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Defining lower airway bacterial infection in children with chronic endobronchial disorders

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Abstract

Background: Differentiating lower airway bacterial infection from possible upper airway contamination in children with endobronchial disorders undergoing bronchoalveolar lavage (BAL) is important for guiding management. A diagnostic bacterial load threshold based on inflammatory markers has been determined to differentiate infection from upper airway contamination in infants with cystic fibrosis, but not for children with protracted bacterial bronchitis (PBB), chronic suppurative lung disease (CSLD), or bronchiectasis.

Methods: BAL samples from children undergoing bronchoscopy underwent quantitative bacterial culture, cytologic examination, and respiratory virus testing; a subset also had interleukin-8 examined. Geometric means (GMs) of total cell counts (TCCs) and neutrophil counts were plotted by respiratory pathogen bacterial load. Logistic regression determined associations between age, sex, Indigenous status, antibiotic exposure, virus detection and bacterial load, and elevated TCCs (>400 × 10^3 cells/mL) and airway neutrophilia (neutrophils >15% BAL leukocytes).

Results: From 2007 to 2016, 655 children with PBB, CSLD, or bronchiectasis were enrolled. In univariate analyses, Indigenous status and bacterial load ≥10^5 colony-forming units (CFU)/mL were positively associated with high TCCs. Viruses and bacterial load ≥10^4 CFU/mL were positively associated with neutrophilia; negative associations were seen for Indigenous status and macrolides. In children who had not received macrolide antibiotics, bacterial load was positively associated in multivariable analyses with high TCCs at ≥10^4 CFU/mL and with neutrophilia at ≥10^5 CFU/mL; GMs of TCCs and neutrophil counts were significantly elevated at 10^4 and 10^5 CFU/mL compared to negative cultures.

Conclusions: Our findings support a BAL threshold ≥10^4 CFU/mL to define lower airway infection in children with chronic endobronchial disorders.

KEYWORDS
antibiotic therapy, bronchiectasis, chronic suppurative lung disease, diagnostic threshold, protracted bacterial bronchitis

Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; CFU, colony-forming units; CI, confidence interval; CSLD, chronic suppurative lung disease; GM, geometric mean; HREC, human research ethics committee; HRCT, high resolution computed tomography; IL, interleukin; IQR, interquartile range; NT, Northern Territory; PBB, protracted bacterial bronchitis; Qld, Queensland; RCH, Royal Children's Hospital in Brisbane; RDH, Royal Darwin Hospital; RSV, respiratory syncytial virus; STGGB, skim-milk tryptone glucose glycerol broth; TCC, total cell count.

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1 | INTRODUCTION

Accurately identifying bacteria originating from the lower airways in patients with known or suspected chronic endobronchial infection is important in clinical practice and research. Its utility includes determining whether infection is indeed present, helping to guide antibiotic choices, and identifying the microbiological impact of novel therapies and vaccines. While sputum specimens are used for these purposes in adults, bronchoalveolar lavage (BAL) is employed in those who are unable to expectorate, such as young children[1] and mechanically ventilated adults.[2] However, as contamination from the upper airways often occurs, quantitative culture for BAL[1,2] is usually employed when bacteria are identified.

Despite the widespread use of quantitative BAL culture, the threshold density of bacteria used to diagnose infection varies. In a study involving both adults with a clinical diagnosis of lower airway infection and healthy controls, quantitative bacterial culture of BAL fluid was found to be 100% specific for infection using a threshold value for positive cultures of 10^4 colony-forming units (CFU)/mL.[3] However, other adult studies have used thresholds of 10^3-10^4 CFU/mL[4] or 10^5 CFU/mL[5] for making an etiologic diagnosis of pneumonia. Similarly, BAL studies involving children have also used different thresholds, usually 10^4 CFU/mL[6–8] or 10^5 CFU/mL,[1,9,10] and included children with cystic fibrosis (CF), bronchiectasis, refractory or recurrent pneumonia, or a chronic wet cough.

