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Chronic Wet Cough in Children and Further Exploration of Protracted Bacterial Bronchitis

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Abstract

Chronic wet cough (i.e. lasting > 4 weeks) is common in children and is associated with significant burden on children, families and the healthcare system. Although some clinicians view chronic cough in children as trivial, for parents, chronic cough is associated with significant quality of life impairment, which improves when the cough resolves. Further, chronic cough in children may signify the presence of underlying lung disease, such as bronchiectasis.

Prior to this thesis, my supervisors were the first to define the clinical entity of protracted bacterial bronchitis (PBB) in children. PBB is characterised by chronic wet cough, response to antibiotics (suggestive of bacterial infection of the airways) and absence of pointers indicating an alternative cause for cough. This early research demonstrated the existence of a recognisable pattern of lower airway inflammation and bacterial infection in children with chronic wet cough. However, many questions relating to chronic wet cough and PBB remained.

This thesis explored the clinical, demographic, immunological and microbiological characteristics of children with chronic wet cough and PBB, with specific focus on viruses and viral-bacterial interactions. A longitudinal 2-year cohort study was also undertaken to describe the natural history of PBB. The medium-term risk of bronchiectasis (BE), in children with PBB, was evaluated; with the over-arching aim of identifying a link between these two conditions and identifying specific risk factors for a diagnosis of bronchiectasis.

The objectives of the thesis were, to:

1. Examine and contrast the lower airway findings of children with different types of cough (wet and dry) compared to no cough, with respect to infection and inflammation.

2. Evaluate the sensitivity, specificity and predictive values of nasopharyngeal aspirate (NPA) for detecting viruses in bronchoalveolar lavage (BAL); and determine the relationship between infection and lower airway neutrophilic inflammation in children with non-acute respiratory illness.

3. Evaluate the major human adenovirus genotypes/species, and relationships to bacterial co-infection, in children with PBB and BE.

4. Delineate the clinical and laboratory characteristics of children with PBB with a specific focus on viruses.

5. Determine the 2-year outcomes and risk factors for BE and recurrent PBB, in children with PBB.

6. Undertake a systematic review on the efficacy of short-course antibiotic therapy for bronchiectasis in children and review specific treatment options for cough in children in general practice.

Five of these objectives have been published (as 6 papers) and presented as chapters. The remaining chapter (objective 5) has been submitted to a peer-reviewed journal. An overview of the major findings, with respect to each chapter is detailed below:

Objective-1 is a study on 232 children undergoing bronchoscopy for evaluation of respiratory symptoms. Children were grouped using a cough nature symptom-based approach into “wet cough,” “dry cough” and “no cough”. Children with wet cough were more likely to have lower airway infection (bacterial, viral and viral-bacterial). Viral-bacterial co-infection was associated with maximal lower airway neutrophilic inflammation.

Objective-2 included extended viral panel analyses on paired NPA and BAL specimens from 75 children with chronic respiratory symptoms. We found that the agreement between NPA and BAL, with respect to virus detection using PCR, was dependant on viral species. A good agreement was seen for adenoviruses and poor agreement for rhinoviruses. Adenovirus, but not rhinovirus, positivity in the airway was associated with lower airway neutrophilic inflammation.

Objective-3 examined BAL samples from 245 children with PBB and BE and found a strong association between human adenovirus (HAdV) positivity on PCR and bacterial lower airway infection. Genotyping, performed on a subset of HAdV positive samples, showed that HAdV species C is the major detectable species in the BAL of children with PBB and BE.

Objective-4, which included 104 children with PBB, showed that PBB typically affects very young boys and is characterised by prolonged wet cough and parent-reported wheeze. Compared to controls (n=49), children with PBB had higher rates of childcare attendance, elevated NK-cell levels in blood and increased virus detection rates (particularly adenovirus) in BAL.

Objective-5 showed that in 161 children with PBB, 106 of whom were followed for 2 years, bronchiectasis was diagnosed in 13 (8.1%). On multi-variate analysis, risk factors for BE diagnosis at 2-years were: recurrent episodes of PBB (p=0.003) and *H. influenzae* lower airway infection in the BAL (p=0.013).

Objective-6 consists of two publications. The first is a review of drug treatments of childhood cough. The second is a systematic review of the efficacy of short-course (i.e. ≤4 weeks) antibiotics for treatment of bronchiectasis in adults and children. In this review, we concluded that there is insufficient evidence, in the current literature, to support the use of short course antibiotics in bronchiectasis and further RCTs are needed.

The limitations of the studies have been outlined in their respective chapters. The studies arising from this thesis provide new knowledge in relation to:

- The significance of wet cough, as a symptoms and sign, in relation to lower airway infection and inflammation.
- The agreement between NPA and BAL with respect to virus detection on PCR; and, the association between pathogen type (viral and/or bacterial) and neutrophilic lower airway inflammation.
- Further description of PBB, including systematic evaluation of clinical, demographic, infective and inflammatory characteristics.
- Identification of the potential role of adenovirus C in chronic suppurative lung diseases in children.
- The medium-term risk of BE diagnosis in children with PBB; and, risk factors for BE.
- Evaluation of current evidence supporting the use of short course antibiotics in adults and children with BE.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed papers:

1. **Wurzel DF,** Marchant JM, Yerkovich ST, Upham JW, Masters IB, Chang AB. *Prospective Characterisation of Protracted Bacterial Bronchitis (PBB) in Children.* Chest 2014 Jun 1;145(6):1271-8. doi: 10.1378/chest.13-2442.
2. **Wurzel DF,** Mackay IM, Marchant JM, Upham JW, Yerkovich ST, Petsky H, Vaughan-Smith H, Chang AB. *Adenovirus C is associated with chronic suppurative lung diseases in children.* Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America 2014 May 9. doi:10.1093/cid/ciu225 epub ahead
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4. **Wurzel DF,** Marchant JM, Chang AB. *Drug treatment of childhood coughs*. Australian Prescriber 2014;37:115-9
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4. American Thoracic Society, Philadelphia 2013: Poster discussion, “Further Description of Protracted Bacterial Bronchitis in Children.”
5. American Thoracic Society, Philadelphia 2013: Poster discussion “Nasopharyngeal Aspirate (NPA) Is The Ideal Viral Sampling Method In Children With Chronic Respiratory Symptoms.”
6. Thoracic Society of Australian and New Zealand, Darwin 2013: Oral presentation “Wet cough in children: Infective and Inflammatory Characteristics in Broncho-alveolar lavage fluid.”
7. Thoracic Society of Australian and New Zealand, Darwin 2013: Poster presentation “Protracted Bacterial Bronchitis in children: results of a large prospective cohort study.”
8. Asia Pacific Society Respirology, Hong Kong 2012: Oral presentation “Wet cough in children: Infective and Inflammatory Characteristics in Broncho-alveolar lavage fluid.”
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1. **Wurzel DF**, Marchant JM, Clarke JE, Yerkovich ST, Upham JW, Masters IB, Chang AB. *Wet cough in children: Infective and inflammatory characteristics in broncho-alveolar lavage fluid.* Pediatric Pulmonology 2014, 49: 561–568. doi:10.1002/ppul.22792 - incorporated into Chapter 2 as section 2.1.

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Julia E Clarke	Critical review of the manuscript
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Ian B Masters	Data collection (10%) Critical review of manuscript
Peter J Baker	Data analysis (10%)

S Anderson-James	Data collection (80%) and study coordination (80%) Critical review of manuscript
Anne B Chang	Co-conceptualised the study (40%) Wrote and edited the paper (20%) Critical review of the manuscript

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Julie M Marchant	Co-conceptualised the study (10%) Critical review of manuscript

John W Upham	Co-conceptualised the study (10%) Critical review of manuscript
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Helen Petsky	Study coordination (80%) Data collection (80%) Critical review of the manuscript
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Ian B Masters	Critical review of manuscript
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Anne B Chang	Wrote and edited the manuscript (20%)

Contributions by others to the thesis

In addition to the invaluable contributions of my supervisors and co-authors, as outlined above, a number of others have contributed significantly to this thesis. Dr Helen Petsky and Ms Sophie Anderson-James coordinated the overall “cough” study including recruitment of patients, data collection, data entry and follow-up of cough diaries (with the exception of the 2-year follow-up component). Ms Carol Willis was primarily responsible for data entry and database management. Associate Professor Ian Mackay and Ms Claire Wang were responsible for viral processing of respiratory samples that included PCR for the extended viral respiratory panel and molecular typing of adenovirus positive samples. Dr Heidi Vaughan-Smith and Jemima Beissmarth undertook *H. influenzae* characterisation.

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Keywords

Adenovirus, airway inflammation, bronchiectasis, children, coinfection, *Haemophilus influenzae,* nasopharyngeal aspirate, protracted bacterial bronchitis, respiratory bacteria, respiratory viruses.

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LIST OF ABBREVIATIONS

BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BE	Bronchiectasis
BLP	beta-lactamase positive
BLN	beta-lactamase negative
CF	cystic fibrosis
CFU	colony forming units
CGRP	calcitonin gene-related peptide
hMPV	human metapneumovirus
IL-8	interleukin-8
MMP-9	matrix metalloproteinase-9
NGF	nerve growth factor
NPA	nasopharyngeal aspirate
OSA	obstructive sleep apnoea
PBB	protracted bacterial bronchitis
PCR	polymerase chain reaction
RSV	respiratory syncytial virus
TAC1	tachykinin, precursor 1
TAC3	tachykinin, precursor 2
TCC	total cell count
TLR	toll-like receptor
NTHi	Non-typeable *Haemophilus influenzae*

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 CHRONIC COUGH

Chronic cough is a common reason for referral to paediatric respiratory physicians and is associated with significant impairment in quality of life for children and families [1]. Several inter-related factors influence the presence, nature and duration of cough in an individual child. The median age of children included in paediatric cough studies is 2-3 years, hence, young age is likely to be an important risk factor for chronic cough in the paediatric population [2-4]. Cough quality i.e. whether it is wet or dry, is known to be the most important single predictor of a specific cause for cough in children [5] and is a valuable tool in clinical medicine and research.

1.1.1 DEFINITION OF CHRONIC COUGH

The definitions of chronic cough in children vary. In Australian [6] and the US [7] cough guidelines, chronic cough in children is defined as cough lasting >4 weeks. Acute and protracted acute cough are defined as cough lasting <2 and 2-4 weeks, respectively [8]. In contrast, British guidelines define chronic cough in children as cough lasting >8 weeks [9]. The rationale for the Australian and US approach is several-fold. Prospective studies on young children with acute cough suggest that in approximately 90%, cough has resolved by 3 weeks [10, 11] and morbidity is reduced if intervention for chronic cough occurs within 30 days e.g. in the case the foreign body aspiration [12].

In addition to chronicity as a means of classifying cough, cough can be divided according its quality i.e. ‘wet’ versus ‘dry’.

1.1.2 'WET' VERSUS 'DRY' COUGH

The sound produced during cough is caused by turbulent flow of expired air causing vibration of large airways and laryngeal structures [13] with shearing of secretions from within the airways. In contrast, laminar flow through smaller airways (ie. <2mm in diameter) is inaudible to the ear. The rheological properties and the cilial transportability of mucous also influence the sound of cough. [14]. The exact amount of secretions per airway calibre needed to produce wet cough in children is unknown. Animal studies on cats have shown that as little as 0.5ml of mucus instilled into the trachea is sufficient to alter cough sound [15]. Similar studies in humans have not been performed.

In young children, 'wet' (or 'moist') cough is the preferred terminology used to describe cough associated with a presumed increase in lower airway secretions. The equivalent term used in adults is 'productive cough.' The terms evolved differently as, unlike adults, young children rarely expectorate sputum. 'Dry cough' implies no, or minimal, lower airway secretions.

In the evaluation of children with chronic cough, presence of 'wet' vs 'dry' cough is a key clinical sign and symptom used by respiratory specialists. It has utility in predicting a specific cause of cough and the likelihood of response to treatment [16, 17]. The validity of wet cough has also been shown in research studies [18-20]. When cough is wet, this implies the presence of increased airway mucous, which is usually infected with bacteria [18]. There is good agreement between parents' versus clinicians' assessment of wet cough (K=0.75, 95% CI, 0.58-0.93) with good sensitivity and specificity with lower airway findings of increased secretions on bronchoscopy (0.75 and 0.79 respectively) [19].

