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Comparison of bronchoscopy and bronchoalveolar lavage findings in three types of suppurative lung disease

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Abstract

Background: Endobronchial suppuration is present in children with protracted bacterial bronchitis (PBB), bronchiectasis, and cystic fibrosis (CF). However, no studies have directly compared bronchoscopy and bronchoalveolar lavage (BAL) findings across these conditions within a single center using the same techniques and with shared community pathogens.

Aim: To determine; (i) the bronchoscopic findings and BAL microbiology and cellularity among children with these conditions and; (ii) the relationship between bacterial pathogens, airway cellularity and aberrant macroscopic bronchoscopic findings.

Methods: We retrospectively reviewed all bronchoscopy data (undertaken over 6.5-years) from our center in children (<6 years; $n = 316$) meeting definitions of PBB ($n = 125$), bronchiectasis ($n = 138$), and CF ($n = 53$).

Results: The children's median age was 26-months (Interquartile range (IQR) = 16-43). Children with PBB and bronchiectasis had higher rates of *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* infection, whereas children with CF had frequent *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections. Novel findings include detection of cytomegalovirus and Epstein-Barr virus (EBV) (by polymerase chain reaction) in children with PBB (26%, 17%, respectively) and bronchiectasis (27%, 29%). Median airway neutrophil percentage was significantly higher in CF (68%; IQR = 42-83) compared to PBB (36%; IQR = 18-68) and bronchiectasis (22%; IQR = 8-64) ($P < 0.0001$), despite lower rates of infection. Presence of malacia did not significantly impact on infection or inflammation.

Conclusion: In this first study to directly compare bronchoscopic data among young children with PBB, bronchiectasis, and CF, microbiological patterns of airway infections and neutrophilia varied. Our findings of cytomegalovirus and EBV detection in children with PBB and bronchiectasis require confirmation and further evaluation.

KEYWORDS

airway microbiology, bronchiectasis, bronchoalveolar lavage (BAL), children, cystic fibrosis (CF), protracted bacterial bronchitis (PBB)

1 | INTRODUCTION

Endobronchial bacterial airway infection is found in children with protracted bacterial bronchitis (PBB), bronchiectasis and cystic fibrosis

(CF).¹ Children with these conditions frequently require repeated courses of antimicrobials in response to exacerbation of symptoms.^{2,3} These airway infections, according to Coles' "vicious cycle" hypothesis, can lead to impaired airway clearance and ongoing neutrophilic

inflammation, consequent airway damage, and eventually long-term to the development of bronchiectasis.^{4,5} This hypothesis is currently the most accepted basis for the development of bronchiectasis, and early adequate therapeutic intervention is needed to prevent the occurrence or aggravation of bronchiectasis in children with chronic wet cough.⁶

Thus, data on specific microbes that may play a role in its disease pathogenesis, is arguably important.⁷ For example, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are commonly identified in older children and adults with CF.⁸ In contrast, *Haemophilus influenzae* is the most common identified microorganism in PBB² and bronchiectasis.⁹ However, in young children with CF, a high prevalence of *H. influenzae* has been shown.^{8,10} Multiple studies, both single- and multicenter, have described bronchoscopy and bronchoalveolar lavage (BAL) findings in children with PBB, bronchiectasis and/or CF,^{11–13} but no studies have examined this data among all three conditions in a single center in young children using the same methods. One study, conducted by van der Gast et al,⁷ compared lower airway microbiomes among children with PBB, bronchiectasis and CF, but pediatric PBB and bronchiectasis specimens came from Brisbane, whereas the CF and adult bronchiectasis specimens were from the USA. Undertaking a comparison of the lower airway microbiology and inflammation in young children with endobronchial suppuration from a single center to ensure standardized specimen collection methods and similar microbial and environmental exposure would increase our insight into the pathogenic role of airway infections among various types of suppurative lung disease.