Determining the appropriate diagnostic threshold in clinical practice is complex and it is not feasible to undertake a randomized controlled study where the decision to treat is determined solely by BAL microbiologic data. A prospective, observational study involving infants and young children with CF examined associations between bacterial load (determined by serial BAL dilutions) and inflammatory markers (total and differential cell counts and interleukin (IL)-8 concentrations) and established that a threshold ≥10^5 CFU/mL should be used in children with CF.[1] This recommendation was supported by subsequent BAL-based studies in young CF children examining inflammatory responses within the lower airways to varying densities of different microorganisms.[11,12] Although one of these studies included control children with other chronic respiratory problems (some had chronic cough),[11] no other published studies in children have systematically evaluated inflammatory indices associated with different bacterial loads in children without CF.[13]

Our earlier study in children with bronchiectasis described significantly elevated inflammatory markers when nontypeable *Haemophilus influenzae* bacterial load (determined by quantitative culture) exceeded 10^4 CFU/mL (versus ≤10^4 CFU/mL).[14] However, associations with lower and higher thresholds were not examined and the other two main respiratory pathogens in pediatric bronchiectasis, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, were not investigated. Further, the associations between bacterial load and inflammatory markers in children with chronic suppurative lung disease (CSLD) or protracted bacterial bronchitis (PBB) are also unknown. PBB, CSLD, and bronchiectasis are believed to form a continuum of chronic endobronchial disorders of increasing severity.[15] We, therefore, examined total cell counts (TCCs) and neutrophil counts associated with bacterial load in 655 children with these chronic endobronchial disorders, and 67 disease controls, to determine the appropriate threshold to define infection. IL-8 concentrations in BAL fluid were measured in a small subset of children with CSLD/bronchiectasis. Based on our previous study,[14] we hypothesized that compared with negative cultures, BAL samples with bacterial pathogen loads ≥10^4 CFU/mL are associated with elevated inflammatory indices (elevated airway TCC, neutrophils, and/or IL-8) in children with chronic endobronchial disorders.

2 | MATERIALS AND METHODS

2.1 | Subjects, study design, and sample collection

BAL fluid was collected from children enrolled in ongoing prospective studies of chronic cough at the Royal Children's Hospital (RCH, now Lady Cilento Children's Hospital) in Brisbane, Queensland (Qld) and the Royal Darwin Hospital (RDH) in the Northern Territory (NT), Australia. Children with PBB, CSLD, or bronchiectasis were included. The definitions for these conditions are standardized[16,17] and described in the Supplementary file. Children undergoing bronchoscopy for other reasons, such as investigation of stridor or suspected structural airway abnormalities, were included as controls. Data on vaccinations and recent or current antibiotic use were collected.

The Human Research Ethics Committees (HRECs) of the NT Department of Health and Menzies School of Health Research (HREC 07/63) and Children's Health Qld Hospital and Health Services (HREC 03/17) approved the studies and each child's parent or caregiver provided written informed consent.

Flexible bronchoscopy was performed under general anesthesia as described previously and when the child was not acutely unwell.[9,10] BAL fluid was obtained from the most abnormal lobe(s), as seen on HRCT scan or during bronchoscopy, in accordance with international guidelines.[18] BAL fluid from the first lavage was placed on ice and sent to the laboratory where it was plated within 2 h for bacterial culture at RCH. At RDH, 0.5 mL BAL aliquots were transferred to cryovials containing 0.5 mL of concentrated skim-milk tryptone glucose glycerol broth (STGGB) and stored at −80°C; these were thawed subsequently and processed, and respiratory pathogens isolated and identified, as described previously.[6]

2.2 | Laboratory methods

Quantitative BAL culture based on serial dilutions is labor intensive and not offered by many laboratories as routine practice.[13] Many hospital pathology laboratories (eg, RCH) report instead semi-quantitative culture results based on plating of a standard 10 μL BAL aliquot. Colony counts (up to 100) and streak zones were used to report results as 10^3, 10^4, etc for known pathogens including *H influenzae*, *S pneumoniae*, *M catarrhalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and other bacteria (eg, other alpha-hemolytic streptococci,

Haemophilus parainfluenzae), each of which were identified by standard procedures described previously.[6,9] The lower limit of detection is 10^2 (one colony from 10 μL BAL fluid). Bacterial growth on primary plates was also semi-quantified in our Darwin research laboratory using 10 μL loops and a protocol developed originally for nasopharyngeal swab cultures[19]: score 0, no colonies; 1, <20 colonies; 2, 20-49 colonies; 3, 50-99 colonies; 4, ≥100 colonies within the primary inoculum only; 5, 6, and 7, growth extending into the first, second, and third streak zones, respectively. To allow calculation of a threshold value of 10^3 CFU/mL, we re-cultured BAL (diluted 1:1 in STGGB, from NT children) with growth scores of 1. Specimens with ≥5 colonies were re-scored as 2 to represent ≥10^3 and <10^4 CFU/mL. To adjust for the 1:1 dilution factor for NT children, a score of 3 was included as ≥10^4 and <10^5 CFU/mL. Since scores based on streak zones represent uncountable colonies, these were grouped and defined as ≥10^5 CFU/mL.

