Clin. Lab. 2026;72:XXX-XXX

ORIGINAL ARTICLE

A Differential Diagnostic Nomogram for *Mycoplasma pneumoniae* and *Bordetella pertussis* in Pediatric Upper Respiratory Infections

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ABSTRACT

Background: **Differentiating *Mycoplasma pneumoniae* (MP) from *Bordetella pertussis* (BP) infections in children at primary care hospitals is challenging due to similar symptoms and overlapping epidemic seasons. This study evaluated the use of C-reactive protein (CRP) levels and lymphocyte counts from a single blood test to quickly distinguish between MP and BP infections.**
Methods: **The study included a derivation cohort (n = 301) and a validation cohort (n = 87). Logistic regression analyses identified predictors for differentiating MP and BP infections, leading to the development of a prognostic scoring model and nomogram. The model's effectiveness was assessed using the area under the receiver operating characteristic curve (AUC) and calibration analysis.**
Results: **CRP and lymphocyte count were key factors in distinguishing MP from BP infections ($p < 0.001$). A straightforward scoring model was developed utilizing these predictors. The score was significantly lower in the BP group compared to the MP group (-0.36 ± 0.17 vs. 2.09 ± 0.13; $p < 0.001$). The model exhibited excellent discriminative capability (AUC = 0.866), surpassing the performance of the neutrophil-to-lymphocyte ratio (NLR) (AUC = 0.704), CRP (AUC = 0.783), lymphocyte count (AUC = 0.838), and neutrophil count (AUC = 0.593). These findings were also validated in a separate cohort. Subsequently, a nomogram was constructed for clinical application.**
Conclusions: **The proposed predictive scoring model and nomogram, which incorporate CRP and lymphocyte count, offer an accessible and practical tool for differentiating between MP and BP infections in pediatric patients with respiratory tract infections in primary care outpatient settings.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250836)

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Manuscript accepted October 17, 2025

KEYWORDS

Mycoplasma pneumoniae, Bordetella pertussis, nomogram, C-reactive protein, lymphocyte counts

INTRODUCTION

Mycoplasma pneumoniae (MP) and *Bordetella pertussis* (BP) are common global pathogens causing respiratory infections in children [1,2]. MP often begins as a mild upper respiratory tract infection but can lead to severe pneumonia if untreated, accounting for up to 40% of community-acquired pneumonia in children over five [3-4]. Despite vaccination efforts, BP remains a public

health concern, with rising cases, especially among older children and teenagers [5,6].

Differentiating between MP and BP infections early on is challenging due to their similar symptoms, such as rhinorrhea, sore throat, low-grade fever, and a persistent dry cough, as well as their overlapping seasonal prevalence. Accurate identification is crucial because of differing antimicrobial resistance and treatment strategies. Delayed differentiation can result in inappropriate antibiotic use, prolonged illness, and higher complication risks.

Culture remains the benchmark for the detection of both pathogens; however, its extended procedural duration constrains its utility in clinical practice. In contrast, polymerase chain reaction (PCR) offers a swift and efficacious diagnostic alternative, gaining widespread recognition for this application [7,8]. Despite its advantages, the adoption of PCR in primary care settings is limited by the necessity for specialized equipment, technical expertise, and laboratory infrastructure [9]. As a result, clinicians in resource-limited regions frequently rely on empirical diagnosis, which can lead to inappropriate antibiotic use. This practice not only contributes to the escalation of antimicrobial resistance but also delays the initiation of targeted treatment. To bridge this diagnostic gap, we have developed a pragmatic diagnostic tool utilizing routine laboratory parameters.

C-reactive protein (CRP), an acute-phase reactant, shows significant elevation in MP infections [10], whereas BP infections induce specific alterations in lymphocyte counts through distinct immunomodulatory mechanisms [11]. Based on these pathophysiological insights, we propose that a combined model incorporating both inflammatory and immune markers could offer clinically significant differentiation between these infections.

Herein, we present the development and validation of a novel CRP-lymphocyte-based scoring system for distinguishing between MP and BP infections, specifically designed for primary care practice. Our final model demonstrates robust performance using only routinely available laboratory tests in community hospitals.

