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Original Article

Construction and validation of a predictive model for severe pneumonia risk using respiratory pathogen nucleic acid Ct values combined with host immune biomarkers

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Abstract: Objectives: We developed a new predictive model to more accurately assess the risk of patients developing severe pneumonia (SP) after hospital admission. Methods: We retrospectively analyzed patients with pneumonia admitted between June 2022 and May 2024. According to the 2019 American Thoracic Society/Infectious Diseases Society of America guideline, patients were classified into SP and non-severe pneumonia (NSP) groups. Basic clinical information at admission and laboratory results, including complete blood count, coagulation function, biochemical parameters, and bacterial co-infection, were collected. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the cycle threshold (Ct) values of respiratory pathogen nucleic acids to estimate the pathogen load. Host immune biomarkers were measured in fasting serum collected on the morning following the first positive pathogen detection. Results: Among 241 patients (NSP group=139, SP group=102), patients with SP showed significantly lower pathogen Ct values (influenza A virus [IVA]: 24.32 ± 4.56 vs. 28.45 ± 3.21, $P<0.001$), higher C-peptide levels (2.72 ± 0.84 vs. 2.25 ± 0.68 ng/mL, $P<0.001$), and higher ferritin levels (590.67 ± 102.78 vs. 498.32 ± 110.45 μg/L, $P<0.001$). The area under the curve (AUC) was 0.906 in the training set and 0.926 in the test set, indicating high predictive accuracy of the model for SP risk. Conclusions: This study demonstrats that a predictive model combining quantitative pathogen load with host immune-metabolic biomarkers can effectively predict the risk of severe pneumonia.

Keywords: Severe pneumonia, prediction model, pathogen load, host immune biomarkers, inflammatory markers, metabolic biomarkers

Introduction

Pneumonia is an acute respiratory infection affecting the lungs, characterized by inflammation of lung tissue and typically caused by bacteria or viruses [1]. Patients commonly exhibit symptoms such as cough and fever, while severe cases may progress to respiratory distress and sepsis [2]. Severe pneumonia (SP) is associated with a high incidence of disease and mortality, posing a serious global public health challenge [3, 4]. Therefore, accurately determining the risk of a disease progression at an early stage is critical for improving patient outcomes. In recent years, with continuous advances in antiviral and antibacterial therapies and improvements in supportive treatments, the effectiveness of clinical management has significantly improved [5, 6]. Molecular diagnostic techniques, such as quantitative real-time polymerase chain reaction (qRT-PCR), have been increasingly applied to more accurately reflect pathogen load through cyclic threshold (Ct) values [7]. In addition, growing recognition of abnormal host immune responses, such as the use of biomarkers including ferritin to assess the severity of inflammation, has provided new reference indicators for clinical evaluation [8].

At present, the evaluation tools used in clinical practice have some shortcomings in integrating pathogen characteristics with the immune status of patients. Previous predictive models

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often fail to correlate pathogen load, such as Ct values from nucleic acid testing, with key host immune markers when predicting the risk of SP. This limitation makes it more difficult to identify high-risk patients at an early stage. In this study, we aimed to construct a new predictive model that incorporates both respiratory pathogen nucleic acid Ct values and host immune biomarkers to more accurately predict the risk of SP, thereby overcoming the limitations of existing evaluation tools.

Host immune responses have a significant impact on the clinical outcome of pneumonia. When the immune system overreacts or becomes dysregulated, it can lead to tissue damage and organ failure, which are characteristic of severe pneumonia (SP) [9]. Biomarkers such as ferritin reflect high levels of systemic inflammation and are often associated with macrophage overactivation and cytokine storms [10]. C-peptide levels are commonly used to assess pancreatic β-cell function; however, under severe inflammatory conditions, they can also serve as reference indicators of stress-induced hyperglycemia, which is frequently associated with poor prognosis for infectious diseases such as pneumonia [11]. On the other hand, a high pathogen load, particularly of influenza A virus (IVA) and respiratory syncytial virus (RSV), often indicates more severe disease. In nucleic acid of testing, a lower Ct value generally represents a higher viral load and is often associated with more pronounced clinical manifestations [12]. Ct values not only reflect pathogen burden at the early stage of infection but may also be related to the functional status of the host immune response, a relationship that can be further explored by assessing relevant biomarkers [13]. Indeed, there is a continuous and dynamic interaction between pathogens and the host immune system, providing important insights into the pathogenesis of SP. Combining these complementary factors may therefore effectively improve the ability to predict the risk of SP.

We aimed to develop and validate predictive models for SP risk by integrating qRT-PCR Ct values of several common respiratory pathogen species with host immune biomarker levels. Our innovation lies in the use of quantitative pathogen load indicators together with specific host immune response markers to construct a practical predictive tool. This approach is expected not only to support early clinical decision-making, but also to more precisely identify patients at high risk of SP, thereby enabling more targeted monitoring and intervention.

Materials and methods

Study design and patients

This study was a retrospective analysis of 241 hospitalized patients diagnosed with pneumonia at Yingshan County Hospital of Traditional Chinese Medicine between June 2022 and May 2024. Patients were included if they tested positive for at least one of six respiratory pathogens by quantitative real-time polymerase chain reaction (qRT-PCR) upon admission and met the diagnostic criteria for pneumonia established by the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) in 2019 [14]. Exclusion criteria were age younger than 18 years, liver failure, chronic dialysis, suspected active pulmonary tuberculosis, thrombosis or pulmonary embolism, primary immunodeficiency diseases, current immunosuppressive therapy, ongoing antineoplastic drug treatment or radiotherapy, and death within 24 hours of admission. Complete clinical records and laboratory test results were available for all included patients.

Patients were classified into non-severe pneumonia (NSP) group (n=139) and a severe pneumonia (SP) group (n=102) according to the 2019 ATS/IDSA guidelines for severe pneumonia (SP) [14]. All eligible patients (n=241) were randomly assigned to a training set (n=193) and a test set (n=48) at an 8:2 ratio. The training set included 107 NSP cases and 86 SP cases, while the test set consisted of 32 NSP cases and 16 SP cases.

Data collection and detection methods

Basic patient information and clinical data at admission were extracted from the hospital's electronic medical record system. These included demographic characteristics, comorbidities, laboratory tests result obtained within 48 hours of admission, and bacterial co-infection status (defined as positive sputum or blood culture results). The specific laboratory tests and detection methods are described below.

(1) Complete blood count, coagulation function, and biochemical analysis: An automated hematology analyzer (XN-1000, Sysmex, Japan), coagulation analyzer (CA-1500, Sysmex, Japan), and automated biochemical analyzer (Cobas 8000, Roche Diagnostics, Switzerland) were used to measure white blood cell count (WBC), neutrophil count (Neu), lymphocyte count (L), hemoglobin (Hb), platelet count (PLT), fibrinogen (FIB), D-dimer (D-D), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), procalcitonin (PCT), and C-reactive protein (CRP).