Real-time polymerase chain reaction assays were used to detect a conventional panel of respiratory viruses: adenovirus, human metapneumovirus, influenza A and B, parainfluenza 1-3, and respiratory syncytial virus (RSV).[10] Rhinoviruses and human coronaviruses were tested in a subset of children.

Determination of total and differential cell counts was performed on the second lavage as described previously using a standardized method.[9,20] IL-8 concentrations in BAL fluid were measured in a subset of NT children using an in-house dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA™).[21]

2.3 | Statistical analyses

Stata version 14.2 (StataCorp, College Station, TX) was used for all analyses. Since BAL cell counts were not normally distributed, they were logarithmically transformed and the results reported as geometric means (GMs) with 95% confidence intervals (CIs). Neutrophil percentages were not normally distributed as raw or transformed data, and are reported as median and interquartile range (IQR). GMs of TCCs and neutrophil counts were plotted by bacterial load, with cell counts from control children included for comparison. Bacterial load was categorized as negative (no growth of any of the five pathogens), or as growth of any pathogen at 10^2 (≥10^2 and <10^3), 10^3 (≥10^3 and <10^4), 10^4 (≥10^4 and <10^5), or 10^5 (≥10^5) CFU/mL, respectively. Univariate logistic regression was used to determine associations between age, sex, Indigenous status, exposure to beta-lactam and macrolide antibiotics, presence of viruses and bacterial load, with high TCC (>400 × 10^3 cells/mL), airway neutrophilia (neutrophils >15% BAL leukocytes)[21] and high IL-8 concentrations (>250 pg/mL).[22] Variables significant in univariate analyses ($P < 0.05$) were included in multivariable analysis. Bacterial load was included as an ordinal variable.

2.4 | Reference values

In the absence of universal agreement on pediatric BAL reference values, we defined the above study-specific thresholds to help differentiate our results from age-matched "normal" children or other controls. Values from five studies of BAL cellularity in children who underwent bronchoscopy for various clinical indications, excluding respiratory tract infections, or surgery for non-pulmonary or non-inflammatory conditions, were collated by a European Respiratory Society Task Force.[18] Neutrophil percentages ranged from 0% to 3% in two studies and 0% to 17% in two studies, and a fifth study reported interquartile range (IQR) 0.6-3.5%.[18] For this study we retained our prior definition of airway neutrophilia as >15%.[21] In the earlier CF study, GMs of TCCs and neutrophils for control, negative and low growth cultures were 115-170 × 10^3 and 3-25 × 10^3 cells/mL, respectively, and GMs of IL-8 concentrations were 27-100 pg/mL.[1] Our definitions of high TCC (>400 × 10^3 cells/mL) and high IL-8 concentrations (>250 pg/mL) are more than twice the upper limit of these values.

3 | RESULTS

3.1 | Study populations

NT children (n = 257) were enrolled from July 2007 to August 2016 and Qld children (n = 398, plus an additional 67 disease controls) were enrolled from July 2008 to August 2016 (Table 1). The median ages of the cohorts were similar, although subgroups within the cohorts differed in age and Indigenous status. In those with bronchiectasis, NT children were younger and mostly Indigenous compared to Qld children. The cohorts also differed in their recent antibiotic exposure; 52% of NT children had received macrolide antibiotics (mostly azithromycin) in the 2-weeks preceding bronchoscopy, compared to 7% of Qld children. Most children in both cohorts (96% NT, 88% Qld) had received two or more doses of a pneumococcal conjugate vaccine.

3.2 | Bacterial pathogens

H influenzae, cultured from 398/655 (61%) BAL samples, was the dominant pathogen in children with chronic endobronchial disorders. *S pneumoniae* was the next most prevalent pathogen, present in 184 (28%) samples, followed by *M catarrhalis* in 154 (24%) samples. *S aureus* and *P aeruginosa* were cultured less frequently in children with chronic endobronchial disorders, detected in 65 (10%) and 21 (3.2%) BAL samples respectively. In the 67 disease control children, *S aureus* was cultured from 15 (22%) children, followed by *H influenzae* (13, 19%), *S pneumoniae* (11, 16%), *M catarrhalis* (6, 9.0%), and *P aeruginosa* (1, 1.5%). Combined pathogen bacterial loads for NT and Qld children with PBB, CSLD, or bronchiectasis, and disease control children, are presented in Table 2.

