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ORIGINAL RESEARCH

Predicting Time to First Rejection Episode in Lung Transplant Patients Using a Comprehensive Multi-Indicator Model

Youpeng Chen[1,*], Enzhong Li[2,*], Qingqing Yang[1], Zhenglin Chang[1], Baodan Yu[1], Jiancai Lu[1], Haojie Wu[1], Peiyan Zheng[1], Zhangkai J Cheng[1,*], Baoqing Sun[1]

[1]Department of Clinical Laboratory, National Center for Respiratory Medicine, National Clinical Research Center for Respiratory Disease, State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, 510140, People's Republic of China; [2]Department of Endocrinology, First Affiliated Hospital of Guangzhou Medical University, Guangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhangkai J Cheng; Baoqing Sun, Department of Clinical Laboratory, National Center for Respiratory Medicine, National Clinical Research Center for Respiratory Disease, State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, 510140, People's Republic of China, Email jasontable@gmail.com; sunbaoqing@vip.163.com

Background: Rejection hinders long-term survival in lung transplantation, and no widely accepted biomarkers exist to predict rejection risk. This study aimed to develop and validate a prognostic model using laboratory data to predict the time to first rejection episode in lung transplant recipients.

Methods: Data from 160 lung transplant recipients were retrospectively collected. Univariate Cox analysis assessed the impact of patient characteristics on time to first rejection episode. Kaplan-Meier survival analysis, LASSO regression, and multivariate Cox analysis were used to select prognostic indicators and develop a riskScore model. Model performance was evaluated using Kaplan-Meier analysis, time-dependent ROC curves, and multivariate Cox regression.

Results: Patient characteristics were not significantly associated with the time to the first rejection episode. Six laboratory indicators—Activated Partial Thromboplastin Time, IL-10, estimated intrapulmonary shunt, 50% Hemolytic Complement, IgA, and Complement Component 3—were identified as significant predictors and integrated into the riskScore. The riskScore demonstrated good predictive performance. It outperformed individual indicators, was an independent risk factor for rejection, and was validated in the validation dataset.

Conclusion: The riskScore model effectively predicts time to first rejection episode in lung transplant recipients.

Keywords: lung transplantation, rejection, prognostic model, laboratory indicators

Introduction

Lung transplantation (LTx) has become an established treatment option for patients with end-stage, non-malignant lung diseases. Since the 1980s, it has evolved from a rare procedure to a widely accepted therapeutic choice for patients with advanced lung diseases, with a steady increase in the number of lung transplants performed annually.[1] However, despite the significant improvements in surgical techniques and post-transplant care, long-term survival among lung transplant recipients remains limited.[2] The latest report from the International Society for Heart and Lung Transplantation (ISHLT) registry study reveals a median patient survival of only 6.6 years.[3]

Several factors contribute to the suboptimal long-term outcomes in lung transplant recipients, including chronic lung allograft dysfunction (CLAD), infections, and malignancies.[4,5] CLAD, which encompasses both bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome, is the leading cause of late mortality and morbidity following LTx.[4] Infections, particularly those caused by bacteria and fungi, are a major cause of early mortality in lung transplant

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patients.[6,7] Additionally, the long-term use of immunosuppressive therapy increases the risk of malignancies, further compromising the survival of lung transplant recipients.[8]

Another significant barrier to successful long-term outcomes in LTx is acute rejection, which occurs at a high incidence in lung transplant recipients.[9] Acute rejection episodes, especially those that are recurrent or severe, have been strongly associated with an increased risk of CLAD development and mortality.[10,11] Therefore, early detection and prompt management of acute rejection are of utmost importance in preserving graft function and improving patient survival.[12] Currently, the gold standard for diagnosing acute rejection is transbronchial lung biopsy (TBLB) followed by histological assessment.[13,14] However, TBLB is an invasive procedure that carries the risk of complications such as bleeding, pneumothorax, and infection. Furthermore, the histological evaluation of biopsy samples is subject to inter-observer variability and may not always reflect the overall state of the graft. These limitations highlight the need for non-invasive and reliable methods to predict and monitor acute rejection in lung transplant recipients.

To date, there are no widely accepted biomarkers or models for predicting the risk of rejection in lung transplant patients. In this study, we aimed to address this unmet need by developing and validating a prognostic model that predicts the time to first rejection episode in lung transplant recipients using readily available clinical and laboratory data. We conducted a comprehensive analysis of 69 laboratory indicators in a retrospectively collected cohort of lung transplant patients. Through this analysis, we identified six key indicators that were strongly associated with the risk of rejection: Activated Partial Thromboplastin Time (APTT), Interleukin-10 (IL-10), estimated intrapulmonary shunt, 50% Hemolytic Complement (CH50), Immunoglobulin A (IgA), and Complement Component 3 (C3). Based on these findings, we developed a risk score (riskScore) that integrates the levels of these six indicators to provide a personalized assessment of the risk of rejection for each patient. We demonstrate that the riskScore is a sensitive and robust predictor of rejection, outperforming individual indicators and exhibiting good discriminatory ability in both the training and validation datasets.

The development of this prognostic model represents a significant step towards personalized risk stratification in lung transplant recipients. By identifying high-risk patients, clinicians can implement closer monitoring, initiate earlier interventions, and optimize immunosuppressive regimens, potentially leading to improved outcomes. The application of this model in clinical practice could guide personalized management strategies and ultimately contribute to enhancing long-term survival and quality of life in lung transplant recipients.

Materials and Methods

Study Design

In this retrospective study, we aimed to investigate the impact of patient background characteristics on the prognosis of lung transplant recipients and to develop a prognostic model for predicting the time from LTx to the first rejection episode based on clinical indicators (Figure 1). The study was conducted at the First Affiliated Hospital of Guangzhou Medical University, and data were collected from patients who underwent LTx at this institution. Univariate Cox analysis was performed to assess the influence of patient background characteristics on post-transplant outcomes. To construct the prognostic model, the dataset was randomly divided into a training set (70%) and a validation set (30%). Kaplan-Meier survival analysis was used to preliminarily screen indicators associated with the time to first rejection episode, followed by LASSO regression to further select variables from the identified indicators. Finally, multivariate Cox analysis was conducted to determine the significant predictors of time to first rejection episode and to establish the prognostic model, which was then validated using the validation set.