In contrast to the validity of wet cough as a symptom and sign, parent-reported wheeze poorly reflects true wheeze and asthma [21, 22] and has a low level of agreement with doctor assessment of wheeze [23]. A possible link between wheeze and bacterial infection of the lower airways in children has been shown [24]. Further exploration of the symptom of wheeze and its co-existence with wet cough is needed and hence addressed in this thesis.

The mechanisms responsible for the development of wet cough are further discussed in Section 1.3.

1.1.3 THE BURDEN AND COST OF CHRONIC COUGH IN CHILDREN

The burden of chronic cough in children is significant. An Australian study examining the prevalence of persistent cough (defined in this study as >3 weeks) showed that 5-10% of school-aged children between 6-12 years reported its presence [25]. It is likely that prevalence is even higher in pre-school aged children. This assumption is based on the indirect observation that the majority of children with chronic cough seen in respiratory specialist clinics are young (median age 2-3 years) [2, 3].

The frequency of doctor consultations for cough is substantial and cough constitutes the most common reason for new medical visits [26]. Previous research has shown that children have multiple doctor visits for cough before referral is made to a respiratory specialist [1]. An Australian study by Marchant et al found that over a half (53%) of children had >10 medical consultations (for cough) and >80% had 5 visits in the 12 months preceding specialist evaluation [1].

Chronic cough has been shown to be associated with significant morbidity [25, 27], financial burden and reduction in quality of life for parents and children [28] [1]. Parents worry about the impact of their child's cough. An Australian study by Marchant et al [1] involving 190 families, showed that parents were most concerned about the cause of their child's cough and whether it indicated a serious illness. Other major concerns included: their child not sleeping well and cough causing damage to their child's chest. Such parental worries largely resolved after resolution of the cough with treatment [1]. Other studies have had concordant findings with parental concerns largely relating to sleep disturbance [29], discomfort and fear that the cough may cause permanent damage to their child's chest or that their child may choke or die [28].

Studies on adults with chronic report increased rates of anxiety and depression in those with cough when compared to healthy controls [30, 31]. This is not the case for parents of children with chronic cough however, whose worries and concerns resolve after the successful treatment of their child's cough [1].

In addition to the impact on quality of life for families, cough confers significant financial burden and substantial resource utilisation. The cost of medications (prescribed and over the counter), doctor visits, medical investigations and work absenteeism, although difficult to quantify, is likely to be in the order of several millions of dollars per year in Australia.

1.2 ANATOMY AND PATHOPHYSIOLOGY OF COUGH

In order to understand the pathophysiology of cough in the context of the paediatric patient, we will briefly summarise the basic principles of cough and respiratory physiology.

1.2.1 WHAT IS COUGH?

Cough is a forced expulsive manoeuvre against a closed glottis that produces a characteristic sound [32]. Simplistically, cough is a protective reflex that aims to clear the host respiratory tract from secretions or potential irritants e.g. aspirated upper airway secretions or gastric contents, inhaled particulate matter or infectious organisms [33, 34]. For example, when cough is suppressed e.g. during general anaesthesia, secretions accumulate and atelectasis and pneumonia can result. However, cough may also play a detrimental role. Forceful or persistent coughing can result in rib fracture, vomiting, syncope and reduction in quality of life [1]. Further, airway mucosal trauma may cause, or result from, repetitive coughing and lead to development of chronic cough [33]. Further, cough pathway sensitisation can perpetuate cough when its original cause has resolved. Cough also facilitates respiratory droplet spread and transmission of organisms, such as influenza or *Bordetella pertussis,* between individuals [1, 33]. Hence, effective treatment of cough requires consideration of the multitude of factors involved in its aetio-pathogenesis.

The cough reflex is a complex pathway involving cough receptors, afferent and efferent sensory limbs, cough centre and effector muscles. Cough has three distinct phases: 1) an initial deep inspiration, 2) a brief, strong expiration against a closed glottis and 3) glottic opening, with nasopharynx closure, and forceful expulsion of air through the mouth producing a characteristic sound [35].

Cough receptors are located throughout the respiratory tract and are particularly abundant in the larynx, carina and at proximal bronchial bifurcations. When cough receptors are activated, the vagus nerve provides afferent sensory pathways that lead to cough centres in the brainstem, whilst efferent pathways supply the respiratory tract muscles responsible for effecting cough [36]. A functional MRI study on adults, using capsaicin inhalation to

stimulate cough, demonstrated activation of several areas of the cerebral cortex during cough [37]. This suggests that the cerebral cortex may also influence cough production. While cough receptors are also present outside the respiratory tract in some, further discussion of cough receptors is beyond the scope of my thesis. A recent review of the physiology of cough is available [34].

1.2.2 IN-UTERO AND EX-UTERO LUNG AND CHEST WALL DEVELOPMENT

In utero lung development is complex and begins just prior to day 28 of gestation [38]. Although most of the lung structure is present at birth, development continues until the postnatal period. In utero, four processes occur in lung development to enable the lung to perform its function of gas exchange. The first involves *branching* of the airways to form conducting and respiratory airways. *Septation* then divides the airpaces to enable *alveolisation* and lung *vascularisation*. This latter process accompanies the development of bronchi [39]. Alveolar multiplication begins at around 27 weeks gestation, increasing rapidly until 2-3 years of age then continuing at a slower rate until 8-10 years, before reaching adult levels [38].

Other post-natal changes include an increase in the diameter of the large airways and a relative increase in peripheral airways size, compared to central airways, as well as an increase in lung volumes with age. Additionally, the rib cage changes to a more vertical orientation. This change in rib orientation is influenced by the transition to an upright posture. The ribs are pulled in a caudal direction, elongating the thoracic cavity [39]. During this time, rib ossification also occurs with both factors acting to improve overall lung biomechanics resulting in an increase in thoracic volume and functional residual capacity (FRC) [38]. Prior to 2 years of age, chest wall compliance relative to lung compliance is

high as rib cage ossification is incomplete and respiratory muscles are underdeveloped. By age 2, chest wall and lung compliance are similar, as seen in adults [39].

An additional factor impacting on respiratory muscle efficiency in young children is the angle of insertion of the diaphragm [40]. The horizontal (as opposed to oblique in adults) angle of insertion of the diaphragm into the chest wall results in the chest wall being drawn inward rather than downwards, making drawing air into the chest cavity less efficient in young children than in older children and adults [39]. Together with the high compliance of the infant rib cage this significantly influences susceptibility to respiratory muscle fatigue [41]. This is particularly relevant during REM sleep when the splinting effect of the intercostal muscles on the chest wall is inhibited and paradoxical inward movement of the rib cage occurs during inspiration [42]. REM sleep comprises over 50% of total sleep time in term infants, and a larger percentage in preterm infants [43]. A recent study on 22 infants showed that these paradoxical movements resolved by 3.3 years of age [44].

The characteristic biomechanics and smaller airways caliber of young children represent a respiratory disadvantage. In addition to their impact on breathing efficiency, these factors are likely to interfere with the adequacy of secretion clearance, particularly from smaller airways. This latter factor may be relevant to the pathogenesis of cough and indeed to the development of chronic suppurative lung diseases in young children, though this remains speculative.

1.2.3 PHYSIOLOGY OF COUGH

Cough is generally considered a respiratory reflex whose aim is to protect the lungs. Its presence usually signifies an underlying condition or irritant exposure [45]. The pathophysiology of cough is complex. A detailed description of the anatomy and

neurophysiology of cough is beyond the scope of this thesis and is covered comprehensively in a recent review [34]. In this chapter, I will provide a brief summary of the aspects of cough physiology relevant to the thesis topic.

Age influences several key physiological domains relevant to cough in children. A schematic overview of these age-dependent physiological domains is presented and explained in detail in a paper by Chang et al [46]. To summarise, these domains include physiology relating to 1) cough 2) the respiratory system as a whole 3) other body systems eg. the immune system and 4) general physiology [46]. Although presented as separate entitites, Chang explains that these domains are in fact overlapping and inter-related. These domains influence cough aetiology, interpretation of investigation results and response to treatment [47].

Individual factors governing the timing, nature, frequency and effectiveness of cough are protean. These include, but are not limited to, anatomic, neurologic, metabolic, immunologic and hormonal contributors. As mentioned, many of these influences are age-dependant, but gender is also important. For example, gender does not influence cough sensitivity in childhood [48], however, post-pubertal females have greater cough sensitivity than males [49], suggesting a gender or hormonal influence.

With regards to neurological influences, the cough reflex is immature and pharyngeal coordination under-developed at birth. It is weak in infants, and more so in premature infants, particularly those who have required intubation and mechanical ventilation for a prolonged period who may have abnormal development of suck and swallow rhythms [50]. Children with co-existent neurological impairment are at further risk of swallowing dysfunction.

As young children are more prone to aspiration events as compared to older children, close attention is given to the consistencies of food provided to them to ensure that these are appropriate for the child's stage of development. For example, parents are advised that children should not consume whole nuts until the age of 5 years, because of risk of aspiration.

For many reasons, young children are generally unable to expectorate sputum, even when secretions are abundant [19]. Hence, the term 'wet' or 'moist' cough is used in children, as opposed to 'productive' cough that is used in adults.

From an anatomical perspective, children differ from adults in a number of ways. Understanding these differences helps to explain disease pathogenesis. Factors such as small airway calibre, lack of collateral ventilation [51], tracheal cartilage plasticity [52] and rib orientation disadvantage airway physiology in infants and young children. Overall, these factors may lead to an increased propensity to small airways obstruction/closure and atelectasis, and, secondarily, to mucociliary clearance impairment. This is of particular relevance to conditions associated with airway mucous hyper-secretion e.g. chronic suppurative lung diseases such as PBB and bronchiectasis and aspiration lung diseases that cause additional, intra-luminal airway obstruction [47].

The anatomical and physiological differences between adults and children may contribute to certain acute paediatric conditions, such as a bronchiolitis and croup, entities that do not usually occur in adults. Similarly, adult conditions such as chronic obstructive pulmonary disease are not recognised entities in children. Protracted bacterial bronchitis is a paediatric diagnosis not described in adult literature on cough. This thesis further explores

this common paediatric condition and provides further insight into aetiological factors that contribute to its origin.

The literature review will now focus on chronic wet cough and the clinical entity of protracted bacterial bronchitis.

1.3 CHRONIC WET COUGH

As mentioned in section 1.1.2, chronic wet cough in children implies cough that is associated with increased lower airways secretions and present for >4 weeks. In this section, I will briefly review the mechanisms by which mucous over production occurs and its role in airway clearance.

Three histopathological mechanisms can potentially cause mucous overproduction leading to wet cough. These include: 1) overexpression of mucin genes leading to increased secretion; 2) hyperplasia, hypertrophy or metaplasia of secretory cells (i.e. goblet cells, submucosal glands and possibly Clara cells); 3) oversecretion of stored mucin from secretory cells [45].

From a functional perspective, cough, together with mucociliary clearance, encourages the flow of mucous in the airways in order to facilitate its removal. Based upon a physical model by Scherer and Burtz, involving turbulent airflow through a straight tube with a mucous-like fluid, cough improved mucociliary clearance of mucus down to the level of the 12^{th} airway generation [53].

In general, voluntary cough manoeuvres are considered ineffective in the absence of mucous hypersecretion [45]. Indeed, Yeates et al showed no effect of voluntary coughing on mucous flow in healthy adults [54]. This is believed to be due to the fact that the mucous layer is substantially thinner in health (<20μm) compared to in bronchitis (>200μm) [55, 56]. Hence, cough, and more importantly wet cough, usually signifies presence of disease [5] and is the most sensitive pointer indicating a specific cause of cough [LR 26.2 (95% CI, 3.8-181.5)] [20]. Further, a study by Goyal et al showed that children whose chronic wet cough is non-responsive to 4 weeks' appropriate antibiotic therapy are more likely, than those whose cough responds to antibiotics, to have bronchiectasis on CT chest [57]. However, to date, no aetiological studies have specifically used chronic wet cough as an inclusion criteria, to determine the relative frequency of individual diagnoses.