3.3 | Viruses

Data for the conventional panel of eight respiratory viruses (adenovirus, human metapneumovirus, influenza A and B, parainfluenza 1-3, and RSV) were available for 163 NT and 387 Qld cases, and 65 controls. At least one virus was detected in BAL samples from 26% of children with chronic endobronchial disorders when they were clinically stable compared to 14% of controls (Table 1). Of 62 Qld children tested, rhinoviruses were detected in 17/50 (34%) with PBB

TABLE 1 Demographic, antibiotic use, pneumococcal conjugate vaccine status, and virus detection data by center and chronic endobronchial disorder

		NT		Qld			
	All children with chronic endobronchial disorders	CSLD	BE	PBB	CSLD	BE	Controls
Number	655	22	235	203	13	182	67
Male	382 (58%)	12 (55%)	130 (55%)	130 (64%)	6 (46%)	104 (57%)	45 (67%)
Median age in years (IQR)	2.3 (1.5-4.3)	2.8 (1.5-3.6)	2.3 (1.6-3.7)	1.7 (1.1-3.2)	2.5 (1.8-3.9)	3.5 (2.1-6.0)	1.6 (0.7-3.7)
Indigenous[a]	288/645[a] (45%)	14 (64%)	221 (94%)	15/200[a] (7.5%)	1 (7.7%)	37/175[a] (21%)	4 (6.0%)
Beta-lactam antibiotics[b]	79/646[b] (12%)	1 (4.6%)	36/234[b] (15%)	19/202[b] (9.4%)	3 (23%)	20/175[b] (11%)	3 (4.5%)
Macrolide antibiotics[b]	160/646[b] (25%)	12 (55%)	122/234[b] (52%)	8/202[b] (4.0%)	2 (15%)	16/175[b] (9.1%)	1 (1.5%)
PCV vaccinated[c]	587/645[c] (91%)	20 (91%)	227 (97%)	185/200[c] (93%)	10 (77%)	145/175[c] (83%)	44/66[c] (67%)
Virus detected[d]	141/550[d] (26%)	1/14[d] (7%)	22/149[d] (15%)	69/194[d] (36%)	1 (7.7%)	48/180[d] (27%)	9/65[d] (14%)

BE, bronchiectasis; CSLD, chronic suppurative lung disease; IQR, interquartile range; NT, Northern Territory; PBB, protracted bacterial bronchitis; PCV, pneumococcal conjugate vaccine; Qld, Queensland; RSV, respiratory syncytial virus.

[a]10 Qld children had records missing for Indigenous status.

[b]Recorded as current antibiotics (Qld) or taken in the 2-week preceding bronchoscopy (NT) (eight Qld children and one NT child had missing antibiotic data).

[c]≥2 doses of any PCV (11 Qld children had missing vaccination data).

[d]Any of adenovirus, human metapneumovirus, influenza virus A/B, parainfluenza virus 1-3, or RSV (13 Qld children had missing virus data, standard eight viruses tested for 163 NT children only).

or bronchiectasis and 2/12 (15%) controls, while human coronaviruses were not detected in any of the BAL samples from these children. In 163 NT children with CSLD/bronchiectasis, rhinoviruses were detected in 41 (25%) and human coronaviruses in one child.

3.4 | Inflammatory markers

Airway cellularity data for all children with chronic endobronchial disorders and disease controls are presented in Table 3. NT children with bronchiectasis had the highest TCCs (37% had $>400 \times 10^3$ cells/mL) and Qld children with PBB had the highest neutrophil counts (78% had airway neutrophilia). The GM of IL-8 in 67 NT children with CSLD/bronchiectasis was 130 (95%CI 88-193) pg/mL; 23 (34%) children had IL-8 concentrations >250 pg/mL.

3.5 | Inflammatory markers versus bacterial load

GMs (with 95%CIs) of TCCs and neutrophil counts for all children with chronic endobronchial disorders, and disease controls, were plotted against bacterial loads for the combined five respiratory pathogens in Figure 1. Compared to controls, children with chronic endobronchial disorders had significantly higher TCCs and neutrophil counts, even when no pathogens were detected: 218 (95%CI 184-258) and 22 (95%CI 16-30) $\times 10^3$ cells/mL, respectively. Statistically significant differences, compared to negative cultures, were seen for TCCs at 10^5 CFU/mL BAL fluid (339, 95%CI 298-386, $\times 10^3$ cells/mL), and for neutrophil counts at 10^4 CFU/mL (49, 95%CI 32-75, $\times 10^3$ cells/mL) and 10^5 CFU/mL (88, 95%CI 70-109, $\times 10^3$ cells/mL). Although numbers were small and CIs wide, GMs of IL-8 concentrations from 67 NT children with CSLD/bronchiectasis were significantly elevated at 10^5 CFU/mL (449, 95%CI 243-829, pg/mL) compared to negative cultures (105, 95%CI 50-222, pg/mL).