Data Collection

To identify factors influencing the time to first rejection episode after LTx, we retrospectively collected data from electronic health records of 160 patients who underwent LTx at the First Affiliated Hospital of Guangzhou Medical University between 2019 and 2023. We collected data on the time to first rejection episode for each patient. Patient background characteristics, including age at the time of LTx, gender, presence of pulmonary hypertension, transplant type, and primary disease type, were collected. Additionally, 69 clinical laboratory indicators were collected, which can be mainly categorized into Blood Gas Analysis Indicators, Liver Function Indicators, Biochemistry Indicators,

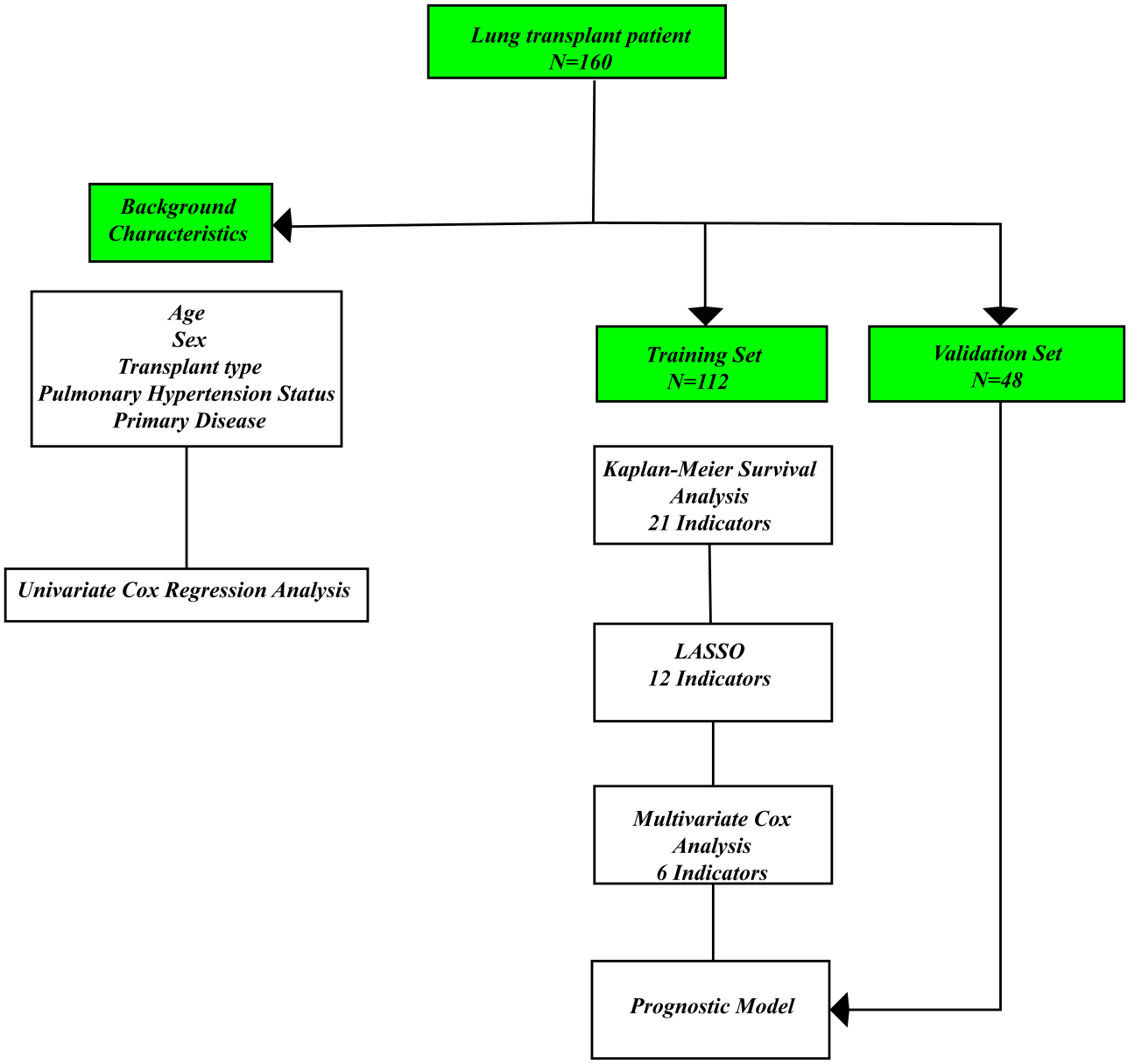

Figure 1 Study Design and Analytical Workflow. This retrospective study investigated the impact of patient background characteristics on the prognosis of lung transplant recipients and developed a prognostic model for predicting the time to the first rejection episode. The study was conducted at the First Affiliated Hospital of Guangzhou Medical University, involving 160 lung transplant patients. Data on demographics, clinical characteristics, and laboratory indicators were collected. The dataset was divided into a training set (70%, n=112) and a validation set (30%, n=48). Univariate Cox regression analyzed the impact of patient characteristics. Kaplan-Meier survival analysis and LASSO regression were used to identify significant predictors, which were further refined using multivariate Cox analysis to develop the prognostic model. The model was validated using the validation set.

Coagulation Function Indicators, Immune Function Indicators, Complete Blood Count Indicators, and Cytokines (Figure 2A). The laboratory data were collected within three days after the lung transplantation surgery. It is important to note that the missing values for each individual laboratory indicator did not exceed one-third of the data for that specific indicator, ensuring that our statistical analyses remain robust and meaningful. For laboratory indicators with missing values, the median of each respective indicator was employed to replace these missing values, preserving the comprehensive integrity of our dataset for analysis. A summary of the participants' statistics is listed in Supplementary Table 1. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University (China) with the ethical approval number ES-2018-119. All organs were donated voluntarily with

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A

Data of 69 clinical indicators

Trait name	Trait name	Trait name
Blood Gas Analysis Indicators	**Liver Function Indicators**	**Immune Function Indicators**
Standard Bicarbonate Concentration	Total Bilirubin	CH50 (50% Hemolytic Complement Activity)
Total Carbon Dioxide Concentration Whole Blood	Total Bile Acids	β2-Microglobulin
pH Value Body Temperature	Serum α-L-Fucosidase	Complement C4
Oxygen Concentration	Albumin	Immunoglobulin A
Atmospheric Pressure Measured	Alanine Aminotransferase	Ceruloplasmin
Carboxyhemoglobin Measured	Direct Bilirubin	Complement C3
Oxygen Saturation Measured	γ-Glutamyl Transpeptidase	Immunoglobulin M
Hemoglobin Concentration Measured	Total Protein	Immunoglobulin G
Carbon Dioxide Partial Pressure Measured	**Biochemistry Indicators**	**Complete Blood Count Indicators**
Reduced Hemoglobin Concentration	Calcium	White Blood Cells
Total Carbon Dioxide Concentration Plasma	Chloride	Hemoglobin
Standard Base Excess	Creatinine	Mean Corpuscular Volume
Methemoglobin Concentration Measured	Glucose	Nucleated Red Blood Cells Absolute
Oxyhemoglobin Concentration Measured	Potassium	Nucleated Red Blood Cells Ratio
Carbon Dioxide Partial Pressure Body Temperature	Sodium	Red Blood Cells
Oxygen Partial Pressure Measured	Lactate	Hematocrit Calculated
pH Value Measured	**Coagulation Function Indicators**	**Cytokines**
Actual Base Excess	Thrombin Time	IL-4 (Interleukin-4)
Intrapulmonary Shunt Estimated	Activated Partial Thromboplastin Time	IL-2 (Interleukin-2)
Capillary Oxygen Concentration	Prothrombin Activity	IFN-γ (Interferon-γ)
Total Oxygen Concentration	Fibrinogen	TNF-α (Tumor Necrosis Factor-α)
Alveolar Arterial Oxygen Tension Ratio Estimated	Prothrombin Time	IL-10 (Interleukin-10)
Alveolar Arterial Oxygen Tension Difference	PT International Normalized Ratio	IL-6 (Interleukin-6)
p50 act T		
p50 act C		
Oxygen Partial Pressure Body Temperature		
Bicarbonate Concentration		

