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Original Article

Early risk identification for recurrent wheezing in children with respiratory syncytial virus infections

Ying Wang[1], Yalin Shi[2], Lijuan Wang[2]

[1]*Department of Pediatric Cardiovascular and Respiratory Medicine, Xianyang Rainbow Hospital, Xianyang 712000, Shaanxi, China;* [2]*Department of Pediatric Respiratory Medicine, Xianyang Rainbow Hospital, Xianyang 712000, Shaanxi, China*

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Abstract: Objective: To evaluate the predictive value of peripheral blood eosinophil (EOS) count and nasopharyngeal microbiota for recurrent wheezing in children following respiratory syncytial virus (RSV) lower respiratory tract infections. Methods: This retrospective study included 614 children with RSV infection and an external validation cohort of 164 children. Clinical data, hematological parameters, and nasopharyngeal microbiota profiles were collected. Logistic regression was used to identify independent predictors of recurrent wheezing. A predictive model was developed and validated using receiver operating characteristic (ROC) and calibration curves. Results: Peripheral blood EOS count, serum 25(OH)D and IgM levels, and nasopharyngeal bacterial colonization (notably *Streptococcus pneumoniae* and *Haemophilus influenzae*) were significantly associated with recurrent wheezing. The predictive model showed moderate-to-good diagnostic performance (AUC = 0.747) and consistent accuracy in the external validation cohort (AUC = 0.741). Conclusion: Peripheral blood EOS count and nasopharyngeal microbiota composition are critical predictors of recurrent wheezing following RSV infection. The predictive model may aid in early risk stratification and personalized intervention to prevent recurrent wheezing in pediatric patients.

Keywords: Respiratory syncytial virus, recurrent wheezing, eosinophils, nasopharyngeal microbiota, predictive model, children

Introduction

Respiratory syncytial virus (RSV) is a prevalent respiratory pathogen in infants and young children, frequently causing acute lower respiratory tract infections, such as bronchiolitis and pneumonia, typically present with cough, tachypnea, and wheezing [1, 2]. RSV infection has been associated with chronic respiratory conditions, including asthma [3, 4]. Some children develop recurrent wheezing after RSV infection, which may progress to chronic wheezing or asthma, thereby imposing substantial health, familial, and societal burdens [5]. Early identification of children at risk for recurrent wheezing is thus a critical research priority in pediatric respiratory research.

Recurrent wheezing, characterized by repeated wheezing episodes following RSV infection, is associated with impaired lung function and chronic airway inflammation [6]. It may progress to chronic obstructive pulmonary disease (COPD) or asthma, adversely affecting growth, development, and quality of life of affected children [7]. Long-term management typically includes inhaled corticosteroids and bronchodilators, which may compromise physical health, immune function, and psychological well-being [8]. Early identification and intervention in high-risk children are essential to prevent long-term complications and improve outcomes.

Peripheral blood eosinophils (EOS) are immune cells implicated in allergic responses, playing a key role in asthma, allergic rhinitis, and eczema [9]. Elevated EOS levels are associated with chronic airway inflammation and asthma exacerbations [10]. Recent studies suggest that increased EOS counts may predict recurrent wheezing, particularly in children with allergic predispositions, where elevated EOS often indicates allergic inflammation [11]. Thus, peripheral blood EOS count may serve as a valuable

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biomarker for predicting the risk of recurrent wheezing following RSV infection.

The nasopharynx, an essential component of the upper respiratory system, filters and warms air while interacting with the immune system [12]. Dysbiosis in the nasopharyngeal microbiota, particularly alterations in dominant bacterial populations, is linked to respiratory infection severity [13]. In RSV-infected children, microbial imbalances may lead to bacterial and viral co-infections, thereby aggravating disease progression. Common nasopharyngeal bacteria, such as *Streptococcus pneumoniae, Haemophilus influenzae*, and *Staphylococcus aureus*, are associated with recurrent wheezing. These pathogens may intensify airway inflammation and interact with persistent viral replication, worsening respiratory outcomes [13, 14]. Elucidating the role of nasopharyngeal microbiota in post-RSV recurrent wheezing is crucial for identifying children at elevated risk.

This study aims to evaluate the predictive value of peripheral blood EOS count and nasopharyngeal microbiota composition for recurrent wheezing among children with RSV lower respiratory tract infections. Through comprehensive analysis of clinical variables, hematological markers, and nasopharyngeal microbiota, we seek to identify key predictors of recurrent wheezing and develop a predictive model. This model may provide clinicians with a reliable risk assessment tool to facilitate early identification and intervention for high-risk children, ultimately reducing recurrence rates.

Methods and materials

Sample size calculation

The sample size was calculated using a formula based on confidence intervals and allowable error to ensure adequate statistical power. According to Zhou et al. [8], the incidence of recurrent wheezing in children following RSV infection was 13.53% (P = 0.1353). With an allowable error of 5% (E = 0.05) at a 95% confidence level (Z = 1.96), the required sample size was estimated using the following formula: $N = Z^2 \times [P \times (1\text{-}P)]/E^2$. This calculation yielded a minimum sample size of 174 children. The final sample size was expanded based on clinical availability, enrollment feasibility, and study logistics.

