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RESEARCH ARTICLE

Dong et al: Lymphocyte subsets predict PQ mortality

Lymphocyte subsets predict mortality in acute paraquat poisoning

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ABSTRACT

Paraquat (PQ) is a highly effective herbicide widely used in agricultural production, known for its strong herbicidal power, rapid action, and minimal environmental pollution. However, it is also highly toxic to humans and animals, with acute lung injury (ALI) being the primary cause of death. While the toxic mechanisms of paraquat have been studied from various perspectives, its effects on lymphocytes and their subsets remain unclear. This study aimed to explore the relationship between lymphocyte dysfunction and mortality in acute paraquat poisoning. A total of 92 patients with paraquat poisoning who visited the emergency department of the Affiliated Lihuili Hospital of Ningbo University between January 1, 2016, and September 30, 2021, were included. Basic demographic and laboratory data within 24 hours of admission were collected. Peripheral blood lymphocyte subsets were analyzed using flow cytometry. To identify independent risk factors for mortality, patients were followed up for 90 days. COX proportional hazards models and LASSO regression were applied to screen for predictive variables and develop a predictive model. All participants provided informed consent, and the study was approved by the relevant ethics committee. Among the 92 patients, 36 died. Compared with the survival group, the death group showed significantly higher white blood cell and neutrophil counts, lymphocyte counts, and CD4+/CD8+ T cell ratios, while the percentage of natural killer (NK) cells was significantly lower ($P < 0.001$). COX regression analysis identified these factors as independent risk factors for mortality: Lymphocyte count: Hazard ratio (HR) = 1.59; 95% confidence interval (CI), 1.02–2.47; $P = 0.04$ Neutrophil count: HR = 1.12; 95% CI, 1.06–1.18; $P = 0.04$CD4+/CD8+ T cell ratio: HR = 2.01; 95% CI, 1.03–3.92; $P = 0.04$NK cell percentage: HR = 0.88; 95% CI, 0.82–0.95; $P = 0.002$. These findings suggest that lymphocyte count, neutrophil count, CD4+/CD8+ T cell ratio, and NK cell percentage are all associated with mortality in paraquat poisoning cases.

Keywords: Lymphocyte subsets; paraquat; PQ; paraquat poisoning; mortality rate.

INTRODUCTION

Paraquat (PQ), chemical known as 1-1-dimethyl-4-4-bipyridinium cation, is a non-selective and highly effective herbicide. Since the 1960s, it has been widely used in agricultural production around the world, especially in developing countries [1]. Due to the lack of specific antidotes and effective treatment measures, the mortality rate of paraquat poisoning is as high as 50% to 90% [2]. In recent decades, with the tremendous progress in the field of immunotoxicology, immunotoxicity has become an important indicator for the evaluation of many pesticide compounds [3]. Paraquat, as an organic heterocyclic herbicide, can cause dysfunction of the body's immune cells. However, current research on PQ's effects on the immune system is inconsistent. Some scholars suggest that PQ can inhibit the body's cellular and humoral immune functions [4-6], while other scholars have proposed that PQ can stimulate the body to exert immune regulatory functions and activate the expression of inflammatory factors [7-9]. Therefore, in order to clarify the immunotoxicity of PQ, it is necessary to further study the effects of PQ on immune cells.

MATERIALS AND METHODS

Study subjects

We retrospectively analyzed 114 patients with paraquat poisoning who were hospitalized in the Emergency Department of Li Huili Hospital, Ningbo University, from January 2016 to September 2021. The inclusion criteria were: (1) aged 18 to 75 years; (2) a history of oral paraquat ingestion; (3) blood or urine PQ concentration greater than or equal to 0.1 mg/L. The exclusion criteria were: (1) combined poisoning with other pesticides or drugs; (2) history of blood disorders, tumors, severe infections, or organ dysfunction; (3) time from poisoning to admission exceeding 48 hours. Based on these criteria, 92 patients were finally included in the study.

Treatment options

All subjects were given a standardized treatment regimen: (1) General treatment: all patients should be given no water, fasting, and no swallowing saliva immediately; and rinse the mouth with new rehabilitation solution and compound chlorhexidine gargle to keep the mouth clean; If the patient's arterial partial pressure of oxygen is greater than 40 mmHg, oxygen therapy is contraindicated. (2) Reduce toxic absorption: all patients should immediately change clothing contaminated with pesticides; Rinse the stomach

repeatedly and thoroughly with water, and thoroughly wash the contaminated skin and mucous membranes. (3) Promote the excretion of poisons: all patients were given furosemide to push diuresis; After admission, the patient should immediately take urine for urine colorimetric examination (the colorimetric agent is composed of 2% sodium thiosulfate and 2mol/L sodium hydroxide, according to the volume ratio of 1:1, stored in a constant temperature refrigerator at -4 °C), the examination results should be compared with the standard colorimetry, and those with positive urine colorimetry should undergo temporary deep venous catheterization blood purification treatment. (4) Reduce the functional damage of various organs and prevent related complications: all patients were treated with intravenous vitamin C, reduced glutathione and ambroxol hydrochloride antioxidant therapy; Omeprazole is given to inhibit gastric acid secretion, protect gastric mucosa, and prevent gastrointestinal bleeding; Ceftazidime is given to prevent infection; Patients with positive urine colorimetry are given intravenous methylprednisolone. (5) Rehydration nutrition support and maintenance of internal environmental homeostasis: according to the patient's urine output, intravenous fluids ranging from 4000ml to 6000ml per day; Electrolytes should be supplemented appropriately according to the patient's electrolyte profile.

Ethical statement

This study was approved by Li Huili Hospital of Ningbo University Institutional Review Board (IRB: KY2023SL371-01)，informed written consent was obtained from the patients or their nearest relatives.

Research methods

Data collection

The following information was recorded for each patient: name, gender, age, hospitalization number, oral paraquat dose, and the time interval between poisoning and admission to our hospital. All patients were sent to our hospital within 48 hours of poisoning, none of the enrolled patients had undergone self-treatment. Laboratory tests were completed within 24 hours of admission, before receiving therapeutic intervention, including blood routine tests, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT),

blood urea nitrogen (BUN), serum creatinine (SCr), immunoglobulin (Ig) G, IgA, IgM, complement (C3, C4), and arterial blood gas analysis. Blood and urine samples were immediately collected for initial urine colorimetric analysis of paraquat (PQ) concentration, followed by further testing at the Shanghai Pesticide Research Institute using high-performance liquid chromatography (HPLC) to measure the precise PQ concentration in blood and urine.

Additionally, all patients were assessed upon admission with the Acute Physiology and Chronic Health Evaluation (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores. A higher SOFA score indicates more severe organ dysfunction, which is associated with a higher risk of mortality in PQ poisoning patients. Flow cytometry was used to detect peripheral blood lymphocyte subsets, including the $CD4^+/CD8^+$ T-cell ratio, B-cell percentage, and NK-cell percentage. All patients were followed up until death or 90 days post-poisoning.