We will briefly describe the range of conditions known to be associated with mucous hypersecretion and hence presenting with chronic wet cough. We will then discuss current knowledge on the clinical condition of protracted bacterial bronchitis.

1.3.1 CAUSES OF CHRONIC WET COUGH

The major causes of chronic cough in children differ to those in adults, hence, paediatric specific cough algorithms should be utilised. In a study involving children with chronic cough (both wet and dry), Marchant et al evaluated the utility of an adult-based algorithm for the diagnosis [2]. This study found that the 3 major causes of chronic cough in adults (asthma, gastro-oesophageal reflux disease and upper airways cough syndrome) differed from those in children (Protracted bacterial bronchitis and natural resolution accounted for 62% of cases)[2]. Hence, empirical therapies for cough, based upon adult algorithms, are inappropriate for use in children.

Chronic wet cough in children has a number of possible causes, ranging from aspiration lung disease and bronchiectasis to atypical infections e.g. tuberculosis. Clinical conditions predisposing to the development of chronic wet cough include: immune-deficiencies (acquired or congenital), cilial motility disorders and CFTR gene mutations. Large airway obstruction e.g. resulting from foreign body inhalation may present with acute onset dry cough followed several days later by wet cough. Clinical management pathways/algorithms, for the assessment and management of chronic cough in children, have recently been developed. These are well validated and have been shown to improve clinical outcomes [16].

1.3.2 POPULATIONS AT RISK

The median age of referral of children to respiratory specialists for the evaluation of chronic cough ranges from 2.8 - 4.5 years in Australian studies with male children being over-represented in these cohorts [5, 16]. The longer-term significance of chronic wet cough with regards to the risk of bronchiectasis has not been investigated in a prospectively designed study.

Racial and ethnic factors may be important to chronic cough. Although prevalence data is limited, rates of chronic wet cough are higher in Indigenous compared to non-Indigenous populations; for example, in a cross-sectional survey of middle-school children, chronic productive cough was reported in 26% of Alaskan native children compared to only 7% of Seattle children [58].

Bronchiectasis is an important cause of chronic wet cough and diagnoses are increasing both within Australia and the US [59, 60]. Indigenous populations living within resource-

rich countries appear to be at increased risk, compared to non-Indigenous populations. Examples of populations with high rates of bronchiectasis include: Aboriginal and Torres Strait Islander children in Australia, Maori and Pacific Islanders in New Zealand and Alaskan children in the United States [61-63].

The prevalence of non-CF bronchiectasis is estimated at 14.7 per 1,000 Aboriginal children aged <15 years [64], based on data collected from children in Central Australia [65]. This is equivalent to approximately 1 in 68 children, which is 40 times higher than that of cystic fibrosis among non-Indigenous Australian children according to data published in 2000 [66]. It is likely that this is an under-estimate of the true rate of non-CF bronchiectasis for two reasons. First, cough is often under-reported by those from remote Indigenous communities, hence bronchiectasis would be missed in a proportion [67]. Second, recent data on the point prevalence of CF births has shown a 17% reduction in the live-birth prevalence of CF since 1989, coinciding with the introduction of newborn screening in Australia [68]. Hence, the disparity in prevalence of non-CF and CF bronchiectasis is likely higher than estimated but is also projected to continue to rise. Research into risk factors for non-CF bronchiectasis in Indigenous populations suggests that recurrent episodes of lower respiratory tract infection [61], together with environmental irritants e.g. smoke exposure [69], are likely contributors.

Air pollution (both indoor and outdoor) has been shown to be associated with cough [70]. Environmental irritants, similar to tobacco smoke exposure, are believed to have additive effects in terms of contribution to respiratory disease, rather than being the sole aetiology [35]. This is supported by research studies that have shown that cough resolution is achievable in those with high exposure rates [71]. However, it is beyond the scope of my PhD work to examine the effects of airway pollution other than accounting for tobacco

smoke exposure in the cohorts of children examined. Where relevant, age and ethnicity were also taken into account in my PhD studies.

1.3.3 Bronchoscopy, bronchoalveolar lavage and wet cough

Examining the lower airways is a key element in furthering our understanding of chronic wet cough in children. There is limited data on the lower airways of children with chronic cough. As many of these studies have included children with PBB, these are described together in section 1.4. My PhD work (Chapter 2) involves examining the BAL of children with chronic wet cough.

In 2006, Marchant et al conducted a cohort study involving 108 children with cough >3 weeks to examine the most common aetiologies [2]. The authors described similar, characteristic lower airway findings in 40% of children presenting with chronic cough (of which 89% had chronic wet cough). These findings included: lower airway bacterial infection (with *H. influenzae*, *M. catarrhalis* and/or *S. pneumoniae*) and intense neutrophilic inflammation. Cough in these children responded to 2-weeks of appropriate antibiotic therapy, resulting in resolution of their cough [2]. This clinical entity was named protracted bacterial bronchitis or PBB and resembled the previous, poorly-defined condition known as chronic bronchitis of childhood [72]. The following section 1.4 provides an up-to-date review of current knowledge on PBB.

1.4 PROTRACTED BACTERIAL BRONCHITIS (PBB)

Protracted Bacterial Bronchitis (PBB), first defined by our research group in 2006 [2], is now a well-recognised clinical entity [6, 9, 38, 73, 74]. It is characterised by chronic wet

cough (lasting >4 weeks), response to 2 weeks' of appropriate antibiotics and absence of indicators to suggest an alternative cause of cough [6]. PBB is the commonest cause of chronic wet cough in Australian children as published in single and multicentre studies on 108 [2] and 346 children with chronic cough, respectively [16, 27]. In the latter study, the 3 most common causes of primary of cough were: PBB (41.0%), asthma/reactive airways disease (15.9%), and bronchiectasis (9.0%).

PBB is also a major cause of chronic wet cough in the UK and US and has been the subject of research by several groups [75-77]. However, these studies were limited by retrospective design, absence of a control group or small number of study participants. For example, a retrospective study by Zgherea et al showed that 46% of children with chronic wet cough had positive bacterial cultures on bronchoalveolar lavage, however, no comparator/control group was included [77]. This raised the need for further research on this topic. Further, the potential role of viruses in PBB is unknown. These research gaps were addressed in this thesis.

Although bronchoscopic assessment provides definitive evidence for lower airway bacterial infection and facilitates a diagnosis of PBB, bronchoscopy is not always feasible in the clinical context, particularly in primary care settings. Hence, clinical criteria for PBB diagnosis in children were devised to obviate the need for bronchoscopy [6]. Bacterial infection of the lower airways is inferred by the observation of a clinical response to appropriate antibiotics. 'Appropriate antibiotics' refers to antimicrobials sufficiently broad-spectrum to cover common respiratory pathogens, including beta-lactamase producing *H. influenzae* strains, *M. catarrhalis* (inherently beta-lactamase positive) and *S. pneumoniae*. Amoxycillin combined with clavulanic acid is the usual first choice in PBB.

The definition of PBB, and its iterations over time, is detailed in Chapter 1.4.2.1.

1.4.1 Lower Airway Findings In PBB

Bacterial Infection

Early studies on wet cough and chronic bronchitis in adults showed that bacterial infection was a common finding. In a study in adults published in the Lancet in 1957, 25 of 61 (41%) sputum samples from adults with chronic bronchitis showed infection with respiratory bacteria. The most common bacterial species isolated were *H. influenzae* and *S. pneumoniae*, with the same organisms predominating in the sputum of adults with bronchiectasis [78].

An earlier study by Boyd, published in 1931, described 56 children with bronchiectasis and reported recurrent or chronic bronchitis to be the second most common cause of bronchiectasis in children, after bronchopneumonia [79]. Boyd showed that *S. pneumoniae and H. influenzae* were amongst the commonest organisms identified on lung aspiration or suction. The study concluded that the 2 most important factors in the development of bronchial dilatation were: 1) obstruction of a bronchus, especially if partial; and 2) infection [79].

Studies on the lower airways of children are limited as children are generally unable to expectorate sputum, and hence we rely on lower airway sampling techniques. Recent research into the lower airways of children with chronic cough has been limited to retrospective studies and small cohorts. Fitch et al published a prospective study on 23 children with chronic unexplained cough and found that those with chronic cough had significantly higher neutrophils in their BAL compared to controls and postulated that this

was due to underlying persistent airways infection [80]. Subsequent research further supported this finding. In 2007, in a retrospective chart review of 81 patients with PBB, in which bronchoscopy was performed in 19 patients, Donnelly et al observed that neutrophils were the major cell line in the BAL samples and that *H. influenzae* and *S pneumoniae* were again the most common organisms seen. The airways of children with PBB appeared oedematous and had presence of secretions [75]. These findings were consistent with Marchant et al's observations in the original studies in which PBB was first defined [2].

In 2012, Zgherea et al published a retrospective chart review of 197 children with chronic wet cough undergoing evaluation. The majority (55%) were 0-3 years of age and most (56%) had purulent bronchitis (as opposed to non-purulent bronchitis) on bronchoscopy. Positive bacterial cultures were also a major feature, being present in almost half (46%). Lower airway neutrophilia was seen, with the severity of the neutrophilia being more pronounced in those with purulent bronchitis, compared to non-purulent bronchitis [77].

Kompare et al's retrospective study of 70 children, published in the same year had concordant findings. The link between bacterial infection and neutrophilia of the lower airways was once again observed, with an additional finding of high rates of airway malacias (74% had tracheo- and/or bronchomalacia) on bronchoscopy [76].

Hence, the triad of chronic wet cough (that responds to antibiotics), bacterial infection and neutrophilic inflammation of the lower airways had been supported by studies both within Australia and internationally. There is also notable similarity in these key features of PBB and those of non-CF bronchiectasis. Lower airway bacterial infection is widely accepted as detrimental to the lung and, together with neutrophilic airway inflammation and impairment

of mucociliary clearance, constitute major components of Cole's "vicious cycle" hypothesis on the pathogenesis of bronchiectasis [81].

Growth of one or more bacterial species, at a density consistent with infection (e.g. $\geq 10^4$ cfu/ml), is present in the lower airway secretions of most children with PBB. However, this is not an absolute criteria for a diagnosis of PBB. The reasons for this are explained later in this section. The 3 bacterial species most commonly identified in the BAL of children with PBB include: *Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis* [2]. Research into *S. pneumoniae* serotypes isolated from the airways of children with PBB has shown that both vaccine and non-vaccine serotypes are present, suggesting that serotype replacement is unlikely to be the sole explanation for the specific strains identified [82]. The majority of *H. influenzae* isolates from the nasopharynx and lower airways of children with non-CF bronchiectasis are NTHi [83]. It is assumed that this is also true for children with PBB, although prior to this thesis, this had not been studied.

The effectiveness of antibiotic therapy in the treatment of chronic wet cough has been shown in retrospective studies [2, 75]. Further, a Cochrane review published in 2005 [84] evaluated the utility of antibiotics in the treatment of chronic cough (>75% had moist cough). In the 2 included studies with a total of 140 participants, antibiotics reduced the proportion of children not cured at follow-up, with number needed to treat (NNT) of 3 (95% CI 2 to 4). More recently, a double-blinded randomised controlled trial by Marchant et al [85] showed that chronic wet cough in children responded to amoxycillin-clavulanate antibiotics, with a NNT of 3 (95%CI 2 to 4). This study also showed that requirement for further antibiotics was significantly lower in those receiving antibiotics, with NNT of 4 (95% CI 3-5) [85]. Hence, the effectiveness of antibiotics in the treatment of wet cough has been demonstrated.

It is nevertheless important to note that a proportion of children with PBB have negative bacterial cultures of their BAL [2]. There are a number of factors that may account for this. First, antibiotic exposure at the time of sampling may suppress colony counts to below the threshold to be classified as 'infection'; second, inadequate bronchoalveolar lavage technique with poor return or very localised sampling may miss the infected portion of the lung [86]; and third, sub-optimal sample handling e.g. a delay in processing, inadequate storage, plating etc may result in failure to culture an organism that may be present. This may be of particular relevance in the case of fastidious organisms e.g. nontypeable *H. influenzae* (NTHi) [87].