(2) Nucleic acid testing for six respiratory pathogens: The six respiratory pathogens tested included influenza A virus (IVA), influenza B virus (IVB), respiratory syncytial virus (RSV), adenovirus (Adv), human rhinovirus (HRV), and Mycoplasma pneumoniae (MP). Nasopharyngeal swabs or sputum samples were collected and immediately placed in pathogen transport medium (Cat. No. VS-02, Tianlong Biotechnology, China). Nucleic acid extraction was performed using an automated nucleic acid extraction system (MagNA Pure 96, Roche Diagnostics, Switzerland) with the corresponding extraction kit (06388980001, Roche Diagnostics, Switzerland). Amplification and detection were carried out using a qRT-PCR instrument (LightCycler 480, Roche Diagnostics, Switzerland) and a multiplex respiratory pathogen detection kit (RT-101, BioGerm Medical Technology, China). A Ct value ≤38 was considered positive, with lower Ct values indicating higher initial pathogen loads.

(3) Host immune marker detection: Peripheral venous blood samples (5 ml) were collected in the morning after an overnight fast on the day following the first positive nucleic acid test result. Serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum levels of C-peptide and ferritin were measured using an electrochemiluminescence immunoassay analyzer (Cobas e602, Roche Diagnostics, Switzerland).

Feature selection and predictive model development

First, the nucleic acid cycle threshold (Ct) values of six respiratory pathogens and host immune markers from the training set (193 cases) were included as candidate feature variables for model development. The Least Absolute Shrinkage and Selection Operator (LASSO) regression was initially applied for dimensionality reduction and preliminary feature selection. Subsequently, multivariate logistic regression analysis was performed to further refine the model, ensuring that clinically relevant variables were considered, including those with *P*-values close to, but not below the conventional significance threshold. A binary outcome variable indicating progression to severe pneumonia (SP; 1=SP, 0=NSP) was used to construct a nomogram-based prediction model using multivariate logistic regression. This approach allowed evaluation of all potentially meaningful predictors, including variables with borderline statistical significance, for their contribution to the model. Model performance was assessed using three methods. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the model's discriminative ability. Calibration curves were used to assess the agreement between predicted and observed risks. In addition, decision curve analysis (DCA) was performed to estimate the potential net clinical benefit of the model. Could provide in clinical practice. Finally, the predictive performance of the model was validated using an independent test dataset consisting of 48 samples.

We use 10-fold cross-validation to select the regularization parameter λ for LASSO regression. A set of candidate λ values was prepared and evaluated through grid search. The λ value corresponding to the smallest mean square error (MSE) was selected as the optimal parameter. Because some data were missing, multiple imputation methods were applied. For each imputed dataset, a predictive model was constructed and analyzed, resulting in five sets of estimates. These results were then combined using Rubin's rules to obtain more robust overall estimates. Variance inflation factor (VIF) were calculated for each predictor to assess multicollinearity, with a VIF value greater than 5 indicating potential collinearity. When this occurred, the relevant variable was either removed or combined with related variables to form a composite indicator. In addition, correlations among Ct values of different pathogens were examined. All correlation coefficients were below 0.7. Indicating that no serious multicollinearity was present in the data.

We intentionally selected only six respiratory pathogen cycle threshold (Ct) values and host immune biomarkers as candidate features in our model. This choice was made primarily to focus on our core research objectives: assessing the specific predictive value of quantitative pathogen load and host immune-metabolic responses without interference from other clinical or routine laboratory variables. Although significant differences were observed between the NSP and SP groups across several demographic and laboratory indicators, we considered these variables more likely to reflect potential confounding factors or downstream pathophysiological processes. Including all statistically significant variables in the model could introduce issues related to overfitting or multicollinearity, thereby obscuring the interpretation of key pathogen-host interactions. Therefore, our variable selection strategy emphasized operationally meaningful biomarkers that were readily available early after admission, and closely aligned with our targeted hypotheses regarding the underlying mechanisms at this stage of disease progression.

Ethical statement

This retrospective study was conducted in strict compliance with medical ethical standards and the principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (IRB) of Yingshan County Hospital of Traditional Chinese Medicine. As the study used existing anonymized clinical data, involved no additional interventions, and all personally identifiable information was removed during data processing to protect patient privacy, the IRB granted an exemption from obtaining informed consent.

Statistics analyses

Statistics analyses were performed using SPSS statistical software (Version 29.0; SPSS Inc., Chicago, IL, USA). Statistical significance was defined as at a two-tailed *P*-value < 0.05. The Shapiro-Wilk test was used to assess the normality of continuous variables. All continuous variables were confirmed to follow a normal distribution and are therefore presented as mean ± standard deviation (M ± SD). Between-group comparisons of continuous variables were conducted using independent samples *t*-tests. Categorical variables are expressed as frequencies and percentages [n (%)] and were compared between groups using the χ^2 test.

Result

Model construction and preliminary validation

Baseline characteristics in the training set: In the training set, comparisons between the non-severe pneumonia (NSP) and severe pneumonia (SP) groups revealed several significant differences (**Table 1**). Patients in the SP group were significantly older than those in the NSP group (P<0.001). There was no significant difference in sex distribution between the two groups (P=0.237). However, body mass index (BMI) was significantly lower in the SP group compared with the NSP group (P=0.001). With respect to smoking status, the SP group included more current smokers and fewer never smokers than the NSP group (P=0.003). Among comorbidities, the prevalence of hypertension (P=0.028) and diabetes (P=0.023) was significantly higher in the SP group. Laboratory examinations showed that patients with SP had significantly higher levels of white blood cell count (WBC), neutrophil count (Neu), D-dimer (D-D), alanine aminotransferase (ALT), aspartate aminotransferase (AST), fibrinogen (FIB), lactate dehydrogenase (LDH), procalcitonin (PCT), and C-reactive protein (CRP), as well as a significantly higher rate of bacterial co-infection (all *P*<0.05). In contrast, lymphocyte count (L) was significantly lower in the SP group compared with the NSP group (P<0.001). Overall, patients with SP tended to be older and have lower BMI values and were more frequently affected by comorbid conditions such as hypertension and diabetes mellitus. Laboratory indicators suggested a more pronounced inflammatory response and a higher risk of bacterial co-infection, indicating a more complex and severe clinical condition in these patients.

