMM, generalized additive mixed model; ICU, intensive care unit; IQR, interquartile range; LODS, Logistic Organ Dysfunction System; LOS, length of hospital stay; LPS, lipopolysaccharide; MAP, mean arterial pressure; MIMIC, Medical Information Mart for Intensive Care; OASIS, Oxford Acute Severity of Illness Score; OR, odds ratio; PaO<sub>2</sub>/FiO<sub>2</sub>, arterial oxygen partial pressure/fraction of inspired oxygen; PEEP, positive end-expiratory pressure; ROC, receiver operating characteristic; RR, respiratory rate; SD, standard deviation; WBC, white blood cells.

## Ethics Approval and Consent to Participate

The establishment of this database was approved by the Institutional Review Boards of the Massachusetts Institute of Technology, and consent was obtained for the original data collection. The study was reviewed and considered exempt by the Ethics Committees of the Second Affiliated Hospital of Chongqing Medical University (NO. 2021-113).

## Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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