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Original Research

SIGNS AND SYMPTOMS OF CHEST DISEASE

Prospective Characterization of Protracted Bacterial Bronchitis in Children

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Background: **Prior studies on protracted bacterial bronchitis (PBB) in children have been retrospective or based on small cohorts. As PBB shares common features with other pediatric conditions, further characterization is needed to improve diagnostic accuracy among clinicians. In this study, we aim to further delineate the clinical and laboratory features of PBB in a larger cohort, with a specific focus on concurrent viral detection.**

Methods: **Children with and without PBB (control subjects) undergoing flexible bronchoscopy were prospectively recruited. Basic immune function testing and lymphocyte subset analyses were performed. BAL specimens were processed for cellularity and microbiology. Viruses were identified using polymerase chain reaction (PCR) and bacteria were identified via culture.**

Results: **The median age of the 104 children (69% male) with PBB was 19 months (interquartile range [IQR], 12-30 mo). Compared with control subjects, children with PBB were more likely to have attended childcare (OR, 8.43; 95% CI, 2.34-30.46). High rates of wheeze were present in both groups, and tracheobronchomalacia was common. Children with PBB had significantly elevated percentages of neutrophils in the lower airways compared with control subjects, and adenovirus was more likely to be detected in BAL specimens in those with PBB (OR, 6.69; 95% CI, 1.50-29.80). Median CD56 and CD16 natural killer (NK) cell levels in blood were elevated for age in children with PBB (0.7×10^9/L; IQR, 0.5-0.9 cells/L).**

Conclusions: **Children with PBB are, typically, very young boys with prolonged wet cough and parent-reported wheeze who have attended childcare. Coupled with elevated NK-cell levels, the association between adenovirus and PBB suggests a likely role of viruses in PBB pathogenesis.**

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Abbreviations: CFU = colony-forming units; HAdV = human adenovirus; HCoV = human coronavirus; IQR = interquartile range; NK = natural killer; PBB = protracted bacterial bronchitis; PCR = polymerase chain reaction; PyV = polyomavirus; RAST = radioallergosorbent test

Chronic cough in children is associated with significant morbidity[1] and a high emotional burden on parents.[2] Protracted bacterial bronchitis (PBB) is recognized as a major cause of chronic cough in children.[3-5] In a multicenter study of 346 children newly referred for chronic cough, PBB was the most common etiologic diagnosis.[6] Prior to 2006, when the entity of PBB was first characterized by our group,[7] bacterial bronchitis was underappreciated as a cause of chronic cough in otherwise healthy children.[8] Several international groups have subsequently studied PBB, with concordant findings.[9-11] PBB has now been incorporated into cough guidelines and education resources in many countries.[3,4,12-14]

The original diagnostic criteria for PBB included (1) wet cough for ≥ 4 weeks, (2) identifiable lower-airway bacterial infection on BAL culture, and (3) response to antibiotics (amoxicillin/clavulanate) with resolution of cough within 2 weeks.[7] For clinical feasibility, criterion 2 was later substituted with "absence of specific pointers to indicate an alternative specific cause of cough,"[15] resulting in criteria that were applicable to both primary and tertiary care settings.

While defining PBB has been transformative in our understanding and management of chronic wet cough in children, many questions remain. To date, studies on PBB have been small[7] and/or retrospective[9-11]; and some argue that PBB is poorly defined,[16] with others calling for more in-depth studies.[13] Furthermore, children with coexistent viruses detected in BAL specimens were excluded from our original PBB cohort.[7] A large prospective study with further clinical and

laboratory descriptors will assist clinicians to differentiate PBB from other causes of cough in children. We hypothesized that PBB would be associated with a well-defined set of clinical characteristics and that virus rates in BAL specimens would be increased compared with those of control subjects. In our study of 104 children with PBB, we, thus, provide more extensive clinical, laboratory, and BAL characterization of PBB.