In earlier studies on PBB [2], children with respiratory viruses in BAL were excluded and hence, prior to this thesis, the role of viruses in PBB had not been explored. Chapter 2.2 explores the role of viral co-infections in the BAL of children with chronic wet cough.

Viral Infection

Prior to this thesis, most studies on PBB have focused on bacterial infection of the airways [2, 4, 75-77]. Respiratory viruses, such as respiratory syncytial virus (RSV), influenza viruses and rhinoviruses are commonly associated with acute cough and self-limiting respiratory illnesses in children. They are also associated with acute exacerbations of non-CF bronchiectasis in children [88], Adenovirus can persist in the airway and has been linked to chronic respiratory diseases such as bronchiectasis and bronchiolitis obliterans [89]. The role of respiratory viruses in the pathogenesis of PBB, and indeed chronic cough, are a focus of this PhD thesis. I also focus on and review the potential role of adenoviruses in this context.

Adenovirus is a common cause of upper and lower respiratory tract infections in children. Although most infections are mild or asymptomatic [90], studies reporting on lower respiratory tract infections in hospitalised infants and young children have found adenovirus to be responsible for 7-15% of cases with high rates of viral-viral co-infection (60-80%) [91-94].

The role of adenoviruses in the pathogenesis of chronic respiratory diseases, such as bronchiectasis and bronchiolitis obliterans, is well-described [89, 95-98]. A meta-analysis published by Edmond et al, examined the long-term sequelae from childhood pneumonia, and showed that adenovirus pneumonia was associated with the highest risk of sequelae (54.8% [95%CI 39.2%-70.5%]). These sequelae included: restrictive lung disease, obstructive lung disease, bronchiectasis, chronic bronchitis, asthma or a combination of conditions [99].

Adenovirus can cause chronic or persistent infection in the airways. Longitudinal studies where children were followed over a 1-year period, have shown that 81% of children with repeated isolation of adenovirus in their upper airway carry the same strain, suggesting chronic rather than repeated infection [100]. Macek and colleagues demonstrated persistence of HAdV in the lower airways of 9 of 11 children with persistent asthma who underwent repeated lower airway sampling [101]. HAdVs can cause latent infection within lymphocytes [102], tonsillar tissue [103] and the lung [104]. These findings support the hypothesis that adenovirus may play a role in the pathogenesis of certain chronic respiratory disorders [102] and this is explored further, in my PhD, in relation in PBB.

I will briefly discuss viral-bacterial co-infection in relation to respiratory tract disease.

Viral bacterial co-infection

Viral bacterial co-infection of the lung is associated with increased severity of lower respiratory tract symptoms [105, 106]. Further, numerous studies in animals [107-110] and several studies in humans support the concept of viral potentiation of bacterial infection [111-113]. There are several mechanisms by which viral infections increase the likelihood of secondary bacterial infection.

In the upper respiratory tract, viral infection predisposes to secondary bacterial infection via impairment of the epiglottal-cough reflex, interruption of the muco-ciliary function and disruption of ciliary ultrastructure with loss of cilia and ciliated cells [114]. Changes to cilial beat frequency also occur impairing the ability to remove fluid, particles and bacteria from the middle ear, sinuses and lower respiratory tract [108-110]. In addition to structural and consequently functional impairment, certain viruses e.g. adenoviruses and herpes viruses, modulate the host immune response to enable persistence of virus within the airways [115]. Prolonged faecal excretion of adenovirus 1 and 2 (for up to 515 days) has been shown in children suggesting persistence within human enteral tissues as well as the respiratory tract [116].

Enhanced adherence of bacterial pathogens is seen in virus infected or damaged cells. Viral proteins on the cell surface can act as bacterial receptors. Also, loss of airway epithelial cells exposes the basement membrane, thereby enabling more sites for bacterial binding [117-119]. Clinical studies correlating viral bacterial co-infection and bronchitis in children are lacking. Potential synergy between viruses and bacteria has been documented in other acute respiratory diseases such as pneumonia [120] and RSV

bronchiolitis [121]. We postulate that viral-bacterial co-infection and microbial synergy is also a key feature of PBB.

To date, the most convincing evidence for microbial synergy between common respiratory pathogens has arisen from animal models of otitis media. Dramatic increases in bacteria positive otitis media were found with co-infection between adenovirus and *S. pneumonia* (67%), as compared to each pathogen alone (4% and 21% respectively) [107]. Similar synergy has been shown in animal studies of otitis media between adenovirus and non-typeable *H. influenzae* [108]. This study also showed that timing of infection was important. Adenovirus prior to *H. influenzae* infection resulted in the greatest mucosal inflammation and tympanic membrane dysfunction [108]. The relevance of these findings with regards to the respiratory tract of humans warrants further study.

Neutrophilic Inflammation

Neutrophilic lower airway inflammation is an important feature of a number of chronic respiratory diseases. Lower airway neutrophilic inflammation is a common and almost universal finding in children with PBB. This has been shown in numerous studies of the lower airway secretions of children with PBB [2, 75-77]. With regards to cellularity of the lower airway, no significant findings in relation to lymphocytes or eosinophils have been seen in children with PBB [2]. A study by Zimmerman et al, examining eosinophil counts in induced sputum of children with and without asthma with post-infectious cough, showed eosinophil levels in sputum to be significantly higher in those with untreated allergic asthma [122]. Similar findings have not been seen in children with PBB. The influence of infection on lower airway cellularity and presence of neutrophilic airway inflammation requires further study and was explored in Chapter 2.

Less commonly studied markers of lower airway inflammation, including: toll-like receptor (TLR 2 and 4) mRNA and inflammatory cytokines (interleukin-8 and matrix metalloprotease-9) have been shown to be increased in BAL fluid of children with PBB [4], suggesting innate immune system activation in children with this condition, however, these studies were preliminary. A further study on the innate immune system and PBB is included in appendix 2.

1.4.2 NOMENCLATURE

'Original' versus 'revised' definition of PBB

The original definition of PBB was first published in 2006 [2] as described in chapter 1.3.3. This study included 108 young children referred to respiratory specialists for assessment of chronic cough. The aims of the study were to identify the major causes of chronic cough in children and to evaluate the utility of an adult-based cough algorithm in the diagnosis of cough in children. This study was first to identify the clinical triad of PBB. The definition of PBB has evolved over time [2].

The original definition of PBB included the following criteria: 1) prolonged wet cough lasting >3 weeks 2) presence of bacterial infection of the lower airways as seen on BAL and 3) with resolution of cough with 2 weeks of appropriate antibiotic therapy [2]. In 2010, the definition was adapted for increased feasibility, to include: 1) presence of isolated chronic wet cough lasting >4 weeks 2) resolution of cough with appropriate antibiotic treatment and 3) absence of pointers suggestive of an alternative specific cause of cough [6]. For increased feasibility and applicability to both primary and tertiary care settings, the BAL component was omitted in the revised definition of PBB [123]. This was further

supported by a randomised controlled trial on children with chronic cough (n=50) that showed significantly higher cough-resolution rates in children receiving 2-weeks of amoxycillin clavulanate antibiotics (48%), compared to placebo (16%) [85].

Recurrent versus non-recurrent PBB

The importance of differentiating recurrent versus non-recurrent PBB in an individual child is based upon the theory that those with more frequent episodes of protracted wet cough are at greater risk of bronchiectasis. This is substantiated by studies on adults, newly diagnosed with bronchiectasis, that show that most (60-80%) have a history of chronic wet cough since childhood [124, 125]. Chronic bacterial infection and concomitant neutrophilic inflammation of the lower airways, key features of PBB, are also the major features described in Cole's vicious circle hypothesis on the pathogenesis of bronchiectasis. Damage to airway epithelium (e.g. secondary to acute or chronic respiratory infection) impairs mucociliary clearance and predisposes to chronic bacterial infection thus initiating an inflammatory response that further inhibits mucociliary clearance, resulting in a vicious circle phenomenon [81].

Young children presenting with PBB frequently experience repeated episodes. A recent retrospective review of 44 children (median age 2.7 years) of which 33 had PBB, 25 (76%) had further relapse/s over an unspecified median follow-up period [126]. An earlier review of 81 children with clinical PBB found that 41 (51%) were symptom free after 2 courses of antibiotics, however 11 (13%) needed 6 or more courses of antibiotics [75]. No studies have examined the relationship between recurrent episodes of PBB and later diagnosis of bronchiectasis. Hence, this important question comprised a major aim of my PhD thesis.

In this PhD, we have defined recurrent PBB as >3 episodes of PBB per year. This cut-off was chosen, based upon the observation in clinical practice that children with more frequent episodes of PBB are less likely to "grow-out" of their wet cough episodes. It is unknown, however, whether these clinical observations were indeed important and hence, the postulated link between recurrent PBB and bronchiectasis is examined in chapter 5.

1.5 THE IMMUNE SYSTEM AND PBB

Studies on children undergoing evaluation for chronic cough have included basic immune function testing and children with major immune-deficits were excluded from earlier studies on PBB. The rationale for this approach was that children with major immune-deficiencies are closely monitored for bronchiectasis, a well-known complication of these conditions. The focus of PBB research is therefore on immune-competent, otherwise healthy children, in whom the index of suspicion for chronic suppurative lung diseases is low.

It is postulated that a defect in the innate immune system, or possibly immaturity of the adaptive immune system, predisposes to the development of PBB in children [4]. Prior to this thesis, studies examining the immune system in PBB focused on aspects of innate immunity, such as toll-like receptors and cytokines [4]. Another study explored the relationship between bacterial infection, neuropeptides and innate immune mediators in BAL fluid [127]. Both studies concluded that further studies in this area were warranted.

1.5.1 INNATE IMMUNITY

The innate immune system comprises a complex network of molecules, receptors and inflammatory mediators. In health, the innate immune system fulfills two major functions: 1) to provide rapid recognition and primary defense against invading pathogens and 2) to

communicate with (and shape) the subsequent adaptive immune system response [128]. The innate immune system differentiates between 'self' and 'non-self' to protect the host from invading organisms. In disease, any of the aforementioned processes may be altered. The innate immune system is pre-programmed and, unlike, the adaptive immune system has no specific memory. Major cell lines involved in the innate immune response include neutrophils, natural killer cells, mast cells, basophils and the complement system [128].

The important and complex role of the innate immune system, in lung health and disease, is recognised [129]. Few studies, however, have examined the innate immune system of children with PBB. In a small study, Marchant et al [4] found increased median cytokine levels (IL-8 and active MMP-9) and TLR-2 and TLR-4 mRNA expression in the BAL of children with PBB when compared to controls who were cough-free or had natural resolution of cough. TLR-2 and TLR-4 are known for their role in responding to bacterial infection. This finding was in association with the presence of positive bacterial cultures from lower airway secretions and increased total cell count and percentage neutrophils. IL-8 and MMP-9 were also increased in BAL and correlated with neutrophil levels. IL-8 is known to potentiate MMP-9. Hence, this study's findings, of significant innate immune system up-regulation in children with PBB, suggested enhanced, rather than deficient, innate immune response in these children. Further, a positive feedback loop, initiated by the presence of bacterial infection, may exist between the 2 cytokines studied as well as recruited airway neutrophils, leading to a state of chronic inflammation in the airway [4].

In another study, Grissell et al [127], examined the relationship between bacterial infection of the lower airways in children with gene expression for TLRs 2, 3, 4; chemokine receptors (CCR3, CCR5, CXCR1); neurotrophins and neurokinin genes (TAC1, TAC3,

CGRP, NGF). This study found that, in children with bacterial colonization of their lower airways, there was an airway inflammatory response, with increased BAL neutrophils, IL-8 protein, and CXCR1 expression. Further, Substance P (TAC1) and TLR4 RNA expression were reduced in the presence of bacterial infection. Overall, these findings suggested possible co-regulation of neuropeptides with the innate immune system, a relationship that may suggest impaired clearance of bacteria from the airways of children with PBB [127].

The above provides clues to the role of the host immune response in diseases such as PBB. While this importance is acknowledged, it is beyond the scope of my PhD to further examine these aspects.