Study population

This retrospective study included 614 children diagnosed with RSV lower respiratory tract infections and hospitalized at Xianyang Rainbow Hospital between April 2020 and December 2022. An external validation cohort comprising 164 children was enrolled from January to December 2023. This study was approved by the Ethics Committee of Xianyang Rainbow Hospital.

Inclusion and exclusion criteria

Inclusion Criteria: (1) Clinical diagnosis of RSV lower respiratory tract infection [15], encompassing bronchitis, pneumonia, or other manifestations involving lower respiratory symptoms. (2) No prior history of wheezing. (3) Complete clinical data, including routine clinical assessments and sufficient peripheral blood and nasopharyngeal samples for laboratory testing.

Exclusion Criteria: (1) Severe immune deficiencies or chronic respiratory conditions (e.g., chronic bronchitis, asthma). (2) Concurrent bacterial, viral, or fungal respiratory infections at admission. (3) Severe systemic diseases (e.g., heart disease, kidney disease, endocrine disorders).

Definition of recurrent wheezing

Wheezing was defined as bronchial obstruction lasting for at least 24 hours, with a symptom-free interval of at least one week preceding each episode. Recurrent wheezing was defined as three or more wheezing episodes within 12 months [8].

Clinical data collection

Clinical data were extracted from the hospital's electronic medical records and follow-up documentation. The following variables were collected:

Demographic Information: Age (stratified as < 3.5 years or ≥ 3.5 years), sex, birth weight (underweight < 2.5 kg, macrosomia ≥ 4 kg), delivery method (cesarean section), breastfeeding status, and primiparity.

Allergic and Environmental Factors: History of eczema, family history of asthma, personal allergy history, and residential location (urban

vs. rural) to evaluate their potential influence on recurrent wheezing.

Laboratory Data: Hematological and Biochemical Markers: Procalcitonin (PCT), C-reactive protein (CRP), white blood cell count (WBC), neutrophil percentage (NEC%), lymphocyte percentage (LYM%), platelet count (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase-MB (CKMB), lactate dehydrogenase (LDH), and 25-hydroxyvitamin D (25(OH)D). Immunological Markers: Immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), and eosinophil count (EOS). Microbiological Data: Nasopharyngeal microbiota, including Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus, to explore their role in recurrent wheezing post-RSV infection.

Laboratory testing

Hematology: White blood cell count, eosinophil count, neutrophil percentage, lymphocyte percentage, and platelet count were measured using the Sysmex XS-500i or XN-350 automated blood analyzer (Sysmex Corporation, Japan). Biochemical Analysis: Serum CRP, ALT, AST, CK, CKMB, and LDH levels were quantified using the Hitachi 008AS biochemistry analyzer (Hitachi, Japan). Serum PCT and 25(OH)D levels were assessed using the Changguang Huayi AE240 analyzer (Changguang Huayi, China). Immunological Testing: IgA, IgM, and IgG levels were evaluated using the Hitachi 008AS biochemistry analyzer (Hitachi, Japan). Microbiological Analysis: Nasopharyngeal bacterial cultures were performed using the bioMérieux VITEK 2 automated microbiology system (bioMérieux, France) to identify *Streptococcus pneumoniae, Haemophilus influenzae*, and *Staphylococcus aureus*.

Follow-up

All participants were followed for 12 months post-discharge using either telephone or outpatient follow-up. Telephone follow-up was conducted every three months to assess respiratory symptoms, wheezing episodes, and medical visits. Outpatient follow-up at 1, 3, 6, and 12 months was scheduled to monitor recurrent wheezing and associated symptoms.

Outcome measures

Primary outcomes: 1) Incidence and severity of recurrent wheezing, including the need for hospitalization; 2) Predictive accuracy of the model based on clinical variables, hematological markers, and nasopharyngeal microbiota.

Secondary outcomes: 1) Associations between clinical variables and recurrent wheezing; 2) Relationships between immunological markers and recurrent wheezing; 3) Impact of nasopharyngeal microbiota composition on recurrent wheezing.

Statistical analysis

Data analysis was performed using SPSS 26.0 and R 4.3.3. Descriptive statistics were used to summarize clinical and laboratory variables. Normally distributed data were reported as means ± standard deviations (Mean ± SD), while non-normally distributed data were presented as medians with interquartile ranges (Median [IQR]). Data normality was assessed using the Kolmogorov-Smirnov test. For group comparisons, independent sample t-tests were applied to normally distributed variables, and Mann-Whitney U tests were used for non-normally distributed data. Categorical variables were compared using chi-square tests. Univariate logistic regression was employed to identify potential predictors of recurrent wheezing, followed by multivariate logistic regression to develop a predictive model and estimate odds ratios (OR) with 95% confidence intervals. Model performance was evaluated using receiver operating characteristic (ROC) curve analysis (pROC package in R), with the area under the curve (AUC) as the primary metric of discrimination. Nomograms were generated using the rms package to visualize regression results. Calibration curves, constructed via the Bootstrap method, assessed the agreement between predicted and observed outcomes. Statistical significance was defined as $P < 0.05$.