MATERIALS AND METHODS

Study design and patients

The study involved the enrollment of two distinct cohorts. Cohort 1 (n = 301) functioned as the derivation cohort and included pediatric outpatients diagnosed with either MP infection (n = 161) or BP infection (n = 140) at the Second Affiliated Hospital of Anhui Medical University between November 2023 and June 2024. The primary objective of this cohort was to identify independent predictors for differentiating between MP and BP infections and to develop a prognostic scoring model and nomogram. To conduct a more detailed analysis of the age distribution among children with MP or BP infection, the cohort was stratified into three distinct groups corresponding to developmental stages: infants/toddlers (< 3 years), preschool children (4 - 6 years), and school-age children (7 - 14 years). Cohort 2 (n = 87), comprising pediatric outpatients diagnosed with either MP (n = 39) or BP (n = 48) infection at the People's Hospital of Chizhou, functioned as the validation cohort. This cohort was utilized to externally assess the model's discriminative performance. All cases were confirmed through quantitative real-time PCR (qPCR) testing. The inclusion criteria were: 1) first-time outpatient visits; 2) age under 14 years; and 3) laboratory-confirmed single pathogen infection (either MP or BP). Exclusion criteria encompassed: 1) high risk or confirmed co-infections and 2) missing C-reactive protein (CRP) or complete blood count parameters. The study protocol was approved by the hospital's ethics committee and was conducted in accordance with the 1975 Declaration of Helsinki.

Clinical data

In this study, clinical data were obtained by analyzing whole blood samples from patients to measure C-reactive protein (CRP), white blood cell (WBC) count, neutrophil count, and lymphocyte count, utilizing the SYSMEX XN-9000 hematology analyzer with reagents matched to the original manufacturer specifications.

In accordance with established collection protocols, oropharyngeal swab specimens were obtained from pediatric outpatients who were enrolled in the study. Nucleic acid extraction was subsequently performed on these swab samples. The presence of MP and BP was then assessed through quantitative real-time PCR (qPCR) utilizing the Agilent Technologies Mx3000P System (USA).

Statistical analysis

Statistical analyses were executed using SPSS 20.0, GraphPad Prism 6.01, and MEDCALC software. Normally distributed continuous variables are presented as mean ± standard deviation and compared using Student's *t*-test, while non-normally distributed variables are expressed as median (interquartile range) and compared using the Mann-Whitney U test. Categorical variables are presented as frequencies with percentages and were compared using the chi-squared test or Fisher's exact test as appropriate. Multivariate logistic regression analysis was employed to identify independent prognostic factors. R software (version 4.5.1) was used to construct the nomogram. A two-tailed p-value of ≤ 0.05 was considered indicative of statistical significance. The model's performance was assessed using receiver operating characteristic (ROC) curves.

RESULTS

Clinical characteristics

Table 1 delineates the baseline characteristics of the derivation cohort, revealing no significant differences in

Table 1. Baseline characteristics of the deriving cohort enrolled in this study.

Variables	Total (n = 301)	MP (n = 161)	BP (n = 140)	Univariate logistic regression		Multivariate logistic regression	
				OR (95% CI)	p	0R (95% CI)	p
Gender (male/female)	162/139	88/73	74/66	0.930 (0.591 - 1.465)	0.755		
Age (years)	6.31 ± 2.22	6.35 ± 2.40	6.26 ± 2.01	1.019 (0.920 - 1.128)	0.723		
CRP (mg/L)	2.90 (0.80 - 6.75)	5.00 (2.6 - 11.43)	1.10 (0.50 - 2.60)	1.189 (1.115 - 1.268)	< 0.001	1.119 (1.053 - 1.190)	< 0.001
WBC ($\times 10^9$/L)	8.81 ± 3.12	7.59 ± 2.38	10.21 ± 3.30	0.711 (0.643 - 0.787)	< 0.001		
Neutrophil ($\times 10^9$/L)	4.72 (3.61 - 6.21)	4.61 (3.41 - 5.45)	5.13 (3.81 - 7.07)	0.829 (0.742 - 0.925)	0.001		
Lymphocyte ($\times 10^9$/L)	2.51 (1.76 - 3.32)	1.99 (1. 50 - 2.51)	3.26 (2.64 - 4.30)	0.265 (0.189 - 0.372)	< 0.001	0.320 (0.225 - 0.456)	< 0.001
Platelet ($\times 10^9$/L)	271.94 ± 70.68	248.83 ± 62.18	298.51 ± 70.73	0.989 (0.985 - 0.993)	< 0.001	0.997 (0.992 - 1.002)	0.203

Data presented as means ± SD or medians (P25 - P75). CRP: C-reactive protein, WBC: white blood cells, MP: *M. pneumoniae*, BP: *B. pertussis*, OR: odds ratio, p: p-value.

Table 2. The age-specific distribution patterns of MP and BP infections.