Materials and Methods

The Queensland Children's Health Services human research ethics committee (HREC/03/QRCH/17) approved the study, and written informed consent was obtained from each parent/guardian. Children undergoing flexible bronchoscopy and BAL for a clinical indication between March 2008 and November 2012, excluding those with cystic fibrosis, were eligible for inclusion. Of 343 potentially eligible children, 104 fulfilled clinical and BAL criteria for PBB, and 49 with chronic respiratory symptoms (without PBB) were allocated as control subjects. Any child with symptoms or signs of acute lower respiratory tract infection (eg, high fever, shortness of breath or tachypnea, recent-onset wheeze, rattle or crepitations on chest auscultation), was deemed (by an anesthetist) to be unfit for anesthesia and thereby excluded.

All caregivers completed a standardized clinical questionnaire including the following: cough descriptors (eg, cough score[17] and duration), current antibiotic treatment, and demographic factors (eg, age, sex, number of siblings, prior radiograph-confirmed pneumonia, indigenous status, childcare attendance, tobacco smoke exposure). Baseline examination findings were collected. Participants had prospective follow-up with cough diaries to confirm PBB diagnosis (via antibiotic response).

Basic immune function tests were performed on peripheral blood samples, including CBC count, immunoglobulin levels (IgG, IgA, IgM, and IgE), IgG subclasses, and specific antibody (IgG) responses to *Haemophilus influenzae* type b and *Clostridium tetani*. Lymphocyte subsets (including natural killer [NK] cells) and antigen-specific IgE (radioallergosorbent test [RAST]) for common environmental allergens (eg, cat, dog, molds and yeasts, house dust mite, common grass mix) were undertaken in a subset. Assays were performed at Queensland Health Pathology.

Bronchoscopy and BAL were performed as previously described (e-Appendix 1).[7] Multiplex polymerase chain reaction (PCR) techniques were used to detect human adenoviruses (HAdVs), respiratory syncytial virus, metapneumovirus, influenza viruses A and B, and parainfluenza viruses in BAL specimens.[18] A random subset had extended viral panel testing for rhinoviruses, human bocavirus, human coronaviruses (HCoVs) (HCoV-NL63, OC43, 229E, HKU1), and polyomaviruses (PyVs) (KIPyV and WUPyV) using PCR techniques as described elsewhere.[18-22]

Tracheomalacia was defined as tracheal deformity at end expiration that was maintained during spontaneous respiration, but which could be altered by the passage of the bronchoscope or positive airway pressure. Bronchomalacia was defined as an appearance of deformity in the right or left main-stem bronchi and/or their respective divisions at the lobar or segmental level.[23]

Statistical Analyses

Statistical analyses were carried out using IBM SPSS version 20.0 (IBM). Medians and interquartile ranges (IQRs) were reported as data that were non-normally distributed. Comparisons of categorical variables were performed using the Pearson χ^2 test or Fisher exact test (if expected value was <5). For continuous variables, the Mann-Whitney *U* test was used for two-group comparisons and Kruskal-Wallis test for comparisons of more than two groups. Univariate logistic regression was used to calculate ORs. A two-tailed *P* value $<.05$ was considered statistically significant.

Results

At the time of recruitment, median cough duration in the 104 children with PBB was 28 weeks (IQR, 6-57 weeks) and median cough score was 3 (IQR, 1-3), indicative of frequent coughing.[17] The primary indications for bronchoscopy in the control subjects were stridor, cough, and other respiratory symptoms (Fig 1). Of the nine control subjects who reported cough on the day of bronchoscopy, the median duration was 6 weeks (IQR, 3-19 weeks). All children with PBB had a normal physical examination without evidence of chronic lung disease. Children with PBB were more likely to have attended childcare (Table 1).