1.5.2 ADAPTIVE IMMUNITY

In defending against invading pathogens, the role of the adaptive immune system is to respond to signals from the innate immune system to initiate antibody (B cell) and cellular (helper and cytotoxic T cell) mechanisms of immune defense [128]. Complex processes occur: including antigen presentation and T or B cell recognition; followed by cell priming; activation and differentiation resulting in release of antibodies into the blood or tissues (from activated B cells) or activated T cells leaving lymphoid tissue and localisation at the disease site [128]. Unlike the innate immune system, the adaptive immune system displays immunological memory, hence, if previous exposure to a pathogen type has occurred, subsequent detection and removal is enhanced. Similar to the innate immune system, aberrations in the function of the adaptive immune system can lead to impaired immunity, with resultant disease [128].

Forerunner studies on adaptive immunity in PBB have included measurement of major antibody types and subtypes (IgG and IgG subtypes, IgA, IgM and IgE) [5] [2]. The

majority of children had intact immune function, and any child with major immune deficiency was excluded from further studies. However, these previous cohorts were small (n ranging from 38-45) and incomplete immune assessments were described [4]. Given this research gap, my PhD work addressed describing basic immune function and atopy in a much larger cohort of children with PBB (Chapter 5). Availability of such data will help clinicians to better understand the complex interplay between the developing immune system and PBB.

1.6 POTENTIAL SIGNIFICANCE OF PBB

PBB and bronchiectasis share many similarities. Indeed, their clinical presentations are alike i.e. recurrent and/or protracted episodes of wet cough responsive to antibiotics, coupled with lower airway findings of neutrophilia and bacterial infection. This observation led to a hypothesis by Chang that the 2 conditions were linked and represented a clinical spectrum (see Figure 1 below) [51].

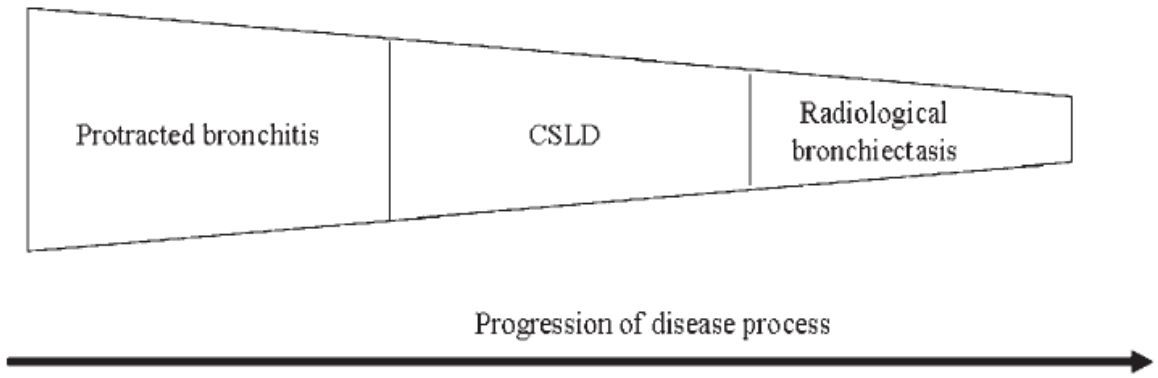

Figure 1.1: Figure reproduced with permission from A Chang.

No studies to date however, have prospectively examined the temporal relationship between PBB and BE to provide definitive support for the proposed link. Hence, this question represented the overarching aim of my thesis.

1.7. CURRENT TREATMENT APPROACH TO CHRONIC WET COUGH AND PBB

Chronic cough pathways, for the assessment and management of chronic cough in children, assist in the accurate diagnosis of chronic cough, resulting in earlier resolution of cough [16, 27].

If a child presents with chronic wet cough lasting >4 weeks, a thorough history and examination is undertaken. Specific cough pointers, suggestive of an underlying diagnosis, are sought. These include a history of developmental delay, feeding difficulties, recurrent lower respiratory tract infection, wheeze, previous treatment response etc. Examination includes assessment for chest wall deformity, clubbing, abnormality on chest auscultation and growth parameters. Chest X-ray (in all) and lung function tests (if school-aged or above) are performed. In the absence of indicators to suggest an alternative specific cause for cough (e.g. asthma, viral bronchitis) a 2-week trial of antibiotic therapy with an appropriate antibiotic (e.g. amoxycillin-clavulanate) is indicated. A randomised controlled trial published by Marchant et al in 2012 supports this approach. This showed that antibiotics (amoxycillin-clavulanate) were associated with higher cough resolution rates compared to placebo. In 50 children with wet cough lasting >3 weeks, antibiotics were associated with a cough resolution rate of 48% versus 16% in those receiving placebo ($p=0.016$) [85].

Follow-up is then undertaken at 2-3 weeks to ensure cough resolution and confirm a diagnosis of PBB. If a child has a partial response to antibiotics, a further 2-4 weeks' course is usually given and further aspects of the history are elicited e.g. adherence to

treatment and appropriateness of dose, with reassessment of the original diagnosis. Subsequent investigations and follow-up are based upon individual clinician practice.

1.8 WHY THIS THESIS IS ABOUT PBB AND CHRONIC WET COUGH

Chronic wet cough commonly affects children and places substantial burden on families and the healthcare system. Hence, accurate identification of the aetiology of cough is important to ensure that the most effective treatment is instituted. Using clear-cut diagnostic criteria, my supervisors were first to define the clinical entity of PBB providing the platform for further research on this important topic.

This thesis aims to further expand on current knowledge in relation to wet cough and PBB with a focus on the role of viral and bacterial infection. The medium-term significance of PBB with respect to development of bronchiectasis is also evaluated. We further describe the clinical profile of PBB and explore potential aetiological factors from the perspective of the environment, infection and the immune system. The overarching aim of this research was to delineate the natural history of PBB in children to evaluate the postulated link to bronchiectasis.

1.9 ADDRESSING CLINICAL RESEARCH GAPS

Several research groups have explored PBB in children however many questions remained. Chronic wet cough is the principle clinical feature of PBB. Further exploration of this “grass roots” symptom of PBB, and its relationship to lower airway neutrophilic inflammation and infection, warranted further exploration. Second, bacterial infection is a

key feature of PBB however the role of viral infection (and viral-bacterial co-infection) had not been investigated. Third, to date, descriptions of the clinical profile of children with PBB were based on small cohorts and further, in-depth characterisation was needed. Lastly, and arguably the most significant question, was whether PBB represent a risk for future bronchiectasis. This question warranted evaluation in a large prospective cohort study. Addressing these research gaps formed the basis of this PhD manuscript.

1.10 HYPOTHESIS AND AIMS

The over-arching aim of my thesis was to determine whether PBB is antecedent to a diagnosis of bronchiectasis, and if so, whether identifiable risk factors exist for a diagnosis of bronchiectasis. The principle hypothesis of this thesis was that PBB and bronchiectasis represent a clinical spectrum.

In order to address the aforementioned research gaps, the specific aims of the studies were to:

1. Examine and contrast the lower airway findings of children with different types of cough (wet and dry) compared to no cough, with respect to infection and inflammation.
2. Evaluate the sensitivity, specificity and predictive values of NPA for detecting viruses in bronchoalveolar lavage (BAL); and determine the relationship between infection and lower airway neutrophilic inflammation in children with non-acute respiratory illness.
3. Evaluate the major human adenovirus genotypes/species, and relationships to bacterial co-infection, in children with PBB and BE.
4. Delineate the clinical and laboratory characteristics of children with PBB with a specific focus on viral detection.

5. Determine the 2-year outcomes and risk factors for BE and recurrent PBB, in children with PBB.
6. Undertake a systematic review on the efficacy of short-course antibiotic therapy for bronchiectasis in children and review specific treatment options for cough in children in general practice.

1.11 THESIS DESIGN

The studies included within this PhD thesis were designed to address key research gaps, as already outlined. Chapter 2 explores the principle clinical feature of PBB i.e. wet cough. It evaluates the significance of wet cough, compared to dry and no cough, in relation to presence of lower airway infection (bacterial and/or viral) and neutrophilic airway inflammation. This study explores the significance of viral-bacterial co-infection and provides the foundation for further studies.

Viral infections are an important cause and contributor to respiratory disease pathogenesis. The relationship between viruses in the upper, versus the lower, airways, is poorly studied. Chapter 3 evaluates the relative yield and agreement between viruses detected on NPA compared to BAL. It relates these findings to presence of neutrophilic inflammation of the lower airways.

The link between adenovirus and bronchiectasis is established. Chapter 4 investigates the adenovirus genotypes present in the lower airways of children with PBB and bronchiectasis. It also examines the potential link between adenovirus and bacterial infection of the lower airways.

Previous studies on PBB have been small and/or retrospective. Chapter 5 further describes the clinical profile of children with PBB to assist clinicians in the more accurate diagnosis of this condition. The natural history of PBB, with respect to the development of bronchiectasis, is unknown. Chapter 6 explores the medium-term outcome of children with PBB and investigates potential risk factors for a diagnosis of bronchiectasis at 2-years.

Chapter 7 discusses the current literature relating to cough treatments. Chapter 7.1 is a Cochrane review that examines the efficacy of short-course antibiotics in the treatment of bronchiectasis in children and adults. Chapter 7.2 is a review of available cough remedies in children, with relevance to general practice.

The final chapter (Chapter 8) summarises the thesis' major research findings in the context of current literature. Potential areas for further research are also explored.

CHAPTER 2

2.1 INTRODUCTION TO CHAPTER 2

In Chapter 1.3.1 we have outlined the major diagnostic entities causing chronic wet cough in children. In clinical medicine, accurate diagnosis of these conditions can be challenging. Pre-school aged children, who comprise the majority of the cohort presenting with chronic cough, are usually unable to perform lung function testing, expectorate sputum for analysis or provide a detailed and accurate history. Hence, diagnosis is frequently based upon indirect findings. Further, data specifically examining the relationship between cough nature i.e. wet versus dry cough, and lower airway infection and inflammation is lacking.

This study included a large cohort of young children presenting to a tertiary respiratory specialist clinic for investigation of chronic respiratory symptoms, the majority of whom had chronic cough. The remaining children were undergoing evaluation for indications such as persistent wheeze, stridor or noisy breathing. Children were divided according to a cough-nature, symptom-based approach into 3 groups (‘wet cough,’ ‘dry cough’ and ‘no cough.’) We compared rates of lower airway infection (with bacteria, viruses and viral-bacterial co-infection) in the 3 groups and explored the relationship with lower airway neutrophilic inflammation.

2.2 Wet cough in Children: Infective and Inflammatory Characteristics in Bronchoalveolar Lavage Fluid

Wet Cough in Children: Infective and Inflammatory Characteristics in Broncho-Alveolar Lavage Fluid

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Summary. Wet cough is a common feature of many disease processes affecting children. Our aim was to examine the relationships between cough nature, lower airway infection (bacterial, viral, and viral–bacterial) and severity of neutrophilic airway inflammation. We hypothesized that viral–bacterial co-infection of the lower airway would be associated with wet cough and heightened neutrophilic airway inflammation. We prospectively recruited 232 children undergoing elective flexible bronchoscopy. Participants were grouped using a cough nature symptom-based approach, into wet, dry or no cough groups. Broncho-alveolar lavage (BAL) and clinical data, including presence, nature, and duration of cough and key demographic factors, were collected. Children with wet cough (n = 143) were more likely to have lower airway bacterial infection (OR 2.6, $P = 0.001$), viral infection (OR 2.04, $P = 0.045$) and viral–bacterial co-infection (OR 2.65, $P = 0.042$) compared to those without wet cough. Wet cough was associated with heightened airway neutrophilia (median 19%) as compared to dry or no cough. Viral–bacterial co-infection was associated with the highest median %neutrophils (33.5%) compared to bacteria only, virus/es only and no infection (20%, 18%, and 6%, respectively, $P < 0.0001$). Children with wet cough had higher rates of lower airway infection with bacteria and viruses. Maximal neutrophilic airway inflammation was seen in those with viral–bacterial co-infection. Cough nature may be a useful indicator of infection and inflammation of the lower airways in children. **Pediatr Pulmonol.**

Key words: airway inflammation; bacterial infections; bronchitis; respiratory tract; viral infections; co-infection.