Age group (years)	MP infection (n = 161)	BP infection (n = 140)	p-value
< 3	20 (12.4%)	6 (4.3%)	0.017
4-6	70 (43.5%)	79 (56.4%)	0.111
7-14	71 (44.1%)	55 (39.3%)	0.519

MP: M. pneumoniae, BP: B. pertussis.

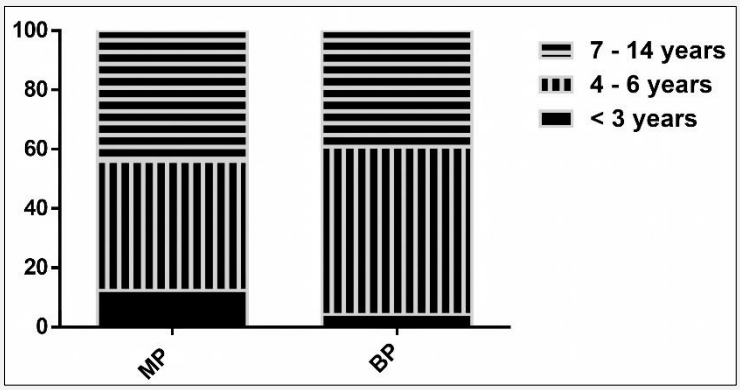

Figure 1. The bar graph displays the age-specific distribution patterns of MP and BP infections among pediatric patients.

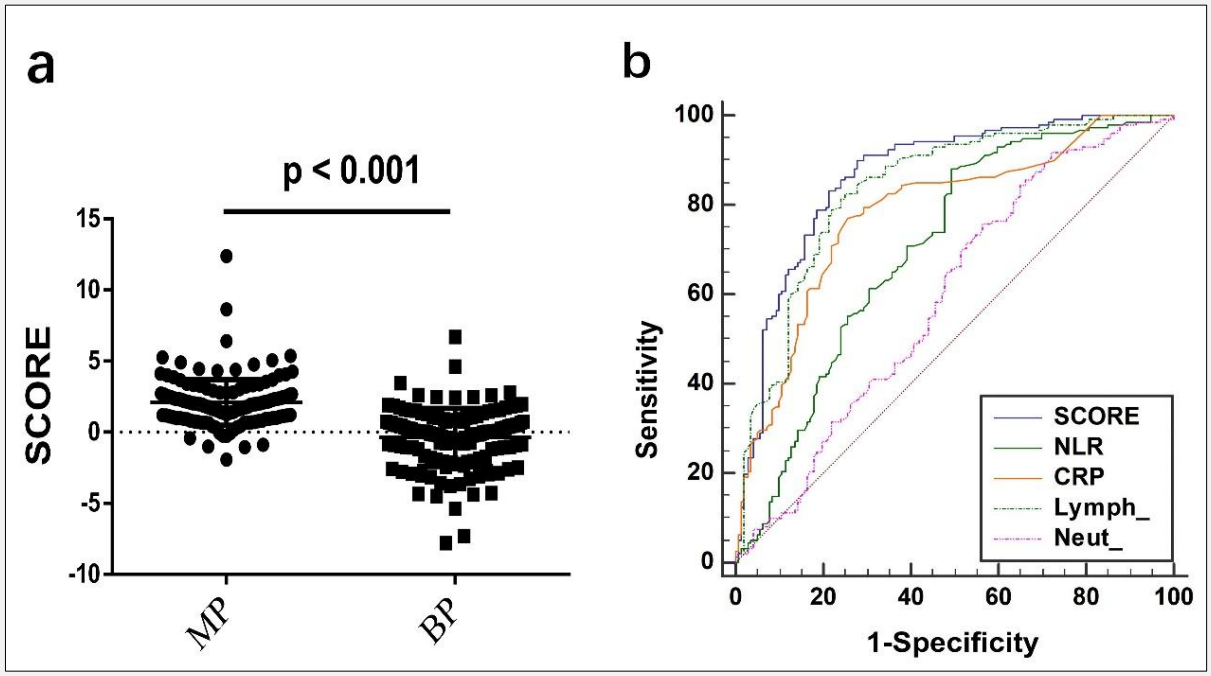

Figure 2. Prediction score for identifying MP and BP infection in the derivation cohort.

a: The score distribution of MP and BP infections in child patients in the derivation cohorts. b: ROC analysis shows the performance of SCORE.
Lymph: lymphocyte count, Neut: neutrophil count.