3.6 | Factors associated with lower airway inflammation

In children with chronic endobronchial disorders, associations with high or low cell counts were not detected for age, sex or use of beta-lactam antibiotics (Table 4). Statistically significant associations using univariate analysis were seen for Indigenous status (positively associated with high TCC, negatively associated with airway neutrophilia), virus detection (positively associated with airway neutrophilia), macrolide antibiotic use (negatively associated with airway neutrophilia), and bacterial load (positively associated with high TCC at 10^5 CFU/mL and airway neutrophilia at 10^4 and 10^5 CFU/mL). In the small subset of 67 NT children with IL-8 data, bacterial load $\geq 10^5$ CFU/mL was positively associated with >250 pg/mL (odds ratio 5.49, 95%CI 1.39-21.6). In multivariable analysis, bacterial load $\geq 10^5$ CFU/mL remained independently associated with high TCC and airway neutrophilia (Table 4). Compared to $\geq 10^5$ CFU/mL, a threshold of $\geq 10^4$ CFU/mL had higher sensitivity, but lower specificity for all three markers of inflammation (Supplementary Table S1).

TABLE 2 Lower airway respiratory bacterial pathogen load in children with chronic endobronchial disorders

Bacterial load (CFU/mL BAL)[a]	Disease controls	All children with chronic endobronchial disorders	Received macrolide antibiotics within previous 2 weeks		Difference (*P*-value)[b]
			Yes	No	
Negative	34 (51%)	170 (26%)	54 (34%)	116 (24%)	**0.014**
≥10^2 and <10^3	1 (1.5%)	53 (8%)	24 (15%)	29 (6%)	<**0.001**
≥10^3 and <10^4	1 (1.5%)	45 (7%)	19 (12%)	25 (5%)	**0.003**
≥10^4 and <10^5	11 (16%)	79 (12%)	24 (15%)	55 (11%)	0.217
≥10^5	20 (30%)	308 (47%)	39 (24%)	261 (54%)	<**0.001**
Total	67	655	160	486	

BAL, bronchoalveolar lavage; CFU, colony-forming units.
[a]Any of *H influenzae*, *S pneumoniae*, *M catarrhalis*, *S aureus*, or *P aeruginosa*.
[b]Two-sample test of proportions for 646 children with chronic endobronchial disorders and available antibiotic use data who did or did not receive macrolide antibiotics; bold values, $P < 0.05$.

3.7 | Effect of macrolide antibiotics on bacterial load and inflammatory markers

Lower airway bacterial loads and cell counts in children with chronic endobronchial disorders were stratified by recent macrolide antibiotic exposure (Tables 2 and 3). Children who received macrolide antibiotics had a significantly lower bacterial load and fewer neutrophils than those who did not receive macrolide antibiotics.

GMs of cell counts for children who did not receive macrolide antibiotics are plotted against bacterial load for the combined five respiratory bacterial pathogens in Figure 2. Compared to negative cultures (TCC 198, 95%CI 161-244, ×10^3 cells/mL and neutrophils 24, 95%CI 17-35, ×10^3 cells/mL), significant differences were seen for TCC and neutrophil counts at 10^4 CFU/mL (344, 95%CI 244-484, and 66, 95%CI 38-114, ×10^3 cells/mL, respectively) and 10^5 CFU/mL (330, 95%CI 288-379, and 92, 95%CI 73-116, ×10^3 cells/mL, respectively). Similarly in univariate analysis, bacterial load ≥10^4 CFU/mL was positively associated with high TCC and airway neutrophilia. In multivariable analysis, bacterial load ≥10^4 CFU/mL was independently associated with high TCC, while bacterial load ≥10^5 CFU/mL was independently associated with airway neutrophilia (Supplementary Table S2).