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Conflict of interest: None.

Contributor's Statement Page:

- Danielle F. Wurzel: Dr. Wurzel was responsible for data analysis and manuscript preparation and was involved in data collection.
- Julie M. Marchant: Dr. Marchant co-conceptualized the study and assisted in data analysis and manuscript preparation.
- Julia E. Clark: Dr. Clark critically reviewed the manuscript and assisted in interpretation of findings.
- Stephanie T. Yerkovich: Dr. Yerkovich assisted in data analysis and manuscript preparation.
- John W. Upham: Professor Upham assisted in manuscript preparation.
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- Anne B. Chang: Professor Chang conceptualized the study and was involved in all aspects of the study design, data collection, data analysis and manuscript preparation.
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INTRODUCTION

Chronic wet cough is the presenting feature of several different disease processes in children, ranging from viral respiratory tract infections to bronchiectasis.[1] Wet cough is an important discriminating sign in children presenting for evaluation of respiratory symptoms.[2] Unlike the symptom of wheeze,[3] wet cough is reliably reported by parents when compared to clinician's assessment and bronchoscopic findings.[4]

The association between chronic wet cough, bacterial infection and extent of lower airway suppuration has been described in prospective[2,5] and retrospective studies.[6–8] However, these studies have been limited by lack of a comparative group or small cohorts. Furthermore, there are no studies to date that report on common respiratory viral pathogens associated with chronic wet cough, nor on the rates of lower airway infection in children without cough. Beyond the field of cystic fibrosis (CF), research into the role of viral–bacterial co-infection and cough in children is scarce.

Neutrophilic inflammation of the lower airways is common in children with chronic wet cough.[9] It is also a key factor in Cole's ''vicious circle'' hypothesis on the pathogenesis of bronchiectasis whereby infection, inflammation, and impairment of mucociliary clearance mechanisms are responsible for airway destruction.[10] The effectiveness of antimicrobials in the treatment of chronic wet cough in children has been shown in cohort studies[7,8,11] and further supported by a recent double-blinded randomized controlled trial.[12]

In this study, 232 children were assigned to symptom-based cough groups and associations between cough presence and nature (i.e., wet or dry), lower airway infection (viral and/or bacterial), and inflammation were evaluated. We hypothesized that neutrophilic inflammation of the lower airways is greater in children with wet cough, as compared to those with dry cough or no cough, and is most intense in the presence of viral–bacterial co-infection.

ABBREVIATIONS:

BAL	Broncho-alveolar lavage
BALF	Broncho-alveolar lavage fluid
BLP	Beta-lactamase positive
BLN	Beta-lactamase negative
CF	Cystic fibrosis
RSV	Respiratory syncytial virus
hMPV	Human metapneumovirus
PCR	Polymerase chain reaction
PBB	Protracted bacterial bronchitis
TCC	Total cell count

MATERIALS AND METHODS

Study Design

Any child referred by a pediatric respiratory physician for elective flexible bronchoscopy (between March 2008 and November 2011) was eligible for inclusion. The single exclusion criterion was CF, as determined by sweat chloride level and/or CF gene mutation analysis. Two hundred fifty-four children were enrolled and BAL data was available for 232 children. Cough category, that is, ''wet,'' ''dry,'' or ''no cough'' was determined by parental assessment (on the day of bronchoscopy) as previously done[2,11] and validated.[4]

The majority of children enrolled in the study had recurrent or chronic (>4 weeks) cough (71.1%). Other common indications for bronchoscopy included: stridor or recurrent croup (7.8%), wheeze or noisy breathing (3.4%) and chronic X-ray changes or recurrent pneumonias (3.1%). Thirty-six (15.5%) participants had radiologically proven bronchiectasis. All children underwent pre-anesthetic assessment by an anesthetist who did not allow performance of bronchoscopy in children with an acute respiratory illness. The Queensland Children's Health Services Ethics Committee approved the study and all parents provided written informed consent.

A standardized clinical questionnaire was undertaken on the day of the bronchoscopy. This included current respiratory symptoms (with detailed questions regarding cough presence or absence and cough nature), past medical history and relevant demographic factors including household cigarette smoking.

Flexible bronchoscopy (Olympus; Tokyo, Japan) was performed under general anesthesia. BAL was obtained as per European Respiratory Society guidelines.[13] Sterile normal saline solution, in three aliquots of 1 ml/kg (maximum 20 ml), was instilled into the most affected area or into the right middle lobe in patients with generalized disease. Gentle suction was applied and the sample was collected in a mucus trap. To reduce upper airway contamination, suction through the bronchoscope was avoided until the tip was wedged in the appropriate segment.

The first aliquot was used for microbiological assessment; the second and third were pooled for cellularity studies. Microbiological assessment included quantitative aerobic bacterial cultures, specific testing for mycobacterial species using inoculation of Lowenstein–Jensen media, and PCR techniques for mycoplasma.

Positive bacterial culture was defined as growth of $\geq 10^5$ colony-forming units (cfu)/ml.[9] Bacteria considered as significant respiratory pathogens included: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Enterobacteriaceae, Mycobacteria, Aspergillus species, and *Mycoplasma pneumonia*.[14]

Detection of respiratory viruses was undertaken using polymerase chain reaction (PCR), as previously described,[15] to detect respiratory syncytial virus (RSV), adenovirus, parainfluenza virus (Types 1–3), influenza and human metapneumovirus (hMPV). A total cell count (TCC) was performed and cell differential profile (minimum 400 cells counted—modified Wright's stain, Diff Quik, Lab Aids, Narrabeen, NSW, Australia) as described in prior studies.[9]

Statistical Analyses

Statistical analysis was carried out using IBM SPSS Statistics (Version 20.0, IBM Corp., Armonk, NY). Medians and inter-quartile ranges were reported as data were non-normally distributed. Mann–Whitney's *U*-test was used for two group comparisons and Kruskal–Wallis test for >2 groups comparisons of continuous variables. Pearson's chi-square test was used for categorical variables, with continuity correction used for 2 × 2 analyses. Children were divided into three groups for the purposes of analysis: "wet cough," "dry cough," and "no cough." For calculation of odds ratios and confidence intervals, "dry cough" and "no cough" groups were combined as a single group and compared to the "wet cough" group. For all analyses, we used two-sided tests, with *P*-values < 0.05 denoting statistical significance.

RESULTS

Participants

The median age of the 232 children (151 male, 81 female) recruited to the study was 26.5 months (IQR 15, 62). Children with dry cough were significantly older than those with wet or no cough and were more likely to be receiving corticosteroid medication. The median duration of cough overall was 28 weeks (8, 58); that in the wet cough group was 28 weeks (11.5, 56.3) and dry cough was 52 weeks (6, 108). There were no other significant differences in baseline characteristics between groups (Table 1).

Of the 232 BAL results, 115 (49.6%) had positive bacterial cultures ($\geq 10^5$ cfu/ml) and/or one or more virus detected. Of the total group, 94 (40.5%) cultured one or more bacterial species, 50 (21.6%) were PCR positive for one or more virus and 29 (12.5%) had co-infection with both bacteria and virus/es (Fig. 1).

Bacteria

The proportion of children that had positive culture ($\geq 10^5$ cfu/ml) of one or more bacterial species varied significantly between groups; 70/143 (49%) in the wet cough group, 5/18 (27.8%) in the dry cough group, and 19/71 (26.8%) in the no cough group ($P = 0.004$). *H. influenzae* was the predominant pathogen in both the wet and dry cough groups, being present in 49 of 143 (34.2%) of the wet cough group and 4 of 18 (22%) of the dry cough group and 7 of 71 (9.9%) of the no cough group ($P = 0.001$). A large proportion of *H. influenzae* strains isolated were B-lactamase positive, with 17/49 (34.7%) in the wet cough group, only 1/7 (14.3%) in the no cough group, and none in the dry cough group ($P = 0.012$).

M. catarrhalis was the second most common organism in the wet cough group, present in 26/143 (18.2%), followed by *S. pneumoniae* in 22/143 (15.4%) and *S. aureus* in 8/143 (5.6%). In the dry cough group, *S. pneumoniae* was present in 1 of 18 (5.6%). In the no cough group, *M. catarrhalis* was isolated in 7 of 71 (9.9%), *S. aureus* in 3 of 71 (4.2%), *S. pneumoniae* 4 of 71 (5.6%) and *Escherichia coli* 1 of 71 (1.4%). Of the 209 children where data for *M. pneumoniae* PCR were available, 4 were positive (3 with wet cough, 1 with no cough). None in the "dry cough" group had *M. pneumoniae*.

Antibiotic use in the 24 hr prior to the bronchoscopy appeared to be associated with lower rates of bacterial infection, however, this was not statistically significant ($P = 0.062$). Only nine of 36 (25%) children who received antibiotics were positive for bacteria compared to 84/194 (43.3%) who did not receive antibiotics being positive (two missing data-points for antibiotic usage).

TABLE 1— Background Characteristics of Study Participants According to Cough Group

Characteristic	Wet cough, n = 143, n (%)	Dry cough, n = 18, n (%)	No cough, n = 71, n (%)	*P*-value[a]
Sex, M:F (% male)	94:49 (66)	11:7 (61)	46:25 (65)	0.926
Median age, mo (IQR)	26 (15, 60)	66 (31, 159)	25 (11, 59)	**0.001**
Household tobacco smoke exposure	55 (39)	2 (12)	22 (32)	0.069
Siblings—1+	115 (80)	16 (89)	55 (78)	0.551
Indigenous status[b]	9 (6)	1 (6)	7 (10)	0.837
Bronchiectasis (radiologically proven)	24 (17)	2 (11)	10 (14)	0.759
Antibiotics prior 24 hr	28 (20)	2 (12)	6 (9)	0.092
Corticosteroid use (oral or inhaled)	n = 118, 22 (19)	n = 11, 5 (46)	n = 64, 8 (13)	**0.031**

[a]*P*-value tests whether all three groups have the same percentage, median, or frequency. *P*-values < 0.05 considered significant and denoted in bold.
[b]Three in wet cough group and one in no cough group had unknown indigenous status.

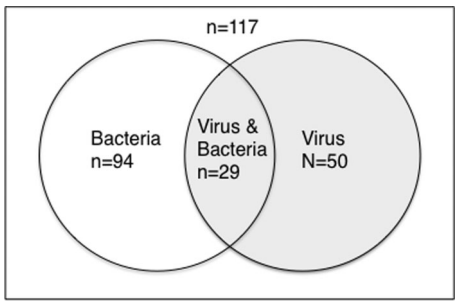

Fig. 1. Venn diagram illustrating relative number of participants demonstrating lower airway infection (bacterial, viral, and viral–bacterial) in BALF.

Viruses

One or more viruses were detected in 37/143 (25.9%) of the wet cough group, 1/18 (5.6%) of the dry cough group, and 12/71 (16.9%) of the no cough group ($P = 0.074$). Adenovirus was the most common virus isolated in the BAL samples across cough categories. In those with wet cough, 21 of 143 (14.7%) were positive for adenovirus, with 1/18 (5.6%) and 5/71 (7%) positive in the dry and no cough groups ($P = 0.183$). Parainfluenza was the second most common virus, present in 9/143 (6.3%), none and 3/71 (4.2%) in the wet, dry, and no cough groups, respectively ($P = 0.478$). RSV was present in 6 (4.2%), 0 and 2 (2.8%) participants in each of the wet cough, dry cough and no cough groups respectively ($P = 0.616$). Influenza was present uncommonly, with 2 (1.4%) in the wet cough and 2 (4.2%) in the no cough groups ($P = 0.636$). hMPV was isolated in a single participant in the wet cough group (0.7%).

To account for seasonal influences on viral carriage, the season in which the bronchoscopy was performed was compared to each individual virus. Parainfluenza was the only virus whose presence was associated with a particular season, being detected significantly more frequently in winter ($P = 0.038$).