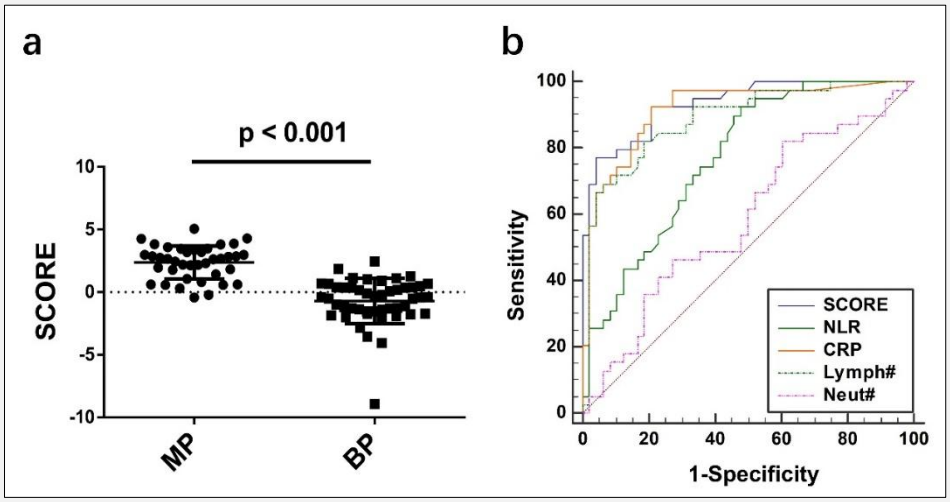

Figure 3. Prediction SCORE for identifying MP and BP infection in the validation cohort.
a: The score distribution of MP and BP infections in child patients in the validation cohort b: ROC analysis shows the performance of the SCORE. Lymph#: lymphocyte count, Neut#: neutrophil count.