Statistical methods

SPSS 26.0 statistical software was used for data analysis. Normally distributed data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and the independent sample t test was used for comparison between two groups, while one-way ANOVA was used for comparison among multiple groups. Non-normally distributed data were expressed as medians and quartiles, and the rank sum test was used for inter-group comparison. Chi-square test was used for constituent ratio. The Kaplan-Meier method was used for survival curve analysis. The COX proportional hazard model was used to evaluate whether the indicator was an independent risk factor. Variables with a univariate analysis significance level ($P < 0.1$) were included in the multivariate risk model for further analysis, and the forward stepwise method was used to eliminate the collinearity between variables. $P < 0.05$ indicated that the difference was statistically significant. The nomogram prediction model was established based on multivariate COX and LASSO COX regression, and the calibration curves, decision curves, C-index charts, and ggrisk plots were constructed to evaluate model performance.

RESULTS

Patient characteristics

According to the inclusion and exclusion criteria described above, a total of 114 PQ poisoning patients were initially identified, with no cases lost to follow-up. However, 22 patients were excluded for not meeting the criteria, leaving 92 patients for the study (Figrue 1). All patients were followed until death or 90 days post-poisoning. Among them, 56 patients (60.9%) survived, while 36 patients (39.1%) died. The cohort consisted of 37 males (mean age: 26.75 years) and 55 females (mean age: 31.50 years). Comparisons between the survival and death groups revealed the following: (1) the PQ oral ingestion volume, plasma PQ concentration, urinary PQ concentration, APACHEII and SOFA scores, WBC and neutrophil count, CRP, BUN, SCr, AST, and ALT in the death group were significantly higher than those in the survival group ($P < 0.001$) ; (2) the Consultation time, ESR, albumin, pH value, and partial pressure of carbon dioxide (PCO_2) were significantly different between the two groups ($P < 0.05$) ; (3) there were no statistical differences in age, gender, hematocrit, red blood cell distribution width, platelet counts, monocyte count, and partial pressure of oxygen (PO_2) between the two groups ($P > 0.05$, Table 1).

Relationship between lymphocyte subsets and prognosis

Compared with the survival group, the lymphocyte count ($P < 0.001$), CD4 + /CD8+ T cell ratio ($P < 0.007$) and lymphocyte-to-monocyte Ratio (LMR, $P<0.004$) of the death group were significantly increased, and the NK cell percentage and platelet-to-lymphocyte Ratio (PLR) were significantly decreased ($P < 0.001$). There was no correlation found between the B cell percentage and death ($P > 0.05$, Table 2). These data are incomplete because some patients do not complete all immunoglobulin and complement tests within 24 hours of admission. We excluded this subset of patients, and we were able to ensure that the data sets analysed were of high quality, thereby increasing confidence in the results of the studies and reducing the selection bias associated with missing data, which could affect the interpretation of the results. In this part, we only performed statistical analysis on 58 patients with complete clinical data on immunoglobulin. The results showed that there was no statistical difference in IgG,

IgA, IgM, C3, and C4 levels between the survival group and the death group ($P > 0.05$, Table 3).

We incorporated variables such as neutrophil count, monocyte count, lymphocyte count, $CD4^+/CD8^+$ T cell ratio, B cell percentage, NK cell percentage, PLR, and LMR into the univariate COX proportional hazards model. The results showed that neutrophil count, lymphocyte count, $CD4^+/CD8^+$ T cell ratio, B cell percentage, NK cell percentage, PLR and LMR were all associated with death. We further included these significant variables in the multivariate COX proportional hazards model and found that neutrophil count (HR=1.12; 95% CI, 1.06-1.18), CD4+/CD8+ ratio (HR=2.01; 95% CI, 1.03-3.92), NK cell percentage (HR=0.88; 95% CI, 0.82-0.95), and lymphocyte count (HR=1.59; 95 % CI, 1.02-2.47) were independent risk factors for death (Table 4).

Relationship between different indicators in LASSO COX regression and mortality risk in patients with PQ poisoning

We included the eight variables from the previous regression model into the LASSO COX regression analysis (Figure 2). The 1-s.e. standard line suggested that lymphocytes, NK cells, and neutrophils were independent risk factors for the prognosis of PQ poisoning patients (Figure 3). We constructed diagnostic ROC curves for the prognosis of PQ poisoning patients based on the variables included in the multivariate COX and LASSO C The AUC values for both models were greater than 0.9, indicating that both models had good predictive performance ($P = 0.119$), suggesting similar diagnostic efficiency between the two models (Figure 4). Furthermore, we performed survival analysis by dividing lymphocytes, NK cells, and neutrophils into quartiles. The results also indicated that lymphocytes, NK cells, and neutrophils were significantly correlated with the mortality risk in PQ poisoning patients. Lymphocytes and neutrophils were associated with an increased risk of mortality, while NK cells were linked to a reduced risk (Figure 5). Based on this, we constructed a nomogram prediction model for the 30-day mortality risk of PQ poisoning patients, incorporating lymphocytes, NK cells, and neutrophils (Figure 6). As a clinical decision support tool, nomograms have high clinical applicability. It provides clinicians with an intuitive and easy way to quantify risk assessment by integrating multiple prognostic factors. In the

management of patients with acute PQ poisoning, nomograms can help doctors quickly assess the survival probability of patients through emergency blood biochemistry, so as to guide treatment decisions and resource allocation. The Linear Predictor equation of the model is as follows: Linear Predictor = $(0.442 \times \text{value}_{\text{lymphocyte}}) + (0.118 \times \text{value}_{\text{Neutrophils}}) + (-0.109 \times \text{value}_{\text{NK}})$. In addition, we also performed a model calibration curve analysis, and the results showed that our predicted probability was highly consistent with the observed probability, indicating that the model has high accuracy. (Figure 7). The ROC curve's AUC value of 90.8% suggested that the model has excellent discriminatory ability (Figure 8).

DISCUSSION

In this study, the data of 92 patients with PQ poisoning were statistically analyzed and it was found that there were significant differences in lymphocyte count, CD4 + /CD8 + T cell ratio and NK cell percentage between the death group and the survival group. More importantly, lymphocyte count, CD4 + /CD8 + T cell ratio and NK cell percentage can be used as objective and valuable indicators to predict acute PQ poisoning. These results show that lymphocyte count, CD4 + /CD8 + T cell ratio and NK cell percentage are all related to the death of patients with PQ poisoning.

Lymphocytes are important cellular components of the body's immune response function. Paraquat poisoning can induce its immune dysfunction, but there are few studies on the effects of PQ on the lymphocyte system. Our study found that absolute lymphocyte count can be used as an independent predictor of death from paraquat poisoning, and a relatively high initial level of lymphocyte count is positively correlated with the 90-day mortality rate in patients with PQ poisoning. However, the exact mechanism of the relationship between lymphocyte count and mortality in patients with PQ poisoning is still unclear, so we further analyzed the characteristics of lymphocyte subsets in patients with PQ poisoning.