Thus, in 335 children aged <6-years, we aimed to determine; (i) the similarities and differences of the macroscopic bronchoscopic findings and the BAL microbiology and cellularity among children with PBB, bronchiectasis and CF and; (ii) the relationship between bacterial pathogens and airway inflammation, cellular counts, and aberrant macroscopic bronchoscopic findings. We hypothesized that airway microbiology and neutrophilic

inflammation of the lower airways are similar in young children with these various endobronchial suppuration categories.

2 | MATERIALS AND METHODS

2.1 | Study design

Retrospective review of all bronchoscopy data from the Royal Children's Hospital, Brisbane (now called the Lady Cilento Children's Hospital, Brisbane). The Queensland Children's Health Services Ethics Committee approved the study prior to commencing the study.

Children were identified from the bronchoscopy reports data folder, where every child who underwent bronchoscopy was systematically captured. Any child, aged <6 years of age who underwent an elective flexible bronchoscopy including BAL between March 2010 and November 2016 was eligible for inclusion. Children who underwent bronchoscopy for research purposes, or if no BAL data were available, were excluded. In total, 355 children met a-prior definitions of PBB (history of chronic wet cough (>4 weeks) and resolution of cough on antibiotic treatment within 2 weeks,² $n = 126$), bronchiectasis (on chest CT scan proven and without CF, $n = 138$) or CF (positive sweat test and/or CF gene mutation present, $n = 71$) were included and assigned to groups based on these diagnoses (Figure 1). The clinical decision to undertake a chest high-resolution computed tomography (c-HRCT)-scan was made by the child's attending physician, the cHRCT-scan results were used in retrospect to identify different cohorts. If a child had multiple bronchoscopies performed, the first bronchoscopy was included in the analyses, unless a cHRCT-scan was undertaken at the subsequent bronchoscopy (up to 1 year before or after the index bronchoscopy). If bronchiectasis was radiologically diagnosed more than a year post-bronchoscopy, or a