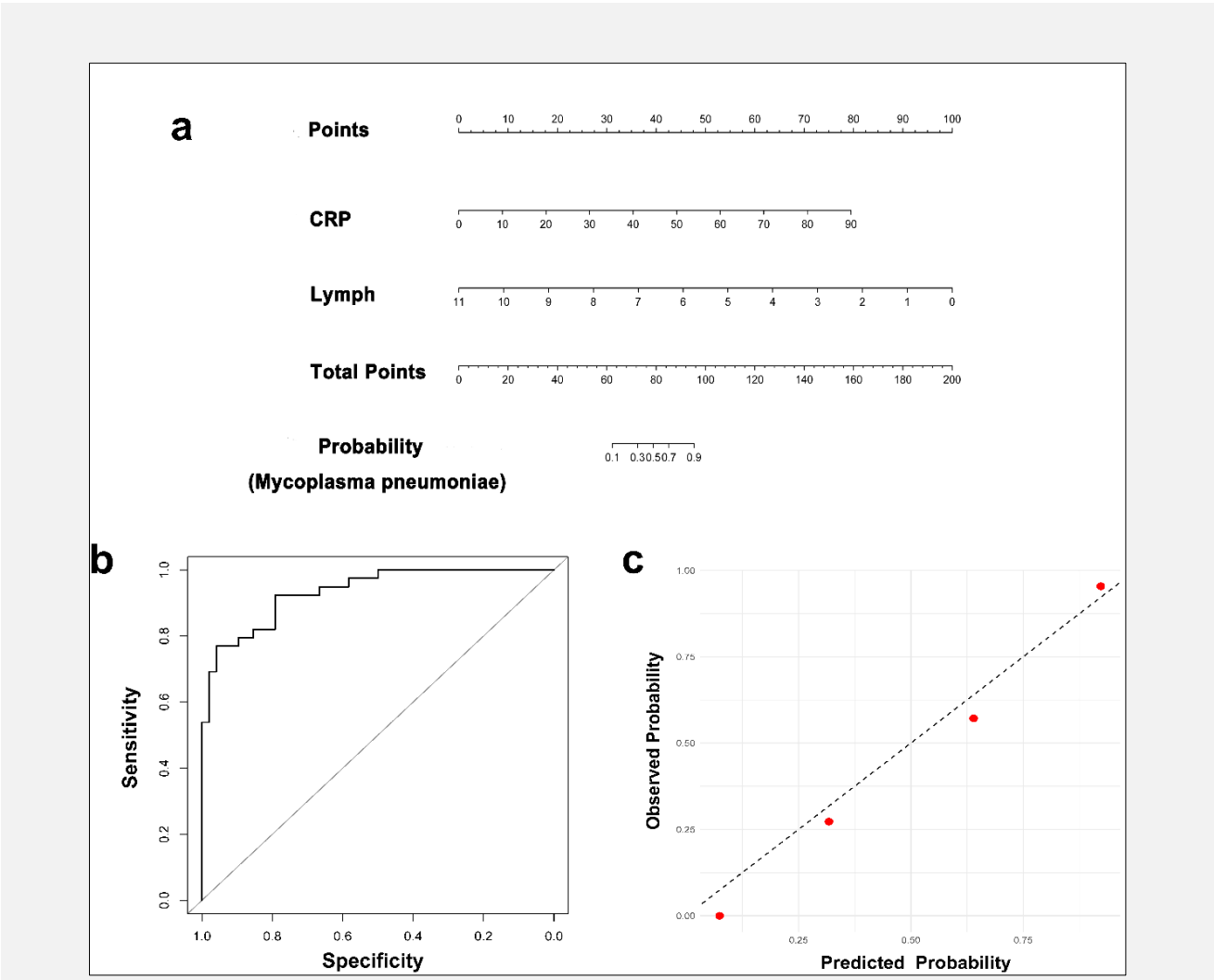

Figure 4. Nomogram and its performance.

a: The nomogram incorporating CRP and lymphocyte count was constructed based on the training cohort, b: Discriminative performance evaluation of the nomogram were measured using the area under the receiver operating characteristic (ROC) curve based on the validation cohort, c: Calibration plot in validation cohort.

age or gender ratio between the MP and BP infection groups. Notably, patients with MP infections exhibited significantly elevated levels of C-reactive protein (CRP) ($p < 0.001$), alongside reduced levels of white blood cell (WBC) count, neutrophil count, lymphocyte count, and platelet count (all $p < 0.01$) in comparison to those with BP infections. An analysis of the age-specific distribution patterns of MP and BP infections among pediatric patients was conducted (Figure 1). MP infections were predominantly observed in preschool and school-age children, with prevalence rates of 43.5% and 44.1%, respectively. Conversely, BP infections were most prevalent in the preschool group, with a peak rate of 56.4% (Table 2).

Establishment and evaluation of a prediction model for identifying MP and BP infection based on CRP and peripheral blood cell count

In our study, we conducted both univariate and multivariate logistic regression analyses on the derivation cohort (Cohort 1) and identified CRP, WBC, neutrophil count, lymphocyte count, and platelet count as independent prognostic indicators for distinguishing between MP and BP infections, with all variables showing statistical significance ($p < 0.01$), as detailed in Table 1. To address multicollinearity, variables with a variance inflation factor (VIF) greater than 5 were excluded. The final predictors, CRP and lymphocyte count, were selected for building the model.

Utilizing these findings, we formulated a predictive model represented by the equation: SCORE = 3.49 + 0.11 × CRP - 1.14 × Lymphocyte count. This SCORE effectively integrates inflammatory intensity (CRP) and immune status (lymphocyte count), providing enhanced discriminative capability for differentiating between MP and BP infections. The score was significantly elevated in the MP group compared to the BP group (2.09 ± 0.13 vs. -0.36 ± 0.17, $p < 0.001$) (Figure 2a). The model achieved an area under the curve (AUC) of 0.866, with a sensitivity of 90.06%, specificity of 72.14%, and a cutoff value of 0.62, demonstrating superior predictive performance relative to the neutrophil-to-lymphocyte ratio (NLR) (AUC = 0.704, $p < 0.001$), CRP (AUC = 0.783, $p = 0.001$), lymphocyte count (AUC = 0.838, $p = 0.029$), and neutrophil count (AUC = 0.593, $p < 0.001$) (Figure 2b).

SCORE performance in the validation cohort

We conducted a retrospective validation study on a cohort of outpatient children (n = 87) with BP infections (n = 48) and MP infections (n = 39) to assess the efficacy of the SCORE model in differentiating between MP and BP infections. Within this cohort, the SCORE was significantly elevated in the MP group compared to the BP group (2.38 ± 0.21 vs. -0.71 ± 0.26, $p < 0.001$) (Figure 3a). The model exhibited an AUC of 0.933 for distinguishing between MP and BP infections, surpassing the performance of the NLR (AUC = 0.770, $p < 0.001$), lymphocyte count (AUC = 0.886, $p = 0.033$), and neutrophil count (AUC = 0.587, $p < 0.001$), and was comparable to CRP levels (AUC = 0.916, $p = 0.573$) (Figure 3b).