T cells originate from pluripotent stem cells in the bone marrow, migrate to the thymus, and differentiate and mature into immune-active T cells under the induction of thymosin. They then migrate to peripheral lymphoid tissues and, after coming into contact with

specific antigenic substances or cells, exert cellular immune functions. Our results show that the ratio of CD4 + /CD8 + T cells in the peripheral blood of patients with PQ poisoning is positively correlated with the mortality rate after PQ ingestion and can be used as an independent predictor of death. Similarly, Hassuneh et al. [10] confirmed through experimental studies on BALB/c mice that PQ activates Th17 cells, promotes Th17 cells to secrete interleukin (IL) -21 and IL-9, and promotes further differentiation of Th17. Another study [11] also found that the levels of Th17 cell-related cytokines IL-17, IL-6, and tumor necrosis factor (TGF)-β in the serum of PQ-poisoned rats were significantly increased, and the expression level of the key transcription factor RORγt mRNA in lung tissue was significantly increased, indicating that Th17-related cytokines are involved in the pathological process of PQ poisoning. On the contrary, after intervention with IL-17 antibodies, the poisoning reaction and pathological damage of lung tissue in rats were alleviated, and the levels of serum Th17-related cytokines IL-17, IL-6, and TGF-β and the expression level of RORγt mRNA in lung tissue were all reduced, further confirming its proinflammatory effect. In addition, after extensive literature search, we found that three patients with PQ poisoning who had been infected with human immunodeficiency virus (HIV) were reported successively [12-14]. After standardized treatment, these three patients all had a good prognosis, and their survival was not caused by too little PQ intake. Their common feature is that the level of CD4 + T cells in the body is very low, but the specific cause is still unclear.

The characteristic surface marker of B cells is membrane surface immunoglobulin, which acts as a specific antigen receptor (B cell antigen receptor, BCR) and activates B cells by recognizing different antigen epitopes, differentiates into plasma cells, and then produces specific antibodies to exert humoral immune function [15]. In this paper, we observed that there was no significant correlation between the percentage of B cells and the mortality rate of patients with PQ poisoning. In addition, the IgG, IgA, IgM, C3, and C4 of the 58 patients with PQ poisoning we counted were not related to the death of PQ poisoning. In our study, we observed no significant correlation between the percentage of B cells and mortality in patients with paraquat (PQ) poisoning. In addition, there were no significant differences in immunoglobulin (IgG, IgA, IgM) and

complement components (C3, C4) levels between the survival and death groups. This finding is somewhat surprising considering the known role of B cells and immunoglobulins in the humoral immune response and the fact that PQ may affect immune system function. Possible reasons for the lack of significant changes include the fact that immunoglobulin and complement levels were measured within 24 hours of admission. The immune response to PQ toxicity may be delayed, and early measurements may not capture the full extent of humoral immune activation. In some cases, immunoglobulin production and activation of the complement system may occur later in the course of the disease, after the first 24 hours. In addition, PQ toxicity may primarily trigger a cellular immune response rather than a humoral immune response. Observed changes in T cell subsets (e.g. increased CD4/CD8 ratio) and NK cell percentage suggest that the cellular immune system is more actively involved in the pathogenesis of PQ toxicity. This cellular dominance could explain why changes in B cells and immunoglobulins are less pronounced. Further research is needed on the molecular mechanisms by which PQ affects B cell and immunoglobulin production. This may involve in vitro studies or animal models to explore the direct effects of PQ on B cell function and antibody production. Bamdad Riahi et al. [16] also reported that PQ had no significant effect on the levels of C3, IgM, and IgG in the blood. These results indicate that PQ has no significant effect on complement system proteins or antibodies induced by B lymphocytes. However, some studies have found [17] that reactive oxygen species (ROS) can significantly reduce the production of IgM in mouse spleen lymphocytes. Hassuneh et al. [10] also found that PQ can significantly inhibit the proliferation of B cells induced by mitogens in vitro, resulting in a significant decrease in IgM and IgG in mice, but did not cause a decrease in IgA. This is consistent with the results of Okabe et al. [18], who also found that the level of IgA in serum increased and the level of IgM decreased 1, 2, and 3 weeks after PQ poisoning. The above results are inconsistent with our conclusions, which may be because this study is a clinical retrospective experimental analysis, with patients as clinical experimental subjects, with many interfering factors, and the use of hormones also inhibits the body's immune response to a certain extent. In addition, we only collected and counted the

humoral immune indicators of poisoned patients within 24 hours of admission. Whether PQ poisoning in the middle and late stages will cause changes in the body's humoral immunity still needs further research in the future.

NK cells can directly kill certain tumor cells and virus-infected cells without the need for pre-sensitization by antigen stimulation, so they play an important role in the body's anti-tumor, early anti-viral or intracellular bacterial infection immune response [19]. In addition, they also activate other cells to participate in innate and adaptive immune regulation responses by producing cytokines such as interferon (INF)-γ and TNF-α [20, 21]. In this study, we found that a low initial percentage of NK cells was associated with the 90-day mortality of patients with PQ poisoning and could be used as an independent factor to predict death from acute PQ poisoning. This is consistent with the results of Riahi et al. [4] who also found that PQ could inhibit the proliferation response of mouse spleen cells to the mitogen PHA (Phytohemagglutinin-A), thereby reducing the number of NK cells and inhibiting the production of cytokines IFN-γ and IL-4. Similarly, Lim et al. [22] confirmed that the NK cell activity of mice in the experimental group was significantly lower than that in the control group, and the greater the PQ dose, the more obvious the inhibition of mouse NK cell activity. Another study [16] found that PQ at a dose of 1 mg/kg/d could significantly inhibit the function of mouse spleen cells, and the number of NK cells also decreased significantly. The above research results show that PQ can inhibit NK cell function and reduce the number of NK cells.

The mechanism of acute paraquat poisoning is not fully understood. There are currently five main theories. (1) Lipid peroxidation: PQ is reduced to free radicals through single electron reduction assisted by NADP H, which reacts with molecular oxygen to form bipyridyl cations and superoxide anions. The latter are further oxidized to hydrogen peroxide and hydroxyl free radicals, thereby inducing lipid peroxidation and directly damaging major cellular components [23]. (2) DNA damage: It is mainly manifested in base changes and chain breaks. Studies have reported that PQ can cause sister chromosome exchange, unscheduled DNA synthesis, and positive comet assays [24-26]. (3) Mitochondrial damage: Mitochondria are the main site of PQ-related oxygen

free radical production. PQ can inhibit NADH-Q reductase activity and damage mitochondrial electron transfer, thereby causing changes in cellular bioenergetics [27]. Mitochondrial damage can also cause a large amount of calcium ion influx, leading to intracellular calcium overload and aggravating cell damage. (4) Enzyme imbalance: Imbalance between tissue inhibitors of collagenase and metalloproteinase, excessive gel decomposition activity and apoptosis of alveolar epithelial cells are jointly involved in the occurrence of pulmonary fibrosis [28]. (5) Cell activation and cytokine network: Many macrophages, neutrophils and monocytes infiltration can be observed in the alveolar cavity and alveolar wall of patients with PQ poisoning. In this study, we also found that T lymphocytes and NK cells are simultaneously involved in the occurrence of acute paraquat poisoning.