B

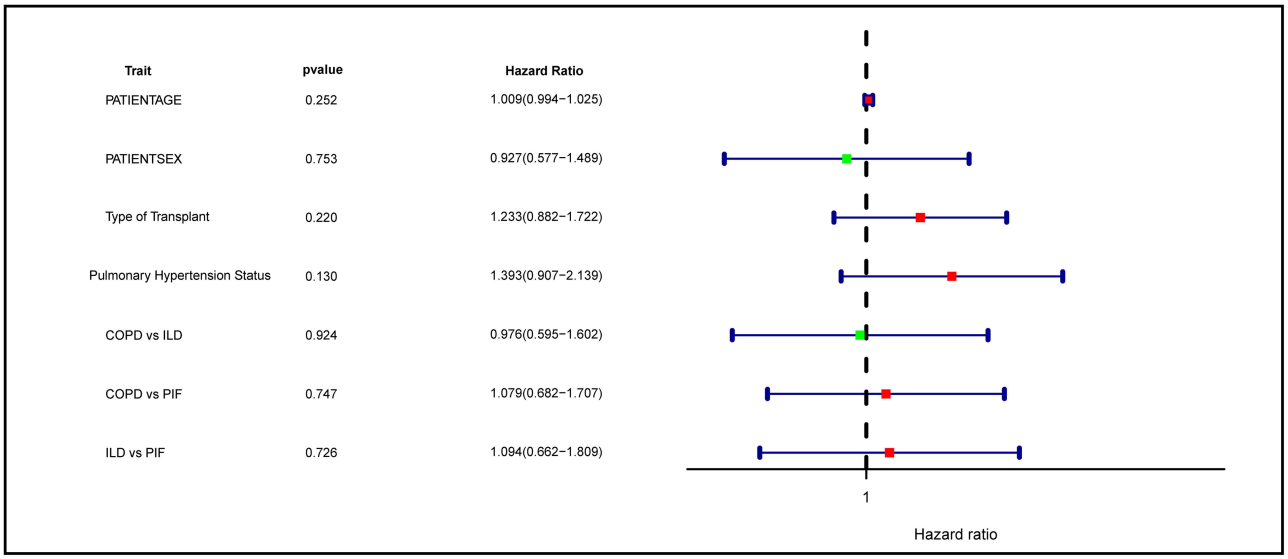

Figure 2 Data Collection and Univariate Cox Analysis. (**A**) Data of 69 Clinical Indicators: This panel categorizes the 69 clinical laboratory indicators collected from 160 lung transplant patients at the First Affiliated Hospital of Guangzhou Medical University between 2019 and 2023. The indicators are grouped into seven categories: Blood Gas Analysis Indicators, Liver Function Indicators, Biochemistry Indicators, Coagulation Function Indicators, Immune Function Indicators, Complete Blood Count Indicators, and Cytokines. (**B**) Univariate Cox Regression Analysis of Patient Characteristics: This panel presents the results of the univariate Cox analysis performed to investigate the impact of various patient characteristics on the time to the first rejection episode after lung transplantation. Factors analyzed included age, gender, type of transplant (single or double lung), pulmonary hypertension status, and primary disease types (Chronic Obstructive Pulmonary Disease (COPD), Interstitial Lung Disease (ILD), and Pulmonary Interstitial Fibrosis (PIF)). The analysis demonstrated that none of these characteristics were significantly associated with the time to the first rejection episode (all p-values > 0.05). The hazard ratios and p-values for each factor are displayed in the figure.

written informed consent, and the procedures were conducted in accordance with the Declaration of Istanbul. All patient data were anonymized and de-identified prior to analysis.

Univariate Cox Analysis of Background Characteristics

We conducted univariate Cox analysis based on the participants' background characteristics to investigate the impact of these factors on patient prognosis. For the primary diseases, we focused on Chronic Obstructive Pulmonary Disease (COPD), Interstitial Lung Disease (ILD), and Pulmonary Interstitial Fibrosis (PIF) while excluding other diseases from the analysis. This decision was based on the following considerations: firstly, these three diseases accounted for a large proportion of the study sample, which is conducive to obtaining reliable statistical results. Secondly, COPD, ILD, and PIF are common primary diseases leading to LTx and have a significant impact on post-transplant prognosis. Therefore, analyzing the relationship between these diseases and the time to rejection episode has clinical significance. In contrast, other primary diseases had relatively small sample sizes, which may make it difficult to obtain statistically significant results.

Development and Validation of a Prognostic Model for Rejection-Free Survival in Patients

To evaluate the model's performance and avoid overfitting, we randomly divided the original dataset into two parts: a training dataset and a validation dataset. We used the sample() function in R to perform random sampling. The proportion of the validation dataset was set to 30%, meaning that 30% of the original dataset was randomly selected as the validation dataset, while the remaining 70% was used for model training. This random splitting method ensures that the training and validation datasets have similar data distributions, allowing for a fair evaluation of the model's performance on unseen data.

To identify clinically meaningful laboratory indicators associated with rejection-free survival, we conducted rejection-free survival analysis on 69 clinical laboratory indicators of the patients. We employed the Kaplan-Meier method to estimate the rejection-free survival probabilities and construct rejection-free survival curves for each indicator. To determine the optimal cutoff point for categorizing each indicator's level, we utilized the surv_cutpoint function from the survminer package (version 0.4.9) in R. This function implements the maximally selected rank statistics method, which searches for the cutpoint that maximizes the difference in rejection-free survival between the resulting two groups. By identifying the optimal cutoff point, we can dichotomize the continuous indicators into high and low levels. The statistical significance of the difference in rejection-free survival rates between the two groups (high and low levels) was assessed using the Log rank test. A p-value less than 0.05 was considered statistically significant, indicating a significant difference in rejection-free survival between the groups.

We performed Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis to select the most informative indicators for predicting rejection-free survival. The optimal penalty parameter (λ) was determined through 10-fold cross-validation. Indicators with non-zero coefficients at the optimal λ were considered as the final prognostic indicators. Subsequently, we fitted a multivariate Cox proportional hazards model using the selected prognostic indicators and performed stepwise regression using the step() function with both forward and backward selection to obtain the final model. Each patient's risk score was calculated as the sum of the products of each indicator's level and its corresponding coefficient from the Cox model. Patients were then stratified into high-risk and low-risk groups based on the surv_cut-point function.

