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 × 103 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 ≥105 colony-forming units (CFU)/mL were positively associated with high TCCs. Viruses and bacterial load ≥104 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 ≥104 CFU/mL and with neutrophilia at ≥105 CFU/mL; GMs of TCCs and neutrophil counts were significantly elevated at 104 and 105 CFU/mL compared to negative cultures.

Conclusions: Our findings support a BAL threshold ≥104 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 and mechanically ventilated adults. However, as contamination from the upper airways often occurs, quantitative culture for BAL 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 10⁴ colony-forming units (CFU)/mL. However, other adult studies have used thresholds of 10⁵-10⁶ CFU/mL or 10⁵ CFU/mL for making an etiologic diagnosis of pneumonia. Similarly, BAL studies involving children have also used different thresholds, usually 10⁴ CFU/mL or 10⁵ CFU/mL 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 ≥10⁵ CFU/mL should be used in children with CF. 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. Although one of these studies included control children with other chronic respiratory problems (some had chronic cough), no other published studies in children have systematically evaluated inflammatory indices associated with different bacterial loads in children without CF.

Our earlier study in children with bronchiectasis described significantly elevated inflammatory markers when nontypeable Haemophilus influenzae bacterial load (determined by quantitative culture) exceeded 10⁴ CFU/mL (versus ≤10⁴ CFU/mL). 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 (CSDL) or protracted bacterial bronchitis (PBB) are also unknown. PBB, CSDL, and bronchiectasis are believed to form a continuum of chronic endobronchial disorders of increasing severity. 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 CSDL/bronchiectasis. Based on our previous study, we hypothesized that compared with negative cultures, BAL samples with bacterial pathogen loads ≥10⁴ 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, CSDL, or bronchiectasis were included. The definitions for these conditions are standardized 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. BAL fluid was obtained from the most abnormal lobe(s), as seen on HRCT scan or during bronchoscopy, in accordance with international guidelines. 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.

2.2 | Laboratory methods

Quantitative BAL culture based on serial dilutions is labor intensive and not offered by many laboratories as routine practice. 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 10³, 10⁵, 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 10² (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 cultures19: 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 10³ 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 ≥10² and <10³ CFU/mL. To adjust for the 1:1 dilution factor for NT children, a score of 3 was included as ≥10³ and <10⁴ CFU/mL. Since scores based on streak zones represent uncountable colonies, these were grouped and defined as ≥10⁵ 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 10² (≥10² and <10³), 10³ (≥10³ and <10⁴), 10⁴ (≥10⁴ and <10⁵), or 10⁵ (≥10⁵) 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 × 10³ 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 × 10³ and 3-25 × 10³ cells/mL, respectively, and GMs of IL-8 concentrations were 27-100 pg/mL.1 Our definitions of high TCC (>400 × 10³ 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
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 × 10³ 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) × 10³ cells/mL, respectively. Statistically significant differences, compared to negative cultures, were seen for TCCs at 10⁵ CFU/mL BAL fluid (339, 95%CI 298-386, ×10³ cells/mL), and for neutrophil counts at 10⁴ CFU/mL (49, 95%CI 32-75, ×10³ cells/mL) and 10⁵ CFU/mL (88, 95%CI 70-109, ×10³ 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 10⁵ 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 10⁵ CFU/mL and airway neutrophilia at 10⁴ and 10⁵ CFU/mL). In the small subset of 67 NT children with IL-8 data, bacterial load ≥10⁵ CFU/mL was positively associated with >250 pg/mL (odds ratio 5.49, 95%CI 1.39-21.6). In multivariable analysis, bacterial load ≥10⁴ CFU/mL remained independently associated with high TCC and airway neutrophilia (Table 4). Compared to ≥10⁵ CFU/mL, a threshold of ≥10⁴ CFU/mL had higher sensitivity, but lower specificity for all three markers of inflammation (Supplementary Table S1).