Respiratory pathogen nucleic acid testing in the training set: In Comparison cycle threshold (Ct) values for six respiratory pathogens between the NSP group and SP groups revealed significant differences for all pathogens examined (**Table 2**). Significantly lower Ct values were observed in the SP group, for including influenza A virus (IVA; *P*<0.001), respiratory syncytial virus (RSV; *P*<0.001), adenovirus (Adv; *P*=0.034), human rhinovirus (HRV; *P*=0.013),

Table 1. Comparison of basic data between two group in the training set

Indicators	NSP group (n=107)	SP group (n=86)	t/χ^2	P
Demographics				
Age (years)	60.35 ± 14.62	72.82 ± 13.49	6.097	<0.001
Male/Female [n (%)]	62 (57.94%)/45 (42.06%)	57 (66.28%)/29 (33.72%)	1.401	0.237
BMI (kg/m^2)	23.45 ± 3.12	21.87 ± 3.45	3.342	0.001
Smoking status [n (%)]			11.679	0.003
Current smoking	35 (32.71%)	41 (47.67%)		
Previous smoking history	10 (9.35%)	16 (18.60%)		
Never smoking	62 (57.94%)	29 (33.72%)		
Comorbidity				
Hypertension [n (%)]	31 (28.97%)	38 (44.19%)	4.805	0.028
COPD [n (%)]	18 (16.82%)	17 (19.77%)	0.279	0.598
Diabetes [n (%)]	10 (9.35%)	18 (20.93%)	5.159	0.023
Coronary heart disease [n (%)]	6 (5.61%)	6 (6.98%)	0.153	0.695
Cerebral infarction [n (%)]	3 (2.80%)	8 (9.30%)	2.635	0.105
Renal disease [n (%)]	3 (2.80%)	4 (4.65%)	0.087	0.768
Hepatitis [n (%)]	2 (1.87%)	4 (4.65%)	0.476	0.490
Laboratory examination				
WBC (×10^9/L)	7.85 ± 2.31	11.24 ± 3.56	7.652	<0.001
Neu (×10^9/L)	5.62 ± 1.14	9.37 ± 2.21	14.289	<0.001
L (×10^9/L)	1.45 ± 0.42	1.18 ± 0.27	5.372	<0.001
Hb (g/L)	128.45 ± 18.32	131.67 ± 20.14	1.161	0.247
PLT (×10^9/L)	215.67 ± 68.32	191.24 ± 52.45	2.810	0.005
FIB (g/L)	3.45 ± 1.12	3.92 ± 1.27	2.714	0.007
D-D (μg/mL)	0.85 ± 0.22	0.94 ± 0.27	2.279	0.024
ALT (U/L)	28.45 ± 8.67	31.92 ± 9.34	2.670	0.008
AST (U/L)	32.12 ± 5.24	34.17 ± 5.12	2.726	0.007
TBIL (μmol/L)	12.45 ± 3.32	13.27 ± 4.21	1.479	0.141
DBIL (μmol/L)	6.12 ± 1.27	5.85 ± 1.34	1.443	0.151
ALP (U/L)	65.67 ± 25.45	72.34 ± 22.12	1.916	0.057
LDH (U/L)	245.67 ± 75.32	387.45 ± 80.34	12.617	<0.001
PCT (ng/mL)	0.39 ± 0.05	0.52 ± 0.15	7.977	<0.001
CRP (mg/L)	32.64 ± 6.92	48.75 ± 15.36	9.693	<0.001
Co-infection with bacteria [n (%)]	26 (24.30%)	61 (70.93%)	41.876	<0.001

NSP, non-severe pneumonia; SP, severe pneumonia; BMI, body mass index; COPD, chronic obstructive pulmonary disease; WBC, white blood cells; Neu, neutrophil; L, lymphocyte; PLT, platelet; Hb, hemoglobin; FIB, fibrinogen; D-D, D-dimer; ALT, alanine transaminase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; PCT, procalcitonin; CRP, C-reactive protein.

and *Mycoplasma pneumoniae* (MP; *P*=0.013), indicating higher pathogen loads in patients with SP. Influenza B virus (IVB) also showed a significant difference, with higher Ct values observed in the NSP group (P=0.018). Overall, these findings suggest that patients with non-severe pneumonia tend to have higher Ct values, and thus, lower pathogen loads, across multiple respiratory pathogens compared with patients with severe pneumonia.

In the comparison of respiratory pathogen positivity rates between the NSP group and SP groups, significant differences were observed for all six pathogens (**Figure 1**). The positivity rates for IVA (*P*<0.001), IVB (*P*=0.042), RSV (*P*<0.001), Adv (*P*=0.041), HRV (*P*=0.045), and MP (*P*=0.025) were significantly higher in the SP group than in the NSP group. Overall, IVA, RSV, Adv, HRV, and MP were more frequently detected in patients with SP, indicating that

Table 2. Comparison of six respiratory pathogens nucleic acid Ct values between two group in the training set

Indicators	NSP group (n=107)	SP group (n=86)	t	P
IVA	28.45 ± 3.21	24.32 ± 4.56	7.111	<0.001
IVB	30.12 ± 4.32	28.48 ± 5.21	2.387	0.018
RSV	26.78 ± 3.45	23.34 ± 4.12	6.305	<0.001
Adv	32.15 ± 5.21	30.37 ± 6.34	2.136	0.034
HRV	29.87 ± 4.56	27.95 ± 5.78	2.518	0.013
MP	31.23 ± 5.67	29.09 ± 6.12	2.508	0.013

NSP, non-severe pneumonia; SP, severe pneumonia; IVA, influenza virus A; IVB, influenza virus B; RSV, respiratory syncytial virus; Adv, adenovirus; HRV, human rhinovirus; MP, mycoplasma pneumoniae.

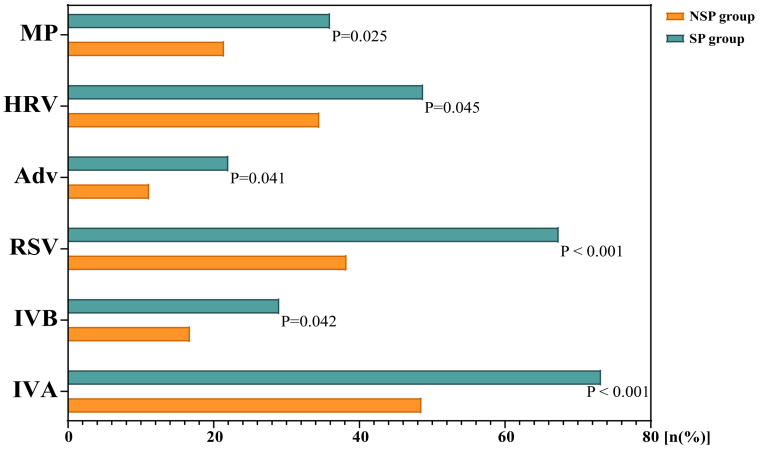

Figure 1. Comparison of positivity rates for six respiratory pathogens between the two groups in the training set. NSP, non-severe pneumonia; SP, severe pneumonia; IVA, influenza virus A; IVB, influenza virus B; RSV, respiratory syncytial virus; Adv, adenovirus; HRV, human rhinovirus; MP, mycoplasma pneumoniae.