Viral–Bacterial Co-Infection

Viral–bacterial co-infection was identified in 23/143 (16.1%) of children with wet cough, 1/18 (5.6%) with dry cough, and 5/71 (7%) with no cough ($P = 0.110$). The most common viral–bacterial combination was adenovirus and *H. influenzae*, with 13 of 29 (44.8%) having this combination, of which the majority, 10 of 13 (76.9%) had wet cough (Table 2). Due to small participant numbers in the no cough group, dry and no cough categories were combined into a single category in Table 3.

TABLE 2— Patterns of Viral–Bacterial Co-Infection and Number With Wet Cough

Co-infection (viral–bacterial)	No. of children	No. with wet cough
Adenovirus and *H. influenzae*[a]	13	10
RSV and *H. influenza*[b]	4	4
Parainfluenza and *H. influenzae*[c]	3	2
Adenovirus and *S. pneumoniae*	2	2
Parainfluenza and *M. catarrhalis*[d]	2	2
Adenovirus and *M. catarrhalis*[e]	1	0
Parainfluenza and *S. pneumoniae*	1	0
Parainfluenza and *M. pneumonia*	1	1
Influenza and *M. catarrhalis*	1	1
Influenza and *S. aureus*	1	1
Total	29	23

[a]Four also had *M. catarrhalis*, 3 had *S. pneumonia*, 1 *S. aureus*.
[b]Two also had *S. pneumoniae*, 2 had *M. catarrhalis*.
[c]One also had *S. aureus* and *S. pneumonia*.
[d]One also had adenovirus.
[e]One also had *E. coli*.

Airway Cellularity

Cellularity data were available for 212 participants. Median TCC differed significantly across cough groups with wet cough having the highest median TCC, followed by the dry cough and no cough groups (Table 4). Wet cough also had the highest median %neutrophils, and the lowest median %macrophages. Only 30 (14.2%) participants had any eosinophils identified (range: 1–16%). Of these children, 14 (6.6%) had clinically significant eosinophilia defined as %eosinophils on BAL >2.5%.[16] Nine had wet cough, of which three were receiving inhaled corticosteroids and/or beta-agonists. There was no significant difference in eosinophil count across cough groups ($P = 0.759$).

When grouped in accordance with infection category (Fig. 2), median %neutrophils were highest in the viral–bacterial co-infection group followed by the bacterial, viral and no infection groups ($P < 0.0001$). Conversely, median %macrophages were highest in the no infection group and lowest in the viral–bacterial co-infection group ($P = 0.002$). There was no significant difference in median %lymphocytes across groups ($P = 0.349$). No significant differences in %eosinophils were seen across infection groups ($P = 0.111$), there were insufficient numbers to generate a boxplot.

Children with no cough and bacterial infection had similar airway %neutrophils and TCC to those with no cough without bacterial infection. Airway %neutrophils (median 4% "no bacteria" vs. 4.5% "bacteria present," $P = 0.976$) and TCCs (median 137×10^6/L vs. 155×10^6/L, $P = 0.37$) showed no significant difference.

Corticosteroid use was identified in 35 patients at the time of BAL but did not influence airway cellularity (results not shown).

TABLE 3— Cough Categories Versus Lower Airway Infection ("Dry Cough" and "No Cough" Groups Combined)

	Wet cough			
	Yes, n = 143, n (%)	No, n = 89, n (%)	Odds ratio (95% CI)	*P*-value[a]
No infection	59 (41.3)	58 (65.2)	0.38 (0.22, 0.65)	**<0.0001**
Any bacterial species	70 (49)	24 (27)	2.60 (1.47, 4.60)	**0.001**
H. influenzae	49 (34.3)	11 (12.4)	3.65 (1.78, 7.50)	**<0.0001**
H. influenzae (BLP)	17 (11.9)	1 (1.1)	11.87 (1.55, 90.86)	**0.017**
H. influenzae (BLN)	32 (22.4)	10 (11.2)	2.28 (1.06, 4.90)	**0.035**
M. catarrhalis	26 (18.2)	7 (7.9)	2.60 (1.08, 6.28)	**0.033**
S. pneumoniae	22 (15.4)	5 (5.6)	3.06 (1.11, 8.39)	**0.030**
S. aureus	8 (5.6)	3 (3.4)	1.70 (0.44, 6.58)	0.443
Any viral species	37 (25.9)	13 (14.6)	2.04 (1.02, 4.10)	**0.045**
Adenovirus	21 (14.7)	6 (6.7)	2.38 (0.92, 6.15)	0.073
Parainfluenza	9 (6.3)	3 (3.4)	1.93 (0.51,7.31)	0.336
RSV	2 (1.4)	6 (6.7)	0.53 (0.10, 2.66)	0.436
Influenza	2 (1.4)	2 (2.2)	0.62 (0.09,4.46)	0.632
Viral–bacterial co-infection	23 (16.1)	6 (6.7)	2.65 (1.04, 6.80)	**0.042**
Adenovirus and *H. influenza*	10 (7)	3 (3.4)	2.16 (0.58, 8.06)	0.383

BLP, beta-lactamase positive; BLN, beta-lactamase negative.
[a]*P*-values < 0.05 denoted in bold.

DISCUSSION

Findings from this large prospective cohort study indicate that rates of lower airway infection with bacteria and/or viruses are significantly higher in children with wet cough compared to those with dry or no cough. In addition, airway neutrophilia is higher in children with wet cough and most heightened in the presence of viral–bacterial co-infection. The use of a symptom-based approach increases the relevance and utility of our findings to clinical practice.

Our prior studies reporting BAL findings in children with wet cough have largely focused on protracted bacterial bronchitis (PBB), one of the most common causes of chronic wet cough in children,[11] findings which have subsequently been supported by retrospective studies.[7,8,17] This study's cohort of children was recruited after our previously studied cohorts and included those with a range of clinical indications. One of the strengths of this study is the use of cough groupings, that is, "wet cough," "dry cough," and "no cough," in place of diagnostic categories. It is well known that significant symptom overlap exists between common pediatric respiratory conditions causing cough.[7,11] Our rationale was that symptom-based categories are likely to be more objective and repeatable than diagnostic categories. Children with CF were excluded due to their exaggerated airway inflammatory responses and unique microbiota.[18,19] We included children with non-CF bronchiectasis, the majority of whom had idiopathic bronchiectasis, as their airway cellularity and microbiota are similar to children with PBB.[20] Our justification for including these children was to reflect the diverse spectrum of disease presentation with wet cough and increase the value and general applicability of our findings.

Persistent lower airway neutrophilic inflammation is known to be important in the pathogenesis of many

TABLE 4— Differential BAL Cell Count According to Cough Category

	Wet cough, n = 132	Dry cough, n = 15	No cough, n = 65	*P*-value[a]
Total cell count ($\times 10^6$/L)	200 (108, 381)	185 (101, 414)	140 (82, 268)	**0.016**
Neutrophil %[b]	19 (6, 53)	11 (8, 36)	4 (2,11)	**<0.0001**
Macrophage %	63 (39, 83)	74 (44, 88)	83 (69, 92)	**<0.0001**
Lymphocyte %	9 (5, 17)	8 (3, 15)	7 (5, 15)	0.612
Eosinophil %[c]	0 (0, 0)	0 (0, 0)	0 (0, 0)	

All values within cough categories given as median (IQR).
Median cell counts/% rounded to nearest whole number.
Due to a lack of standardization of BAL sampling and processing methods, there is currently no international consensus on normal BAL cell counts in children.[13] We have quoted cutoffs used in our center.
[a]*P*-values calculated using Kruskal–Wallis test to compare medians across three groups. *P*-values < 0.05 denoted in bold.
[b]Neutrophilia defined as >6.5% neutrophils in BAL fluid.[11]
[c]Eosinophilia defined as >2.5% eosinophils in BAL fluid.[16]

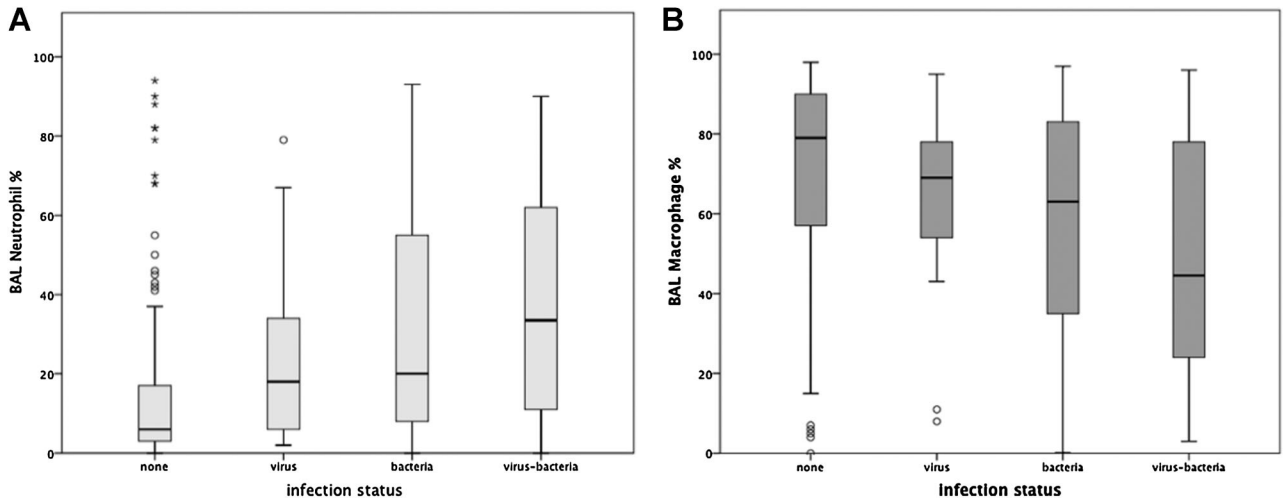

Fig. 2. A and B: Boxplots illustrating median (A) %neutrophils and (B) %macrophages according to infection presence and type on BAL; no infection, virus, bacteria and both virus and bacteria. Of the outliers (represented by asterisks/open circles in (A), most had wet cough (11 of 16). Five had elevated (>2.5%) eosinophils on BAL. Cough improved or resolved in 9 who received antibiotics. It is likely that some of these children had undetected bacterial infection (i.e., $<10^5$ cfu/ml), asthma or other conditions such as aspiration, to explain these elevated neutrophil counts.

respiratory diseases.[21,22] Neutrophilic inflammation is a common pathological feature of diseases such as COPD, bronchiectasis, and severe asthma.[22] Its effect on the growing lung may be particularly relevant. It is postulated that recurrent or prolonged episodes of wet cough are antecedent to bronchiectasis,[20,23] with neutrophilic inflammation being the presumed mechanism. Therefore, our findings of neutrophilic inflammation in symptom-based ''wet cough'' groupings may be clinically relevant and important.

Research on viral–bacterial co-infection in the lower airway epithelium suggests a role for increased pathogen adherence and delayed clearance,[24] with impairment of mucociliary clearance being an important contributor.[25] Clinically, viral–bacterial co-infection is thought to play an important role in the pathogenesis of respiratory diseases such as severe RSV bronchiolitis,[26] pneumonia,[27] otitis media,[28,29] and sinusitis.[30,31] We have shown that viral–bacterial co-infection is more common in children with (compared to those without) wet cough, and that it causes a heightened neutrophilic airway response. It is possible that viral–bacterial co-infection may also play a role in the pathogenesis of bacterial bronchitis and further research is needed.

Interestingly, in this study, the same organisms were identified in children with and without wet cough (Table 3) albeit with varying frequencies. We observed higher rates of beta-lactamase positive *H. influenzae* in the wet cough compared to the no wet cough group. This finding may be explained by antibiotic selection pressure (these children may have had greater cumulative antibiotic exposure), however, we did not specifically examine for this. Lower airway infection rates in the cough-free group were also interesting. Over a quarter (27%) of cough-free children had bacteria detected with no significant increase in their median %neutrophils in BAL (median neutrophils: 4% in those without bacteria vs. 4.5% in those with bacteria). This indicates that bacteria were present without signs of active infection, that is, cough or neutrophilia. Upper airway contamination of the bronchoscope may explain this finding, however, the higher threshold of $\geq 10^5$ cfu/ml for diagnosis of bacterial infection used in this study should minimize such interference. Viruses were detected in 16.9% of the cough-free children despite having no acute viral lower respiratory tract infection symptoms. Together, these findings support recent literature suggesting that the lung has a characteristic microbiota, both in health and disease,[32,33] and is unlikely to be sterile, as previously believed.