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

|                      | **All children with chronic endobronchial disorders** | **NT** | **Qld** |
|----------------------|------------------------------------------------------|--------|---------|
|                      | **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) |
| Indigenous³           | 288/645⁴ (45%) | 14 (64%) | 221 (94%) | 15/200⁴ (7.5%) | 1 (7.7%) | 37/175⁴ (21%) | 4 (6.0%) |
| Beta-lactam antibiotics³ | 79/646⁵ (12%) | 1 (4.6%) | 36/234⁵ (15%) | 19/202⁵ (9.4%) | 3 (23%) | 20/175⁵ (11%) | 3 (4.5%) |
| Macrolide antibiotics³ | 160/646⁵ (25%) | 12 (55%) | 122/234⁵ (52%) | 8/202⁵ (4.0%) | 2 (15%) | 1678⁵ (9.1%) | 1 (1.5%) |
| PCV vaccinated³        | 587/645⁵ (91%) | 20 (91%) | 227 (97%) | 185/200⁵ (93%) | 10 (77%) | 145/175⁵ (83%) | 44/66⁵ (67%) |
| Virus detected³        | 141/550⁶ (26%) | 1/14⁶ (7%) | 22/149⁶ (15%) | 69/194⁶ (36%) | 1 (7.7%) | 48/180⁶ (27%) | 9/65⁶ (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.

*¹Ten Qld children had records missing for Indigenous status.
²Recorded as current antibiotics (Qld) or taken in the 2-week preceding bronchoscopy (NT) (eight Qld children and one NT child had missing antibiotic data).
³≥2 doses of any PCV (11 Qld children had missing vaccination data).
⁴Any 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).
1. 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, ×10³ cells/mL and neutrophils 24, 95%CI 17-35, ×10³ cells/mL), significant differences were seen for TCC and neutrophil counts at 10⁴ CFU/mL (344, 95%CI 244-484, and 66, 95%CI 38-114, ×10³ cells/mL, respectively) and 10⁵ CFU/mL (330, 95%CI 288-379, and 92, 95%CI 73-116, ×10³ cells/mL, respectively). Similarly, in univariate analysis, bacterial load ≥10⁴ CFU/mL was positively associated with high TCC and airway neutrophilia. In multivariable analysis, bacterial load ≥10⁴ CFU/mL was independently associated with high TCC, while bacterial load ≥10⁵ CFU/mL was independently associated with airway neutrophilia (Supplementary Table S2).

2. DISCUSSION

In 655 children with chronic endobronchial disorders, elevated TCC and airway neutrophilia were consistently associated with respiratory pathogen bacterial load ≥10⁵ CFU/mL. These associations were seen by plotting GMs of cell counts against bacterial load and by using

### 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 |
|------------------------------|------------------|--------------------------------------------------|----------------------------------------------------|-----------------------|
| Negative                     | 34 (51%)         | 170 (26%)                                        | Yes 54 (34%)                                      | 116 (24%) 0.014       |
| ≥10² and <10³                | 1 (1.5%)         | 53 (8%)                                          | Yes 24 (15%)                                      | 29 (6%) <0.001        |
| ≥10³ and <10⁴                | 1 (1.5%)         | 45 (7%)                                          | Yes 19 (12%)                                      | 25 (5%) 0.003         |
| ≥10⁴ and <10⁵                | 11 (16%)         | 79 (12%)                                         | Yes 24 (15%)                                      | 55 (11%) 0.217        |
| ≥10⁵                         | 20 (30%)         | 308 (47%)                                        | Yes 39 (24%)                                      | 261 (54%) <0.001      |
| Total                        | 67               | 655                                              | 160                                                   | 486                   |

BAL, bronchoalveolar lavage; CFU, colony-forming units.