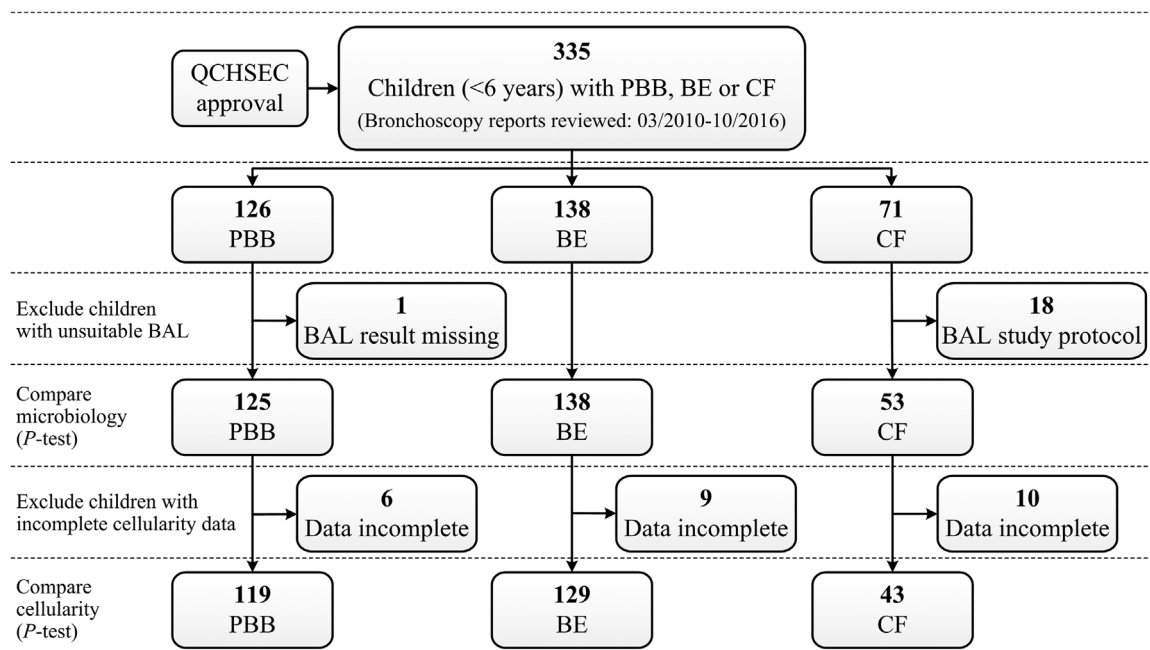


FIGURE 1 Study flow diagram. QCHSEC, Queensland Children's Health Services Ethics Committee; BE, bronchiectasis

cHRCT-scan revealing no bronchiectasis was performed more than a year before bronchoscopy, radiological findings were excluded ($n = 9$).

In our center, all flexible bronchoscopy (Olympus; Tokyo, Japan) was performed under general anesthesia and BAL undertaken in accordance with the ERS guidelines as previously described.¹⁴ None of the children were acutely unwell. Briefly, sterile saline instilled in three aliquots of 1 mL/kg (maximum 20 mL) into the two most affected area in localized disease or right middle lobe and left lingula in generalized disease.¹⁴ The first aliquot was used for microbiological assessment, which mostly included quantitative aerobic bacterial cultures, specific testing for mycobacterial species using inoculation of Lowenstein-Jensen media and polymerase chain reaction (PCR) for mycoplasma and various respiratory viruses. The second and third lavage aliquots were pooled and used for cellular analysis, including total and differential cell counts.

Data, including patient characteristics, indication for bronchoscopy, and aberrant macroscopic findings (presence of laryngo-, tracheo-, or bronchomalacia (>50% airway diameter reduction during spontaneous exhalation), were retrieved from bronchoscopy reports. When cHRCT-scans were performed, radiological reports and images were reviewed to determine the presence of bronchiectasis.¹⁵ BAL quantitative microbiological and cytological data were retrieved from the hospital's laboratory information system (Auslab, Citadel Health; Melbourne, Australia).

Lower airway bacterial infection was defined as a positive culture ($\geq 10^4$ colony-forming units (cfu)/mL BAL)² growing recognized respiratory pathogens, including *Streptococcus pneumoniae*, β -hemolytic streptococci, haemophilus species, *Moraxella catarrhalis*, *S. aureus*, and Enterobacteria. Two bacteria, *Stenotrophomonas maltophilia* and *P. aeruginosa*, were considered infection at a lower bacterial growth threshold ($\geq 10^1$ cfu/mL).¹⁶ Respiratory infection was also considered present if any fungi or mycobacteria were isolated or viruses and mycoplasma (by PCR) were detected.

2.2 | Statistical analyses

IBM SPSS Statistics (Version 23.0, IBM Corp.; Armonk, NY) was used for statistical analyses. As data were non-normally distributed, continuous outcomes were reported as medians and interquartile ranges (IQR). Statistical comparison between groups for categorical variables was performed using chi-square tests,

with continuity correction for 2×2 analyses, or Fisher's exact test if any values were < 5 . Continuous variables were compared between two groups using Mann-Whitney's *U*-test and using Kruskal-Wallis test for comparison of > 2 groups. Furthermore, to explore the factors influencing neutrophil percentage among the different groups, variables were, based on the strength of their univariate association with neutrophil percentage, selected ($P < 0.2$) and included in multiple linear regression analyses with backward elimination. For all analyses, 2-sided tests were used with P -values < 0.05 considered statistically significant.