4 | DISCUSSION

In 655 children with chronic endobronchial disorders, elevated TCC and airway neutrophilia were consistently associated with respiratory pathogen bacterial load ≥10^5 CFU/mL. These associations were seen by plotting GMs of cell counts against bacterial load and by using

TABLE 3 Lower airway cellularity in children with chronic endobronchial disorders

	Disease controls	All children with chronic endobronchial disorders	Received macrolide antibiotics within previous 2 weeks		Difference[a]
Number	67	655	Yes (*n* = 160)	No (*n* = 486)	*P*-value[b]
TCC[c]	123 (97, 155)	292 (268, 319)	308 (263, 361)	288 (260, 319)	0.629
Neutrophils[c]	5 (3, 7)	47 (40, 56)	27 (19, 37)	56 (47, 67)	<**0.001**
Percent neutrophils[d]	4.0 (2.0, 8.0)	20 (6.7, 55)	8.3 (3.0, 32)	24 (8.7, 59)	<**0.001**
					P-value[e]
High TCC[f]	6/66 (9%)	206/612 (34%)	48/140 (34%)	156/463 (34%)	0.897
Neutrophilia[g]	5/65 (8%)	336/613 (55%)	50/143 (35%)	281/461 (61%)	<**0.001**

BAL, bronchoalveolar lavage; CI, confidence interval; GM, geometric mean; IQR, interquartile range; TCC, total cell count.
[a]Between 646 children with chronic endobronchial disorders and available antibiotic use data who did or did not receive macrolide antibiotics.
[b]Two-sample Wilcoxon rank-sum (Mann-Whitney) test; bold values, $P < 0.05$.
[c]GM (95% CI) × 10^3 cells/mL.
[d]Median (IQR).
[e]Two-sample test of proportions; bold values, $P < 0.05$.
[f]TCC >400 × 10^3 cells/mL.
[g]Airway neutrophils >15% BAL leukocytes.

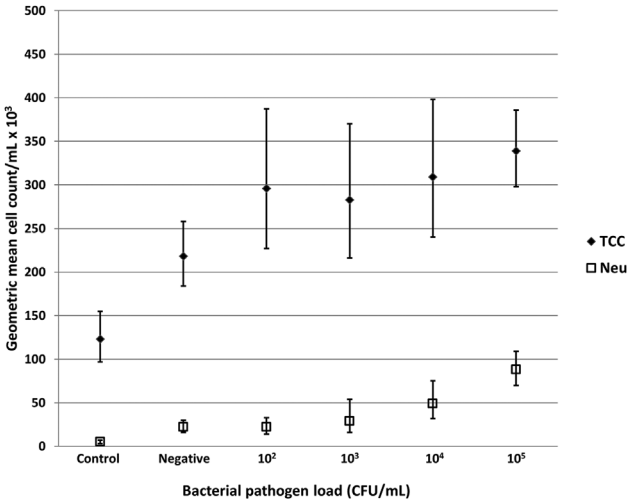

FIGURE 1 Paired airway cellularity and respiratory bacterial pathogen load data from 610 children with chronic endobronchial disorders, and 66 control children. CFU, colony-forming units; Neu, neutrophils; TCC, total cell count. Error bars represent 95% confidence intervals. Pathogens included any of *H influenzae*, *S pneumoniae*, *M catarrhalis*, *S aureus*, or *P aeruginosa*

logistic regression. When children who had recently received macrolide antibiotics were excluded, there were statistically significant associations between bacterial loads ≥10^4 CFU/mL and high TCC in univariate and multivariable analyses.

Currently, BAL bacterial load thresholds used for diagnosing lower airway infections in children are usually defined as either ≥10^4, >10^4, or ≥10^5 CFU/mL of BAL fluid.[1,6–10,23,24] Our study provides further support for these thresholds. Since infection can be defined as damage to body tissues from the combined effects of multiplying microorganisms and the resulting host inflammatory response, employing one to predict the other represents a circular argument. Unfortunately, there is no "diagnostic gold standard" and both are imperfect predictors. Nevertheless we aimed to determine the most appropriate bacterial load threshold to define lower airway infection by examining associations with inflammatory markers. Based on TCC and neutrophil counts, we conservatively suggest that a threshold of ≥10^4 CFU/mL should be used to indicate the likelihood of lower airway infection in children with PBB, CSLD, and bronchiectasis.

The relationship between lower airway infection and inflammation has been well established in PBB[16] and bronchiectasis.[9,25] Similar data for pre- and post-antibiotic treatment are unsurprisingly limited in children as obtaining lower airway specimens is difficult in those who are either too young or unable to expectorate. In adults with bronchiectasis, treatment with antibiotics significantly reduced bacterial load and concomitant airway inflammation in a dose-dependent manner.[25] Lower airway inflammation is a broad term that includes increased TCC, neutrophils and cytokines (eg, IL-8, free neutrophil elastase, matrix metalloproteinases, TNF-alpha) in lower airway specimens.[25–27] It is possible that employing more sensitive

TABLE 4 Factors associated with high total cell count or neutrophilia in the lower airways of children with chronic endobronchial disorders