After constructing the prognostic model, we employed various methods to assess its performance and clinical utility. Kaplan-Meier survival curves were plotted to compare rejection-free survival between the high-risk and low-risk groups, and the Log rank test was used to evaluate the significance of the differences. We also generated risk curves to visualize the distribution of risk scores and rejection-free status of patients in the training cohort. Time-dependent receiver operating characteristic (ROC) analysis and the area under the curve (AUC) were employed to assess the prognostic performance of the risk score. To further evaluate the independence of the prognostic model, we performed multivariate Cox regression analysis by combining the risk score with clinical patient background characteristic variables, such as age and gender.

To enhance the robustness of our findings, we validated the prognostic model using the validation dataset. Risk scores for patients in the validation cohort were calculated using the same formula derived from the training cohort. Kaplan-Meier survival analysis was conducted to evaluate the predictive value of the model in the validation cohort.

Statistical Analysis

The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables with a normal distribution were expressed as mean ± standard deviation, while those with a non-normal distribution were presented as median (25%, 75% interquartile range). Categorical variables were expressed as percentages. For continuous variables with a normal distribution, the Student's *t*-test was used to compare between groups, while for those with a non-normal distribution, the Wilcoxon rank-sum test was employed. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The Kaplan-Meier method was used to estimate rejection-free survival probabilities, and the Log rank test was employed to compare survival curves between groups. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify prognostic factors associated with rejection-free survival. Lasso regression analysis was conducted to select the most informative indicators for predicting rejection-free survival, with the optimal penalty parameter (λ) determined through 10-fold cross-validation. The prognostic model's performance was evaluated using time-dependent ROC analysis and the AUC. The independence of the prognostic model was assessed by combining the risk score with clinical patient background characteristic variables in a multivariate Cox regression analysis. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.3.1).

Results

Patient Characteristics

A total of 160 patients who underwent LTx were included in this study. We divided them into a training set (n=112, 70%) and a validation set (n=48, 30%). The mean age of the entire cohort was 56.43±10.99 years, with no significant difference between the training and validation sets (p=0.995). The majority of patients were male (n=135, 84.4%), and the gender distribution did not differ significantly between the two groups (p=0.235). The time to first rejection episode was 122.00 (67.50, 392.50) days, with no significant difference between the training and validation sets (p=0.632).

Among the patients, 18.1% had pulmonary hypertension, and 53.8% underwent double LTx. The distribution of these characteristics was comparable between the training and validation sets (p=0.560 and p=0.782, respectively). The most common primary diseases leading to LTx were COPD (27.5%), PIF (25.0%), and ILD (20.0%). The distribution of primary diseases did not differ significantly between the training and validation sets (all p-values > 0.05).

Several laboratory indicators were also assessed, including APTT, IL-10, estimated intrapulmonary shunt, CH50, IgA, and C3. No significant differences were found between the training and validation sets for these indicators (all p-values > 0.05).

In summary, Table 1 describes the overall patient characteristics, including demographics, clinical features, and laboratory indicators. These characteristics were well-balanced between the training and validation sets, providing a solid foundation for the development and validation of a prognostic model for the first rejection episode after LTx.

Univariate Cox Analysis of Patient Characteristics and Time to First Rejection Episode

To investigate the potential impact of patient characteristics on the time to first rejection episode after LTx, we performed univariate Cox analysis based on various factors, including age, gender, type of transplant (single or double lung), pulmonary hypertension status, and primary diseases. For the primary diseases, we focused on three major indications: COPD, ILD, and PIF (Figure 2B). The results of the univariate Cox analysis demonstrated that none of the patient characteristics had a significant association with the time to first rejection episode after LTx (all p-values > 0.05). Specifically, age (p=0.252), gender (p=0.753), type of transplant (p=0.220), pulmonary hypertension status (p=0.130), and the comparisons between COPD vs ILD (p=0.924), COPD vs PIF (p=0.747), and ILD vs PIF (p=0.726) did not show any statistically significant impact on the time to first rejection episode.

Table 1 Patient Characteristics

Variable	Overall N=160(100%)	Training Set N=112(70%)	Validation Set N=48(30%)	P-value
Age	56.43±10.99	56.43±11.05	56.42±10.98	0.995
Gender				
Male	135(84.4%)	97(86.6%)	38(79.2%)	0.235
Female	25(15.6%)	15(13.4%)	10(20.8%)	0.235
Time to First Rejection (days)	122.00(67.50, 392.50)	123.00(72.00, 392.00)	106(57.00, 420.50)	0.632
Pulmonary Hypertension Patients	29(18.1%)	19(17.0%)	10(20.8%)	0.560
Transplant Type, Double	86(53.8%)	61(54.5%)	25(52.1%)	0.782
Primary Disease				
COPD	44(27.5%)	30(26.8%)	14(29.2%)	0.757
PIF	40(25.0%)	28(25.0%)	12(25.0%)	1.000
ILD	32(20.0%)	23(20.5%)	9(18.8%)	0.796
Bronchiectasis	10(6.3%)	7(6.3%)	3(6.3%)	1.000
BO	6(3.8%)	5(4.5%)	1(2.1%)	0.468
Emphysema with Giant Bullae	1(0.6%)	1(0.9%)	0(0.0%)	0.511
IP	17(10.6%)	11(9.8%)	6(12.5%)	0.614
LCH	1(0.6%)	0(0.0%)	1(2.1%)	0.304
LAM	3(1.9%)	2(1.8%)	1(2.1%)	0.899
Pneumoconiosis	4(2.5%)	4(3.6%)	0(0.0%)	0.439
Severe Pneumonia	2(1.3%)	1(0.9%)	1(2.1%)	0.511
Laboratory Indicators				
APTT	38.15±6.54	37.47±6.13	39.89±7.30	0.053
IL-10	5.77±7.94	6.05±8.85	4.98±4.47	0.502
Intrapulmonary Shunt Estimated	8.40(4.80, 15.67)	8.40(4.80, 15.27)	8.90(4.37, 17.15)	0.555
CH50	52.65(43.37, 62.22)	52.60(42.70, 60.70)	52.90(46.40, 64.40)	0.607
IgA	2.79±1.71	2.75±1.72	2.87±1.69	0.689
C3	0.81(0.68, 1.00)	0.82(0.71, 1.00)	0.79(0.65.0.94)	0.905

Notes: Data are presented as mean ± standard deviation, median (25th percentile, 75th percentile), or count (percentage). P-values were calculated using the Student's *t*-test, Wilcoxon rank-sum test, or chi-square test, as appropriate.
Abbreviations: COPD, Chronic Obstructive Pulmonary Disease; ILD, Interstitial Lung Disease; PIF, Pulmonary Interstitial Fibrosis; BO, Bronchiolitis Obliterans; IP, Interstitial Pneumonia; LCH, Langerhans Cell Histiocytosis; LAM, Lymphangioleiomyomatosis; APTT, Activated Partial Thromboplastin Time; IL-10, Interleukin-10; CH50, 50% Hemolytic Complement; IgA, Immunoglobulin A; C3, Complement Component 3.