3 | RESULTS

3.1 | Study population

Of the 335 children identified, 19 were excluded for: CF research study ($n = 18$) and no BAL data recorded ($n = 1$). The median age of the remaining 316 children (192 male, 124 female) at the time of bronchoscopy was 26 months (IQR 16, 43). Children with PBB were significant younger than children with bronchiectasis ($P < 0.0001$) and CF ($P < 0.001$), there was no significant difference in age between the bronchiectasis and CF cohorts ($P = 0.207$). With regards to sex, no significant differences between the three cohorts were observed ($P = 0.610$), nor between the bronchiectasis and CF cohorts ($P = 0.426$). Among all cohorts, 214 children (68%) underwent a cHRCT-scan, which revealed radiologically proven bronchiectasis in 157 children (73%) (Table 1). The main indication to perform a bronchoscopy was "recurrent or chronic cough" ($n = 284$).

3.2 | Airway microbiology

Of the 316 BAL results from children that underwent a bronchoscopy due to clinical indications, 314 (99%) had at least one organism detected, including upper airway flora such as α - and non-hemolytic streptococci, coagulase negative staphylococci and *Neisseria* and *Corynebacteria* species, which are considered as non-pathogenic in immunocompetent children. In total, 253 (80%) BAL results cultured one or more pathogenic bacterial species, 122 (39%) results that underwent PCR for viral detection were positive (Table 2). In our study, 36 children (11%) had

TABLE 1 Characteristics of the included study population categorized by diagnoses

Characteristic	PBB, $n = 125$, n (%)	BE, $n = 138$, n (%)	CF, $n = 53$, n (%)	<i>P</i> -value ^a
Sex, M:F (% male)	77:48 (62)	86:52 (62)	29:24 (55)	0.610
Median age at bronchoscopy, mo (IQR)	18 (12, 29)	31 (20, 47)	40 (23, 50)	<0.0001
cHRCT scan included	38 (30)	138 (100)	38 (72)	— ^b
Bronchiectasis (radiologically proven) ^c	0 (0) [$n = 38$]	138 (100)	19 (50) [$n = 38$]	— ^b
Bronchoscopy, cHRCT scan on same day ^c	28 (74) [$n = 38$]	114 (83)	23 (61) [$n = 38$]	— ^b

BE, Bronchiectasis; mo, months; IQR, interquartile range.

Medians, IQR, and percentages rounded to the nearest whole number.

^a*P*-value tests whether all three groups have the same percentage or median. *P*-values < 0.05 denote statistical significance and are noted in bold.

^bSince bronchiectasis on cHRCT scan was used as a diagnostic criterion, no statistical analyses on difference between cohorts were applied.

^cSquare brackets refer to the denominator for the specific test as not all children had the test undertaken.

only upper airway flora detected, with no significant differences observed between the various cohorts ($P = 0.113$).

Among the three different cohorts, significant differences in the presence of pathogenic bacterial organisms were found, with significantly ($P = 0.001$) fewer children with lower airway infection in the CF cohort (Table 2, Figure 2). In children with PBB and bronchiectasis *H. influenzae* was the most common cultured pathogen, followed by *M. Catarrhalis* and *S. pneumoniae*. *M. Catarrhalis* presence differed significantly between the cohorts ($P = 0.023$), however, this might be confounded by the difference in age between the cohorts, since children with *M. Catarrhalis* present were significantly younger ($P = 0.010$). In CF, in contrast, *H. influenzae* was the third most cultured pathogen, while *P. aeruginosa* and *S. aureus* were more common.

Of the children who underwent respiratory viral PCR-testing ($n = 314$), 107 (34%) had bacterial-viral co-infection detected; prevalence rates differed significantly among the three cohorts ($P < 0.0001$). These numbers might underestimate the actual prevalence, as not all BAL samples were tested for CMV and EBV. The most common bacterial-viral combination identified was *H. influenzae* and adenovirus ($n = 38$, 36%). Respiratory viral infections were seen significantly more frequent in the PBB and bronchiectasis cohorts, compared to the CF cohort ($P < 0.0001$) (Table 2). The most common virus detected in PBB and bronchiectasis was cytomegalovirus (CMV), followed by adenovirus and Epstein-Barr virus (EBV).