	Univariate analyses[a]		Multivariable analyses[a]	
Factor	**High TCC[b]**	**Neutrophilia[c]**	**High TCC[b]**	**Neutrophilia[c]**
Age (years)	0.97 (0.92, 1.03)	0.98 (0.93, 1.04)	na	na
Male	0.90 (0.64, 1.27)	0.92 (0.67, 1.27)	na	na
Indigenous	**1.56 (1.11, 2.20)**	**0.40 (0.29, 0.55)**	**2.07 (1.42, 3.01)**	0.75 (0.49, 1.13)
Beta-lactams[d]	1.36 (0.83, 2.23)	1.17 (0.71, 1.91)	na	na
Macrolides[d]	1.03 (0.69, 1.53)	**0.34 (0.23, 0.51)**	na	0.63 (0.38, 1.06)
Respiratory virus[e]	1.21 (0.80, 1.82)	**1.82 (1.21, 2.75)**	na	1.48 (0.94, 2.33)
Bacterial load (CFU/mL BAL)[f]	No growth Reference		No growth Reference	
	≥10^2 and <10^3		≥10^2 and <10^3	
	1.07 (0.52, 2.22)	0.74 (0.38, 1.46)	0.82 (0.39, 1.73)	0.60 (0.26, 1.38)
	≥10^3 and <10^4		≥10^3 and <10^4	
	1.11 (0.51, 2.42)	1.09 (0.55, 2.19)	0.93 (0.42, 2.06)	1.07 (0.45, 2.57)
	≥10^4 and <10^5		≥10^4 and <10^5	
	1.73 (0.95, 3.13)	**1.86 (1.06, 3.26)**	1.64 (0.89, 3.01)	1.60 (0.85, 3.00)
	≥10^5		≥10^5	
	2.17 (1.40, 3.35)	**4.15 (2.75, 6.27)**	**2.48 (1.58, 3.90)**	**3.11 (1.97, 4.91)**

BAL, bronchoalveolar lavage; CFU, colony forming units; na, not applicable; NT, Northern Territory; Qld, Queensland; RSV, respiratory syncytial virus; TCC, total cell count.

[a]Odds ratio (95% confidence interval); bold values, $P < 0.05$.

[b]TCC >400 × 10^3 cells/mL (data available for 612/655 children).

[c]Neutrophils >15% BAL leukocytes (data available for 613/655 children).

[d]Recorded as current antibiotics (Qld) or taken <2-week preceding bronchoscopy (NT) (data available for 646/655 children).

[e]Any of adenovirus, human metapneumovirus, influenza virus A/B, parainfluenza virus 1-3, or RSV (data available for 550/655 children).

[f]Any of *S pneumoniae*, *H influenzae*, *M catarrhalis*, *S aureus*, or *P aeruginosa*.

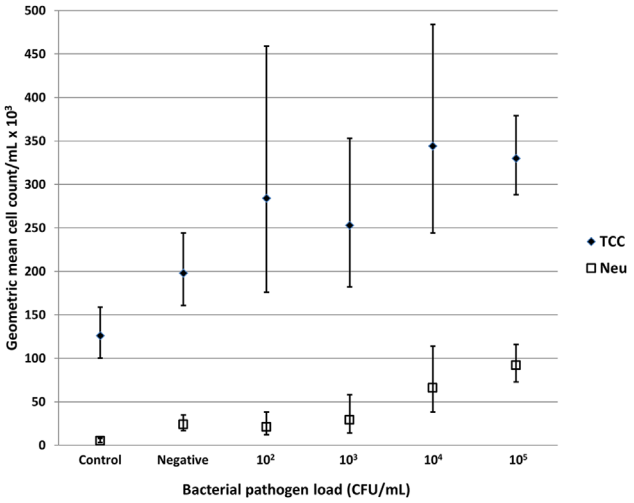

FIGURE 2 Paired airway cellularity and respiratory bacterial pathogen load data from 461 children with chronic endobronchial disorders, and 65 control children, who had not received macrolide antibiotics. Recorded as current antibiotics or taken <2-weeks preceding bronchoscopy. CFU, colony-forming units; Neu, neutrophils; TCC, total cell count. Error bars represent 95% confidence intervals. Pathogens included any of *H influenzae*, *S pneumoniae*, *M catarrhalis*, *S aureus*, or *P aeruginosa*