At present, there are few studies on the effects of paraquat on lymphocytes in the body at home and abroad, and most of the previous studies were conducted in vitro or in animal experiments. This study analyzed the relationship between lymphocyte subsets and the prognosis of patients with PQ poisoning for the first time. However, due to technical deficiencies, we only detected the relative values of lymphocyte subsets, and the relationship between their absolute values and paraquat poisoning is still unclear. In addition, this study cannot fully explain the mechanism of action of lymphocyte subsets in acute paraquat poisoning. Therefore, more in -depth research on molecular mechanisms is needed in the future.

CONCLUSION

In summary，we found the lymphocyte count, neutrophil count, and CD4+/CD8+ T cell ratio are positively correlated with mortality in paraquat poisoning patients, while the NK cell percentage is negatively correlated with mortality. They may serve as independent predictors of death risk in patients with paraquat poisoning.

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Data availability: The data that support the findings of this study are available from

the corresponding author upon reasonable request.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Comparison of baseline and laboratory indicators of patients in the survival group and the death group within 24 hours of admission

Indicators	Survival group (*n*=56)	Death group (*n*=36)	*P* - value
Age (years)	35.50 (26.75, 43.00)	31.50 (26.75, 41.25)	0.889
Gender (Male/Female)	25/31	12/24	0.280
Consultation time (hours)	22.00 (13.00, 30.00)	14.00 (9.38, 20.50)	0.014
Estimated oral PQ ingestion(mL)	10.00 (5.00, 20.00)	68.75 (50.00, 100.00)	<0.001
Plasma PQ concentration (μg /mL)	0.10 (0.10, 0.10)	0.81 (0.44-3.75)	<0.001
Urine PQ concentration (μg /mL)	0.54 (0.10, 2.82)	25.56 (12.04, 69.42)	<0.001
Score			
APACHEII	3.00 (2.00, 5.00)	8.00 (5.00, 12.00)	<0.001
SOFA	2.00 (1.00, 4.00)	5.00 (3.75, 8.00)	<0.001
Complete blood count			
Hematocrit (%)	38.70±5.26	36.51±5.56	0.060
Red cell distribution width (%)	12.75 (12.38, 13.43)	13.05 (12.60, 13.85)	0.146
Platelets (×10^9 /L)	146.00 (109.75, 192.75)	138.50 (87.50, 189.25)	0.347
WBC (×10^9/L)	10.04 (7.31, 14.00)	22.13 (13.20, 26.30)	<0.001
Neutrophils (×10^9 /L)	7.84 (6.12, 12.36)	18.37 (11.08, 22.46)	<0.001

Monocytes (×10^9/L)	0.40 (0.15, 0.60)	0.36 (0.12, 0.82)	0.952
Lymphocytes (×10^9/L)	1.19 (0.66, 1.63)	3.42 (1.55, 4.16)	<0.001
CRP (mg/L)	3.44 (3.30, 6.00)	18.74 (9.22, 52.82)	<0.001
ESR (mm/h)	13.00 (7.00, 19.70)	20.85 (11.00, 26.85)	0.002
Renal function			
BUN (mmol/L)	4.30 (3.15, 6.42)	6.90 (5.25, 10.22)	<0.001
SCr (μmol/ L)	70.05 (47.85, 102.67)	168.70 (124.05, 208.00)	<0.001
Liver function			
Albumin (g/L)	43.55±5.55	40.76±5.33	0.019
AST (U/L)	19.70 (14.90, 25.85)	64.25 (28.85, 339.92)	<0.001
ALT (U/L)	14.40 (9.57, 23.73)	40.40 (14.88, 288.28)	<0.001
Arterial blood gas analysis			
PH	7.42 (7.40, 7.44)	7.40 (7.36, 7.43)	0.034
PCO_2 (mmHg)	36.68±5.61	32.29±8.57	0.004
PO_2 (mmHg)	91.10 (81.30, 102.25)	83.70 (62.88, 103.50)	0.261

Table 2. Comparison of peripheral blood lymphocytes and their subsets between the survival group and the death group

Indicators	Survival group (*n*=56)	Death group (*n*=36)	*P* - value
Lymphocytes (×10^9 /L)	1.2 (0.7, 1.6)	3.4 (1.6, 4.2)	<0.001
CD4+	30.2±8.1	33.0±6.9	0.087
CD8+	29.0±9.8	25.4±9.7	0.081

$CD4^+/CD8^+$ (%)	1.1 (0.8, 1.4)	1.4 (1.0, 1.8)	0.007
B cells (%)	19.79±7.77	23.31±9.33	0.053
NK cells (%)	15.5 (9.0, 21.0)	7.0 (3.0, 10.2)	<0.001
PLR	128.21 (92.88, 201.07)	49.77 (23.52, 74.18)	<0.001
NLR	7.41 (4.34, 13.88)	6.11 (4.02, 9.11)	0.472
LMR	3.48 (1.77, 6.40)	7.19 (3.16, 25.46)	0.004

Table 3. Comparison of immunoglobulin and complement characteristics between the survival group and the death group of 58 patients

Indicators	**Survival group (*n*=35)**	**Death group (*n*=23)**	***P* -value**
IgG (g/L)	11.93±2.50	11.18±3.61	0.353
IgA (g/L)	2.32±0.83	2.46±1.84	0.693
IgM (g/L)	1.36±0.57	1.35±0.95	0.942
C3 (g/L)	1.00±0.23	0.92±0.23	0.205
C4 (g/L)	0.25±0.10	0.22±0.07	0.285

Table 4. Relationship between different indicators in the COX model and the risk of death in patients with PQ poisoning

Indicators	**Univariate COX model**		**Multivariate COX model**	
	HR (95%CI)	***P*- value**	**HR (95%CI)**	***P* -value**
Neutrophils	1.1 8 (1.1 3 -1.2 4)	<0.001	1.1 2 (1.0 6 -1.1 8)	<0.001
Monocytes	1.61 (0.69 -3.76)	0.2 74	1.78(0.58-5.45)	0.312
Lymphocytes	2.57 (1.96 -3.36)	<0.001	1.59 (1.02-2.47)	0.04
$CD4^+/CD8^+$	1.72 (1.09 - 2.70)	0.02	2.01 (1.03 - 3.92)	0.04
B cells	1.03(1 -1.0 7)	0.08 2	1(0.95-1.05)	0.942
NK cells	0.87 (0.8 2 -0.92)	<0.001	0.88 (0.82-0.95)	0.002
PLR	0.99 (0.98-0.99)	<0.001	1 (0.99-1.01)	0.706
LMR	1.02(1.01-1.03)	<0.001	1(0.99-1.02)	0.555