A fungus was detected in 100 of 266 (38%) children who had fungi reported in their microbiology results and fungal growth was relatively

TABLE 2 Lower airway infection amongst the three diagnostic cohorts

	PBB $n = 125$, n (%)	BE $n = 138$, n (%)	CF $n = 53$, n (%)	P -value ^a PBB-BE	P -value ^b BE-CF	P -value ^c all cohorts
No significant pathogenic infection ^d	10 (8)	16 (12)	10 (19)	0.442	0.282	0.113
Pathogenic bacterial infection ^e	109 (87)	111 (80)	33 (62)	0.189	0.015	0.001
<i>H. influenzae</i> ^f	81 (65)	91 (66)	9 (17)	0.948	<0.0001	<0.0001
<i>H. influenzae</i> (BLP)	17 (14)	22 (16)	2 (4)	0.701	0.041	0.076
<i>H. influenzae</i> (BLN)	65 (52)	70 (51)	7 (13)	0.934	<0.0001	<0.0001
<i>M. catarrhalis</i> (BLP)	41 (33)	27 (20)	1 (2)	0.023	0.004	<0.0001
<i>S. pneumoniae</i>	31 (25)	33 (24)	3 (6)	0.978	0.007	0.009
<i>S. aureus</i>	10 (8)	9 (7)	13 (25)	0.836	0.001	0.001
<i>H. parainfluenzae</i>	15 (12)	5 (4)	1 (2)	0.021	1.000 ^g	0.008
<i>P. aeruginosa</i>	4 (3)	5 (4)	15 (28)	1.000 ^g	<0.0001	<0.0001
<i>S. maltophilia</i>	1 (1)	0 (0)	5 (9)	— ^h	— ^h	0.010 ⁱ
Any viral species ^j	58 (47) [$n = 124$]	57 (41)	7 (14) [$n = 52$]	0.444	0.001	<0.0001
Adenovirus ^j	32 (26) [$n = 122$]	23 (17) [$n = 136$]	2 (4) [$n = 52$]	0.094	0.034	0.002
Parainfluenza ^j	10 (8) [$n = 122$]	12 (9) [$n = 136$]	1 (2) [$n = 52$]	1.000	0.117 ^g	0.248
RSV ^j	9 (7) [$n = 122$]	4 (3) [$n = 136$]	2 (4) [$n = 52$]	0.180	0.669 ^g	0.237
CMV ^j	12 (26) [$n = 47$]	13 (27) [$n = 49$]	0 (0) [$n = 19$]	1.000	— ^h	— ^h
EBV ^j	7 (17) [$n = 41$]	8 (19) [$n = 43$]	0 (0) [$n = 19$]	1.000	— ^h	— ^h
Bacterial-viral co-infection ^j	55 (44) [$n = 124$]	48 (35)	4 (8) [$n = 52$]	0.145	<0.0001	<0.0001

BE, Bronchiectasis; BLP, β -lactamase positive; BLN, β -lactamase negative; RSV, respiratory syncytial virus; CMV, Cytomegalovirus; EBV, Epstein-Barr virus. Percentages rounded to the nearest whole number, the number of samples tested for a specific outcome noted in heading or directly behind result if distinct. P -values < 0.05 denote statistical significance and are noted in bold.

^a P -value tests whether the PBB and the BE cohorts have the same prevalence of an outcome.

^b P -value tests whether the BE and the CF cohorts have the same prevalence of an outcome.

^c P -value tests whether all three cohorts have the same prevalence of an outcome.

^dNo significant pathogenic organisms identified (includes testing for bacteria, viruses, fungi, and mycobacteria).

^eSignificant pathogenic bacterial growth defined as growth $\geq 10^4$ cfu/mL (or $\geq 10^1$ cfu/mL for *P. aeruginosa* and/or *S. maltophilia*).

^fTwo children (PBB and BE) grew significantly *H. influenzae* (BLP) as well as *H. influenzae* (BLN).

^gFisher's exact test.

^hDue to an expected value < 1 , no statistics were applied.

ⁱFisher's exact test between PBB and CF cohort.