These findings suggest that, in our study cohort, patient characteristics such as age, gender, type of transplant, pulmonary hypertension status, and the three major primary diseases (COPD, ILD, and PIF) did not significantly influence the time to first rejection episode after LTx. However, the lack of significant associations could be attributed to the relatively small sample size for some subgroups, which might limit the power to detect significant differences. Further studies with larger cohorts may be needed to confirm these findings and explore other potential predictors of the time to first rejection episode after LTx.

Development and Validation of a Prognostic Model for Time to First Rejection Episode After LTx

To develop a prognostic model for predicting the time to first rejection episode based on laboratory indicators in lung transplant patients, we divided the patients into a training dataset and a validation dataset at a ratio of 7:3. In the training dataset, we performed rejection-free survival analysis on 69 laboratory indicators. Through Kaplan-Meier survival analysis, we initially excluded 48 indicators that were not associated with the time to first rejection episode (Supplementary Figure 1) and selected 21 indicators that showed significant associations (Figure 3). Subsequently, we conducted LASSO analysis on these 21 indicators and chose the 12 most informative indicators (Figure 4A). Finally,

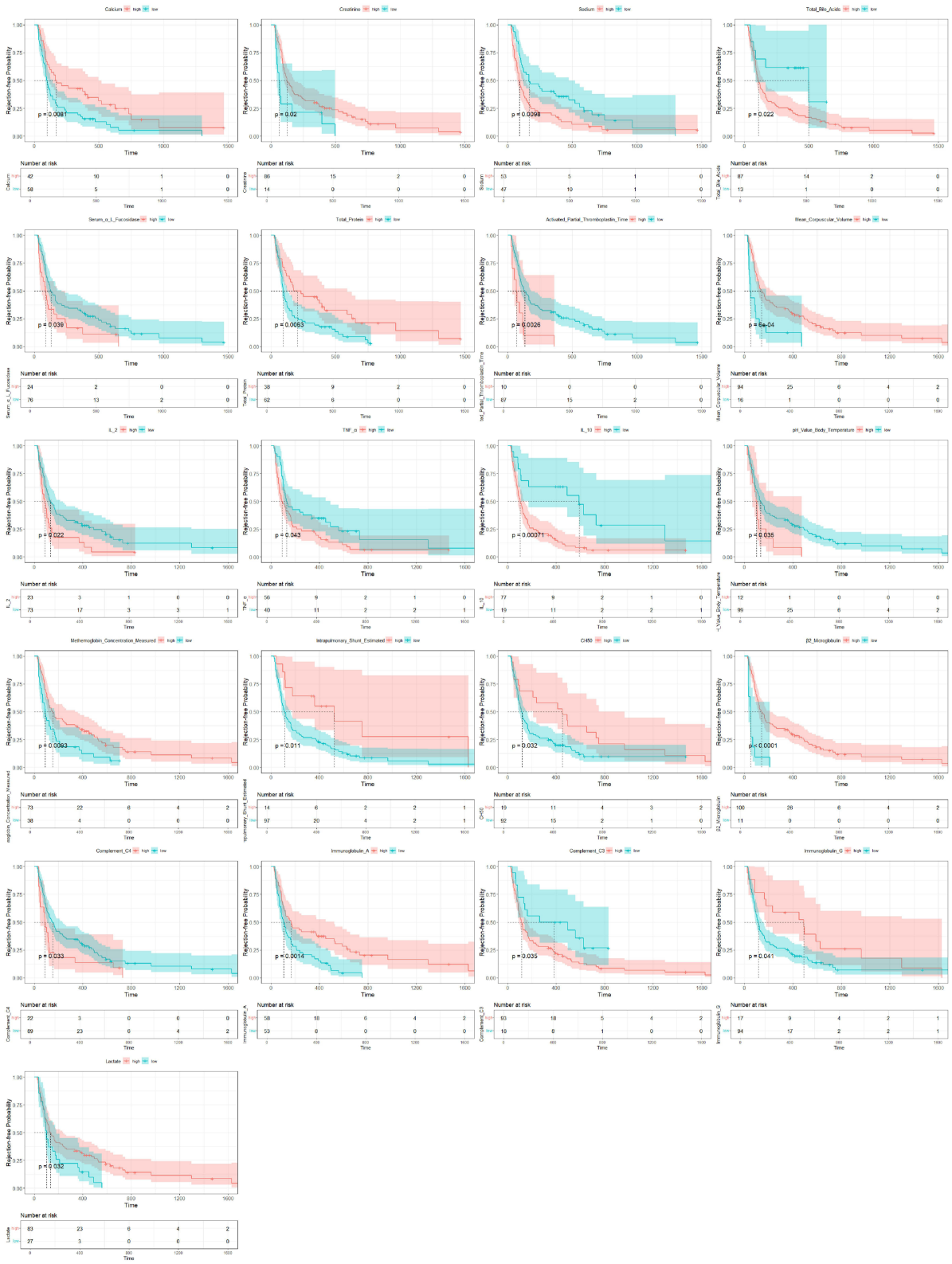

Figure 3 Kaplan-Meier Survival Analysis of Laboratory Indicators for Time to First Rejection Episode. To develop a prognostic model for predicting the time to the first rejection episode based on laboratory indicators in lung transplant patients, we divided the patients into a training set (70%) and a validation set (30%). This figure presents Kaplan-Meier survival curves for 21 laboratory indicators that showed significant associations with the time to the first rejection episode in the training set. Each plot compares the rejection-free survival between two groups categorized by the optimal cutoff points for each indicator. These findings were used to further refine the prognostic model through LASSO regression and multivariate Cox analysis.