Immune Function

Basic immune function test results in children with PBB were normal, except for median NK-cell levels (Table 2). All control data were within normal reported limits (not shown). Median peripheral blood eosinophil counts were low in both groups (PBB group: 0.3×10^9/L [IQR, 0.1-0.6] vs control group: 0.3×10^9/L [IQR, 0.2-0.4]; normal range, 0.10-1.00). Median

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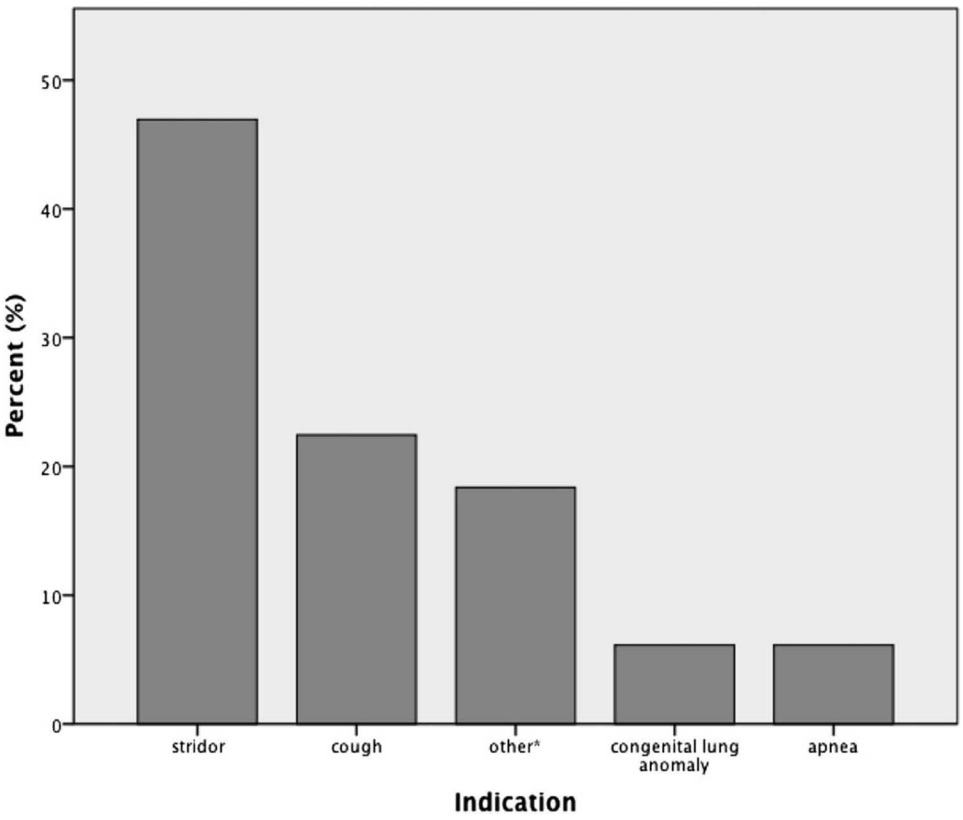

FIGURE 1. Bar graph showing primary indication for bronchoscopy in control subjects. *Includes wheeze, exercise-induced symptoms, imaging abnormality, and hemoptysis.

total IgE levels, in 24 control subjects and 47 children with PBB, were normal (PBB group: 32 kU/L [IQR, 13-149 kU/L]; control group: 58 kU/L [IQR, 13-256]; normal range, < 100 kU/L). Of the 39 children with PBB who underwent RAST for common aeroallergens, 26% were positive for one or more allergens compared with 25% of 20 control subjects ($P = .957$).

Peripheral-blood lymphocyte subsets were tested in 46 children with PBB, of whom 33 (72%) had NK-cell levels above the upper limit of normal for age.[24] Median NK-cell levels ($\times 10^9$/L) were higher in children with PBB than control subjects (PBB group: n = 46; median, 0.72 cells [IQR, 0.50-0.93 cells]; control group: n = 22; median, 0.53 cells [IQR, 0.39-0.89 cells]; $P = .123$). Blood NK-cell levels were more likely to be elevated above the normal for age in HAdV positive, compared with HAdV negative, participants (12 of 13 children vs 30 of 52, respectively; $P = .023$).