^jSquare brackets refer to the denominator for the specific test as not all children had the test undertaken.

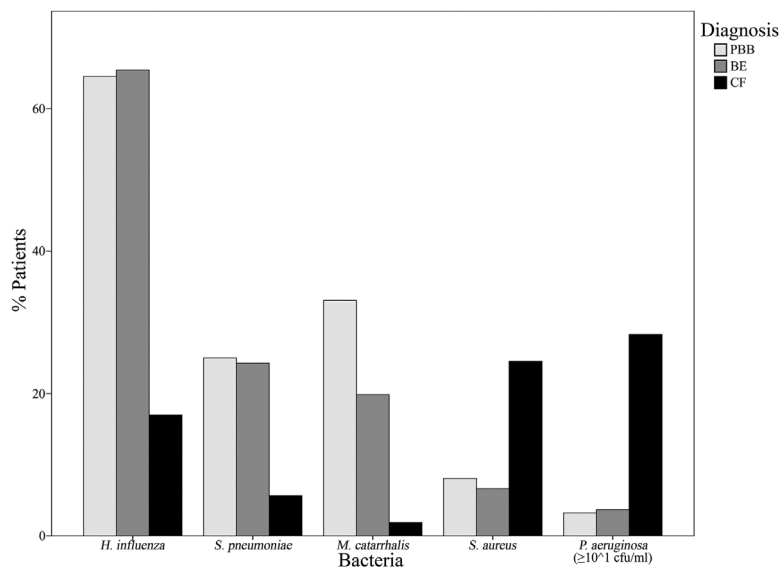


FIGURE 2 Bar graph showing prevalence of most common bacteria identified (growth $\geq 10^4$ cfu/mL), specified per cohort (PBB, bronchiectasis, and CF). For *P. aeruginosa* any growth ($\geq 10^1$ cfu/mL) was considered significant. BE, bronchiectasis

more common in CF (PBB: $n = 29$ (31%); BE: $n = 42$ (35%); CF: $n = 29$ (55%); $P = 0.013$). No mycobacteria, burkholderia species, or mycoplasma were present in the study population.

3.3 | Airway cellularity

For this component, a further $n = 25$ were excluded as the cellularity data available were incomplete (Figure 1). Total cell count was significantly higher in the CF cohort compared to PBB and bronchiectasis, there was no statistically significant difference between the CF and bronchiectasis cohorts ($P = 0.187$) (Table 3). Neutrophilia, defined as $>6.5\%$ neutrophils,¹⁷ was identified in 252 (87%) children. Eosinophilia, defined as $>1\%$ eosinophils,¹⁷ was identified in 47 (16%) children.

Children with PBB and bronchiectasis had significantly lower neutrophil percentages compared to children with CF ($P < 0.0001$) (Table 3) and, inversely related, higher percentages macrophages. Children with PBB had significant higher neutrophil percentages than children with bronchiectasis ($P = 0.043$). To examine whether this was confounded by the age difference between these cohorts,

univariate regression analysis was undertaken, revealing a very weak association between age and neutrophil count ($R^2 = 0.017$, $P = 0.041$). Subgroup analyses revealed no significant association between age at bronchoscopy and neutrophil percentages within the PBB and bronchiectasis cohorts.

Multiple linear regression analyses with backward elimination were undertaken to identify other factors influencing BAL neutrophil percentage. For the entire study population, the only significant factor associated with neutrophilia was total cell count (R^2 for model = 0.338, $P < 0.0001$). Subgroup-analysis revealed the number of different pathogenic bacteria cultured as an additional significant factor in the PBB cohort (R^2 for model = 0.322, $P < 0.0001$). These same factors were also significant in the CF-cohort, with the presence of adenovirus as an additional factor (R^2 for model = 0.399, $P < 0.0001$). In bronchiectasis, total cell count and the presence of EBV were significant associations with increased neutrophil percentages (R^2 for model = 0.438, $P < 0.0001$). Adding positive test results for adenovirus and/or *H. influenzae* in BAL fluid as independent factors,¹¹ lead to a very