Results

Clinical variables and their association with recurrent wheezing

Age differed significantly between groups ($P = 0.015$), with the non-wheezing group showing older age than the recurrent wheezing group. A history of eczema, family history of asthma, and personal allergy history were significantly associated with recurrent wheezing ($P < 0.05$). No significant differences were observed in sex, birth weight, delivery method, breastfeed-

Table 1. Comparison of clinical variables between the recurrent wheezing and non-wheezing groups

Variable	Total	Recurrent Wheezing Group (n = 188)	Non-Wheezing Group (n = 426)	Z/χ^2 Value	*P* Value
Age (Median [IQR])	3.00 [2.00, 5.00]	3.00 [2.00, 5.00]	4.00 [2.00, 6.00]	2.437	0.015
Gender					
Male	374	118	256	0.391	0.532
Female	240	70	170		
Weight (kg)	18.10 [13.50, 22.20]	17.65 [13.20, 20.80]	18.35 [13.70, 22.40]	1.293	0.196
Cesarean Delivery					
Yes	346	103	243	0.270	0.604
No	268	85	183		
Breastfeeding					
Yes	285	85	200	0.158	0.691
No	329	103	226		
Primiparous					
Yes	426	128	298	0.214	0.643
No	188	60	128		
Eczema History					
Yes	133	56	77	10.544	0.001
No	481	132	349		
Family Asthma History					
Yes	99	39	60	4.278	0.039
No	515	149	366		
Allergy History					
Yes	115	47	68	6.999	0.008
No	499	141	358		
Residential Area					
Rural	251	85	166	2.105	0.147
Urban	363	103	260		
Low Birth Weight					
< 2.5 kg	16	4	12	0.244	0.621
≥ 2.5 kg	598	184	414		
Macrosomia					
< 4 kg	14	3	11	0.570	0.450
≥ 4 kg	600	185	415		

ing, primiparity, residential area, low birth weight, or macrosomia between the two groups (P > 0.05; **Table 1**).

Hematological markers and their association with recurrent wheezing

Significant differences were found in serum 25-hydroxyvitamin D (25(OH)D) levels, peripheral blood EOS count, and levels of IgM and IgA between the recurrent wheezing and non-wheezing groups (P < 0.05). Children with recurrent wheezing exhibited significantly lower 25(OH)D levels (P < 0.001) and higher EOS counts (P < 0.001) compared to those without wheezing. IgM and IgA levels were also elevated in the recurrent wheezing group (P = 0.007 and P = 0.014, respectively). No significant differences were found in other hematological markers, including PCT, CRP, WBC, NEC%, LYM%, PLT, ALT, AST, CK, CKMB, LDH, and IgG (all P > 0.05; **Table 2**).

Nasopharyngeal microbiota distribution

The distribution of nasopharyngeal microbiota differed between the two groups. In the recurrent wheezing group, *H. influenzae* and *S. pneumoniae* were more prevalent, while *S. aureus* distribution remained comparable. Statistical

Table 2. Comparison of hematological indicators between the recurrent wheezing and non-wheezing groups

Variable	Total	Recurrent Wheezing Group (n = 188)	Non-Wheezing Group (n = 426)	Z/t/χ^2 Value	*P* Value
PCT (ng/ml)	0.13 [0.09, 0.17]	0.12 [0.08, 0.17]	0.13 [0.09, 0.17]	1.434	0.152
CRP (mg/L)	4.42 [2.25, 6.93]	4.29 [2.29, 7.04]	4.47 [2.25, 6.89]	0.002	0.999
WBC (10^9/L)	9.38 [6.13, 12.69]	8.77 [5.72, 12.61]	9.52 [6.35, 12.76]	0.896	0.370
NEC%	47.64 [36.86, 59.62]	46.92 [36.95, 59.96]	47.70 [36.64, 59.33]	0.192	0.848
LYM%	41.98 [30.58, 52.39]	41.91 [29.20, 52.57]	42.11 [30.68, 52.09]	0.200	0.842
PLT (10^9/L)	316.00 [251.00, 381.00]	307.00 [248.00, 372.00]	320.50 [253.25, 389.75]	0.979	0.327
ALT (U/L)	15.50 [10.00, 20.00]	17.00 [12.00, 19.00]	15.00 [9.00, 20.00]	1.722	0.085
AST (U/L)	37.14±10.42	36.14±8.72	37.58±11.07	1.579	0.115
CK (U/L)	116.00 [80.00, 154.00]	116.00 [81.00, 147.50]	116.50 [80.00, 156.00]	0.691	0.49
CKMB (U/L)	28.00 [21.00, 35.75]	28.50 [21.00, 35.25]	27.00 [21.00, 35.75]	0.423	0.672
LDH (U/L)	297.00 [251.00, 342.00]	294.00 [253.00, 328.25]	299.50 [250.25, 348.00]	1.052	0.293
25(OH)D [nmol/L]	29.88 [24.41, 35.70]	26.46±8.14	29.94±8.47	4.825	< 0.001
EOS (10^9/L)	0.16 [0.08, 0.28]	0.22 [0.11, 0.38]	0.15 [0.07, 0.24]	4.843	< 0.001
IgA	Elevated	105	192	6.07	0.014
	Normal	83	234		
IgM	Elevated	120	222	7.257	0.007
	Normal	68	204		
IgG	Elevated	38	107	1.584	0.208
	Normal	150	320		