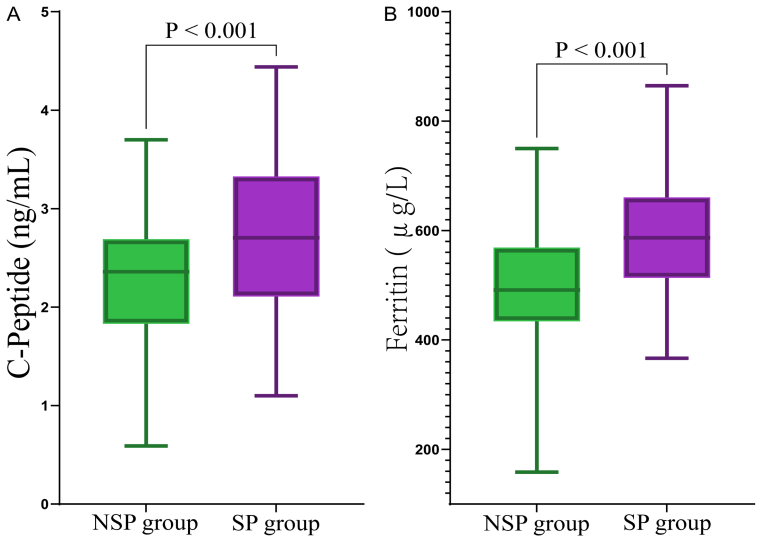

Figure 2. Comparison of host immune biomarker levels between two groups in the training set. A: C-Peptide (ng/mL); B: Ferritin (μg/L). NSP, non-severe pneumonia; SP, severe pneumonia.

these pathogens are more likely to be present in severe cases than in non-severe cases.

Host immune biomarker testing in the training set: In the training set, compared with patients with NSP, those with SP exhibited significantly higher levels of C-peptide and ferritin, with the difference in ferritin levels being particularly pronounced (P<0.001) (**Figure 2**). Similarly, ferritin levels were significantly higher in the SP group than in the NSP group (*P*<0.001). These findings suggest that C-peptide and ferritin levels may serve as important indicators for assessing pneumonia severity and could aid clinicians in more accurately evaluating disease status.

Multivariate logistic regression analysis: In the multivariate analysis combining respiratory pathogen nucleic acid Ct values with host immune biomarkers to predict the risk of severe pneumonia (SP), several variables were significantly associated with the SP risk (**Table 3**). Higher Ct values for IVA were significantly associated with a reduced risk of SP (*P*<0.001), and similar associations were observed for higher Ct values of RSV (*P*=0.001), HRV (*P*=0.034), and MP (*P*=0.028). In contrast, elevated C-peptide levels were significantly associated with an increased risk of SP (*P*=0.006), and elevated ferritin levels showed the same trend (*P*<0.001). Notably, IVB (*P*=0.059) and Adv (*P*=0.072) were not significantly associated with SP risk at the conventional *P*<0.05 threshold. These findings suggest that lower Ct values for specific respiratory pathogens and elevated host immune biomarkers may be

Table 3. Multivariate analysis of respiratory pathogens nucleic acid Ct Values combined with host immune biomarkers in predicting the risk of SP

Indicators	Coefficient	Std Error	Wald Stat	P	OR	OR CI Lower	OR CI Upper
IVA	-0.313	0.062	25.604	<0.001	0.731	0.647	0.825
IVB	-0.086	0.046	3.557	0.059	0.917	0.838	1.003
RSV	-0.210	0.064	10.772	0.001	0.810	0.715	0.919
Adv	-0.071	0.039	3.240	0.072	0.931	0.862	1.006
HRV	-0.091	0.043	4.490	0.034	0.913	0.839	0.993
MP	-0.082	0.037	4.805	0.028	0.921	0.856	0.991
C-Peptide	0.833	0.301	7.650	0.006	2.299	1.275	4.148
Ferritin	0.011	0.003	17.640	<0.001	1.011	1.006	1.016

IVA, influenza virus A; IVB, influenza virus B; RSV, respiratory syncytial virus; Adv, adenovirus; HRV, human rhinovirus; MP, mycoplasma pneumoniae; OR, odds ratio; CI, confidence interval.

Table 4. ROC analysis of respiratory pathogens nucleic acid Ct Values combined with host immune biomarkers in predicting the risk of SP

Indicators	Best threshold	Sensitivities	Specificities	AUC	Youden index	F1 score
IVA	26.415	0.721	0.748	0.784	0.469	0.253
IVB	27.285	0.442	0.766	0.594	0.208	0.444
RSV	24.805	0.651	0.729	0.736	0.380	0.309
Adv	29.620	0.442	0.710	0.574	0.152	0.457
HRV	22.83	0.244	0.972	0.591	0.216	0.510
MP	31.565	0.663	0.495	0.594	0.158	0.345
C-Peptide	2.790	0.465	0.813	0.660	0.278	0.548
Ferritin	553.61	0.686	0.701	0.73	0.387	0.667

IVA, influenza virus A; IVB, influenza virus B; RSV, respiratory syncytial virus; Adv, adenovirus; HRV, human rhinovirus; MP, mycoplasma pneumoniae; AUC, area under the curve.

useful for predicting pneumonia exacerbation and identifying patients at high-risk of SP.

ROC analysis: Receiver operating characteristic (ROC) analysis showed that RSV had an area under the curve (AUC) value of 0.736, indicating good diagnostic performance (**Table 4**). Its sensitivity and specificity were also relatively favorable, suggesting effective discrimination between NSP and SP cases. Ferritin likewise demonstrated good diagnostic ability, with an AUC value of 0.73 and acceptable sensitivity and specificity in distinguishing between NSP and SP. In contrast, the diagnostic performance of several other indicators was less satisfactory, including IVB (AUC=0.594), Adv (AUC=0.574), HRV (AUC=0.591), MP (AUC=0.594), and C-Peptide (AUC=0.660), indicating limited utility in predicting SP risk when used individually. Overall, RSV Ct values and ferritin levels appear to be more reliable indicators for assessing pneumonia severity than the other evaluated variables.

