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Neutrophils are involved in the development and outcomes of plastic bronchitis associated with *Mycoplasma pneumoniae* pneumonia

Xia Huang[1†], Houbing Qin[1†], Rui Zhang[1], Xinyi Jia[1], Deyu Zhao[1*] and Feng Liu[1*]

Abstract

Background Previous research has demonstrated a notable increase in neutrophil counts among pediatric patients with plastic bronchitis (PB) associated with *Mycoplasma pneumoniae* pneumonia (MPP). However, the role of neutrophils in MPP-associated PB remains largely elusive.

Methods This is a nested case-control study that enrolled patients diagnosed with MPP who underwent bronchoscopy in our department during the MPP pandemic from September 2023 to January 2024. We conducted an analysis of clinical characteristics, blood samples, bronchoalveolar lavage fluid (BALF), and cast specimens, correlating these factors with the development and outcomes of PB.

Results Among the 557 patients with MPP included in the study, 21 (3.8%) developed PB. The peripheral neutrophil count was identified as an independent risk factor for PB (OR = 3.113 [95%CI 1.050–9.224], $P = 0.04$) and exhibited strong predictive value for the condition (AUC = 0.885 [95%CI 0.796–0.975], $P < 0.001$). Notably, there was a marked presence of neutrophil infiltration and neutrophil extracellular traps (NETs) formation in the blood, BALF, and cast samples from patients with PB. Furthermore, the levels of neutrophils and NETs correlated significantly with clinical outcomes.

Conclusion A high level of neutrophils poses a risk for PB and demonstrates strong predictive value for its diagnosis. Neutrophils and NETs are closely linked to the clinical outcomes of PB in patients with MPP.

Keywords *Mycoplasma pneumoniae*, Plastic bronchitis, Neutrophils, Neutrophil extracellular traps, Outcomes

Introduction

Mycoplasma pneumoniae pneumonia (MPP) is a prevalent form of community-acquired pneumonia, particularly among children. Starting mid-September 2023, we have witnessed a notable upsurge in MPP cases, which has placed considerable strain on several pediatric emergency departments [1–3]. Even with the administration of suitable antibiotics, some cases of Mycoplasma pneumoniae may escalate to a refractory state, presenting with a range of serious complications. These include necrotizing pneumonia [4], bronchiolitis obliterans [5], and plastic bronchitis (PB) [4, 6, 7]. PB is a rare but

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life-threatening condition characterized by the formation of gelatinous, tree-like airway casts that can lead to severe respiratory obstruction [8]. The diagnosis of PB is typically established through a history of expectorating sputum with a branching pattern or the direct extraction of such casts during bronchoscopy [9]. However, the underlying causes of PB in the context of MPP remains poorly understood.

Prior studies have suggested a possible link to inflammation of the immune system, either locally or throughout the body. In patients with MPP, the PB group exhibited higher levels of neutrophils compared to the non-PB group, which was identified as a significant risk factor for PB [4, 7]. However, these studies are limited by the absence of pathological evidence, and the contribution of neutrophils to the pathogenesis of PB remains unclear. Our previous transcriptome sequencing results of bronchoalveolar lavage fluid (BALF) and blood from patients with MPP suggest that neutrophil extracellular traps (NETs) may play an important role in MPP [10, 11]. Neutrophils, the most prevalent immune cells in the bloodstream, are capable of releasing NETs which can be detected by DNA, myeloperoxidase (MPO), and citrullinated histone 3 (citH3). NETs possess antimicrobial properties but have also been associated with inflammatory processes [12]. However, the association between NETs and PB has yet to be established in the literature.

Consequently, we conducted a nested case-control study within a cohort of MPP patients undergoing bronchoscopy, comprising 21 matched pairs of children with and without PB. The objective was to analyze their biological samples and investigate the role of neutrophils in the development and outcomes of MPP-associated PB.

Methods

Study population

Between September 2023 and January 2024, patients hospitalized within the Department of Respiratory Medicine at the Children's Hospital of Nanjing Medical University were enrolled in the MPP cohort for the study. The research was conducted in accordance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee of Children's Hospital of Nanjing Medical University (Approval number: 202308005-1). Informed consent was obtained from patients or their guardians.

The inclusion criteria included: (1) Age range of 28 days to 15 years; (2) Presence of fever or other respiratory symptoms; (3) Radiographic confirmation of pneumonia; (4) Positive serologic findings, including elevated serum Mycoplasma pneumoniae (MP) immunoglobulin M (IgM) levels or evidence of seroconversion in paired serum samples, along with positive results from MPP polymerase chain reaction assays conducted on nasopharyngeal aspirates; (5) Undergoing treatment with bronchoscopy.

The exclusion criteria included: (1) Patients with pre-existing bronchopulmonary dysplasia, congenital heart disease, immunodeficiency, or hereditary nervous system disorders; (2) Evidence of co-infection with other pathogens as determined by nucleic acid testing, culture of nasopharyngeal aspirates, blood, alveolar lavage fluid, or pleural effusion; (3) Disease duration of 4 weeks or longer prior to hospital admission; (4) Hospital stay of less than 3 days; (5) Incompleteness of the clinical data.

Bronchoscopy was undertaken in cases where lobar consolidation or pulmonary atelectasis showed no improvement or worsened despite receiving appropriate treatment according to the Chinese guidelines for pediatric flexible bronchoscopy (2018) [13].

Nested Case-Control study

Cases were defined as patients who received a diagnosis of PB confirmed by the observation of characteristic branching casts during bronchoscopy. The control group consisted of individuals who, following bronchoscopy, were determined not to have PB. Each case was matched with two control patients and two healthy controls (HC) based on the following criteria: age (within ± 1 year), sex, and the duration since the diagnosis of PB (within ± 2 weeks). This matching process was conducted through random sampling.