Note: WBC: White Blood Cell Count, EOS: Eosinophils, IgA: Immunoglobulin A, IgM: Immunoglobulin M, IgG: Immunoglobulin G, CK: Creatine Kinase, CKMB: Creatine Kinase Myocardial Band, LDH: Lactate Dehydrogenase, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, PCT: Procalcitonin, CRP: C-Reactive Protein, NEC: Neutrophil, LYM: Lymphocyte, PLT: Platelet Count, 25(OH)D: 25-Hydroxy Vitamin D.

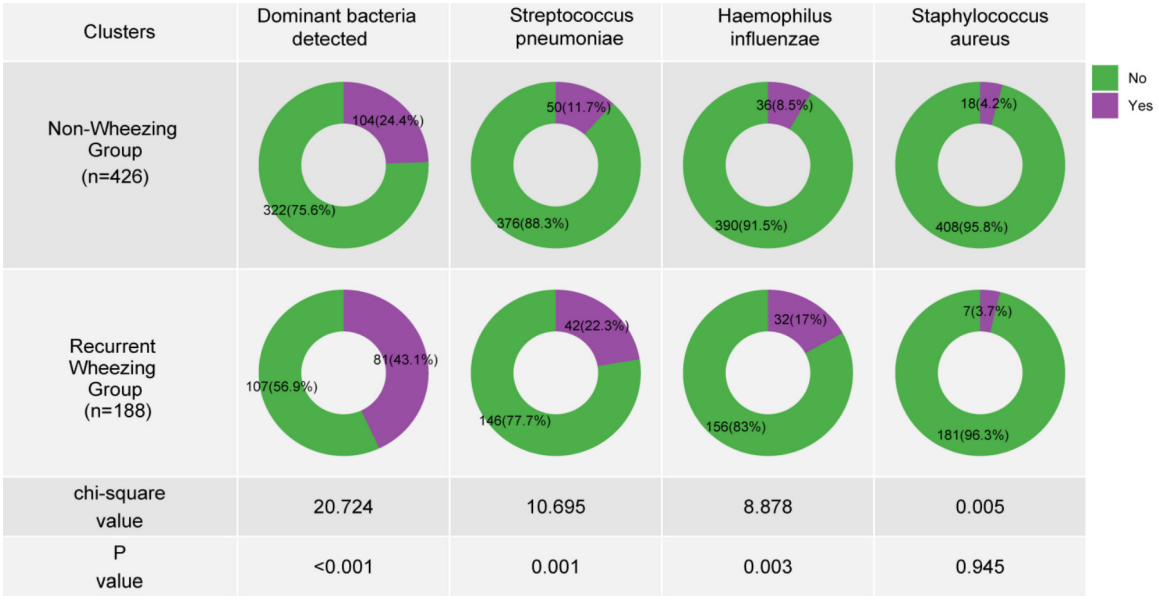

Figure 1. Comparison of dominant bacterial distribution between the recurrent wheezing and non-wheezing groups.

analysis confirmed significant associations between *S. pneumoniae* (P = 0.001) and *H. influenzae* (P = 0.003) and recurrent wheezing, while *S. aureus* was not significantly associated (P = 0.945; **Figure 1**).

Logistic regression analysis for predictive factors

Logistic regression was conducted to identify independent predictors of recurrent wheezing.

Table 3. Variable assignment and definitions

Variable	Assignment Content
Age	< 3.5 years = 1, ≥ 3.5 years = 0
History of Eczema	Yes = 1, No = 0
Family Asthma History	Yes = 1, No = 0
Allergy History	Yes = 1, No = 0
25(OH)D [nmol/L]	< 28.72 = 1, ≥ 28.72 = 0
EOS (10^9/L)	< 0.265 = 1, ≥ 0.265 = 0
IgA	Elevated = 1, Normal = 0
IgM	Elevated = 1, Normal = 0
Streptococcus pneumoniae	Detected = 1, Not detected = 0
Haemophilus influenza	Detected = 1, Not detected = 0
Recurrent Wheezing	Yes = 1, No = 0

Note: 25(OH)D: Vitamin D (25-Hydroxy Vitamin D), EOS: Eosinophils, IgA: Immunoglobulin A, IgM: Immunoglobulin M.