Bronchoscopic and BAL Findings

Of the 104 children with PBB, tracheo- and/or bronchomalacia were present in approximately two-thirds with similar rates in the control group (PBB group, n = 71 [68%] vs control group, n = 26 [53%]; $P = .068$). Laryngomalacia was more common in the control

Table 1—*Background Characteristics of Children in the Cohort*

Characteristic	PBB Group (n = 104)	Control Group (n = 49)	*P* Value[a]
Sex, male to female ratio, % male	72:32 (69)	33:16 (67)	.815
Age, mo (IQR), y	19 (12-30)	20 (8-63)	.967
Household tobacco smoke exposure	39 (38)	15 (33)	.565
Siblings, median (IQR)	1 (1-2)	1 (1-2)	.788
Childcare attendance	48 (91) (n = 53)	15 (58) (n = 26)	.001
Indigenous status[b]	10 (10)	1 (2)	.106
Previous pneumonia	15 (14)	8 (16)	.659
Wheeze (ever)	63 (81) (n = 78)	12 (67) (n = 18)	.192

Data given as No. (%) unless otherwise indicated. IQR = interquartile range.

[a]$P < .05$ considered statistically significant.

[b]Defined as those who identified as aboriginal or Torres Strait Islander.

Table 2—*Basic Immune Function Tests*

Immunologic Parameter	Median (IQR) (n = 104)[a]	Normal Range
Immunoglobulins (g/L)		
IgG	7.1 (5.6-8.5)	3.0-13.0
IgA	0.5 (0.3-0.8)	0.4-1.4
IgM	0.9 (0.7-1.1)	0.5-1.6
IgG subclasses (g/L)		
IgG_1	5.2 (4.1-6.7)	2.9-8.5
IgG_2	0.8 (0.6-1.1)	0.45-2.6
IgG_3	0.3 (0.2-0.4)	0.15-1.13
IgG_4	0.05 (0.02-0.2)	0.03-0.79
Lymphocyte subsets ($\times 10^9$/L) (n = 46)		
CD3 (T cells)	3.4 (2.7-4.4)	2.3-3.5
CD4+/CD3+ (helper T cells)	1.9 (1.4-2.6)	1.9-2.5
CD8+/CD3+ (cytotoxic T cells)	1.2 (0.9-1.6)	0.35-2.5
CD 19 (total B cells)	1.4 (0.9-1.7)	0.43-3.3
CD56 and CD16 (NK cells)	0.7 (0.5-0.9)[b]	0.05-0.52
Complement levels (hemolytic complement assay, CH50), U/mL	n = 70, 794 (725-874)	> 520
Vaccine responses		
IgG *Clostridium tetani*, IU/mL	n = 86, 0.5 (0.2-1.1)	Protective level: > 0.16 IU/mL
IgG *Haemophilus influenza* type B, μg/mL	n = 75, 1.1 (0.3-4)	Short-term protection: > 0.15 μg/mL

IU = International Units; NK = natural killer. See Table 1 legend for expansion of other abbreviation.
[a]Where n < 104, patients were either recruited later in the study or insufficient specimen volume was available to perform the test.
[b]Parameters outside the normal range are denoted in bold.

group (PBB group, n = 10 [9.6%] vs control group, n = 15 [30.6%]; $P = .001$), likely explained by the fact that stridor was the major indication for bronchoscopy in control subjects (Fig 1).