Data collection and sample measurements

Data were prospectively collected from the medical records of the enrolled patients. The clinical information encompassed age, sex, total duration of fever, duration of fever before hospitalization, disease course at the time of bronchoscopy, length of hospital stay, and associated hospitalization expenses. Laboratory data included white blood cell (WBC) counts, neutrophil counts, levels of C-reactive protein (CRP), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and assessments of coagulation function. In addition to these, a range of biological samples were obtained, comprising a blood sample taken within 24 h of hospital admission, BALF, and branching airway casts from patients diagnosed with MPP.

Samples detection

Blood and BALF

The levels of NETs in the plasma and BALF were measured using a Quant-iT PicoGreen® dsDNA kit (Invitrogen MA, USA) and MPO-DNA Elisa Kit (YOBIBIO, China) following the manufacturer's guidelines. DNA quantity was determined based on fluorescence intensity (480 and 520 nm) and MPO-DNA quantity was determined based on the optical density (OD) (450 nm).

Branching airways cast

The cast specimens were treated with 4% paraformaldehyde at room temperature for 24 h, followed by paraffin embedding and staining with hematoxylin and eosin. In order to observe NETs in cast samples, tissue sections embedded in paraffin were subjected to immunofluorescence staining for NETs. The sections were treated with primary antibodies targeting MPO (1:100, 60299–1 g, Proteintech) and citH3 (1:100, ab5103, Abcam) overnight at 4 °C. After that, they were exposed to corresponding secondary antibodies for 2 h at room temperature and then stained with DAPI.

Freshly collected bronchial cast samples were initially fixed in a 2.5% glutaraldehyde solution. Following standard dehydration and sputter coating protocols, the samples were then examined for NETs and cell surface morphology using a scanning electron microscope (Hitachi SU8100), operated at an acceleration voltage of 3.0 kV.

Follow-up

Electronic medical records were utilized to compile data regarding both outpatient and inpatient encounters of pediatric patients throughout the designated follow-up period. Post-discharge, the patients were subjected to weekly monitoring, with chest radiographs being evaluated contingent upon each individual's clinical condition. The duration of the follow-up period was delineated from the date of PB diagnosis to the most recent evaluation of chest imaging. The collected follow-up data encompassed several parameters, including the patient's transfer to the intensive care unit (ICU), the cumulative duration of hospital stays, the hospital expenses, the incidence of necrotizing pneumonia, the frequency of bronchoscopy interventions, and the time elapsed for the resolution of abnormalities on chest imaging. Time to imaging recovery was defined as the interval from the diagnosis of PB to the point at which large infiltrates on chest radiographs were no longer discernible [10]. All follow-up data were available up to May 10, 2024.

Statistical analysis

Statistical analyses were performed using SPSS 26.0 and GraphPad Prism 9.0 software. Quantitative data with skewness were reported as the median (interquartile range: 25th–75th percentiles) and were analyzed using the Wilcoxon-Mann-Whitney test or the Kruskal-Wallis test, as appropriate. Categorical data were expressed as frequencies and analyzed using the chi-square test or Fisher's exact test, depending on the sample size. Variables found to be significant ($P < 0.05$) in univariate analysis were subsequently included in a multivariate logistic regression model to determine potential risk factors associated with PB. The diagnostic accuracy of the model was assessed using receiver operating characteristic (ROC) curves and calculating the areas under the curve (AUCs). Kaplan-Meier analysis was employed to evaluate the time to imaging recovery, with log-rank tests used for statistical comparison. All statistical tests were two-tailed, and statistical significance was set at $P < 0.05$.

Results

Participants and clinical characteristics

A total of 1,125 patients diagnosed with MPP were initially enrolled in the study. Upon application of the inclusion criteria, 557 patients were deemed eligible for the final analysis. PB was diagnosed in 21 cases (3.8%) through bronchoscopy, with male children comprising 52.4% of these PB cases. For each PB case, two patients without PB and two healthy controls (HC) were matched based on age (within ± 1 year), sex, and the interval since the diagnosis of PB (within ± 2 weeks), employing a random sampling method. A schematic representation of the study's participant selection process is depicted in Fig. 1.

The demographic and baseline clinical characteristics of the 63 individuals diagnosed with MPP are detailed in Table 1. The median age of the pediatric patients was 7.7 years, and bronchial casts were predominantly located in the bronchi of the two lower lobes accounting for 66.7% of cases. Significantly elevated median levels of CRP, WBC, neutrophils, and LDH were observed in the PB group when compared to the non-PB group (all $P < 0.05$). Additionally, the PB group exhibited a higher incidence of extrapulmonary complications, including increased ALT levels suggestive of liver damage, serosal effusion, and elevated D-dimer levels indicative of hypercoagulability, compared to the non-PB group (all $P < 0.05$). A comparative analysis of the demographic and baseline clinical features between the MPP and HC groups is presented in Supplementary Table 1.