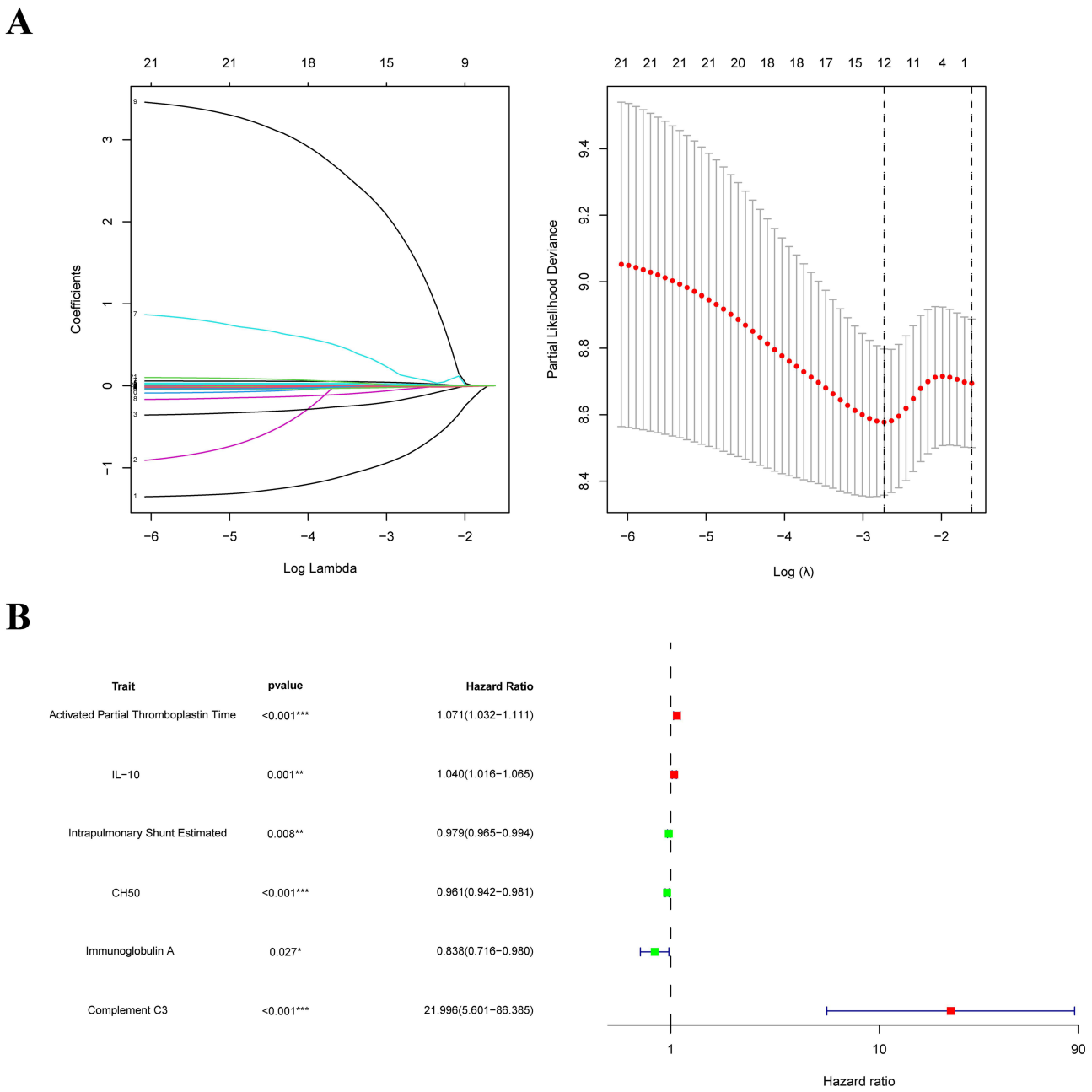

Figure 4 LASSO Regression and Multivariate Cox Analysis for Developing the Prognostic Model. (**A**) LASSO Regression Analysis: The left plot shows the coefficients of the 21 selected indicators as a function of the regularization parameter (log lambda). The right plot shows the 10-fold cross-validation results for tuning parameter selection in the LASSO model, with the dotted line indicating the optimal lambda that results in 12 non-zero coefficients. (**B**) Multivariate Cox Analysis: This plot shows the hazard ratios and p-values of the six key indicators identified through multivariate Cox analysis: Activated Partial Thromboplastin Time (APTT), Interleukin-10 (IL-10), estimated intrapulmonary shunt, 50% Hemolytic Complement (CH50), Immunoglobulin A (IgA), and Complement Component 3 (C3). These indicators were used to construct the final prognostic model for predicting the time to the first rejection episode in lung transplant patients.

through multivariate Cox analysis, we identified 6 indicators (APTT, IL-10, estimated intrapulmonary shunt, CH50, IgA, and C3) to construct the prognostic model (Figure 4B).

The risk score for each patient was calculated as the sum of the products of each indicator's level and its corresponding coefficient from the Cox model. Patients were then divided into high-risk and low-risk groups based on the surv_cutpoint function. Compared to the low-risk group, the high-risk group had a significantly lower rejection-free survival rate (Figure 5A). The risk curve showed that the riskScore performed well in assessing the prognostic outcomes of first rejection episode in lung transplant patients (Figure 5B). The ROC curve demonstrated that the riskScore could

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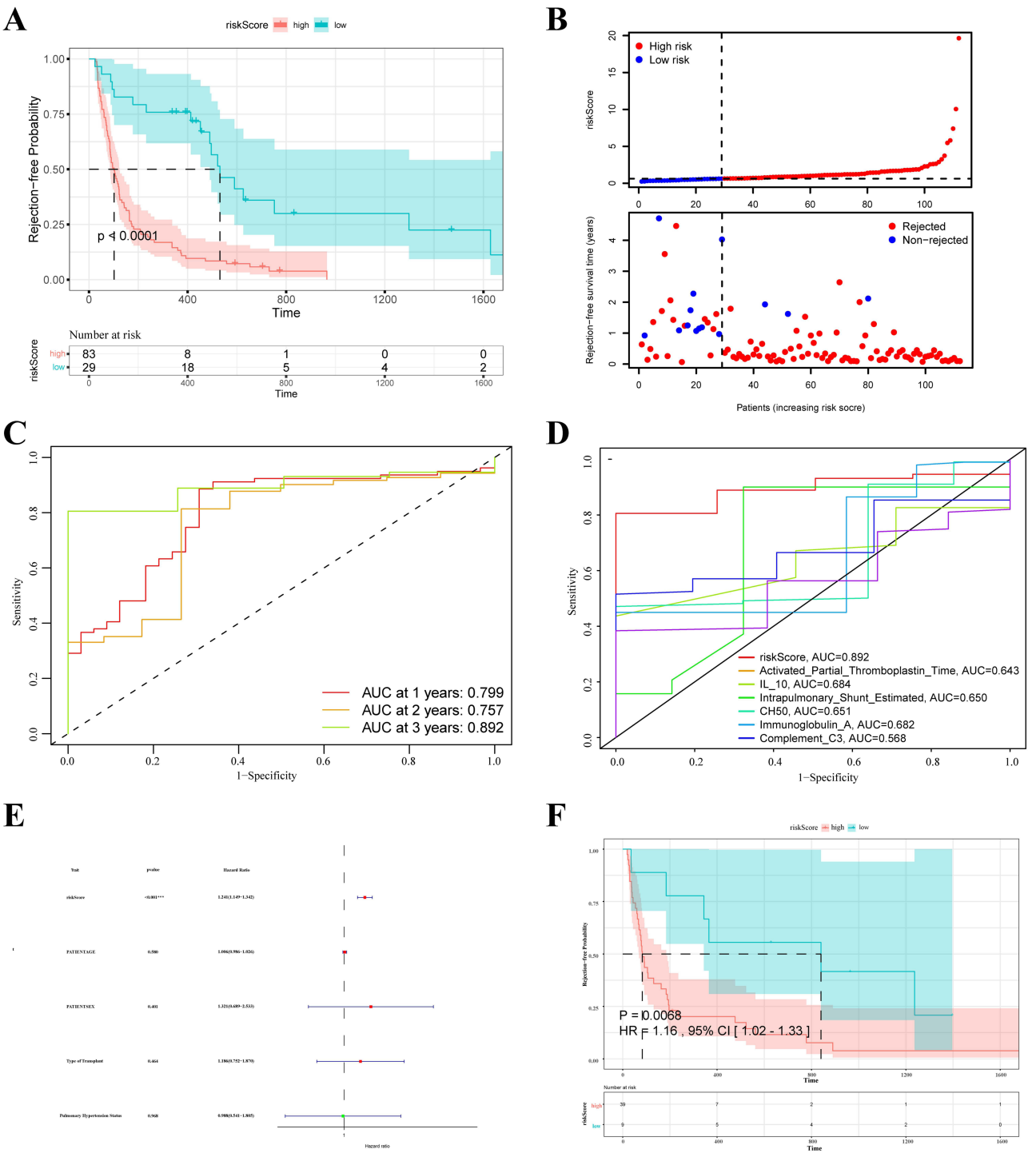