Bacterial infection ($\geq 10^4$ colony-forming units [CFU]/mL) with common respiratory pathogens was present in BAL specimens of all patients with PBB (n = 104, 100%) and 19 control subjects (39%, $P < .001$). Nine children with PBB (9%), and four in the control group (8%) ($P = .933$) received antibiotics in the 24 h prior to bronchoscopy. *H influenzae* was the most common bacterial pathogen in both groups. A minority of strains were β-lactamase positive (PBB group: n = 17 [16%]; control group: n = 4 [8%]; $P = .213$]. One-half of the participants with PBB (n = 52, 50%) had two or more bacterial species at levels $\geq 10^4$ CFU/mL compared with four children in the control group (8%) ($P < .001$). Of those with PBB, most (n = 30; 56%) were found to be infected with *H influenzae* and *Moraxella catarrhalis* on culture. *Staphylococcus aureus* infection was more common in BAL specimens of control subjects than those with PBB (18% vs 11%, respectively; $P = .19$). *Mycoplasma pneumonia* was detected (on PCR) in a single child with PBB (< 1%) and a single control participant (2%). Children with PBB were significantly more likely than control subjects to have coinfection with HAdV and *H influenzae* (PBB group: n = 22 [23%]; control group: n = 1 [2%]; $P = .001$). A random subset of children (n = 27 with PBB, n = 13 control subjects) had extended viral-panel analyses performed. In those with PBB, 18 of 27 samples (67%) were positive for any virus compared with five of 13 (38%) of control subjects ($P = .066$). Additional viruses identified in children with PBB included human rhinovirus (n = 11, 41%), human bocavirus (n = 1, 4%), and HCoV (n = 1, 4%). Additional viruses identified in control subjects included human rhinovirus (n = 2, 15%), WUPyV (n = 1, 8%), and HCoV-C43 (n = 1, 8%). Table 3 presents results of univariate logistic regression for common lower-airway bacteria and viruses.

Median total cell counts were 270×10^6/L (IQR, 150×10^6/L to 520×10^6/L) in PBB vs 102×10^6/L (IQR, 47×10^6/L to 203×10^6/L) in control subjects ($P < .001$). The median percentage of neutrophils was markedly elevated at 42% (IQR, 19%-66%) in children with PBB compared with 4% (IQR, 2%-8%) in control subjects ($P < .001$) and higher in HAdV-positive participants (43%; IQR, 14%-55%) vs HAdV-negative participants (16%; IQR, 5%-55%) ($P = .044$). The median percentage of macrophages was correspondingly depressed at 45% (IQR, 24%-68%) in children with PBB compared with 90% (IQR, 80%-94%) in control subjects ($P < .001$). The percentages of lymphocytes were 9% (IQR, 5%-15%) and 6% (IQR, 3%-10%) in PBB and control groups, respectively ($P = .033$). The median percentage of eosinophils was 0% (IQR, 0%-0%) in both groups.

Discussion

In this large prospective study on PBB in children, we advanced our understanding of PBB by further examining clinical, laboratory, and BAL descriptors in a large cohort of children. In addition to chronic

Table 3—*Univariate Logistic Regression for Lower Airway Bacteria and Viruses*

Infective Findings (BAL)	PBB Group, No. (%) (n = 104)	Control Group, No. (%) (n = 49)	OR (95% CI)	*P* Value
Bacteria				
Haemophilus influenzae	75 (72)	10 (20)	10.09 (4.46-22.82)	< .001
Moraxella catarrhalis	45 (43)	5 (10)	6.71 (2.46-18.30)	< .001
Streptococcus pneumoniae	41 (39)	8 (16)	3.34 (1.42-7.83)	.006
Viruses				
HAdV	22 (23)	2 (4)	6.78 (1.52-30.22)	.012
RSV	5 (5)	0 (0)	…	NS
HMPV	2 (2)	0 (0)	…	NS
IFV	2 (2)	2 (4)	0.24 (0.02-2.71)	.248
HPIV	7 (7)	0 (0)	…	NS
Any virus	n = 92, 35 (38)	n = 45, 4 (9)	6.30 (2.08-19.09)	.001

HAdV = human adenovirus; HMPV = human metapneumovirus; HPIV = human parainfluenza virus; IFV = influenza virus; NS = not significant; RSV = respiratory syncytial virus.

wet cough in PBB, we observed high rates of parent-reported wheeze, and an association between PBB and childcare attendance. Children with PBB demonstrated, within-population normal immunoglobulin levels, antibody responses (to tetanus and *H influenzae* type b vaccines) and propensity to atopy (IgE and RAST); however, many children had elevated NK-cell levels in blood specimens. Tracheobronchomalacia was common, but rates were similar in the control group. The BAL specimens from children with PBB showed a high prevalence of viruses, particularly HAdV, in addition to bacterial infection and airway neutrophilia.