Neutrophils was a risk factor for PB in MPP

Univariate logistic regression analysis identified seven factors—CRP, neutrophils, ALT, LDH, D-dimer, and serosal effusion—as statistically significant risk indicators for the development of PB in patients with MPP, with *P*-values less than 0.05 (Table 1). These variables were subsequently included in a multivariate logistic regression model, which confirmed neutrophils as a significant risk factor for PB within the MPP cohort (odds ratio [OR] = 3.113, 95% confidence interval [CI] 1.050–9.224, $P = 0.04$) (Fig. 2A). The diagnostic significance of neutrophils for PB in individuals with MPP was further underscored by the receiver operating characteristic (ROC) curve analysis, yielding an area under the curve (AUC) of 0.885 (95% CI 0.796–0.975, $P < 0.001$) (Fig. 2B). At a neutrophil count threshold of $> 9.0 \times 10^9$/L, the sensitivity

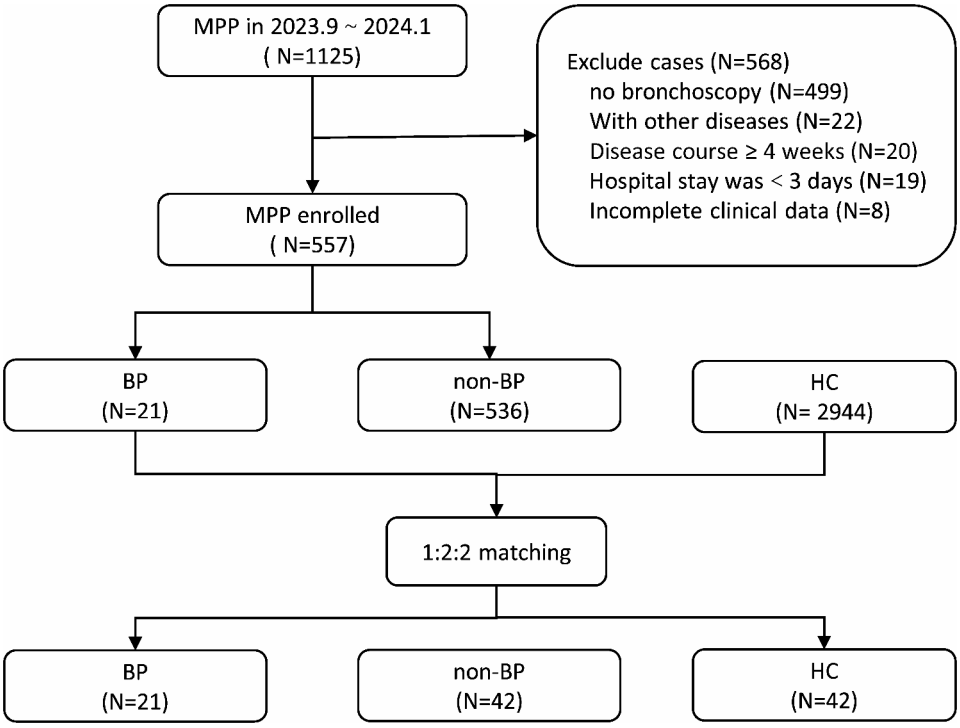

Fig. 1 Flowchart of the study. HC: heathy control; MPP: Mycoplasma pneumoniae pneumonia, PB: Plastic bronchitis

and specificity for PB were determined to be 88.1% and 85.7%, respectively.

Neutrophils and NETs present in diverse biological samples from PB

Chest computed tomography scans of pediatric patients with PB frequently depict consolidation or atelectasis confined to a single lobe (Fig. 3A). Bronchoscopy examination typically reveals inflammation and swelling of the mucous membrane, along with airway obstruction by gelatinous, tree-like casts (Fig. 3B). The presence of NETs in PB was assessed by performing immunofluorescence staining and scanning electron microscopy on the cast samples and quantifying levels of double-stranded DNA (dsDNA), MPO-DNA complex, well-established markers of NET formation, in both peripheral blood and BALF [14]. The results demonstrated a significant presence of NETs within the casts (Fig. 3C-D). Cast samples from 10 children with PB were histologically examined, revealing extensive areas of inflammatory necrosis, an abundance of neutrophils, and a fibrinous network structure (Fig. 3E and Supplementary Fig. 1). Compared to both the non-PB group and HC group, patients with PB exhibited significantly elevated levels of dsDNA and MPO-DNA complex in plasma (390.6 ng/ml vs. 244.1 ng/ml vs. 139.0 ng/ml, $P<0.001$; 2.7 vs. 2.3 vs. 1.8, $P<0.001$) (Fig. 3F-G). Similarly, levels of dsDNA and MPO-DNA complex in BALF were markedly higher in the PB group compared to the non-PB group (386.6 ng/ml vs. 240.6 ng/ml, $P<0.001$;0.9 vs. 0.5, $P<0.001$) (Fig. 3H-I). The levels of NETs in both plasma and BALF were positively correlated with the peripheral blood neutrophil count, as determined by Spearman's rank correlation analysis (all $P<0.05$) (Fig. 3J-M).

Neutrophils and NETs were associated with clinical parameters and outcomes in PB

To examine the correlation between peripheral neutrophil counts, NETs, and a spectrum of clinical parameters in patients with PB, we conducted a correlation matrix analysis, the results of which are presented in Fig. 4. Figure 4 illustrates a significant positive association between levels of neutrophils and NETs with several clinical indices, including CRP, LDH, D-dimer, total fever duration, length of hospital stay, and hospitalization expenses.

Subsequently, we conducted an analysis of the clinical outcomes associated with PB within the MPP patient population. Relative to the non-PB group, the PB group exhibited a notably higher incidence of ICU admissions, necrotizing pneumonia, and the necessity for multiple bronchoscopies. Additionally, patients in the PB group had a significantly extended total fever duration and hospital stay, coupled with higher hospital expenses (all $P<0.05$), as depicted in Fig. 5A-F.