Establishment and evaluation of the predictive model: The nomogram integrated multiple indicators, including IVA, RSV, HRV, MP Ct values, as well as C-Peptide and ferritin levels, to calculate a total risk score (**Figure 3A**). Each indicator was assigned a corresponding score based on its measured value, and the sum of these scores yielded the overall risk score. The probability scale at the bottom of the nomogram was used to estimate the likelihood of severe pneumonia (SP). Thereceiver operating characteristic (ROC) curve analysis showed that the predictive model achieved an area under the curve (AUC) of 0.906 (95% confidence interval [CI]: 0.867-0.938), indicating high diagnostic accuracy (**Figure 3B**). The Youden index points (0.423, 0.841, 0.872) identified threshold values that provided an optimal balance between sensitivity and specificity. Calibration curves were used to compare observed probabilities of SP with model-predicted probabilities (**Figure 3C**). The apparent performance, bias-corrected performance, and ideal performance were

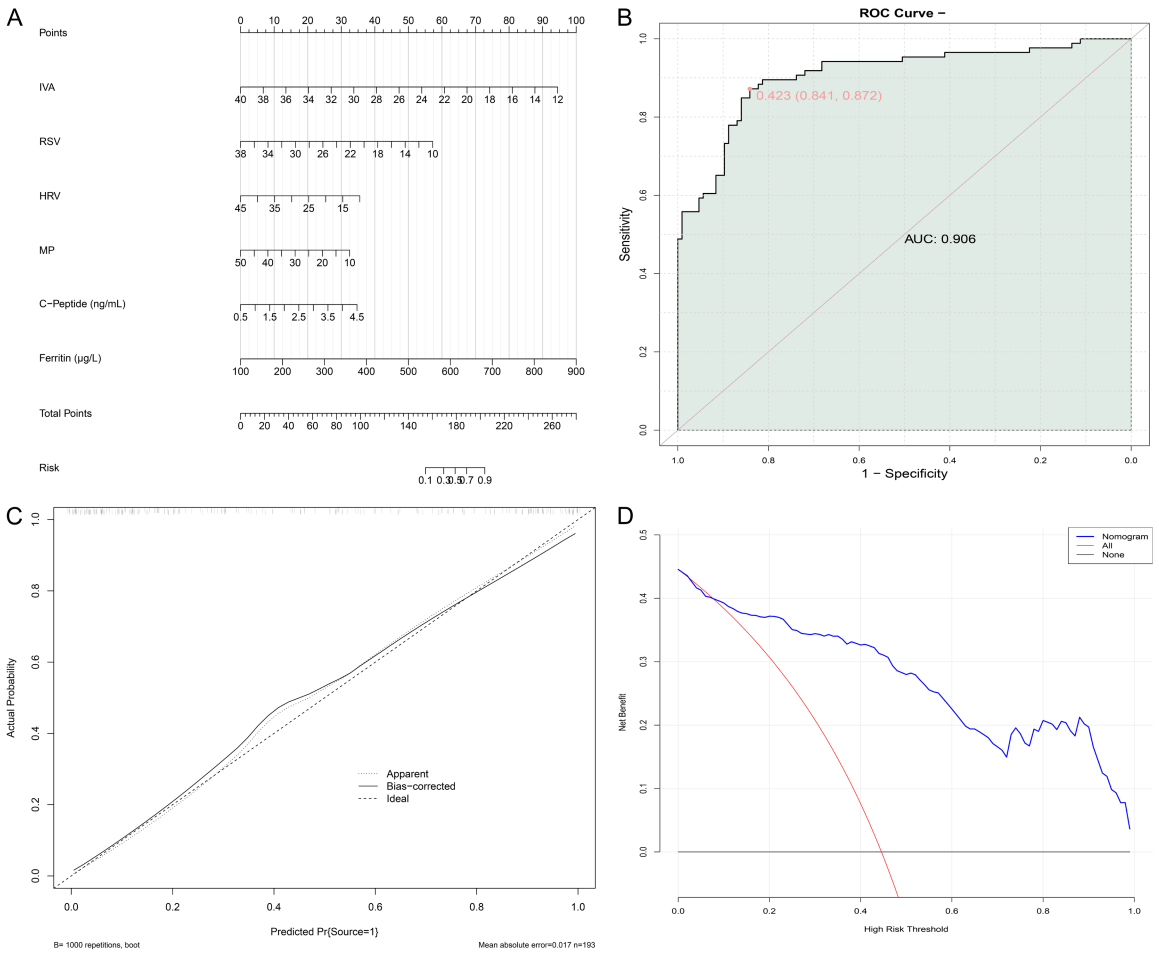

Figure 3. Establishment of a nomogram predictive model combining respiratory pathogen nucleic acid Ct Values and host immune biomarkers to predict the risk of SP. A. Nomogram showing the score of each predictive indicator and the overall risk score; B. ROC curve demonstrating the model's predictive performance, with an AUC of 0.906; C. Calibration curve indicating good agreement between predicted probabilities and observed outcomes; D. DCA showing a positive net clinical benefit for decision-making. SP, severe pneumonia; ROC, receiver operating characteristic; DCA, decision curve analysis; AUC, area under the curve.

represented by three lines, and the mean absolute error of 0.017 indicated good agreement between predicted and observed outcomes. Decision curve analysis (DCA) demonstrated the net clinical benefit of the nomogram across a range of high-risk thresholds (**Figure 3D**). The net benefit of using the nomogram (blue line) remained higher than the strategies of treating all or no patients as high risk (red and gray lines) over a relevant threshold range, indicating that the model provides added value in predicting SP risk.

External validation

Baseline characteristics in the test set: In the test set, several indicators differed significantly between the non-severe pneumonia (NSP) and severe pneumonia (SP) groups (**Table 5**). Age was significantly higher in the SP group than in the NSP group (P=0.009), indicating that older patients were more likely to develop severe pneumonia. Smoking status also differed significantly between groups (P=0.043), with a higher proportion of current smokers in the SP group. laboratory examinations showed that white blood cell count (WBC) was significantly higher in the SP group than in the NSP group (P=0.002), suggesting a more intense inflammatory response in severe cases. Patients in the SP group also exhibited significantly higher levels of neutrophil counts (Neu) and significantly lower lymphocyte counts (L) (P<0.001 for both). At the same time, Platelet count (PLT) and fibrinogen (FIB) levels were significantly reduced (P=0.028 and P=0.027, respectively),