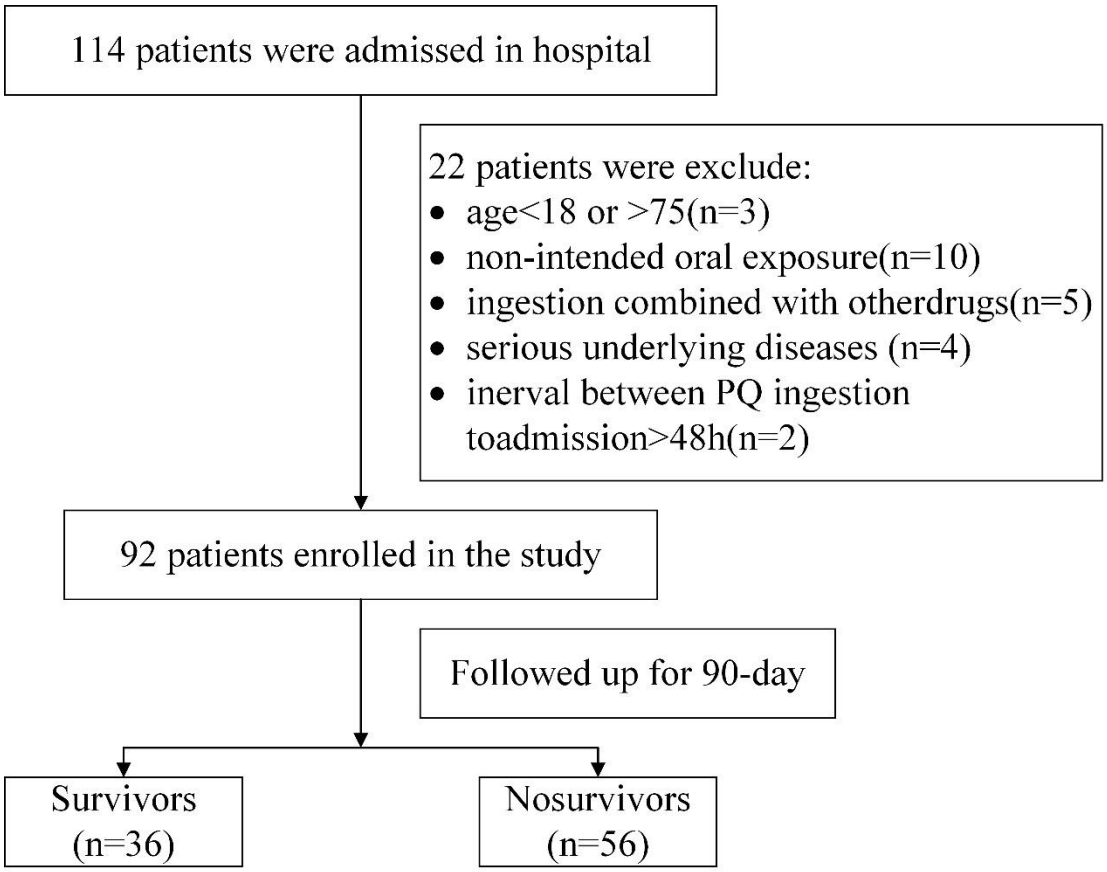

Figure 1. Flow chart.

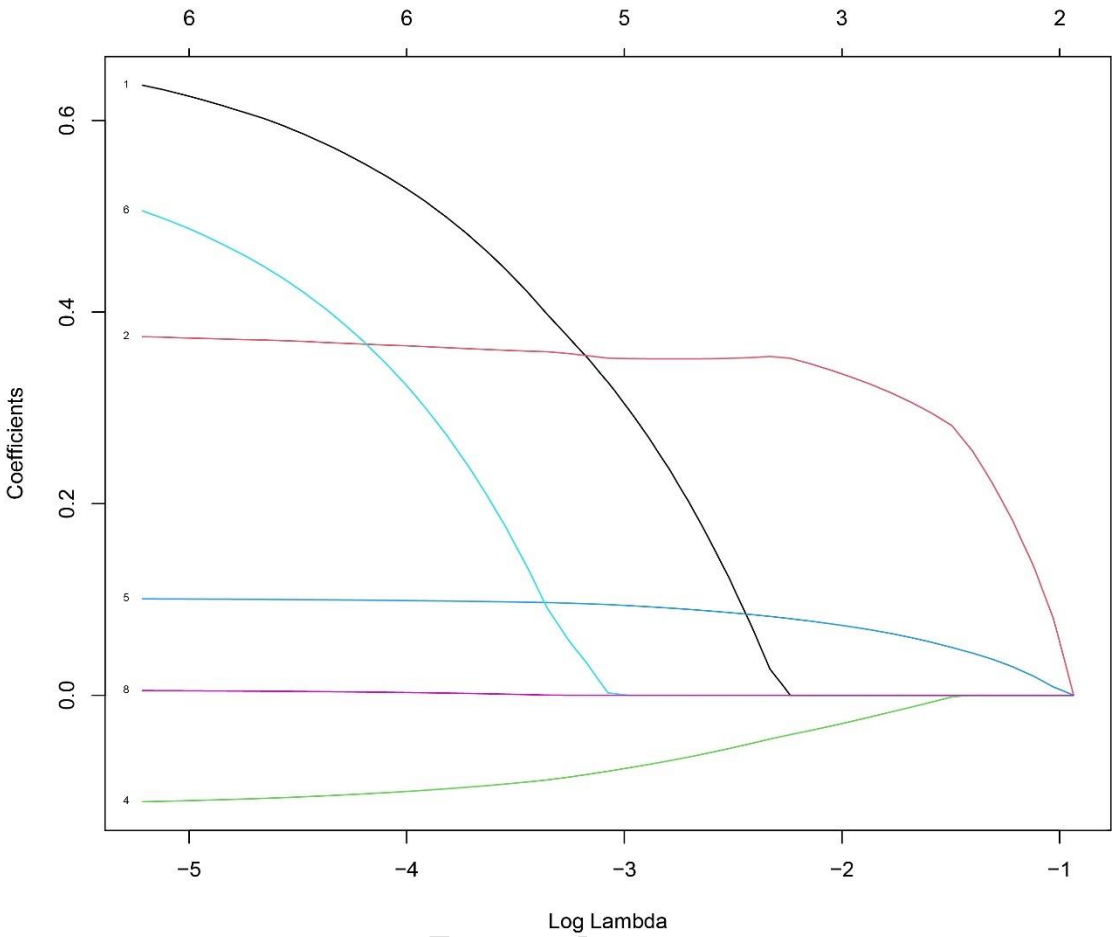

Figure 2. LASSO coefficient profiles of the 8 risk factors.