The MPP cohort was further stratified into two groups based on the median values of peripheral neutrophil counts, plasma dsDNA levels, plasma MPO-DNA levels, BALF dsDNA levels, and BALF MPO-DNA levels,

Table 1 Demographic and baseline clinical characteristics of the patients with MPP

Characteristics	Total (*n* = 63)	non-PB (*n* = 42)	PB (*n* = 21)	*P*
Sex (n, %)				1.000
Male	33 (52.4)	22 (52.4)	11 (52.4)	
Female	30 (52.4)	20 (47.6)	10 (47.6)	
Age (y)	7.7 (6.7–8.9)	7.7 (6.7–9.0)	7.7 (6.6–8.9)	0.793
CRP (mg/L)	9.5 (4.8–25.3)	8.0 (3.2–18.8)	23.8 (6.8–57.1)	**0.022**
WBC ($\times 10^9$/L)	10.5 (8.1–12.7)	9.3 (7.3–11.3)	14.0 (12.1–17.2)	**< 0.001**
Neutrophils ($\times 10^9$/L)	7.1 (5.0–10.3)	6.0 (4.5–8.2)	11.5 (9.3–13.5)	**< 0.001**
Hemoglobin (g/L)	127.0 (12.5–134.0)	128.5 (122.0–135.8)	126.0 (120.3–132.8)	0.240
Platelets ($\times 10^9$/L)	298.0 (249.5–382.5)	301.5 (261.3–398.3)	284.0 (204.3–327.3)	0.232
ALT (U/L)	19.0 (13.0–51.0)	16.0 (13.0–32.5)	31.0 (16.5–79.8)	**0.025**
AST (U/L)	25.0 (20.0–36.5)	24.0 (20.8–33.0)	27.0 (19.0–51.3)	0.593
LDH (U/L)	325.0 (266.5–475.5)	303.5 (261.8–412.3)	403.0 (321.3–726.8)	**0.007**
PT (s)	12.3 (11.6–13.1)	11.9 (11.3–13.2)	12.9 (11.9–13.1)	0.105
APTT (s)	30.1 (27.5–33.4)	30.4 (27.6–32.8)	29.9 (27.2–33.6)	0.971
Fibrinogen (g/L)	3.4 (2.8–3.7)	3.3 (2.8–3.7)	3.4 (3.0–3.8)	0.255
D-dimer (ng/ml)	328.0 (179.5–2122.5)	271.5 (159.0–894.8)	2430.5 (279.3–3443.8)	**0.001**
Course of disease before admission (d)	10.0 (8.0–12.0)	10.0 (7.8–12.0)	10.0 (8.0–12.8)	0.697
Fever time before admission (d)	9.0 (6.0–10.0)	7.5 (6.0–10.0)	9.5 (7.0–11.5)	0.103
Course of disease before bronchoscopy (d)	12.0 (10.0–15.5)	12.0 (11.0–15.0)	12.0 (10.3–16.8)	0.959
Serosal effusion (n, %)				**0.002**
Yes	19 (30.2)	7 (16.7)	12 (57.1)	
No	44 (69.8)	35 (83.3)	9 (42.9)	
PB position (n, %)				
Right upper			2 (9.5)	
Right middle			1 (4.8)	
Right lower			5 (23.8)	
Right main bronchus			1 (4.8)	
Left upper			3 (14.5)	
Right lower			9 (42.9)	

ALT: alanine aminotransferase; AST: aspartate aminotransferase; APTT: activated partial thrombin time; CRP, C-reactive protein; LDH: lactate dehydrogenase; MPP: Mycoplasma pneumoniae pneumonia; PB: Plastic bronchitis; PT: prothrombin time; WBC: white blood cell

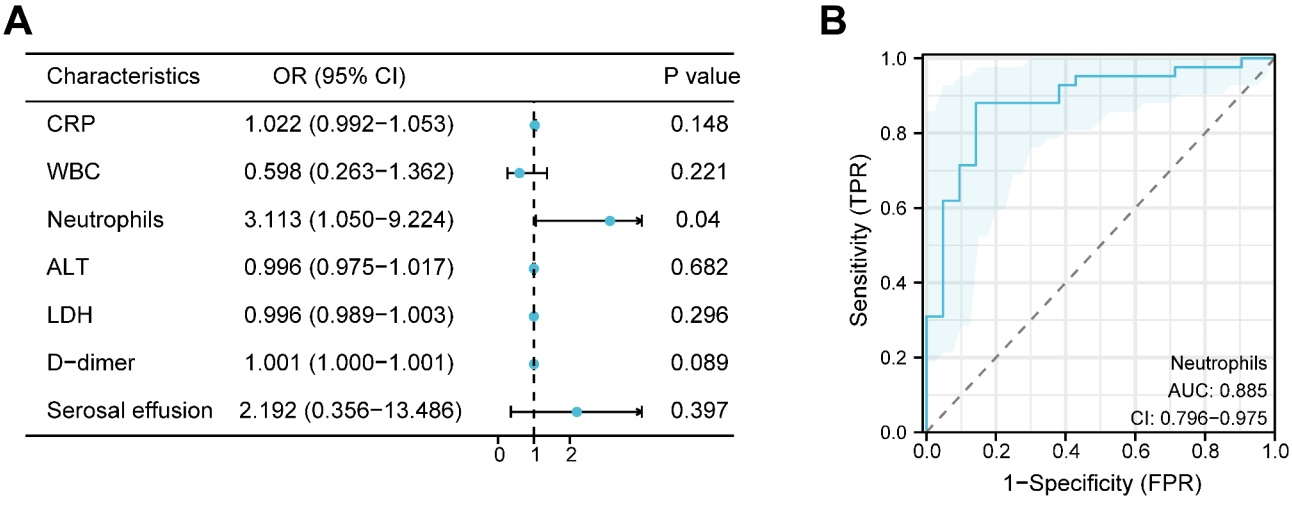

Fig. 2 Risk assessment and predictive value of neutrophils for PB in MPP. (**A**) The forest plot derived from the multivariate logistic regression analysis illustrates that neutrophil counts independently predict the risk of PB development in the context of MPP. (**B**) The ROC curve analysis demonstrated that neutrophil counts possess a significant predictive value in the diagnosis of PB. ALT: alanine aminotransferase; AUC: area under the curve; CRP, C-reactive protein; CI: 95% confidence interval; LDH: lactate dehydrogenase; MPP: Mycoplasma pneumoniae pneumonia; OR: Odds Ratio, PB: Plastic bronchitis; ROC: receiver operating characteristic; WBC: white blood cell