Our study concurs with existing published studies on PBB regarding the demographics of these children. These factors include the young median age of children with PBB,[7] the predominance of boys,[10] and the coexistence of central airway anomalies (tracheo- and/or bronchomalacia).[10] Our new finding of high rates of childcare attendance is likely to have relevance to the rates of virus detection in our PBB cohort, notwithstanding the ongoing immune maturation also occurring at this age. Thus, boys with several months' history of wet cough, who attended childcare, and who were between their first and second birthdays typified our PBB cohort. Wheezing episodes were also commonly reported in children with PBB.

A high proportion of caregivers reported "wheeze ever" in their child (74%). This supports the findings of Saglani et al[25] of high rates of bacterial infection (43%) and neutrophilic inflammation (54%) in the BAL specimens of young children with severe recurrent wheeze, and those of Bisgaard et al[26] in their birth cohort study linking bacteria (in the naso- or hypopharynx) with wheeze. The similar rates of wheeze in children with PBB and the control subjects may relate to the high airway malacia rates also observed. However, it is important to acknowledge the inherent inaccuracy of parent-reported wheeze, which poorly reflects true wheeze and asthma[27,28] and has low-level agreement with doctor assessment.[29,30] This fact is likely to contribute to asthma overdiagnosis, particularly in primary care settings.

Coexistent asthma is unlikely to explain the high rates of parent-reported wheeze in children with PBB in the present study. This is supported by the fact that all children with PBB in this cohort had resolution of their cough with 2 weeks of antibiotic therapy (a criterion for PBB diagnosis). Our findings of low median IgE levels coupled with absence of eosinophilia (both in the lower airway and peripheral blood) provide further laboratory evidence against asthma.

Our previous descriptions of the immune status of children with PBB were limited to basic immunoglobulin levels.[7] In the present study, we further evaluated immune function. We have demonstrated that antibody-mediated responses (to a protein-based [tetanus] and conjugate [*H influenzae* type b] vaccine) and lymphocyte subsets are normal in PBB, with the exception of elevated levels of NK cells $CD16^+$ and $CD56^+$. This is a novel finding, and likely to be associated with recent viral infection. Although the cross-sectional nature of this study precludes making causal associations, it is probable that the high NK-cell levels seen in children with PBB were related to elevated virus detection rates in BAL specimens (38% of BAL specimens on a standard respiratory panel and 67% of BAL specimens on an extended viral panel). Moreover, the association between HAdV in BAL specimens and NK-cell elevation is consistent with published literature suggesting that NK cells play a role in innate immune defense against HAdV.[31] Although we are unable to draw conclusions regarding the significance of HAdV in the airways of children with PBB, it deserves further research. A possible mechanism includes reactivation of HAdV in response to certain stresses, as HAdV can remain in a quiescent state in tonsillar and adenoidal tissue.[32]

In accordance with previous findings,[33] increased total cell counts, neutrophilia, and reduced percentage

of macrophages were observed in the BAL specimens of children with PBB. Notably, airway eosinophilia was absent. Previous research has linked viral-bacterial coinfection to significantly heightened neutrophil levels in the lower airways of children—higher than that of bacterial or viral infection alone.[34] This finding is likely to have relevance to our cohort with PBB, as high rates of viral-bacterial coinfection and greatly elevated percentages of neutrophils in BAL specimens (median, 42%) were apparent. Furthermore, HAdV was significantly more likely to be detected in the lower airways of children with PBB compared with control subjects and was associated with heightened levels of NK cells in blood specimens and with neutrophilia in the airways (indicating a systemic and airway immune response to the virus).