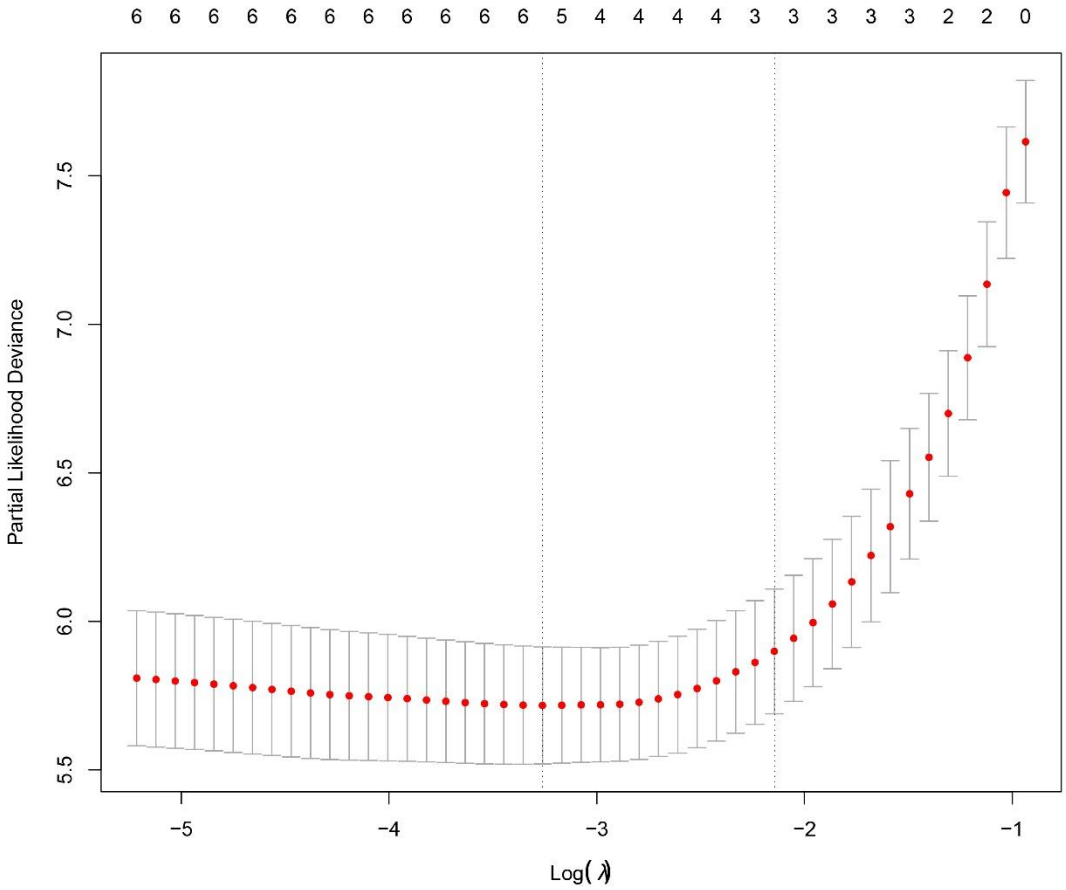

Figure 3. Three risk factors were selected using LASSO Cox regression analysis. The two dotted vertical lines were drawn at the optimal scores by minimum criteria and 1-se criteria (At minimum criteria including CD4/CD8, lymphocytes, NK cells, neutrophils, and monocytes Nuclear cells; At 1-s.e. criteria including lymphocytes, NK cells, and neutrophils.

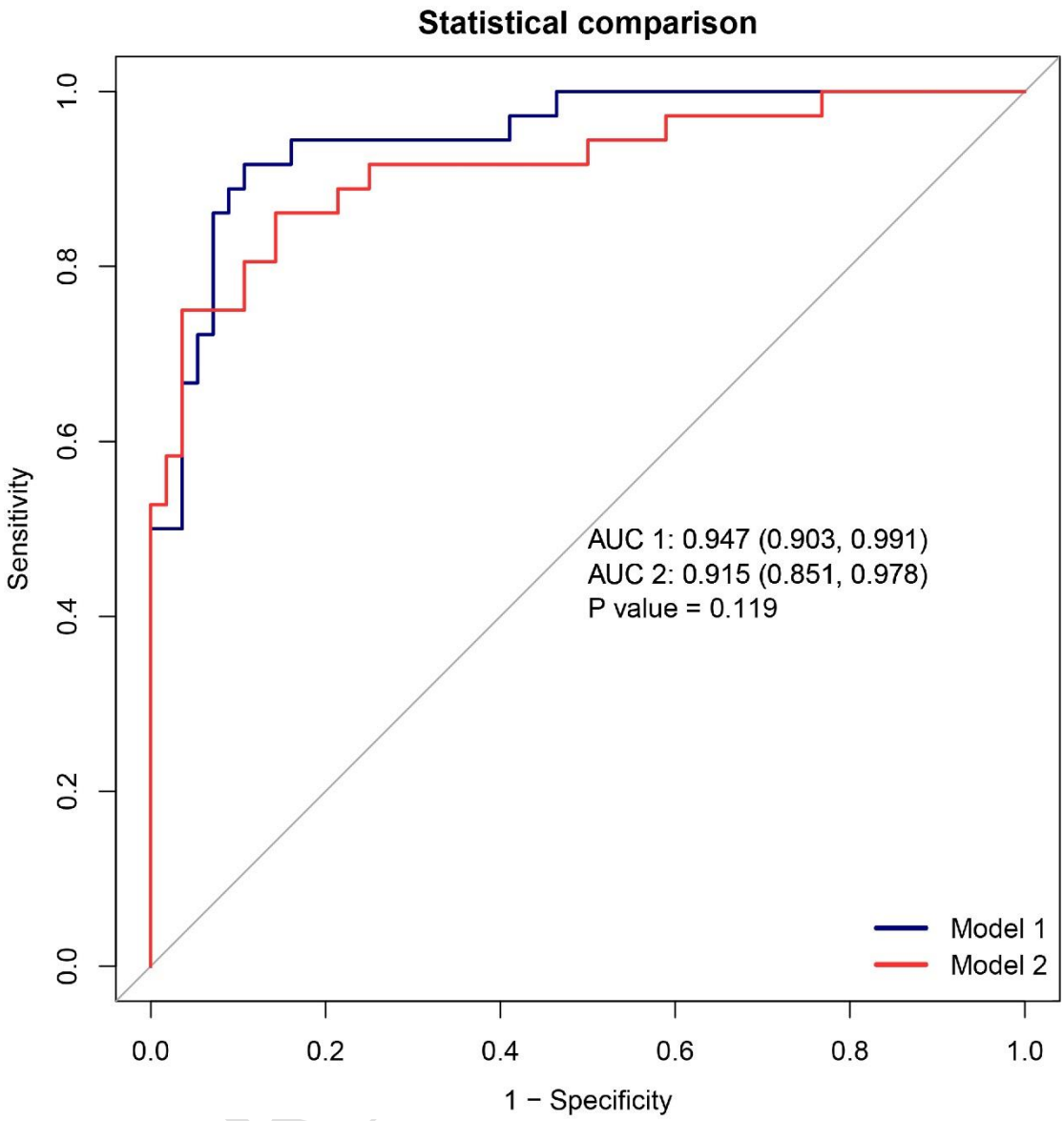

Figure 4. Model 1 represents the COX regression, and Model 2 represents the LASSO regression. Both models have an area under the curve (AUC) value greater than 0.9, indicating good classification performance for predicting mortality in paraquat (PQ) poisoning patients. However, with a P-value of 0.119, which is greater than 0.05, it suggests that the diagnostic efficiency of both models is similar.