Categorical variables were assigned values based on their categories, and continuous variables were dichotomized using clinically relevant cut-off values (**Table 3**). Univariate logistic regression revealed significant associations between recurrent wheezing and the following variables: age (P = 0.004), history of eczema (P = 0.001), family history of asthma (P = 0.040), allergy history (P = 0.009), serum 25(OH)D levels (P < 0.001), EOS count (P < 0.001), IgA (P = 0.014), IgM (P = 0.007), *S. pneumoniae* (P = 0.001), and *H. influenzae* (P = 0.002; **Table 4**).

Multivariate logistic regression identified age (OR = 1.644, P = 0.011), history of eczema (OR = 0.572, P = 0.014), allergy history (OR = 0.521, P = 0.006), 25(OH)D levels (nmol/L; OR = 2.234, P < 0.001), EOS count (10^9/L; OR = 0.288, P < 0.001), IgM (OR = 0.620, P = 0.016), *S. pneumoniae* (OR = 0.401, P < 0.001), and *H. influenzae* (OR = 0.366, P = 0.001) as independent predictors of recurrent wheezing. Family asthma history (OR = 0.733, P = 0.223) and IgA (OR = 0.708, P = 0.076) were not statistically significant in the multivariate model (**Table 5**).

Nomogram for predicting recurrent wheezing risk

A nomogram was developed based on the multivariate logistic regression model to predict the probability of recurrent wheezing. Each variable was assigned a score proportional to its relative contribution to the overall risk. Age (< 3.5 years), history of eczema, allergy history, low 25(OH)D levels (< 28.72 nmol/L), and low EOS count (< 0.265 × 10^9/L) were strongly associated with increased risk, with 25(OH)D and EOS carrying the highest weights. IgM, *S. pneumoniae*, and *H. influenzae* colonization also contributed to the model, albeit to a lesser extent (**Figure 2**).

Model group: Roc and calibration curves

The performance of the predictive model was evaluated using ROC and calibration curves. The ROC curve yielded an area under the curve (AUC) of 0.747, indicating moderate-to-good diagnostic accuracy (**Figure 3A**). Calibration curves, based on 1,000 bootstrap samples, demonstrated strong agreement between predicted and actual probabilities, with the curve closely aligning with the ideal diagonal (**Figure 3B**). The goodness-of-fit test yielded a *P*-value of 0.301, confirming a good model fit. Additional calibration metrics included an average absolute error of 0.015, a mean squared error of 0.00032, and a 0.9 quantile absolute error of 0.028, reflecting high predictive precision. The model's Dxy value was 0.4942 and R^2 was 0.2325, indicating good discriminative power and model stability.

Comparison of predictive factors between model and external validation groups

No significant differences in the incidence of recurrent wheezing were observed between the model group and the external validation group (P = 0.974). Age, history of eczema, allergy history, 25(OH)D levels, EOS count, IgM levels, and detection of *S. pneumoniae* and *H. influenzae* showed similar distributions across both groups (P > 0.05; **Table 6**), confirming the model's consistency. Decision curve analysis (DCA) demonstrated clinical utility across a threshold probability range of 0% to 94%, with a maximum net benefit of 69.38% (**Figure 3C**).

External validation group: ROC and calibration curves

In the external validation cohort, the ROC curve yielded an AUC of 0.741, demonstrating consistent moderate diagnostic performance (**Figure 4A**). Calibration curves confirmed good agreement between predicted and actual outcomes (**Figure 4B**). The goodness-of-fit test yielded a

Table 4. Univariate logistic regression analysis of predictive factors for recurrent wheezing

Variable	Estimate	Std Error	*P* Value	OR	Lower	Upper
Age	0.510	0.178	0.004	1.665	1.178	2.364
History of Eczema	-0.654	0.203	0.001	0.520	0.35	0.776
Family Asthma History	-0.468	0.227	0.040	0.626	0.402	0.983
Allergy History	-0.562	0.214	0.009	0.570	0.375	0.870
25(OH)D [nmol/L]	0.817	0.182	< 0.001	2.263	1.590	3.243
EOS (10^9/L)	-1.176	0.19	< 0.001	0.309	0.212	0.448
IgA	-0.433	0.176	0.014	0.649	0.458	0.915
IgM	-0.483	0.18	0.007	0.617	0.432	0.876
Streptococcus pneumoniae	-0.772	0.231	0.001	0.462	0.294	0.729
Haemophilus influenza	-0.799	0.261	0.002	0.45	0.270	0.753

Note: 25(OH)D: Vitamin D (25-Hydroxy Vitamin D), EOS: Eosinophils, IgA: Immunoglobulin A, IgM: Immunoglobulin M.