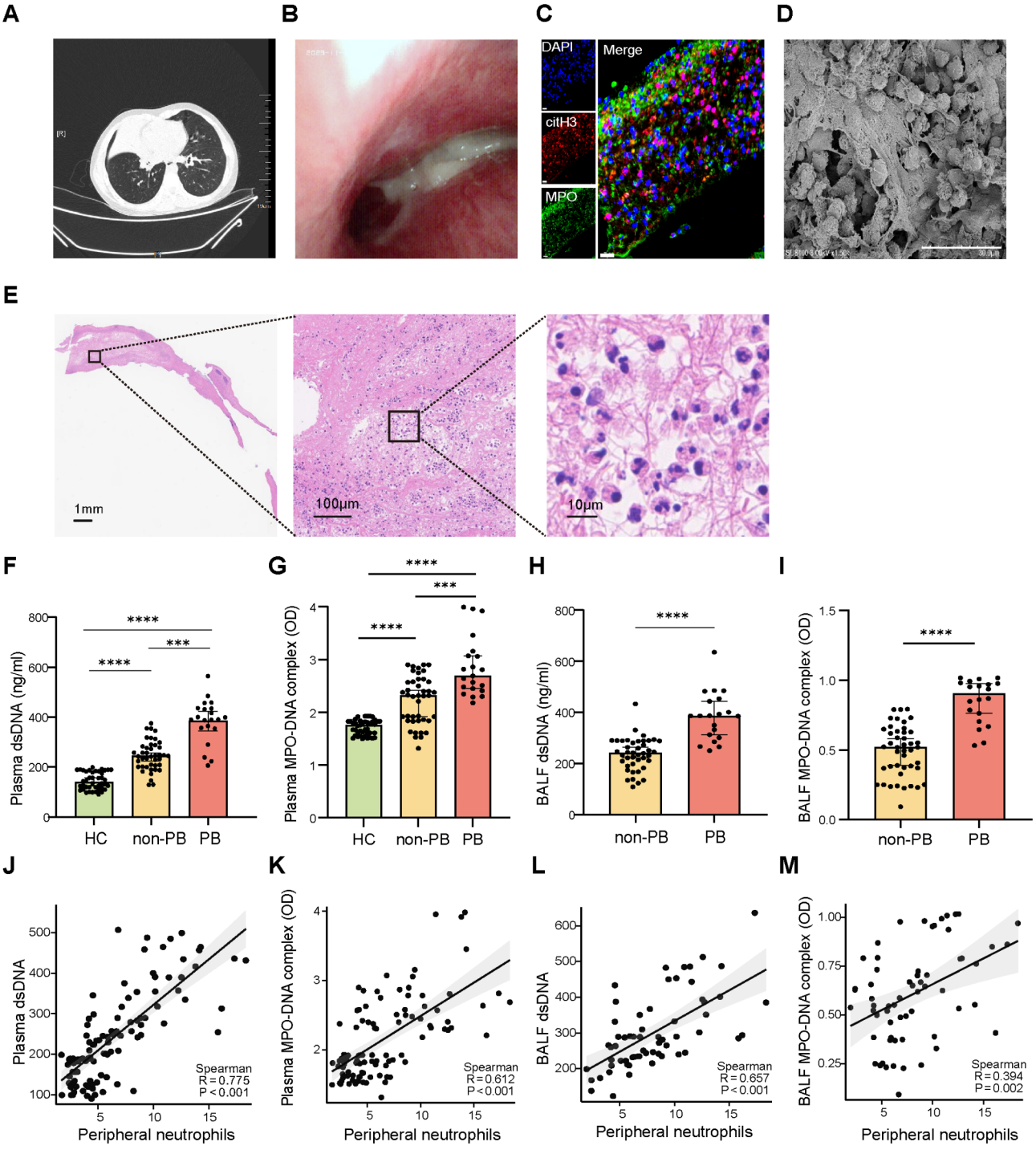

Fig. 3 (See legend on next page.)

respectively. Comparative analysis revealed that the prevalence of PB was substantially higher in the groups with elevated neutrophil counts (≥ 7.1×10^9/L), plasma dsDNA levels (≥ 256.9 ng/mL), plasma MPO-DNA levels (≥ 2.4), BALF dsDNA levels (≥ 266.9 ng/mL) and BALF MPO-DNA levels (≥ 0.6), as compared to their counterparts with lower levels, as illustrated in Fig. 5G-K.