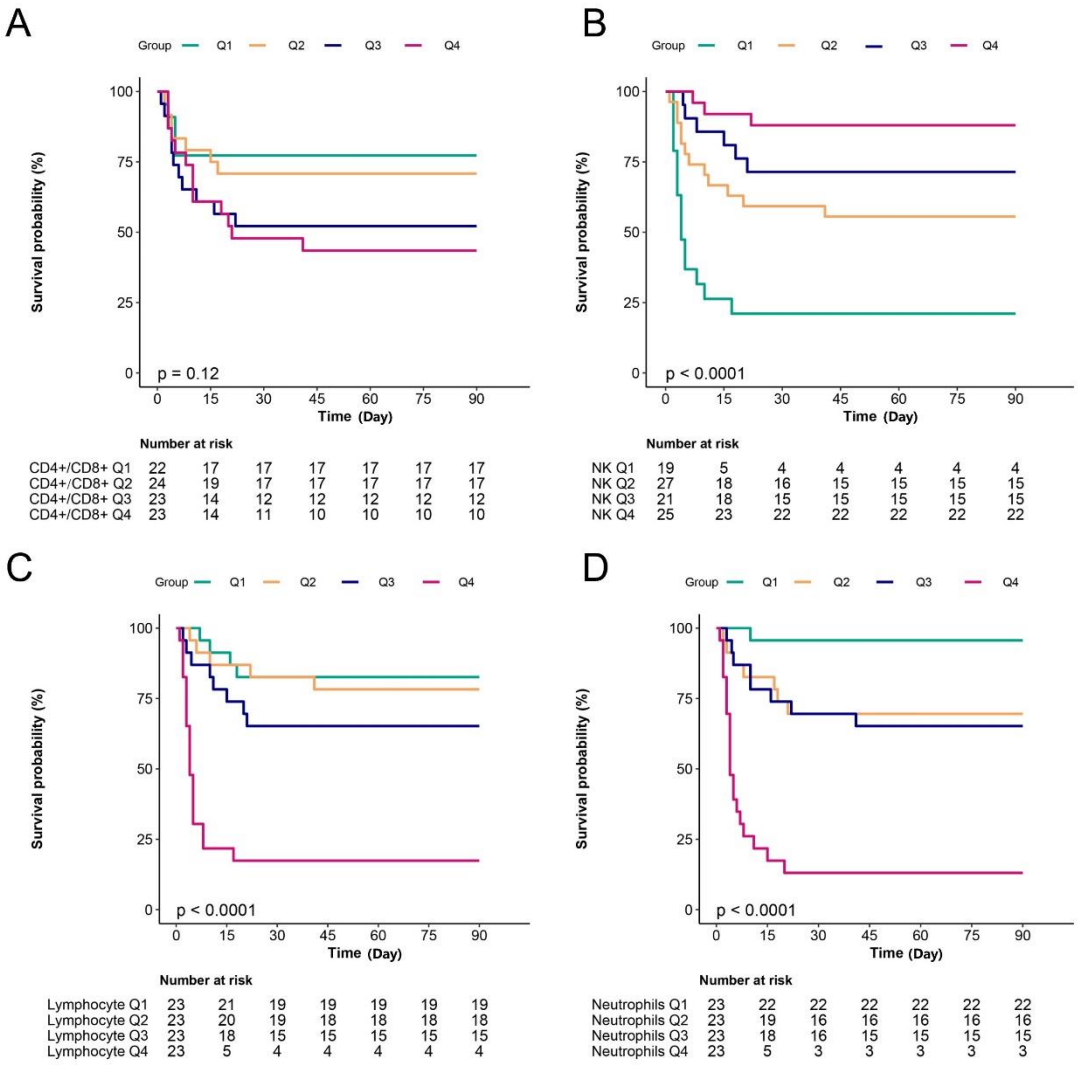

Figure 5. Comparison of survival time among patients in different quartiles of neutrophil, lymphocytes, CD4 $^{+}$/CD8 $^{+}$, and NK cells. Survival curves were plotted using the Kaplan-Meier method for all study subjects, stratified by neutrophil count, lymphocyte count, $CD4^{+}/CD8^{+}$ T-cell ratio, and NK cell percentage. The results further confirm that high levels of neutrophil count, lymphocyte count, and low levels of NK cell percentage are indicative of poor prognosis in patients ($P < 0.001$).

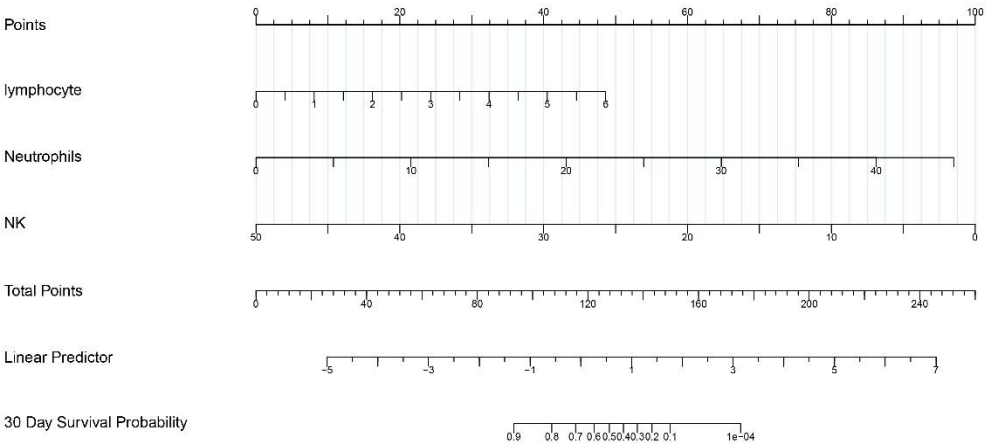

Figure 6. Nomogram for predicting the survival probability of patients based on lymphocyte count, neutrophil count, and NK cell percentage. The corresponding points for each variable are determined based on its specific value. The total points are calculated by summing the points for all variables. The 30-day survival probability is then calculated using the Linear Predictor equation: Linear Predictor = (0.442 × value lymphocyte) + (0.118 × value Neutrophils) + (− 0.109 × value NK).