Table 5. Multivariate logistic regression analysis of predictive factors for recurrent wheezing

Variable	Estimate	Std Error	*P* Value	OR	Lower	Upper
Age	0.497	0.196	0.011	1.644	1.121	2.421
History of Eczema	-0.559	0.227	0.014	0.572	0.367	0.895
Family Asthma History	-0.31	0.254	0.223	0.733	0.447	1.214
Allergy History	-0.652	0.238	0.006	0.521	0.327	0.833
25(OH)D [nmol/L]	0.804	0.198	< 0.001	2.234	1.521	3.308
EOS (10^9/L)	-1.245	0.208	< 0.001	0.288	0.191	0.432
IgA	-0.346	0.195	0.076	0.708	0.482	1.036
IgM	-0.478	0.199	0.016	0.62	0.418	0.914
Streptococcus pneumoniae	-0.914	0.261	< 0.001	0.401	0.240	0.670
Haemophilus influenza	-1.005	0.293	0.001	0.366	0.206	0.652

Note: 25(OH)D: Vitamin D (25-Hydroxy Vitamin D), EOS: Eosinophils, IgA: Immunoglobulin A, IgM: Immunoglobulin M.

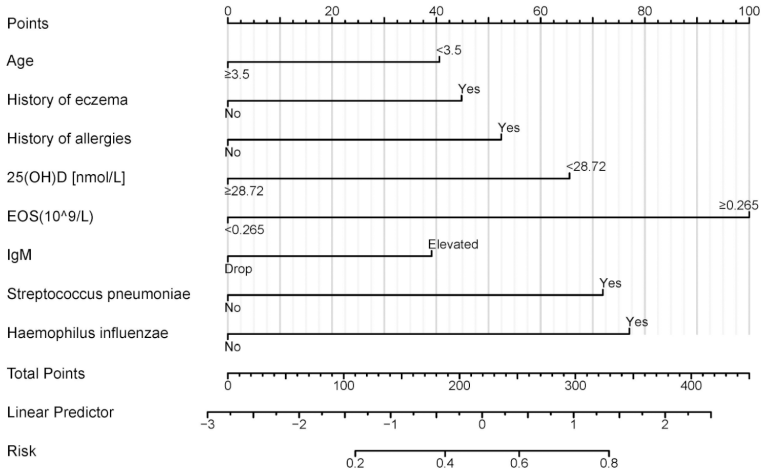

Figure 2. Nomogram for predicting recurrent wheezing risk based on multivariate logistic regression analysis. Note: 25(OH)D: 25-Hydroxy Vitamin D, EOS: Eosinophils, IgM: Immunoglobulin M.

P-value of 0.307, indicating a good fit. Calibration metrics included an average absolute error of 0.015, a mean squared error of 0.00032, and a 0.9 quantile absolute error of 0.028, confirming high model accuracy. The model's Dxy value was 0.4811, and the Nagelkerke R^2 was 0.2267, indicating satisfactory discriminative ability and stability. DCA showed a net benefit across a threshold probability range of 0% to 93%, with a maximum net benefit of 69.51%, affirming the model's practical applicability in clinical settings (**Figure 4C**).

Discussion

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infections in infants and young children, particularly those under the age of three, and is frequently associated with subsequent recurrent wheez-

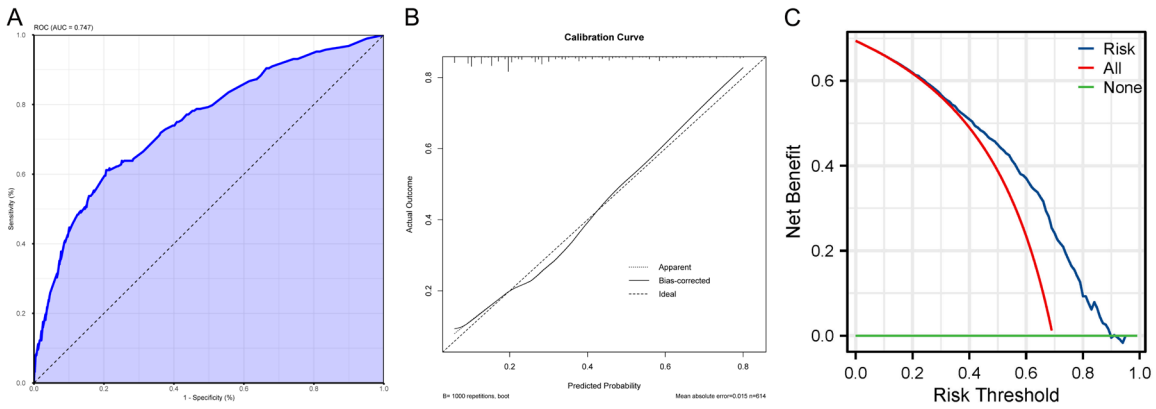

Figure 3. ROC curve and calibration curve for the predictive model in model group. A. ROC Curve Assessing the Predictive Performance of the Developed Model. B. Calibration Curve Assessing the Predictive Accuracy of the Model. C. DCA Curve Assessing the model's clinical benefit. Note: ROC: Receiver Operating Characteristic curve, AUC: Area Under Curve, DCA: Decision Curve Analysis.