### Table 3 Lower airway cellularity in children with chronic endobronchial disorders

| Number | Disease controls | All children with chronic endobronchial disorders | Received macrolide antibiotics within previous 2 weeks | Difference a |
|--------|------------------|--------------------------------------------------|------------------------------------------------------|--------------|
| TCC²   | 67               | 655                                              | Yes 308 (263, 361)                                  | 288 (260, 319) 0.629 |
| Neutrophils³ | 5 (3, 7)         | 47 (40, 56)                                      | Yes 27 (19, 37)                                    | 56 (47, 67) <0.001 |
| Percent neutrophils⁴ | 4.0 (2.0, 8.0) | 20 (6.7, 55)                                    | Yes 8.3 (3.0, 32)                                  | 24 (8.7, 59) <0.001 |
| High TCC⁵ | 6/66 (9%)        | 206/612 (34%)                                   | Yes 48/140 (34%)                                   | 156/463 (34%) 0.897 |
| Neutrophilia⁶ | 5/55 (8%)        | 336/613 (55%)                                   | Yes 50/143 (35%)                                   | 281/461 (61%) <0.001 |

BAL, bronchoalveolar lavage; CI, confidence interval; GM, geometric mean; IQR, interquartile range; TCC, total cell count.

aAny of H influenzae, S pneumoniae, M catarrhalis, S aureus, or P aeruginosa.

bTwo-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.

cGM (95% CI) × 10³ cells/mL.

dMedian (IQR).

Two-sample Wilcoxon rank-sum (Mann-Whitney) test; bold values, P < 0.05:

cGM (95% CI) × 10³ cells/mL.

dMedian (IQR).

Two-sample test of proportions; bold values, P < 0.05:

cGM (95% CI) × 10³ cells/mL.

dMedian (IQR).

Two-sample test of proportions; bold values, P < 0.05:

cGM (95% CI) × 10³ cells/mL.

dMedian (IQR).

Two-sample test of proportions; bold values, P < 0.05:

cGM (95% CI) × 10³ cells/mL.
logistic regression. When children who had recently received macrolide antibiotics were excluded, there were statistically significant associations between bacterial loads \( \geq 10^4 \text{ CFU/mL} \) 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 \( \geq 10^5 \), \( >10^4 \), or \( \geq 10^5 \) CFU/mL of BAL fluid.\(^1\)\(^-\)\(^6\)\(^,\)\(^10\)\(^-\)\(^22\)\(^,\)\(^23\)\(^,\)\(^24\) 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 \( \geq 10^4 \) 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

![FIGURE 1](image-url)  
**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*.

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

| Factor                        | Univariate analyses\(^a\) | Multivariable analyses\(^a\) |
|-------------------------------|---------------------------|-------------------------------|
|                              | High TCC                  | Neutrophilia                  | High TCC                  | Neutrophilia                  |
| 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)             | 1.48 (0.94, 2.33)         | na                            |
| Bacterial load (CFU/mL BAL)\(^f\) | No growth Reference      |                               | No growth Reference      |                               |
| \( \geq 10^2 \) 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)             |
| \( \geq 10^3 \) 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)             |
| \( \geq 10^4 \) 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)             |
| \( \geq 10^5 \)              | 2.17 (1.40, 3.35)         | 4.15 (2.75, 6.27)             | 2.48 (1.58, 3.90)         | 3.11 (1.97, 4.91)             |

**Notes:**  
\(^a\)Odds ratio (95% confidence interval); bold values, \( P < 0.05 \).  
\(^b\)TCC \( >400 \times 10^3 \) 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*.  

BAL, bronchoalveolar lavage; CFU, colony forming units; na, not applicable; NT, Northern Territory; Qld, Queensland; RSV, respiratory syncytial virus; TCC, total cell count.
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 CF using similar indicators of inflammation to those used in our study. 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. The elevations in cell counts seen in our study at 10^4 CFU/mL (not apparent in the CF study) 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. 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. 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. Numerous synergistic virus-bacteria interactions have been documented, particularly between S pneumoniae and influenza virus and RSV. Combinations of bacterial pathogens and respiratory viruses can enhance pathogen transmission and exacerbate disease development. 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.

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, and these respiratory pathogens remain viable in frozen STGGB storage for at least 12 years.

Our finding of a statistically significant negative association between macrolide antibiotics and airway neutrophilic inflammation is consistent with a small Turkish randomized controlled trial, 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. 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. However, macrolide antibiotics reduced nasopharyngeal carriage of respiratory bacterial pathogens in Indigenous children, and this was more pronounced when antibiotic use was frequent or long-term. 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. 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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