Table 5. Comparison of basic data between two group in the test set

Indicators	NSP group (n=32)	SP group (n=16)	t/χ^2	P
Demographics				
Age (years)	61.24 ± 13.85	72.56 ± 12.73	2.741	0.009
Male/Female [n (%)]	19 (59.38%)/13 (40.62%)	11 (68.75%)/5 (31.25%)	0.400	0.527
BMI (kg/m^2)	23.12 ± 2.98	21.45 ± 2.22	1.985	0.053
Smoking status [n (%)]			6.279	0.043
Current smoking	11 (34.38%)	11 (68.75%)		
Previous smoking history	3 (9.38%)	2 (12.50%)		
Never smoking	18 (56.25%)	3 (18.75%)		
Comorbidity				
Hypertension [n (%)]	9 (28.12%)	9 (56.25%)	3.600	0.058
COPD [n (%)]	5 (15.62%)	4 (25.00%)	0.154	0.695
Diabetes [n (%)]	2 (6.25%)	3 (18.75%)	0.698	0.404
Laboratory examination				
WBC (×10^9/L)	8.12 ± 2.45	10.87 ± 3.21	3.304	0.002
Neu (×10^9/L)	5.84 ± 1.21	9.02 ± 2.15	5.518	<0.001
L (×10^9/L)	1.38 ± 0.39	0.95 ± 0.25	4.059	<0.001
Hb (g/L)	127.85 ± 17.32	130.45 ± 19.24	0.473	0.638
PLT (×10^9/L)	220.45 ± 61.27	180.32 ± 50.12	2.265	0.028
FIB (g/L)	3.52 ± 1.08	4.36 ± 1.42	2.285	0.027
D-D (μg/mL)	0.84 ± 0.21	0.98 ± 0.25	2.046	0.047
ALT (U/L)	27.12 ± 8.45	34.56 ± 9.12	2.804	0.007
AST (U/L)	31.45 ± 5.12	36.78 ± 6.87	3.024	0.004
TBIL (μmol/L)	12.67 ± 3.45	14.32 ± 4.12	1.470	0.148
DBIL (μmol/L)	6.24 ± 1.32	5.62 ± 1.28	1.554	0.127
ALP (U/L)	66.12 ± 24.56	71.45 ± 21.87	0.735	0.466
LDH (U/L)	250.12 ± 72.45	380.56 ± 78.32	5.725	<0.001
PCT (ng/mL)	0.41 ± 0.04	0.51 ± 0.12	3.316	0.004
CRP (mg/L)	31.23 ± 8.67	46.45 ± 12.21	4.991	<0.001
Co-infection with bacteria [n (%)]	7 (21.88%)	11 (68.75%)	10.000	0.002

NSP, non-severe pneumonia; SP, severe pneumonia; BMI, body mass index; COPD, chronic obstructive pulmonary disease; WBC, white blood cells; Neu, neutrophil; L, lymphocyte; PLT, platelet; Hb, hemoglobin; FIB, fibrinogen; D-D, D-dimer; ALT, alanine transaminase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; PCT, procalcitonin; CRP, C-reactive protein.

while dimer (D-D) levels were elevated (P= 0.047), indicating coagulation abnormalities. In addition, alanine aminotransferase (ALT; P= 0.007) and aspartate aminotransferase (AST; P=0.004) levels were significantly higher in the SP group, suggesting hepatic involvement. Lactate dehydrogenase (LDH) levels were also markedly elevated (P<0.001), further supporting the presence of tissue damage. Notably, procalcitonin (PCT) levels were significantly higher in the SP group (P=0.004), indicating an increased risk of bacterial co-infection, which was further confirmed by a significantly higher co-infection rate (P=0.002). C-reactive protein (CRP) levels were also significantly elevated (P<0.001), providing additional evidence of systemic inflammation. Compared with the NSP group, patients in the SP group exhibited a more intense inflammatory response, more pronounced immune dysregulation, and signs of multiple organ involvement. These findings contribute to a more accurate assessment of disease severity.

Respiratory pathogen nucleic acid testing in the test set: When comparing respiratory pathogen nucleic acid Ct values between the NSP and the SP groups, significant differences were observed for all pathogens tested (**Table 6**). Specifically, the Ct value of IVA in the SP group

Table 6. Comparison of six respiratory pathogens nucleic acid Ct values between two group in the test set

Indicators	NSP group (n=32)	SP group (n=16)	t	P
IVA	28.67 ± 3.45	25.29 ± 4.21	2.973	0.005
IVB	29.87 ± 4.12	27.05 ± 5.03	2.075	0.044
RSV	27.12 ± 3.21	24.18 ± 3.98	2.755	0.008
Adv	31.89 ± 5.34	28.12 ± 6.21	2.185	0.034
HRV	30.23 ± 4.45	27.12 ± 5.67	2.082	0.043
MP	31.56 ± 5.78	27.45 ± 6.34	2.252	0.029

NSP, non-severe pneumonia; SP, severe pneumonia; IVA, influenza virus A; IVB, influenza virus B; RSV, respiratory syncytial virus; Adv, adenovirus; HRV, human rhinovirus; MP, mycoplasma pneumoniae.

Table 7. Comparison of six respiratory virus positivity rate between two group in the test set

Indicators	NSP group (n=32)	SP group (n=16)	χ^2	P
IVA [n (%)]	13 (40.62%)	12 (75.00%)	5.050	0.025
IVB [n (%)]	3 (9.38%)	5 (31.25%)	2.269	0.132
RSV [n (%)]	10 (31.25%)	10 (62.50%)	4.286	0.038
Adv [n (%)]	2 (6.25%)	4 (25.00%)	1.929	0.165
HRV [n (%)]	9 (28.12%)	7 (43.75%)	1.172	0.279
MP [n (%)]	5 (15.62%)	6 (37.50%)	1.784	0.182

NSP, non-severe pneumonia; SP, severe pneumonia; IVA, influenza virus A; IVB, influenza virus B; RSV, respiratory syncytial virus; Adv, adenovirus; HRV, human rhinovirus; MP, mycoplasma pneumoniae.

Table 8. Comparison of host immune biomarker levels between two group in the test set

Indicators	NSP group (n=32)	SP group (n=16)	t	P
C-Peptide (ng/mL)	2.18 ± 0.65	2.69 ± 0.72	2.463	0.018
Ferritin (μg/L)	492.45 ± 105.32	575.78 ± 95.67	2.661	0.011

NSP, non-severe pneumonia; SP, severe pneumonia.

was significantly lower than that in the NSP group (P=0.005), indicating a higher viral load in patients with SP. Similarly, IVB (P=0.044), RSV (P=0.008), Adv (P=0.034), HRV (P=0.043) and MP (P=0.029) also showed significantly lower Ct values in the SP group. Overall, these findings suggest that patients in the SP group generally had higher pathogen loads across all tested respiratory pathogens, supporting the association between increased pathogen burden and greater disease severity.

When comparing the positivity rates of respiratory pathogens between the NSP and SP groups, significant differences were observed for some pathogens (**Table 7**). Specifically, the positivity rate of IVA was significantly higher in the SP group than in the NSP group (P=0.025), indicating a higher prevalence of IVA infection among critically ill patients. Similarly, the positivity rate of RSV was significantly higher in the SP group (P=0.038). These findings suggest that IVA and RSV infections may be associated with pneumonia severity and could be important contributors to disease exacerbation. In contrast, no significant differences were observed between the two groups for IVB (P=0.132), Adv (P=0.165), HRV (P=0.279), or MP (P=0.182).

Host immune biomarker testing in the test set: In the SP group, C-peptide levels were significantly higher than those in the NSP group (P=0.018), suggesting that elevated C-peptide levels may be associated with disease severity (**Table 8**). Similarly, ferritin levels were significantly higher in patients with SP (P=0.011), indicating a more intense inflammatory response or greater systemic involvement in critically ill patients. Overall, these findings suggest that specific host immune biomarker levels are elevated in patients with SP compared with those with NSP, potentially reflecting a more pronounced metabolic and inflammatory state in severe disease.