Table 6. Comparison of predictive factors for recurrent wheezing between the external validation group and the model group

Variable	Total	External Validation Group (n = 164)	Model Group (n = 624)	χ^2 value	*P* Value
Recurrent Wheezing					
Yes	238	50	188	0.001	0.974
No	540	114	426		
Age					
< 3.5	385	83	302	0.105	0.746
≥ 3.5	393	81	312		
History of Eczema					
Yes	172	39	133	0.338	0.561
No	606	125	481		
Allergy History					
Yes	148	33	115	0.163	0.687
No	630	131	499		
25(OH)D [nmol/L]					
< 28.72	377	80	297	0.009	0.926
≥ 28.72	401	84	317		
EOS (10^9/L)					
< 0.265	210	41	169	0.419	0.518
≥ 0.265	568	123	445		
IgM					
Elevated	423	81	342	2.077	0.15
Normal	355	83	272		
Streptococcus pneumoniae					
Detected	112	20	92	0.817	0.366
Not Detected	666	144	522		
Haemophilus influenza					
Detected	85	17	68	0.067	0.796
Not Detected	693	147	546		

Note: 25(OH)D: Vitamin D (25-Hydroxy Vitamin D), EOS: Eosinophils, IgA: Immunoglobulin A, IgM: Immunoglobulin M.

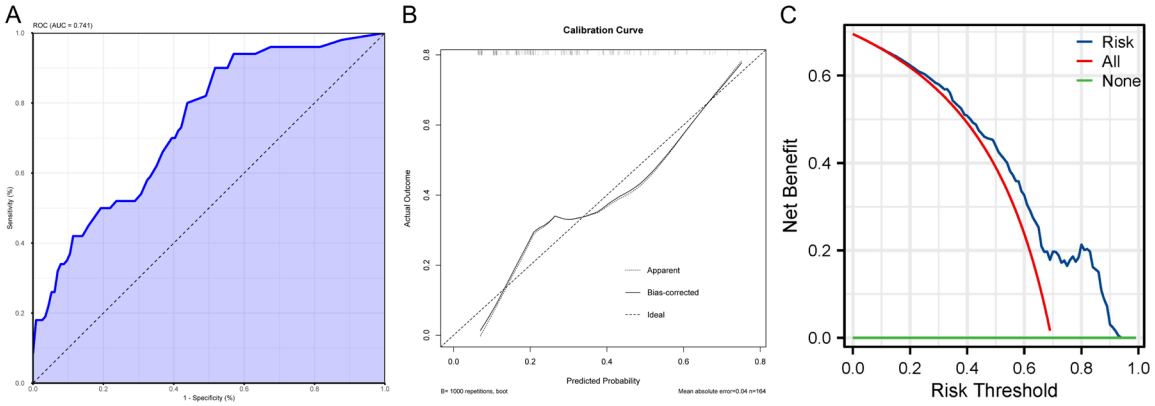

Figure 4. ROC curve and calibration curve for the predictive model in external validation group. A. ROC Curve Assessing the Predictive Performance of the Developed Model. B. Calibration Curve Assessing the Predictive Accuracy of the Model. C. DCA Curve Assessing the model's clinical benefit. Note: ROC: Receiver Operating Characteristic curve, AUC: Area Under Curve, DCA: Decision Curve Analysis.

ing [16]. Recurrent wheezing imposes a significant health burden, potentially leading to chronic airway diseases like asthma or COPD [17]. Despite well-characterized clinical features of RSV infection, accurately predicting the development of recurrent wheezing remains challenging. Emerging evidence suggests that peripheral EOS levels and nasopharyngeal microbiota composition play key roles in the development of recurrent wheezing post-RSV infection. Elevated EOS counts are indicative of allergic inflammation and have been associated with airway hyperresponsiveness [18]. Investigating the predictive roles of EOS and nasopharyngeal microbiota could thus provide clinicians with tools for early risk assessment and tailored therapeutic strategies.

In this study, multivariate logistic regression identified several independent predictors of recurrent wheezing: younger age, history of eczema, allergy history, low serum 25(OH)D levels, elevated EOS counts, increased IgM levels, and colonization by *Streptococcus pneumoniae*, and *Haemophilus influenzae*. Younger age was associated with higher risk, aligning with prior research indicating that immature immune and respiratory systems in young children increase susceptibility to environmental triggers and immune dysregulation, thereby elevating wheezing risk [19]. Early RSV infection, particularly between 0 and 36 months, markedly increases the likelihood of recurrent wheezing later in life [20]. Beigelman et al. [21] suggested that this heightened risk in younger children may result from an exaggerated immune reaction during RSV infection, which initiates chronic airway inflammation and remodeling. These pathophysiological changes may serve as the foundation for recurrent wheezing and subsequent asthma development.