Model development and validation

A nomogram was developed incorporating two independent prognostic factors using the training cohort (Figure 4a). The model demonstrated strong discriminative power, with a C-statistic of 0.866 and a well-calibrated slope of 0.963, indicating minimal overfitting. External validation in an independent cohort further confirmed the model's robustness, maintaining high discrimination (AUC = 0.933) (Figure 4b) and excellent calibration (slope: 0.941, Emax: 3.2%) (Figure 4c). Calibration plots indicated nearly perfect concordance between predicted and observed probabilities in both the training and validation cohorts. These results suggest that the nomogram reliably differentiates between MP and BP infections, supporting its potential clinical utility.

DISCUSSION

Recent years have seen significant changes in MP and BP infections. Global data show a notable rise in pertussis cases post-COVID-19, affecting not just infants and young children but also adolescents and adults [12]. Our study also observed that most BP cases (95.7%) are now in preschool and school-age children, indicating a shift in age distribution. In China, MP infection rates surged to 35.01% in 2023, with macrolide-resistant strains increasing to 87.16% [13]. Notably, these two pathogens showed overlapping epidemic peaks in China. Nonspecific clinical and radiological presentations pose significant challenges for pediatricians in differential diagnosis, often resulting in inappropriate antibiotic treatments. In clinical contexts where MP or BP infection is strongly suspected, early differentiation and timely targeted therapy are essential for effective disease management and improved prognosis. Currently, PCR technology is a highly specific and sensitive detection method widely used in advanced medical centers to quickly distinguish between MP and BP infections. However, many primary healthcare facilities, like community hospitals, often lack the necessary infrastructure, limiting its use.

In response, this study developed and validated an innovative C-reactive protein (CRP)-lymphocyte scoring system, which exhibited high diagnostic accuracy in distinguishing MP from BP infections among pediatric outpatients. To facilitate further clinical application, nomograms were constructed and calibrated. The C-index of training and test cohorts were 0.866 and 0.933, respectively. Both C-reactive protein and lymphocyte count are readily available clinical indicators that can be tested in primary healthcare settings such as community hospitals. This model is both rapid and cost-effective, offering a viable alternative to PCR in resource-limited settings, thus addressing clinical diagnostic needs, and mitigating the misuse of antibiotics.

The biomarker model we developed demonstrates a robust ability to differentiate between MP and BP infections, consistent with established disease mechanisms. Existing research suggests that the pathogenesis of MP infection is linked to both invasiveness and immune dysfunction. During MP infection, the host's immune system initiates a pronounced inflammatory response, resulting in marked increases in interleukin-6 (IL-6) and C-reactive protein (CRP) levels, while excessive inflammation contributes to disease progression and severity [14,15]. Additionally, studies have shown an inverse relationship between the severity of MP infection and lymphocyte counts in pediatric patients [16,17]. Our findings indicate that children with MP infections display significantly elevated CRP levels and reduced lymphocyte counts compared to those with BP-induced respiratory infections. This suggests that MP infection elicits a more intense inflammatory response over a shorter duration, whereas BP infection provokes a more robust immune response, in agreement with previous studies [18]. These differential immune responses partially elucidate why the composite score surpasses the predictive power of individual CRP or lymphocyte count measurements.

This study has several limitations that should be acknowledged. First, due to practical constraints in the outpatient and emergency setting, comprehensive bacterial and viral testing was not feasible, preventing the

complete exclusion of co-infections and potentially introducing bias into the findings. Second, the study's sample was limited to patients from only two centers, highlighting the need for validation through more extensive multicenter data. Additionally, the model's applicability is somewhat limited to specific scenarios, particularly during concurrent MP and BP epidemics when clinical suspicion is high, yet differentiation between the two is challenging.

Despite these limitations, the C-reactive protein (CRP)-lymphocyte scoring system offers a practical, equipment-free method for distinguishing between MP and BP infections in primary healthcare settings. By utilizing existing laboratory infrastructure for rapid and accurate diagnosis, this tool has the potential to significantly enhance rational antibiotic use and improve patient outcomes, particularly in resource-constrained regions.

Source of Funds:
This work was supported by the Provincial Natural Science Research Project of Anhui Colleges (KJ2019A0276).

Data Sharing Statement:
The datasets generated and/or analyzed during the current study are not publicly available due to privacy restrictions but are available from the corresponding author on reasonable request.

Declaration of Generative AI in Scientific Writing:
Generative AI was used in this work only for refining the language. The author takes full responsibility for the intellectual content.

Declaration of Interest:
The authors declare no conflict of interest.

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