TABLE 3 Different cohorts versus BAL cellularity data including differential cell count

	PBB, $n = 119$	BE, $n = 129$	CF, $n = 43$	P-value ^a
Total cell count ($\times 10^6/L$)	260 (120, 450)	270 (150, 645)	430 (175, 1070)	0.039
Macrophage %	50 (22, 70)	54 (23, 78)	25 (12, 51)	0.002
Lymphocyte %	8 (5, 14)	9 (4, 17)	3 (1, 6)	<0.0001
Neutrophil %	36 (18, 68)	22 (8, 64)	68 (42, 83)	<0.0001
Eosinophil %	0 (0, 0)	0 (0, 0)	0 (0, 0)	— ^b

BE, Bronchiectasis.

Data presented as medians (IQR). Rounded to the nearest whole number.

^aP-value tests whether all three groups have the same median. P-values < 0.05 denote statistical significance and are noted in bold.

^bSince median eosinophil counts and IQR equalled 0, no statistics were applied.

minor, non-significant, improved prediction model in PBB ($R^2 = 0.329$) and bronchiectasis ($R^2 = 0.456$).

3.4 | Aberrant macroscopic findings

Tracheo- and/or bronchomalacia was detected in 155 (48%) of the 311 children. There was no difference in sex ($P = 1.000$), but children with malacia were significantly younger ($P < 0.0001$). Malacia was more prevalent in children with PBB (71%) than children with bronchiectasis (47%) ($P < 0.0001$) and was significantly less frequently diagnosed in children with CF (9%) ($P < 0.0001$). Laryngomalacia was more common in children with PBB (11%) than children with bronchiectasis (4%) ($P = 0.034$), and no children with CF had laryngomalacia.

To prevent confounding due to their small numbers, children with CF were excluded from the analyses on malacia. There was no significant difference in BAL infection rates or microbiology between children with and without malacia; *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* were the most frequent identified microorganisms. No differences in cellularity parameters were observed, except for a significant lower macrophage percentage in the non-malacia group ($P = 0.026$), however without concomitant increase in neutrophil percentage.

4 | DISCUSSION

This is the first study comparing the bronchoscopic and BAL findings from 316 young children (<6 years) across three diagnostic categories with endobronchial suppuration (PBB, bronchiectasis, and CF) from a single center and time frame. We found that the pattern of lower airway infection by various microorganisms was distinctly different in children with CF compared to those with PBB and bronchiectasis. Lower airways infection in children with PBB and bronchiectasis were similar with *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* mostly cultured, whereas *P. aeruginosa* and *S. aureus* were common in children with CF. We found, previously unreported, high prevalence rates of CMV and EBV infection in children with PBB and bronchiectasis, although not in children with CF. Airway malacia was more common in children with PBB than bronchiectasis, but its presence did not influence airway infection and/or inflammation.

Our study has several major limitations including the retrospective design, which impairs the ability to assess current disease state, such as current cough and use of antibiotics, thereby impairing the ability to determine the clinical significance of our findings. Another limitation was the lack of an appropriate healthy control group. However, performing bronchoscopy including lavage in healthy children is unfeasible due to ethical issues. A further limitation may be airway sampling, as we generally lavaged only two lobes. As pathogens present elsewhere in the airways might not be identified,¹⁸ our study may have underestimated the lower airway infection prevalence rates. Lastly, viral PCR were not undertaken in all children and we did not include human rhinovirus, a common upper airway pathogen. Thus, it is likely that the prevalence of viral

infection, and thereby the prevalence of bacterial-viral co-infection, has also been underestimated.