The Kaplan–Meier survival curve analysis was applied to estimate the time to imaging recovery among patients with MPP. Variables such as the presence of PB, neutrophil counts, plasma dsDNA, plasma MPO-DNA, BALF dsDNA and BALF MPO-DNA were included in the survival analysis. The presence of PB, elevated neutrophil counts, increased levels of NETs both in plasma

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Fig. 3 Massive infiltration of neutrophils and formation of NETs in plasma, BALF, and bronchial casts were observed in MPP associated PB. (**A**) Chest computed tomography prior to bronchoscopy reveals consolidation and atelectasis in a single lobe of the lung. (**B**) Bronchoscopy revealed mucosal hyperemia and/or edema, as well as the presence of gelatinous, tree-like casts causing obstruction in the airway. (**C**) Representative immunofluorescence microscopy images depict the staining for MPO in green, citH3 in red, and DNA in blue within bronchial casts. The co-localization of DNA with both MPO and citH3 is evident, confirming the presence of NETs. $n = 10$. Scale bar = 20 μm. (**D**) A representative scanning electron microscopy image reveals the presence NET-like structures in close proximity to neutrophils. Scale bar = 30 μm. (**E**) Representative images of hematoxylin-eosin staining of the bronchial casts reveal chromatolysis and the presence of cell-free neutrophils, which are indicative of the formation of NETs. $n = 10$. Scale bar = 100 mm (left),100 μm (middle) and 10 μm (right). (**F**-**G**) Levels of dsDNA and MPO-DNA complex, biomarkers for the presence of NETs, were quantified in the plasma across three distinct study cohorts: patients diagnosed with PB (PB, $n = 21$), patients without PB (non-PB, $n = 42$), and healthy controls (HC, $n = 42$). (**H**-**I**) Levels of NETs were quantified in the BALF across two distinct study cohorts: patients diagnosed with PB (PB, $n = 21$), patients without PB (non-PB, $n = 42$). (J-M) Correlation curves were constructed to delineate the relationship between NETs levels in plasma and the counts of peripheral neutrophils, as well as between NETs levels in BALF and peripheral neutrophil counts

Statistical analysis: (F-I) The graphical representation illustrates the median NETs levels, with each bar accompanied by its corresponding 95% CI. Statistical significance among the groups is indicated by asterisks, using the following convention: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.001$. (H-I) The Spearman rank correlation test was utilized to evaluate the strength and direction of the association between plasma NETs concentrations and peripheral neutrophil counts. BALF: bronchoalveolar lavage fluid. citH3: citrullinated histone 3; CI: confidence interval; dsDNA: double-stranded DNA; MPO: myeloperoxidase; NETs: neutrophil extracellular traps; PB: plastic bronchitis

and BALF were found to be significantly associated with a prolonged recovery time (all $P < 0.05$), as shown in Fig. 5L-Q.

Discussion

PB is a rare, underdiagnosed and potentially fatal complication in children with MPP. We conducted a nested case-control study to delineate the role of neutrophils in pediatric patients with MPP associated PB during the MPP outbreak period from September 2023 to January 2024. Our findings indicate that elevated neutrophil counts are significantly associated with an increased risk of developing PB and are robust predictors for the diagnosis of PB within the MPP cohort. Additionally, neutrophils and NETs are correlated with the clinical outcomes of PB in MPP, suggesting a complex interplay between these cellular components and disease progression.

The precise biological mechanisms that contribute to the development of PB in patients with MPP are unclear at present. Previous studies have shown that epithelial cell injury and increased mucus secretion play an important role in PB formation [4, 15]. *Mycoplasma pneumonia*e infection may cause direct or indirect damage to airway epithelial cells and impaired ciliary function due to direct damage and heightened inflammation, thereby facilitating the development of mucus plugs [16]. Excessive inflammation is a key factor in this process [7]. In this study, children diagnosed with PB were found to have a prolonged fever duration, increased incidence of complications, and elevated levels of inflammatory markers including CRP, neutrophils, and LDH, suggesting an amplified inflammatory response in PB. Neutrophils, which are crucial for the initial defense against infection, have been reported to exhibit increased infiltration in the peripheral blood and BALF in MPP [17, 18], and the count of neutrophils has been found to be correlated with the severity of MPP, necrotizing pneumonia, respiratory failure, and other adverse prognostic outcomes [10, 19, 20]. Consistent with previous findings [7], an elevated level of neutrophils serves as a risk factor for the development of PB and demonstrates strong predictive value in its diagnosis. Furthermore, histopathological examination of bronchial cast samples from 10 children with PB revealed substantial neutrophil infiltration. These findings underscore the significant role of neutrophils in the inflammatory process associated with PB, however, the mechanism through which neutrophils play their role has not yet been studied.

The release of NETs, a critical function of neutrophils, acting as both a beneficial defense mechanism against a spectrum of infectious agents and a potential contributor to pathological inflammation and tissue damage [21, 22]. Transcriptome sequencing of peripheral neutrophils and BALF from pediatric patients with MPP has demonstrated significant activation of the NETs pathway, particularly in cases with severe disease progression [10, 11]. The association between NETs and PB has not yet been documented in the literature. In the present study, analysis of blood, BALF, and tissue samples from children with MPP has revealed the presence of extensive NETs across all biological samples examined. Extensive research has confirmed that NETs can exert significant influence on the airway epithelium, contributing to the pathogenesis of lung damage [23–26]. NETs have been shown to modulate sepsis-related acute lung injury by initiating ferroptosis in alveolar epithelial cells [23, 24]. Additionally, the direct application of NETs may induce apoptosis in alveolar epithelial cells [25], and particulate matter (PM2.5) has been linked to excessive mucus production via the NETs pathway [26], all of which could potentially contribute to the pathogenesis of PB. Further investigation is required to validate these hypotheses.