Figure 5 Performance of the Risk Score Model for Predicting Time to First Rejection Episode in Lung Transplant Patients. (**A**) Kaplan-Meier Survival Analysis: The Kaplan-Meier survival curves show the rejection-free survival rates for high-risk and low-risk groups based on the risk score. The high-risk group had a significantly lower rejection-free survival rate compared to the low-risk group ($p < 0.0001$). (**B**) Risk Score Distribution and Rejection Status: The top plot illustrates the distribution of risk scores, with patients classified into high-risk and low-risk groups. The bottom plot shows the corresponding rejection status (rejected vs non-rejected) for each patient, demonstrating the predictive power of the risk score. (**C**) ROC Curves for the Risk Score: The time-dependent ROC curves at 1 year, 2 years, 3 years, and 4 years demonstrate the sensitivity and specificity of the risk score in predicting the first rejection episode. The area under the curve (AUC) values are 0.799, 0.757, 0.892, and 0.868, respectively. (**D**) Comparison of ROC Curves for Individual Indicators: The ROC curves compare the predictive performance of the risk score against individual indicators. The risk score (red line) outperforms each individual indicator in predicting the first rejection episode. (**E**) Multivariate Cox Regression Analysis: The forest plot displays the hazard ratios and 95% confidence intervals for the risk score and other clinical variables. The risk score is an independent risk factor for rejection (HR 1.241, 95% CI 1.149–1.342, $p < 0.001$). (**F**) Validation in the Validation Dataset: The Kaplan-Meier survival curves for the validation dataset confirm the effectiveness of the constructed prognostic model. High-risk patients show significantly lower rejection-free survival rates compared to low-risk patients.

serve as a sensitive indicator for predicting the first rejection episode in lung transplant patients (1-year AUC: 0.799, 2-year AUC: 0.757, 3-year AUC: 0.892) (Figure 5C). Moreover, the ROC curve showed that the riskScore combining multiple indicators outperformed individual indicators in predicting the first rejection episode in lung transplant patients (Figure 5D). Multivariate Cox regression analysis revealed that the riskScore was an independent risk factor for lung transplant patients (HR 1.241, 95% CI 1.149–1.342, p <0.001) (Figure 5E). Finally, we validated the effectiveness of our constructed prognostic model in the validation dataset (HR 1.16, 95% CI 1.02–1.33, p=0.0068) (Figure 5F).

In conclusion, we developed a prognostic model based on six laboratory indicators to predict the time to first rejection episode in lung transplant patients. The model's performance was validated in an independent dataset, demonstrating its potential clinical utility in identifying high-risk patients and guiding personalized post-transplant management.

Discussion

In this study, we employed univariate Cox analysis to evaluate the potential associations between patients' demographic and clinical characteristics and the time to first rejection episode after LTx. The factors assessed included age, gender, transplant type, pulmonary hypertension status, and primary underlying diseases. The results demonstrated that none of these factors showed a statistically significant correlation with the time to first rejection episode (p>0.05). Previous studies have indicated that lung transplant outcomes are closely related to various clinical factors, such as primary diseases, transplant type, and recipient age. COPD and idiopathic pulmonary fibrosis are the most common indications for LTx,[7,15] but they differ in prognosis. Some studies suggest that COPD patients have relatively higher 1-year survival rates after transplantation, but their 5-year survival rates may be lower than those of patients with other diseases.[1,16] In contrast, patients with IPF and pulmonary hypertension generally have poorer overall prognoses.[16] Regarding transplant type, the overall survival rate of double LTx is usually higher than that of single LTx.[1,17] However, the difference in rejection risk between the two transplant types remains unclear. One study found that double LTx may reduce the incidence of BOS,[18] but this finding requires further validation. Moreover, for patients with pulmonary hypertension, the long-term prognosis does not differ significantly between double and single LTx.[19] Most previous studies have not found a significant association between gender and lung transplant outcomes,[20–22] which is consistent with our findings. The impact of age on transplant outcomes varies among studies. Some research indicates that younger recipients (<35 years old) have a higher incidence of acute rejection,[16] while older recipients (>50 years old) have an increased risk of mortality within the first year after transplantation.[1] However, other studies have not found a clear correlation between age and survival rates.[17]

Although our study did not find significant associations between patients' age, gender, transplant type, pulmonary hypertension status, primary diseases, and the time to first rejection episode, this does not imply that these factors are entirely unrelated to lung transplant outcomes. In fact, previous research has shown that different primary diseases, transplant types, and ages can influence various aspects of lung transplant outcomes, particularly long-term prognostic indicators such as survival rates and chronic rejection. The reasons for these differences may include the characteristics and severity of the diseases themselves, the degree of donor-recipient matching, response to immunosuppressive therapy, and the risk of complications. However, current research on the relationship between these factors and rejection episodes is insufficient, especially regarding their impact on the time to first rejection episode. There is a lack of evidence from prospective, large-sample studies. This may also be one of the reasons why our study failed to detect significant associations. Additionally, the relatively limited sample size in our study, particularly in some subgroups, may have affected the statistical power and made it difficult to detect potential correlations. Future research should involve larger-scale, multicenter, prospective cohort studies to further explore the relationship between patients' demographic and clinical characteristics and rejection episodes, as well as to investigate the underlying mechanisms. Moreover, it is necessary to dynamically assess the associations of these factors with rejection episodes and graft function at different time points after transplantation, which may help to more comprehensively understand their impact on lung transplant outcomes.

To further explore the value of laboratory indicators in predicting lung transplant rejection, we used machine learning methods to integrate multiple indicators and construct a risk assessment model for predicting the time to first rejection episode in lung transplant patients. Through Kaplan-Meier survival analysis, LASSO regression, and multivariate Cox

analysis, we selected 6 indicators with the highest predictive value from 69 candidate indicators, including APTT, IL-10, estimated intrapulmonary shunt, CH50, IgA, and C3. We then constructed a risk score (riskScore) based on the weighted combination of these six indicators. In an independent validation cohort, the riskScore demonstrated good predictive performance, with significantly lower rejection-free survival rates in the high-risk group compared to the low-risk group, suggesting that the model can effectively identify patients at high risk of rejection. Furthermore, the predictive efficacy of the riskScore was superior to that of individual indicators, reflecting the advantage of integrating multiple biomarkers.