ROC prediction model: In this study, the predictive model for assessing the risk of severe pneumonia (SP) demonstrated excellent performance (**Figure 4**). The area under the curve (AUC) was 0.926. Given the relatively limited sample size of the test set, a bootstrap resampling method was applied to enhance the reliability of model evaluation. The resulting 95% confidence interval ranged from 0.872 to 0.965. In addition, a receiver operating characteristic (ROC) curve illustrating the relationship between sensitivity and specificity was generated. Several key points along the curve correspond to different sensitivity and specificity values, which facilitated identification of the

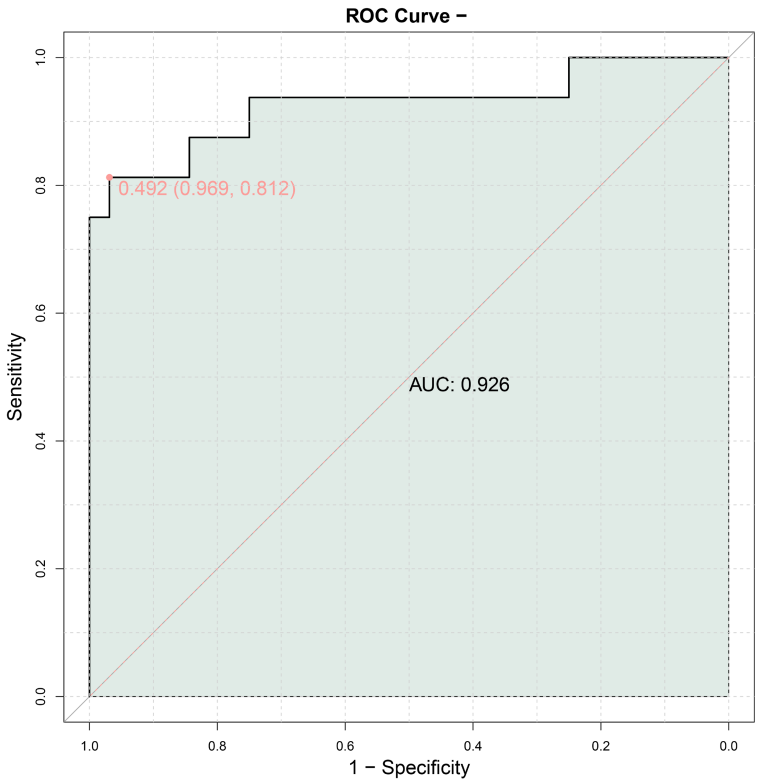

Figure 4. Construction of ROC curves for respiratory pathogen nucleic acid Ct values combined with host immune biomarkers in predicting the risk of SP. SP, severe pneumonia; ROC, receiver operating characteristic; AUC, area under the curve.

optimal diagnostic threshold and further confirmed the model's reliability in distinguishing severe and non-severe pneumonia. Overall, the image further suggested that combining pathogen load with immune biomarkers improves the prediction of severe pneumonia risk.

Discussion

We developed a predictive model that combines respiratory pathogen nucleic acid cycle threshold (Ct) values with host immune biomarkers to predict the risk of progression to severe pneumonia (SP). We found that the quantitative pathogen burden, when integrated with key host inflammatory and metabolic responses, provides more reliable prognostic information than either factor alone. This comprehensive analytical approach is more effective than traditional syndrome-based assessment methods, compensates for the limited predictive power of single biomarkers, and is well suited for early risk stratification at the time of hospital admission. These findings further demonstrate that the pneumonia severity results from a complex interaction between pathogen virulence and host defense mechanisms, which are central to disease progression.

We observed that lower cycle threshold (Ct) values and higher pathogen loads were positively associated with the risk of severe pneumonia (SP) across multiple respiratory pathogens, with this association being particularly evident for influenza A virus (IVA) and respiratory syncytial virus (RSV). This finding is consistent with previous studies showing that high initial pathogen loads can overwhelm the host's innate immune defenses, leading to tissue damage and systemic inflammation [15, 16]. Our results further support the notion that IVA and RSV exhibit the strongest individual correlation with disease severity, both in terms of Ct values and positivity rates. Indeed, these two pathogens have long been recognized as major causes of pneumonia-related hospitalization and poor prognosis in large-scale epidemiological studies [17]. In contrast, multivariate analysis revealed weaker or non-significant associations between IVB, Adv, and HRV and the risk of SP. These differences may reflect inherent variations in infectivity, replication dynamics, or cellular pathogenicity among pathogens [18]. For example, HRV, despite its high prevalence despite its high prevalence, typically causes mild upper respiratory tract symptoms [19]. In our study, the observed association between HRV and disease severity may be attributable to co-infections or increased host susceptibility, rather than intrinsic viral virulence [20]. As an atypical bacterial pathogen, *Mycoplasma pneumoniae* (MP) was appropriately included because of its distinct clinical manifestations and diagnostic characteristics [21]. In the training set, MP was associated with an increased risk of SP, suggesting that it may contribute to severe pulmonary complications, particularly in cases with extrapulmonary involvement or delayed

antibiotic treatment. Taken together, these findings highlight the importance of considering multiple pathogens in clinical diagnosis and risk assessment. Given the heterogeneous biological characteristics of different pathogens individualized evaluation rather than generalized assumptions is essential for accurate risk stratification and management.

We observed that in the larger training set, the prevalence of all six respiratory pathogens was significantly higher in the SP group, whereas in the smaller independent test set, only IVA and RSV remained statistically significant. This inconsistency is likely due to the limited sample size and reduced statistical power of the test set, rather than reflecting a true biological discrepancy. Across both datasets, the positivity rate of multiple pathogens were consistently higher in the SP group, suggesting that pathogen distribution may indeed influence disease severity. Notably, the associations between IVA and RSV and SP were particularly strong and stable, which is consistent with their well-established higher pathogenicity. Future studies with larger sample sizes are warranted to further clarify the roles of different respiratory pathogens in determining the risk of severe pneumonia.

In the receiver operating characteristic (ROC) analysis, human rhinovirus (HRV) demonstrated high specificity but relatively low sensitivity for predicting severe pneumonia (SP). This suggests that HRV alone is not suitable as a standalone predictor of SP, but rather may be useful for excluding non-severe cases. This finding is consistent with the clinical observation that HRV infections typically cause mild respiratory symptoms and rarely progress directly to severe disease. Consequently, when HRV is detected, clinicians may be more confident in excluding it as the primary cause of SP. In our study, patients with relatively clinical presentations were more likely to exhibit HRV infection, which helps explain the limited sensitivity of HRV in identifying SP. These findings highlight the importance of evaluating biomarker data from a broader perspective rather than relying on a single indicator. Although HRV has limited sensitivity, its high specificity is valuable for predictive modeling. Incorporating HRV into a multivariable model can help reduce false-positives predictions and thereby improve overall diagnostic accuracy. In summary, while HRV alone is insufficient for reliable severity assessment, its integration with other biomarkers enhances the model's ability to more accurately evaluate pneumonia severity.