measures or a broader range of airway inflammatory markers may lead to a lower threshold. Nevertheless, a threshold ≥10^5 CFU/mL was established for infants with CF using similar indicators of inflammation to those used in our study.[1] Children were younger in the original CF study (mean age 17-months, range 1-52), the predominant pathogens were *S aureus*, *P aeruginosa*, and *H influenzae*, and 30 of the 150 BAL procedures were conducted in children hospitalized for an acute pulmonary exacerbation.[1] The elevations in cell counts seen in our study at 10^4 CFU/mL (not apparent in the CF study)[1] may be associated with *S pneumoniae* and/or *M catarrhalis*, two pathogens found less commonly in CF patients. Further, simultaneous colonization with *S pneumoniae* and *H influenzae* resulted in a synergistic pro-inflammatory response (increased production of IL-8, the major neutrophil chemokine in the airway) in vitro and in a mouse model.[28] An examination of pathogen-specific thresholds, and pathogen-pathogen interactions in a larger cohort, may help to explain differences in inflammatory responses to bacterial load.

We found other factors, in addition to bacterial load, that were associated with increased TCC and/or neutrophilia. Indigenous children with chronic endobronchial disorders, particularly NT children with bronchiectasis (94% indigenous), had significantly higher TCCs than non-Indigenous children. The high prevalence of bronchiectasis in Australian Indigenous children has long been recognized.[29] In Qld, only 6% of control children and 7.5% with PBB were Indigenous; however, 21% of children with bronchiectasis were Indigenous. This may represent evidence from another Australian setting that Indigenous children are more likely to progress to bronchiectasis. Alternatively, Indigenous children could be less likely to present early with symptoms of chronic endobronchial infection, and the finding that TCCs were higher in Indigenous compared to non-Indigenous children might reflect more advanced disease.

The presence of viruses also influenced airway cell counts as documented previously in children with wet cough and PBB.[10] Numerous synergistic virus-bacteria interactions have been documented, particularly between *S pneumoniae* and influenza virus and RSV.[30] Combinations of bacterial pathogens and respiratory viruses can enhance pathogen transmission and exacerbate disease development.[31] One of our study's limitations is that not all samples were tested for viruses; 85% of children had samples tested for the conventional panel of eight viruses, but only 31% were tested for human rhinovirus and coronavirus. There may, therefore, be residual confounding from unmeasured viruses, although the importance of rhinoviruses as pathogens in children is complicated by their presence in up to 45% of asymptomatic children when using sensitive molecular detection techniques.[32,33]

Differences in culture methods between Qld and NT laboratories represent another limitation. BAL specimens were plated within 2-hours of collection in Qld, whereas NT specimens were stored in STGGB at −80°C before being thawed and processed. However, we have shown that recovery of *H influenzae*, *S pneumoniae*, and *M catarrhalis* from nasopharyngeal swabs stored short-term in STGGB at −70°C is equivalent to direct plating of Amies swabs,[34] and these respiratory pathogens remain viable in frozen STGGB storage for at least 12 years.[35]

Our finding of a statistically significant negative association between macrolide antibiotics and airway neutrophilic inflammation is consistent with a small Turkish randomized controlled trial,[27] which found that children receiving macrolides (compared to controls) had significantly reduced airway TCC, neutrophilia, and IL-8. Similar observations have been described in animal studies.[36] We also found a statistically significant reduction in lower airway bacterial load in children who received macrolide antibiotics. This reduction was not apparent in a smaller study of 104 Indigenous children with bronchiectasis.[37] However, macrolide antibiotics reduced nasopharyngeal carriage of respiratory bacterial pathogens in Indigenous children,[37,38] and this was more pronounced when antibiotic use was frequent or long-term.[39] Indeed, a "cumulative dose-response" relationship was observed whereby increasing azithromycin exposure was associated with decreasing nasopharyngeal carriage of *S pneumoniae*, *H influenzae*, and *M catarrhalis*.[40] Our findings (in this larger study of 655 children) of a statistically significant reduction in respiratory bacterial pathogen load in BAL samples from children exposed to macrolide antibiotics may be showing a similar effect in the lower airways to that seen in the upper airways.

In conclusion, our findings support a threshold of ≥10^4 CFU/mL BAL to define lower airway infection in children with PBB, CSLD, or bronchiectasis. Associations with airway cellularity were stronger at ≥10^5 CFU/mL which may provide greater specificity in the setting of a clinical trial. However when compared to negative cultures, associations with high TCC and neutrophilia were consistently stronger at bacterial loads between 10^4 and 10^5 CFU/mL suggesting lower airway

infection is present. In contrast, there was no evidence of elevated inflammatory indices at bacterial load $<10^4$ CFU/mL. Finally, it is important to acknowledge that in clinical practice when interpreting bacterial culture results, the health of the patient, recent antibiotic exposure, inflammatory indices, and the nature of the pathogen should also be taken into account.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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