Our findings are consistent with previous literature reports to some extent, and also expand the understanding of the role of related indicators in lung transplant rejection. IL-10, as an important anti-inflammatory cytokine, plays a crucial role in immune regulation after LTx. Elevated IL-10 levels have been associated with poor prognosis in lung transplant recipients.[23] IL-10 also plays a critical role in reducing the incidence and severity of primary graft dysfunction after LTx, thereby influencing both short-term and long-term outcomes for transplant recipients.[24] Additionally, IL-10 is important in immune regulation after LTx, with specific gene polymorphisms being associated with reduced infection risk and indirectly affecting the incidence of acute rejection.[25] Our study is the first to incorporate IL-10 into a comprehensive predictive model, further confirming its potential as a biomarker for rejection. Coagulation dysfunction is a common complication after LTx, and prolonged APTT indicates an increased risk of coagulation disorders, which can lead to adverse events such as bleeding or thrombosis.[26] However, the direct association between APTT and acute rejection has not been reported. The APTT indicator included in our study suggests that coagulation dysfunction may indirectly regulate immune responses by affecting the graft microenvironment, but the specific mechanism needs further exploration.

Estimated intrapulmonary shunt is an indicator that reflects the mismatch between alveolar ventilation and blood flow, and is closely related to transplanted lung function. Currently, research on the relationship between estimated intrapulmonary shunt and lung transplant prognosis is limited. Our study incorporated this indicator into the predictive model, providing new insights for exploring its clinical application value. The complement system plays a double-edged role in transplant rejection. On one hand, complement activation promotes inflammatory responses and tissue damage; on the other hand, complement regulatory factors such as C3 are also involved in inducing immune tolerance.[27] CH50, as an indicator of total complement activity, is closely related to C4d deposition and antibody-mediated rejection[28,29]. At the same time, donor-derived C3 is crucial for graft survival.[30] This is consistent with our research results, indicating the value of complement-related indicators in assessing rejection risk. IgA plays an important role in mucosal immune barriers, but its significance in LTx remains unclear. Some studies have shown that immunoglobulin preparations containing IgA and IgM help to clear donor-specific antibodies and prevent rejection.[31] Other studies suggest that IgA autoantibody levels are associated with Primary Graft Dysfunction and survival rates.[32] Our study included IgA in the prognostic model to reveal the synergistic effects of humoral and cellular immunity in rejection.

Recent proteomic and genomic studies have provided new insights into the molecular mechanisms of acute rejection. IL-10, as a key immunosuppressive cytokine, reflects immune tolerance dysregulation,[33] with studies showing elevated pro-inflammatory cytokines (IL-1β, IL-8) and decreased immunosuppressive mediators in transplant BAL fluid.[34] The reduced FOXP3 expression in Tregs[35] further indicates impaired immunoregulatory function. Additionally, APTT is mechanistically associated with endothelial dysfunction,[36] and proteomic studies have revealed increased MMP-9 expression,[37] reflecting microvascular injury and tissue remodeling, which regulate immune cell function and inflammatory responses. These molecular insights not only validate the biological basis of our laboratory-based risk score but also suggest potential therapeutic targets for rejection prevention. Our integration of routine laboratory parameters with these molecular mechanisms provides a clinically applicable approach while maintaining biological relevance in predicting acute rejection.

The strength of our study lies in the integration of multiple indicators reflecting different aspects of the body's immune status to construct a comprehensive predictive model. Compared to single biomarkers, this integrated strategy can more comprehensively assess patients' rejection risk and facilitate precise stratification and individualized management. However, our study also has some limitations. First, the sample size was relatively limited, especially considering the high heterogeneity of the lung transplant population. Future studies need to expand the sample size to improve the robustness of the model. Second, this was a retrospective study, making it difficult to control for various confounding

factors that may affect the interpretation of the results. Prospective cohort studies will help to further validate the predictive performance and clinical application value of the model. Third, differences between transplant centers, such as donor selection, immunosuppression regimens, and rejection diagnostic criteria, may affect the universality of the model. Multicenter studies are needed to verify its external applicability. Finally, the indicators included in the model were mainly hematological and immunological indicators. Future research can attempt to integrate biomarkers from multiple levels, such as histopathology, imaging, and genomics, to construct a more comprehensive and accurate predictive model. Additionally, the immune status of transplant patients is dynamically changing, and future research needs to explore strategies for dynamically monitoring risk changes.

In conclusion, lung transplant outcomes are the result of the combined effects of multiple factors, and the timing and risk of rejection may be influenced by patient individual differences, immune status, treatment regimens, and other factors. Although our study did not find significant associations between patients' demographic and clinical characteristics and the time to first rejection episode, different primary diseases, transplant types, and ages may still affect lung transplant outcomes in different ways. Future larger-scale, prospective studies are needed to further validate the prognostic value of these factors. At the same time, our study developed a lung transplant rejection risk prediction model based on six laboratory indicators, which demonstrated good predictive performance and potential clinical application value. The model integrated biomarkers reflecting coagulation function, cytokines, humoral immunity, and complement activation, providing a new tool for assessing patients' rejection risk. However, its application in clinical practice still requires further validation through large-scale, prospective studies. Future research directions include incorporating more comprehensive omics indicators, exploring the relationship between acute rejection, chronic rejection, and long-term prognosis, dynamically monitoring risk changes, and developing individualized diagnostic and treatment strategies. Through multidisciplinary collaboration and optimization of post-transplant monitoring and intervention measures, it is hoped that the long-term survival and quality of life of lung transplant patients can be further improved.

Abbreviations

APTT, Activated Partial Thromboplastin Time; AUC, Area under the curve; BO, Bronchiolitis Obliterans; BOS, Bronchiolitis obliterans syndrome; C3, Complement Component 3; CH50, 50% Hemolytic Complement; CLAD, Chronic lung allograft dysfunction; COPD, Chronic Obstructive Pulmonary Disease; IL-10, Interleukin-10; ILD, Interstitial Lung Disease; IgA, Immunoglobulin A; IP, Interstitial Pneumonia; ISHLT, International Society for Heart and Lung Transplantation; LAM, Lymphangioleiomyomatosis; LASSO, Least Absolute Shrinkage and Selection Operator; LCH, Langerhans Cell Histiocytosis; LTx, Lung transplantation; PIF, Pulmonary Interstitial Fibrosis; ROC, Receiver Operating Characteristic; TBLB, Transbronchial lung biopsy.

Data Sharing Statement

The datasets used and analyzed during the current study are included in this article and its supplementary information files.

Ethics Approval and Consent to Participate

The Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University waived the requirement for ethical approval and informed consent due to: (1) the retrospective nature of the study; (2) all patient data were anonymized and de-identified prior to analysis; and (3) no additional risks were posed to the patients. All organs were donated voluntarily with written informed consent at the time of donation, and the procedures were conducted in accordance with the Declaration of Istanbul. All patient data were handled in compliance with the Declaration of Helsinki principles for medical research involving human subjects. Patient confidentiality was strictly maintained throughout the study by using coding systems and restricting data access to authorized research personnel only.

Consent for Publication

Not applicable.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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