A link to chronic cough and bacterial bronchitis has been reported previously in airway malacias.[10] A causal relationship (in either direction) may exist between large airway malacias and PBB to explain the high rates of tracheobronchomalacia (68%) in children with PBB. The similar tracheobronchomalacia rates in children with PBB and the control group (53%), and the high rates of bacterial infection (39%) observed in the control group are likely interrelated. These findings reflect the fact that children in the control group had chronic respiratory symptoms leading to bronchoscopy. Another possible explanation for the high rates of bacterial infection in the control group is contamination of the bronchoscope during the procedure. However, our fastidious BAL technique and use of a $\geq 10^4$ CFU/mL cutoff to indicate infection should minimize this likelihood.

A number of limitations of our study merit consideration. First, its cross-sectional nature precluded making causal links relating to the role of viruses in PBB pathogenesis. Second, due to the inherent ethical issues regarding use of control subjects for studies involving bronchoscopy, our comparator group comprised children with chronic respiratory symptoms (other than PBB). This likely reduced the strength of our observations. Third, we had missing data on childcare attendance and wheeze, as these questions were introduced later in the course of the study. Last, extended viral panel analyses were only performed on a small subset of participants, limiting our ability to make inferences regarding the significance of other viruses (eg, rhinovirus).

The major strengths of this study are its prospective design, the large cohort, and the inclusion of a control group. Our lower-airway findings confirm our previous observations in relation to PBB, with the addition of new information regarding environmental exposure (ie, childcare attendance, and virus and wheeze prevalence). Our findings illustrate the symptom overlap between PBB and asthma,[7,9] highlighting the importance of clear-cut diagnostic criteria for PBB. The most convincing evidence for PBB in the children studied was a clear response to 2 weeks of amoxicillin-clavulanate antibiotics—a key clinical factor in the diagnosis of PBB. While bronchoscopy has been undertaken in this study and included in our original description of PBB,[35] we do not advocate that all children with a chronic wet cough should undergo flexible bronchoscopy. In the Australian guidelines for chronic cough in children,[15] the clinical definition of PBB does not include BAL criteria. The validity of this approach was documented in a recent, multicenter, randomized controlled trial on the management of chronic cough in children.[36]

The importance of accurate PBB diagnosis and timely management extends beyond the significant financial and social repercussions of protracted cough in children.[2] Many authors postulate that recurrent episodes of PBB and/or untreated lower airway bacterial infection predispose to later development of bronchiectasis.[37-39] Douros et al[40] described the association between duration of wet cough and abnormalities seen on CT scans. However, the question of whether recurrent PBB is antecedent to later development of bronchiectasis remains unanswered to date, and warrants elucidation in a longitudinal cohort study.

Our study's findings provide further depth to the clinical profile and our overall understanding of PBB. Children with PBB are, typically, very young boys with protracted wet cough and parent-reported wheeze, and without elevated IgE or eosinophilia, who have attended childcare. Elevated rates of viruses in BAL specimens (in particular, HAdV), and heightened levels of NK cells in blood provide both direct and indirect evidence for a role of viruses in PBB pathogenesis. In view of our findings, we postulate that interactions between the innate immune system, environmental exposures, and viruses and bacteria underlie the development of PBB in children, and further research is needed.

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Dr Wurzel: contributed to conceptualization of the study, data analysis and collection, and manuscript preparation; read and approved the final manuscript; and served as principal author.

Dr Marchant: contributed to conceptualization of the study, data analysis, and manuscript preparation and read and approved the final manuscript.

Dr Yerkovich: contributed to preparation and critical review of the manuscript and read and approved the final manuscript.

Dr Upham: contributed to preparation and critical review of the manuscript and read and approved the final manuscript.

Dr Mackay: contributed to the extended viral panel analyses, critically reviewed the manuscript, and read and approved the final manuscript.

Dr Masters: contributed to data collection, critically reviewed the manuscript, and read and approved the final manuscript.
Dr Chang: contributed to conceptualization of the study, all aspects of the study, and manuscript preparation and read and approved the final manuscript.
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Additional information: The e-Appendix can be found in the "Supplemental Materials" area of the online article.

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