Histories of eczema and allergy often indicate a predisposition to allergic immune responses, fostering chronic airway inflammation. Such inflammation is readily triggered during RSV infection, contributing to recurrent wheezing episodes [22]. Children with eczema and allergies typically display a Th2-biased immune response, heightening airway sensitivity. Following RSV infection, this response amplifies airway inflammation, promoting recurrent wheezing [23]. Beigelman et al. [21] reported that a personal history of eczema or allergy significantly elevates the risk of long-term respiratory complications, including asthma, particularly in the context of early RSV infection.

Low 25(OH)D levels are associated with immune dysregulation, as vitamin D modulates T-cell, B-cell, and inflammatory responses [24]. Deficiency may lead to suboptimal or excessive immune reactions to respiratory infections, intensifying airway inflammation and contributing to recurrent wheezing [25]. Furthermore, low 25(OH)D levels are linked to allergic diseases and asthma, and in the context of RSV infection, they may aggravate inflammatory responses, increasing wheezing risk [26]. These findings underscore the potential benefit of maintaining adequate vitamin D levels in children to mitigate post-infectious respiratory complications.

Elevated EOS counts are a hallmark of allergic inflammation and are strongly linked to asthma and other eosinophilic airway disorders [27]. Following RSV infection, increased EOS counts reflect the degree of allergic airway inflammation, promoting recurrent wheezing [28]. Children with elevated EOS counts are more likely to experience heightened airway reactivity and exaggerated responses to environmental or infectious stimuli, increasing the likelihood of wheezing episodes after RSV infection [29].

IgM, an early responder to acute infections, is typically elevated at infection onset. Higher IgM levels suggest a robust immune reaction, potentially reflecting infection severity and immune overactivity [30]. In RSV infections, elevated IgM levels may indicate an intense immune response that triggers persistent airway inflammation, contributing to recurrent wheezing [31]. This elevation may also suggest inadequate immune resolution, sustaining chronic airway inflammation and increasing wheezing risk.

Streptococcus pneumoniae and Haemophilus influenzae, dominant nasopharyngeal bacteria, are closely associated with recurrent wheezing following RSV infection [32]. These pathogens can interact synergistically with RSV, exacerbating airway inflammation and complicating disease progression [33]. The increased prevalence of *S. pneumoniae* and *H. influenzae* in the recurrent wheezing group suggests that nasopharyngeal microbiota dysbiosis plays a critical role in elevating wheezing risk. Previous studies have similarly linked dysbiosis involving these bacteria to recurrent wheezing following RSV infection [34, 35], highlighting their role in disease pathogenesis.

This study developed a predictive model for recurrent wheezing, incorporating clinical variables, hematological markers, and nasopharyngeal microbiota, and assessed its performance using ROC curves. The model's AUC of 0.747 indicates moderate-to-good predictive accuracy for identifying children at risk of recurrent wheezing post-RSV infection. While previous studies have primarily identified risk factors for recurrent wheezing, few have developed specific predictive models for this outcome in RSV-infected children. This model addresses this gap, offering clinicians a practical tool to identify high-risk patients and implement targeted early interventions. External validation further confirmed the model's reliability and generalizability, with consistent predictive factors across the model and validation groups, supporting its potential for broad clinical application.

Despite these insights, several limitations warrant consideration. First, its retrospective design may introduce selection bias, potentially affecting the generalizability of findings. Second, factors such as air pollution and RSV viral load, known to influence recurrent wheezing, were not evaluated. Third, while the sample size was adequate for model development, it may have been insufficient to detect the impact of rare but clinically relevant predictors. Future research should prioritize large-scale, multicenter prospective studies to further validate the model's predictive performance across diverse populations and clinical setting. Additionally, incorporating additional immunological biomarkers, such as IL-5, IgE, and Th2 cytokines, which are associated with allergic and inflammatory responses, may enhance predictive accuracy. Lastly, interventional approaches targeting nasopharyngeal microbiota dysbiosis, such as probiotics or selective antimicrobial strategies, also merit investigation as potential strategies to reduce recurrent wheezing risk following RSV infection.

Conclusion

Peripheral eosinophil count and nasopharyngeal microbiota composition are key predictors of recurrent wheezing following RSV infection. The predictive model, developed based on multivariate logistic regression, demonstrated reliable performance and clinical utility, offering an effective tool for early risk identification. This model may support timely, personalized treatment strategies to mitigate recurrent wheezing and its long-term consequences in pediatric patients.

Disclosure of conflict of interest

None.

Address correspondence to: Lijuan Wang, Department of Pediatric Respiratory Medicine, Xianyang Rainbow Hospital, No. 3 Weiyang West Road, Qindu District, Xianyang 712000, Shaanxi, China. E-mail: juanj_1988@163.com

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