However, the sample size in this study was relatively small. Therefore, further extensive prospective studies and more comprehensive mechanistic investigations

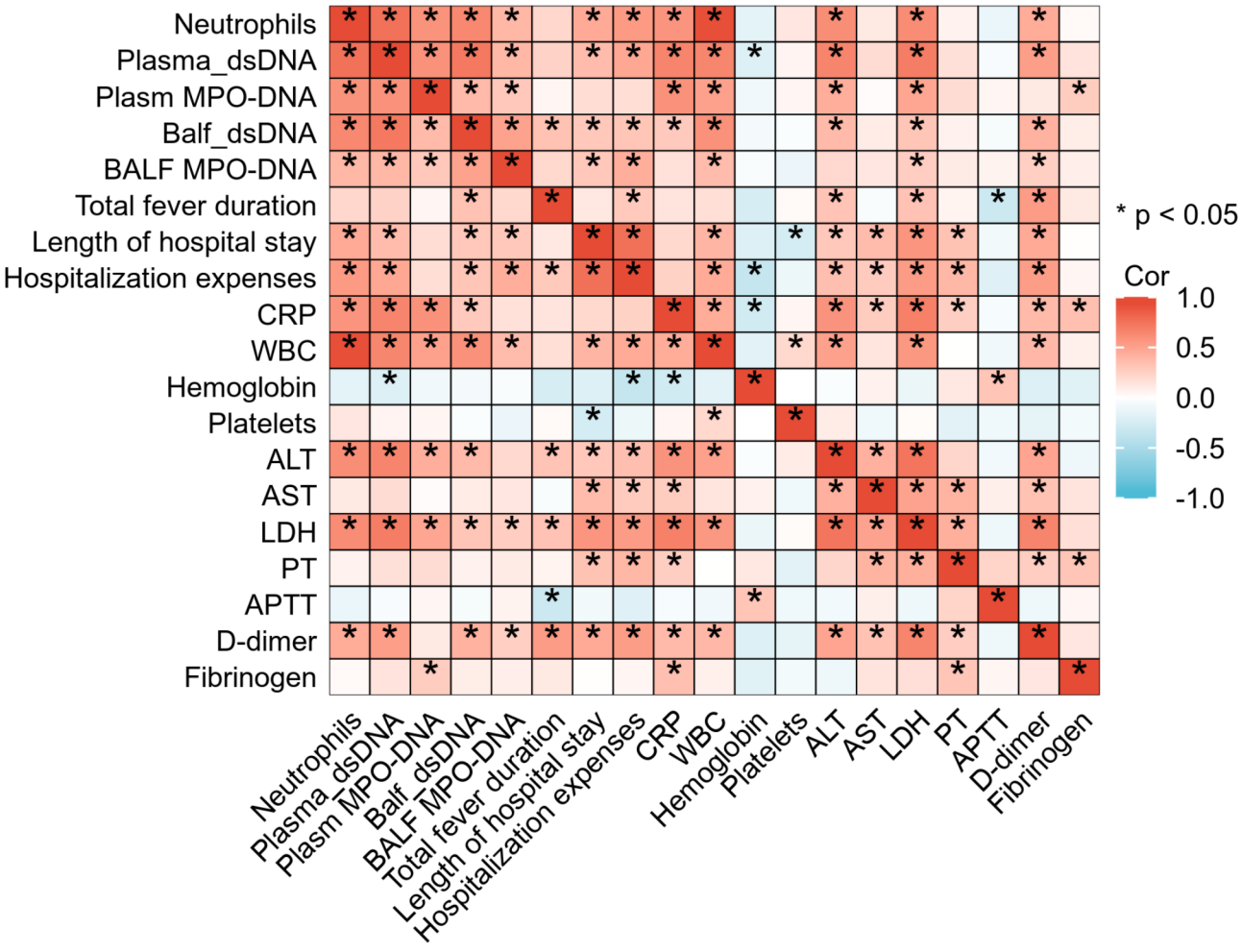

Fig. 4 Analysis of the correlation between peripheral neutrophils, NETs and various clinical parameters. Statistical analysis: The Spearman correlation test was employed to measure the strength and direction of the association between two variables. In the graphical representation, red coloration is utilized to denote a positive correlation, whereas blue coloration signifies a negative correlation. * $P < 0.05$. ALT: alanine aminotransferase; AST: aspartate aminotransferase; APTT: activated partial thrombin time; BALF: bronchoalveolar lavage fluid, CRP, C-reactive protein; dsDNA: double-stranded DNA; LDH: lactate dehydrogenase; MPO: myeloperoxidase; PT: prothrombin time; WBC: white blood cell

are necessary to elucidate the role of neutrophils in the pathogenesis of PB.

Conclusion

In summary, this study has identified that elevated peripheral neutrophil counts serve as a significant risk factor for the development of PB and are closely associated with its clinical outcomes in patients with MPP. Recognizing this correlation can facilitate the early detection of PB and inform the timely implementation of effective treatment strategies, potentially improving patient outcomes in the context of MPP.