Ferritin levels were higher in patients with SP, supporting its role as an early marker of severe inflammation [22, 23]. Marked elevations in ferritin are closely linked to mechanisms such as macrophage activation syndrome and cytokine storm, which are commonly observed in severe viral pneumonias, including those caused by influenza viruses and coronaviruses [24, 25]. In our predictive model, ferritin demonstrated independent prognostic value, highlighting the central role of excessive innate immune activation in the development of organ dysfunction. We also found that elevated C-peptide levels were associated with an increased risk of SP, a finding that has been less extensively studied but may be linically relevant. C peptide is traditionally used as an indicator of endogenous insulin secretion, however, in acute illness, its elevation may reflect stress-induced hyperglycemia, pancreatic β-cell responses to systemic inflammation, or the effects of inflammatory mediators such as interleukin-6 (IL-6) on insulin secretion and resistance [26]. This observation is consistent with previous studies -showing that both diabetes mellitus and stress-related hyperglycemia are closely associated with poor outcomes in pneumonia and sepsis [27]. As an easily measurable biomarker, C-peptide may therefore facilitate early identification of harmful metabolic in clinical practice. In addition, patients with SP exhibited significant alterations in other immune-related indicators, including severe lymphopenia, neutrophilia, and elevated levels of C-reactive protein (CRP), procalcitonin (PCT), and lactate dehydrogenase (LDH). Collectively, these changes reflect immune dysregulation, excessive neutrophil-driven inflammation, tissue injury, and frequent bacterial co-infection. These findings are consistent with the current understanding of the pathophysiological mechanisms underlying severe pneumonia [28].

The significantly increased rate of bacterial co-infection in patients with severe pneumonia (SP) is a key finding of this study. This observation further supports previous evidence that primary infections with pathogens such as influenza virus or respiratory syncytial virus (RSV) can damage lung epithelial cells are impair mucociliary clearance and immune regu-

lation, thereby increasing susceptibility to secondary bacterial infections [29]. In clinical practice, bacterial co-infection is often a major driver of disease exacerbation, with many progressing to sepsis or acute respiratory distress syndrome [30]. Although our predictive model is primarily based on biomarkers obtained at hospital admission, this finding underscores the importance of enhanced dynamic surveillance and timely intervention for bacterial infections in patients identified as high-risk [31]. When the predictive model indicates a high risk of SP, should remain vigilant and closely monitor disease progression, even if initial bacterial culture results are negative [32].

A key innovation of this study is the integration of quantitative pathogen load and host response biomarkers into a unified predictive model. The model demonstrated strong differentiative performance in both the training set and the independent test set, with results that were superior to those of traditional assessment methods, which often rely on a single biomarker or clinical score. To facilitate clinical application, we further developed a nomogram that allows clinicians to estimate individual risk by summing scores derived from routine examinations, making the approach both simple and practical. Within this predictive framework, ferritin levels and respiratory syncytial virus (RSV) Ct values emerged as particularly influential predictors. Consistent with prior evidence supporting their biological and prognostic relevance [33]. Although incorporating additional variables could potentially further enhance predictive accuracy. The current findings highlight the strength of comprehensive, multi-parameter assessment strategies. The model demonstrated good calibration and provided a positive net clinical benefit, supporting its potential utility in real-world clinical settings. We anticipate that such predictive tools may assist clinicians in making more accurate diagnoses and more informed decisions regarding patient management.

When interpreting these findings and considering future directions, several limitations of this study should be acknowledged. First, this was a retrospective analysis conducted at a single medical center; therefore, the conclusions may not be directly generalizable to other populations. Selection bias inherent to the study design cannot be excluded. Although the model demonstrated good performance in internal validation, external validation in difverse hospitals, regions, and patient populations is necessary to more objectively assess its clinical applicability. In addition, immunocompromised patients were excluded from the study, which limits applicability of the model to this high-risk group. Future studies should specifically include immunocompromised population to address this limitation. The relatively small size of the test set, particularly the limited number of severe pneumonia (SP) cases, also represents a major constraint for external validation. Although the dataset was divided into training and test sets using a commonly adopted machine-learning approach (8:2 ratio), and the model achieved a high AUC in the test set, the smaller sample size may have resulted in wider confidence intervals for performance estimates, thereby affecting the precision of generalization assessment. Future research should therefore include a larger number of cases, especially SP cases, to enable more robust external validation. Moreover, biomarkers were measured only at the time of admission. Longitudinal monitoring of these markers could provide more comprehensive prognostic information and improve understanding of treatment response, as dynamic changes may reveal additional clinically relevant insights. While the LASSO method was effective for feature selection, alternative machine learning approaches, such as random forest algorithms, may further enhance model performance or capture nonlinear relationships that logistic regression cannot identify. Comparative analyses of different algorithms could help identify the most clinically appropriate modeling strategies of different algorithms could help identify the most clinically appropriate modeling strategies. At present, the diagnosis of bacterial co-infection primarily relies on culture-based methods, which may underestimate true infection rates due to limited sensitivity. Future studies may benefit from incorporating molecular-based bacterial detection techniques or expanding biomarker panels to improve diagnostic accuracy, which will be a focus of our subsequent research. Finally, the lack of long-term follow-up data precluded evaluation of the model's ability to predict post-discharge outcomes, such as mortality, functional recovery, and long-term sequelae. These aspects of patient prognosis are equally important and warrant further investigation in future studies.

In constructing the predictive models in this study, we intentionally included only pathogen Ct values and selected host immune biomarkers, without incorporating additional clinical parameters. Although this strategy has certain limitations, it also offers important advantages. Specifically, it allows a more intuitive and direct assessment of pathogen, host interactions and enhances interpretability, which is particularly valuable in clinical settings where rapid decision-making based on pathophysiological data is required. Moreover, this approach helps minimize confounding from common variables such as age and comorbidities. At the same time, the exclusion of these established clinical risk factors may limit the overall comprehensiveness of the model, as age and comorbidities are well-recognized predictors of pneumonia severity. Models that integrate all available clinical information may therefore achieve higher overall predictive performance. For this reason, we view our model not as a replacement for existing scoring systems, but rather as a complementary tool designed to elucidate the specific relationship between pathogen burden and host immune metabolic responses.

Conclusion

In our study, respiratory pathogen load and selected host immune-metabolic biomarkers were identified as useful predictors of severe pneumonia (SP) at the time of hospital admission. These findings suggest that interactions between the pathogens and host responses play a critical role in the progression and exacerbation of pneumonia.

Disclosure of conflict of interest

None.

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