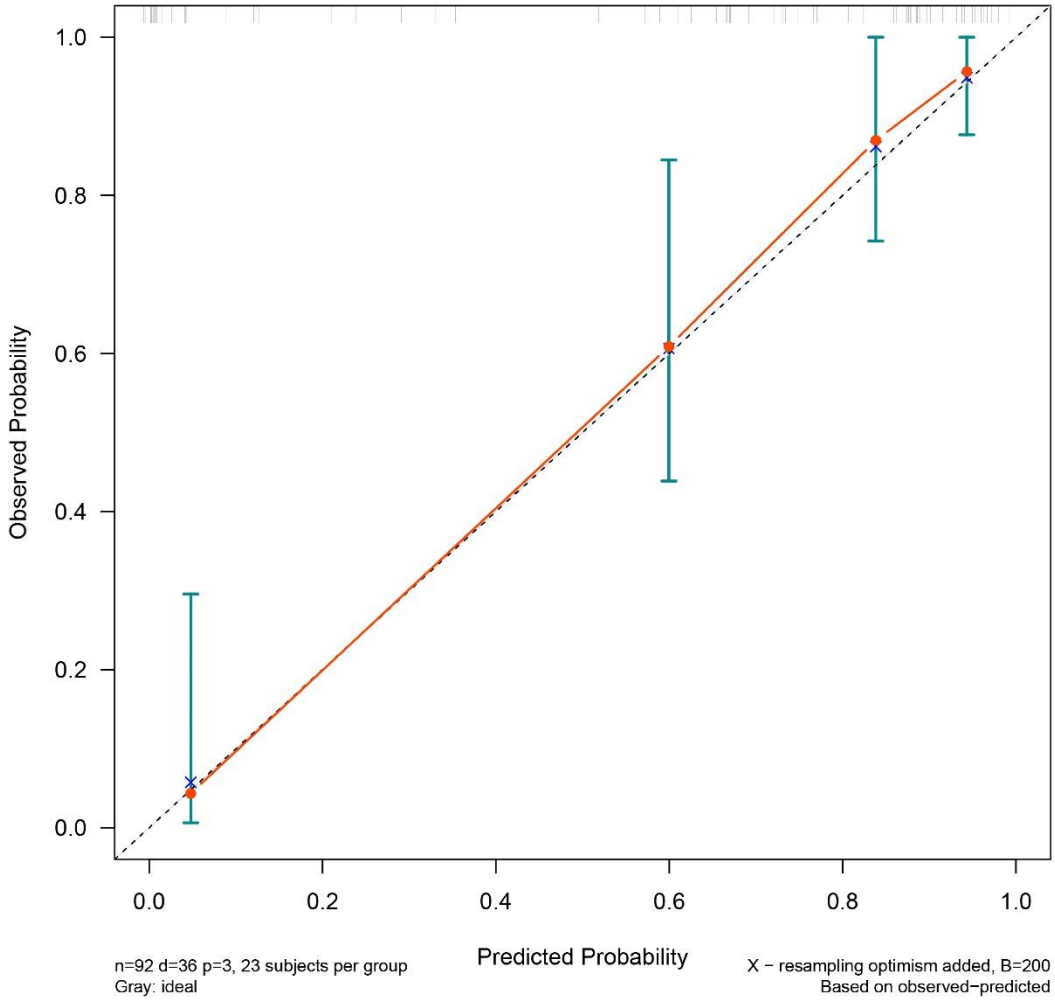

Figure 7. Calibration curve used to evaluate the calibration ability of the model, which reflects the agreement between the predicted survival probability and the actual observed survival probability. The gray line represents the ideal scenario, where the model's predictions align perfectly with the actual results. The blue line represents the calibration curve based on the actual data. This graph illustrates the degree of error between the model's predictions and actual outcomes, indicating that the model has good calibration.

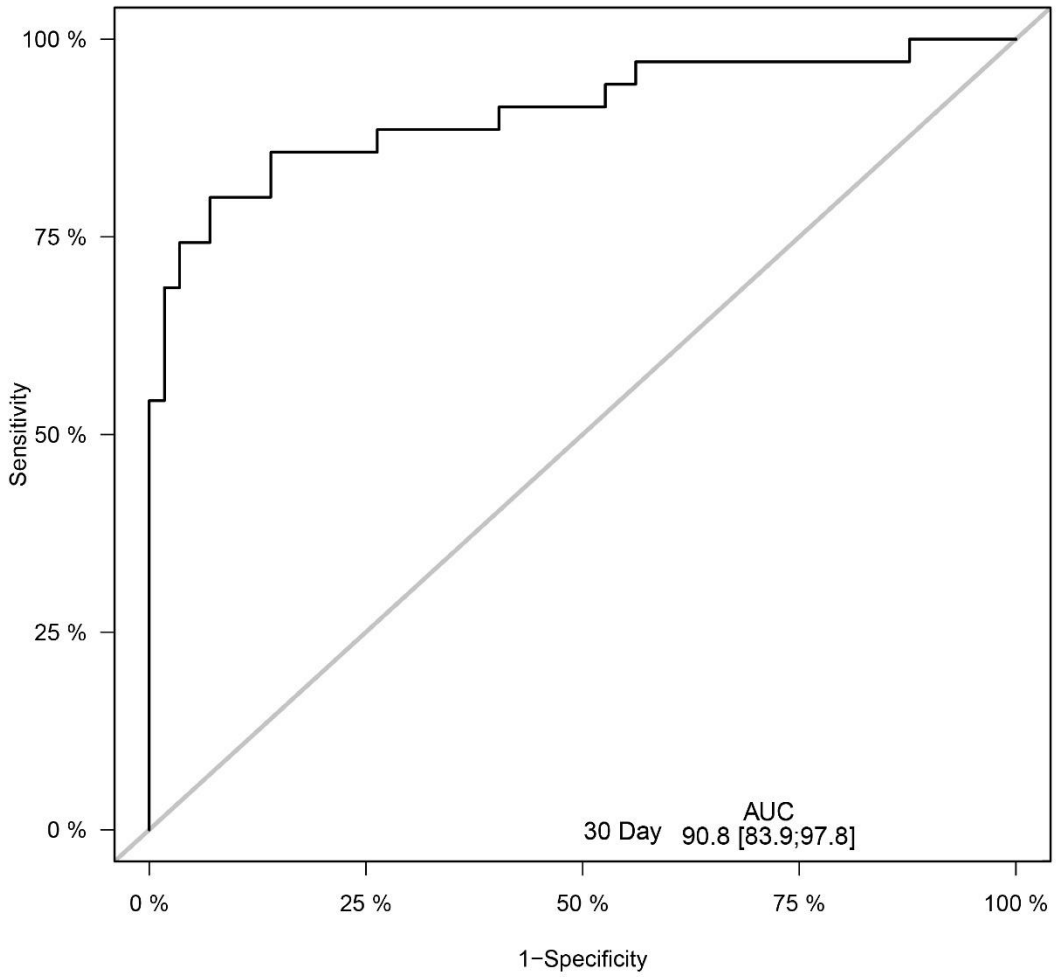

Figure 8. ROC curve for predicting 30-day mortality. This curve assesses the discriminative ability of the model, showing the sensitivity and specificity of the model. The AUC (Area Under the Curve) is 90.8%, indicating high accuracy in predicting 30-day survival probability (with the AUC ranging from 83.9% to 97.8%). The ROC curve demonstrates the model's strong discriminatory ability, and an AUC value close to 1 indicates excellent model performance in predicting 30-day survival.