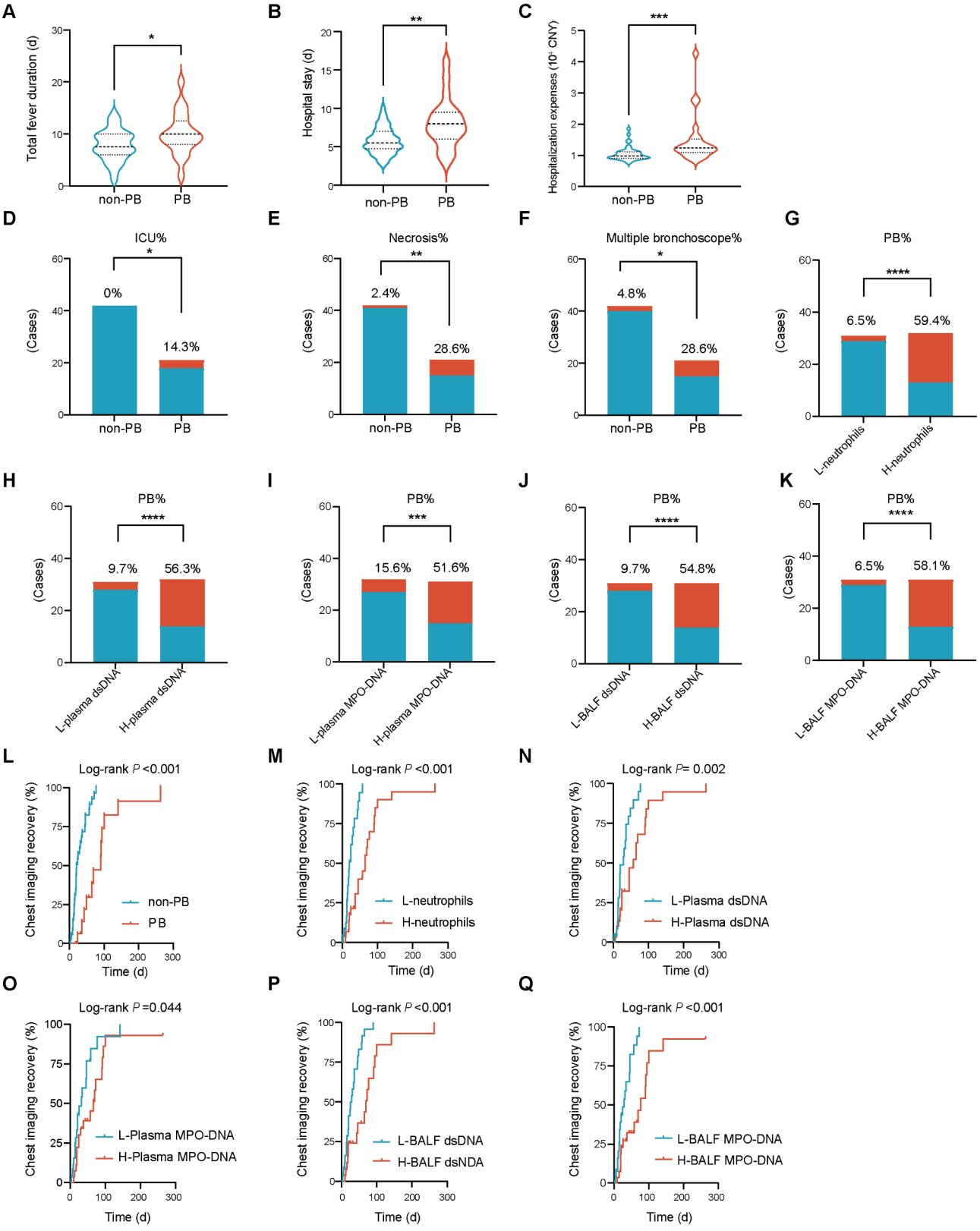

Fig. 5 (See legend on next page.)

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Fig. 5 Clinical outcomes of the patients with PB associated with MPP. (**A**-**C**) Violin plots illustrate the comparative analysis of the total fever duration, hospital stay, and hospitalization expenses between the PB and non-PB groups. (**D**-**F**) Cumulative bar graphs depict the comparative analysis of the proportions of ICU admissions, necrosis, and the requirement for multiple bronchoscopies between the PB and non-PB groups. (**G**-**I**) Cumulative bar graphs illustrate the comparisons of PB proportion between Low-neutrophils and High-neutrophils groups compare the proportions of PB across different groups, specifically between the Low-neutrophils and High-neutrophils groups (**G**), the Low-plasma dsDNA and High-plasma dsDNA groups (**H**), the Low-plasma MPO-DNA and High-plasma MPO-DNA groups (**I**), the Low- BALF dsDNA and High-BALF dsDNA groups (**J**), and the Low- BALF MPO-DNA and High-BALF MPO-DNA groups (**K**). (**L**-**Q**) Kaplan-Meier survival curves evaluate the time to chest imaging recovery, stratified by the presence of PB (**L**), neutrophil counts (**M**), plasma dsDNA (**N**), plasma MPO-DNA (**O**), BALF dsDNA (**P**) and BALF MPO-DNA (**Q**)
Statistical analysis: Quantitative data with skewness were presented as the median with interquartile range (IQR: 25th–75th percentiles) and were subjected to the Wilcoxon-Mann-Whitney rank-sum test to assess statistical differences (A-C). Categorical data are expressed as frequencies and were evaluated using the chi-square test or Fisher's exact test(D-K). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.001$. The log-rank test was utilized to calculate the P values for comparing the survival distributions across these groups (L-Q). BALF: bronchoalveolar lavage fluid; H: high; ICU: intensive care unit; L: low; MPO: myeloperoxidase; NETs: neutrophil extracellular traps; PB: Plastic bronchitis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03167-z.

Supplementary Material 1

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Author contributions
FL and DZ contributed to the study design. XH and HQ contributed to data acquisition, data analyzed and manuscript writing. RZ and XJ collected the clinical data. All authors read and approved the final manuscript.

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Data availability
The raw data is available upon reasonable request from the corresponding authors.

Declarations

Ethics approval
This study was approved by the research ethics committee of our institution (approval number: 202308005-1) and complied with the Declaration of Helsinki.

Informed consent
The parents of all participating children provided written informed consent before inclusion in the study.

Consent for consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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