In spite of the above limitations, the novelty of our study includes describing for the first time, clinically relevant differences within one center, between children with these various forms of endobronchial suppuration, based on culturable bacteria. Our results are consistent with pathogenic bacterial patterns in PBB^{19–21} and bronchiectasis^{22,23} previously described, i.e., *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*. In contrast, young children with CF showed a significantly different pattern, mainly culturing *P. aeruginosa* and *S. aureus*, which accords with previous age-comparable studies.^{24,25} Additionally, viral infections were significantly less common in children with CF than in children with PBB and bronchiectasis, with significantly lower rates observed compared to previous studies in CF.^{13,26} Although lower respiratory infection data for these three groups of children have been reported separately, results from this first single-center study with the same local community pathogens, confirm the significant microbiological differences between PBB and bronchiectasis, compared with CF in the young child (<6 years).

Pulmonary bacterial infection is associated with airway neutrophilia, as we^{16,27,28} and others^{4,8} have previously described. However, a unique finding was that, despite the lower rate of airway infection, the intensity of neutrophilia was significantly higher in the CF group compared to the PBB and bronchiectasis groups. Despite undertaking regression analyses, we could not explain the differences in neutrophil counts between the various cohorts. Important clinical data, for example, current cough and antibiotic treatment, that may explain this finding were unavailable as our study was retrospective. Nevertheless, it also raises questions on the local airway pathobiology of the different diseases, such as increased neutrophilia related to alveolar macrophage phagocytic dysfunction, i.e., efferocytosis.²⁹ While it is known that efferocytosis is impaired in children with PBB and bronchiectasis (compared to healthy controls),²⁹ and CF,³⁰ it remains unknown whether there is gradation of effect among these diseases, as there has not been a direct comparative study.

The association between pathogenic respiratory viruses and increased inflammatory markers we found in our study have been reported previously in PBB,^{11,27} bronchiectasis,³¹ and CF.^{13,26} In this study, we also found an association between EBV-colonization in bronchiectasis and increased neutrophilia, a finding not previously reported. The high prevalence of CMV in PBB and bronchiectasis is also a novel finding and further research is necessary to determine the clinical significance. A high prevalence of airway colonization with CMV (51%) among immunocompetent wheezing children with no underlying disease has been described,³² but inflammatory markers were not assessed in that study. Although CMV and EBV are well known opportunistic pathogens in immunocompromized children, little is known about their role in the airways of healthy children or children suffering from endobronchial bacterial airway infection. As primary immunodeficiency is absent in the children with PBB² and all these children in our current study were assessed for systemic immune deficiency (immunoglobulins G, A, M, and E, full blood count, response to vaccines) as per the Australian bronchiectasis guidelines,³³ these

children did not have classical immunodeficiency. Also, none of the children received specific EBV or CMV treatment, as the children did not have the clinical profile suggestive of an active infection. Latent infection of both these viruses may cause subtle immune dysfunction,^{34,35} but their role in endobronchial suppuration is poorly understood. As we could not undertake serological assessments and did not have any data on viral loads, the interpretation of our findings is substantially limited. Nevertheless, it is an emerging area for further research.

Airway malacia prevalence rates in our study (PBB = 71%; bronchiectasis = 47%; CF = 13%) were in concordance with previous studies on PBB,^{36,37} bronchiectasis,¹² and CF.³⁸ These values exceed the prevalence of one per 2100 newborns in the general pediatric population.³⁹ In support of the results found by Wang et al.,⁴⁰ we found no significant differences in airway infection rates and airway neutrophil percentages between children with and without malacia.

In conclusion, this first direct comparative study determining the bronchoscopic findings among young children with three conditions with endobronchial suppuration (PBB, bronchiectasis, and CF) revealed that a pattern of different lower airway infection exists even during early childhood. Our novel findings of EBV and CMV, with consequent inflammatory response in children with PBB and bronchiectasis, raise the possibility of these viruses influencing the host immunity. Prospective research into the bronchoscopic and BAL findings among children with these conditions is needed to verify our results and examine the role of the viral pathogens CMV and EBV in the initiation and/or perpetuation of chronic suppuration and/or infection.

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