The high prevalence of virus in the BAL has to be interpreted with caution in the context that viruses detected by PCR can be found in up to 33% of asymptomatic children.[34] Nevertheless, the role of viruses in the pathogenesis of chronic cough in children is the focus of further research by our group at present. Preliminary results show that viral infection probably plays an important role in the initiation of conditions such as PBB (results not yet published), the most common cause of chronic wet cough in children.[11,17]

There were a number of relevant negative findings from our study. Firstly, we did not find a statistically

significant inverse relationship between antibiotic use (in the 24 hr prior to bronchoscopy) and significant bacterial growth ($\geq 10^5$ cfu/ml on BAL). However, our study was not powered to detect this difference, and we would expect a significant association with larger participant numbers. Secondly, there was no difference in airway lymphocyte nor eosinophil counts across infection groups, even in the presence of viral infection, nor was there any association between corticosteroid use and cellularity. However, 9 of the 14 children with airway eosinophilia on BAL had wet cough; 3 of these were receiving inhaled corticosteroids and/or beta-agonist at time of bronchoscopy. Although not specifically examined in this paper, bacterial infection has recently been found to be associated with wheeze (and possibly later development of asthma).[35–37] We believe that asthma and PBB co-exist in some children, and the two conditions are not mutually exclusive.

There are some important limitations of this study. Firstly, due to the ethical issues in obtaining control BAL data in children, we used a comparator rather than a control group. This comparator group was children without cough having bronchoscopy for investigation of other respiratory symptomatology, for example, stridor. It is difficult to predict the effect of these ''other respiratory symptomatology'' on the infective and inflammatory properties of the lower airway but we would expect a reduction in the strength of the observed associations. This factor may explain the large number of cough-free children having bacteria and viruses detected. Secondly, we did not undertake PCR for human rhinovirus (HRV), a very common upper airway virus. The importance of HRV in the lower airways of children remains unknown. Thirdly, as bronchoscopies were performed for clinical indications only, the majority of participants had only a single BAL performed. Without multiple lower airway specimens over time, we were unable to attribute causality. Lastly, we did not look specifically at biomarkers of neutrophilic inflammation, such as IL-8.

In conclusion, our study has several important and novel findings. Foremost, wet cough is associated with higher rates of lower airway infection, with bacteria and viruses, compared to dry or no cough, with similar organisms present across cough groups. Bacteria and/or viruses are commonly present in the lower airway of children without symptoms of viral respiratory tract infection and who are cough-free. Viral–bacterial co-infection of the lower airways is associated with a significantly heightened neutrophilic airway response. Lastly, a direct correlation exists between cough nature and neutrophilic airway inflammation; children with wet cough have greater neutrophilic inflammation compared to children with dry cough.

Our symptom-based approach shows that cough nature in children correlates with severity of underlying airway inflammation and neutrophilic response as found in BAL, highlighting the importance of accurate characterization of cough during pediatric respiratory consultations. The finding of high rates of lower airway infection associated with wet cough may assist clinicians in making management decisions and inform future research in this area.

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CHAPTER 3

3.1 INTRODUCTION TO CHAPTER 3

In Chapter 2 we demonstrated that children with wet cough have higher rates of lower airway infection with bacteria, viruses and viral-bacterial co-infection. We have also shown that children with viral-bacterial co-infection have the greatest neutrophilic lower airway inflammation, raising the possibility of microbial synergy. To date, research into chronic wet cough in children has focused on bacterial pathogens. The role of viral infections therefore needed further evaluation.

Nasopharyngeal aspirate (NPA) is a common sampling method for detection of respiratory tract viruses in children. Unlike bronchoalveolar lavage (BAL), a positive NPA does not necessarily reflect presence of virus in the lower respiratory tract. However, BAL is significantly more invasive than NPA. The level of agreement between NPA and BAL for the detection of respiratory viruses in children with non-acute respiratory illness had been poorly studied. Further, the relationship between virus presence in the airways (as detected by PCR) and lower airway neutrophilic inflammation needed further elucidation.

Specific viruses may exert greater influence on airway inflammation and may have relevance to the pathogenesis of certain chronic respiratory disorders in children. In this study, we tested for an extended panel of viruses in paired upper (NPA) and lower (BAL) respiratory tract samples from children undergoing bronchoscopy and BAL for assessment of chronic respiratory symptoms. The final diagnosis in the majority of the children in the study (64%) was PBB or bronchiectasis whilst those remaining (36%) had either no specific diagnosis or an airway abnormality. Of those with no final diagnosis, most (55%) were being investigated for chronic cough (73% had wet cough, 27% had dry cough) whilst the remainder had complaints of stridor, persistent wheeze, noisy breathing, haemoptysis or persistent auscultatory findings.

3.2 Respiratory Virus Detection in Nasopharyngeal aspirate versus Bronchoalveolar Lavage is Dependent on Virus Type in Children with Chronic Respiratory Symptoms

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Respiratory virus detection in nasopharyngeal aspirate versus bronchoalveolar lavage is dependent on virus type in children with chronic respiratory symptoms

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ABSTRACT

Background: The comparative yield of respiratory virus detection from nasopharyngeal aspirate (NPA) versus bronchoalveolar lavage (BAL) is uncertain. Furthermore, the significance of virus detection and its relationship to lower airway neutrophilic inflammation is poorly studied.
Objectives: To evaluate the sensitivity, specificity and predictive values of NPA for detecting respiratory viruses in BAL; and to determine the relationship between viruses and lower airway neutrophilia in children with non-acute respiratory illness.
Study design: 150 paired NPA and BAL samples were obtained from 75 children aged <18 years undergoing flexible bronchoscopy for investigation of chronic respiratory symptoms. Viral studies were performed using polymerase chain reaction (PCR). Cellularity studies were performed on BALs. Diagnostic parameters of NPA compared to BAL and associations between viruses and lower airway %neutrophils were evaluated.
Results: NPA had a higher yield than BAL for detection of any respiratory virus (52 versus 38, respectively). NPA had a high sensitivity (92%) and low specificity (57%) for detecting HRV in BAL with poor kappa agreement value of 0.398 (95% CI 0.218–0.578, $p < 0.001$). NPA had a fair sensitivity (69%) and good specificity (90.3%) for detecting HAdV on BAL, kappa agreement was 0.561 (95% CI 0.321–0.801, $p < 0.001$). HAdV positivity on NPA, compared to negativity, was independently associated with heightened airway neutrophilia [mean difference (95% CI): 18 (1,35); $p = 0.042$].
Conclusions: NPA has a higher yield for respiratory virus detection than BAL, however its diagnostic accuracy is dependent on viral species. Adenovirus positivity is associated with significantly heightened lower airway neutrophilia in children with chronic respiratory symptoms.

1. Background

Accurate identification of respiratory pathogens is important in clinical medicine and research. In bacteriology, upper airway sampling can misrepresent the lower airway microbiota [1,2]. In contrast, there is little such comparative data for virology. Nasopharyngeal aspirates (NPA) or nasal washes are regarded as the specimen of choice for detection of upper respiratory tract viruses by polymerase chain reaction (PCR) [3,4]. Upper airway sampling is relatively simple, can be performed at the bedside and is minimally invasive. In contrast, lower airway sampling

Abbreviations: NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; RVI, respiratory virus infection; HRV, human rhinovirus; HAdV, human adenovirus; RSV, respiratory syncytial virus; IFAV, human influenza A virus; IFBV, human influenza B virus; HPIV1–3, human para-influenza virus 1–3; hMPV, human metapneumovirus; HBoV, human bocavirus; HCoV – NL63, OC43, 229E, HKU1, human coronaviruses; WUPyV, WU polyomavirus; KIPyV, KI polyomavirus.

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in young children is invasive, requiring a bronchoalveolar lavage (BAL).

Traditionally, BAL has been considered the 'gold-standard' for the microbiological diagnosis (viral and bacterial) of lower respiratory tract infection in children. However, as BAL requires specialist input and is often performed under anesthesia, it is reserved for patients with complex or severe disease, e.g., in the setting of intensive care; immune-compromise or in children with chronic or recurrent respiratory tract symptoms. The disparities in ease of collection of NPA versus BAL, raise two important questions. Firstly, what is the comparative yield of NPA compared to BAL for respiratory virus detection in children? Secondly, what is the significance of a positive NPA with respect to lower airway inflammation? Given the lack of studies addressing these important questions [5,6], we evaluated paired NPA and BAL specimens in 75 children.

2. Objectives

To compare the yield and diagnostic parameters of respiratory virus detection on NPA versus BAL using molecular methods, and to evaluate whether virus positivity on NPA correlates with lower airway neutrophilic inflammation and by inference, active viral pulmonary disease.

3. Study design

The Queensland Children's Health Services Ethics Committee approved the study and written informed consent was obtained from each parent or guardian. Children undergoing flexible bronchoscopy, for any clinical indication, as arranged by their treating physician, were eligible. Samples evaluated in this article were obtained from children recruited to a larger cohort study on chronic cough in children [7]. Caregivers completed a standardized clinical questionnaire on the day of bronchoscopy including current respiratory symptoms and relevant demographics. Children with symptoms of significant acute lower respiratory tract infection, e.g., high fever, tachypnea/shortness of breath, wheeze or rattly chest were deemed, by an anesthetist, to be unfit for anesthesia and excluded from the study.

Contemporaneous NPA and BAL sampling was performed under general anesthesia. NPA was collected first, using a disposable catheter connected to a mucous trap. Dry nasopharyngeal suction, via both nares, was performed, followed by suction of 2–3 ml of sterile normal saline directly into the suction catheter to rinse through remaining contents.

Bronchoscopic BAL was then performed using standardized methods as per European Respiratory Society guidelines [8]. Sterile normal saline, in three aliquots of 1 ml/kg (maximum 20 ml), was instilled into the most affected area, or right middle lobe in patients with generalized disease. To minimize upper airway contamination, suction through the bronchoscope was avoided until the tip had entered the distal airways. The first aliquot was used for microbiological testing, the second and third were pooled for cellularity studies.

Viral studies were undertaken using real-time PCR techniques, as described previously [9–13], to detect 16 respiratory virus types and subtypes. These included human rhinoviruses (HRV), human adenoviruses (HAdV), respiratory syncytial virus (RSV), human influenza A virus (IFAV), human influenza B virus (IFBV), human para-influenza virus 1–3 (HPIV1–3), human metapneumovirus (hMPV), human bocavirus (HBoV), human coronaviruses (HCoV – NL63, OC43, 229E, HKU1), WU polyomavirus (WUPyV) and KI polyomavirus (KIPyV). BAL specimens were refrigerated immediately and processed within 24 h. NPA specimens were stored at −70 °C prior to processing.

We defined positive bacterial culture as growth of $\geq 10^5$ colony-forming units (cfu)/ml [10] of any of the following pathogens on BAL: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or Enterobacteriaceae. Total cell count and differential profile (minimum 400 cells counted) were performed on BAL as described previously [14].

3.1. Statistical analyses

Statistical analyses were carried out using IBM SPSS (v20, San Francisco, USA). Medians and inter-quartile ranges were reported as data were non-normally distributed. Kappa analyses were used to assess levels of agreement between sampling techniques. Comparisons of categorical variables were performed using Pearson's χ^2 test or Fisher exact test if any category had an expected value of <5. Mann–Whitney *U* test was used for two group comparisons and Kruskal–Wallis test for >2 groups comparisons of continuous variables. Multiple linear regression was employed to adjust for virus co-detection and bacterial infection. A two-tailed *p*-value <0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values and likelihood ratios were manually calculated.

4. Results

Between February 2010 and May 2012, paired NPA and BAL samples were obtained from 75 children (median age = 33 months, IQR 16, 69; male $n = 50$, 66.7%). One-third of children ($n = 25$, 33.3%) were exposed to household cigarette smoke. Chronic cough (≥4 weeks) was reported in 45 participants (60%) at time of bronchoscopy. The major diagnoses within the cohort included: protracted bacterial bronchitis ($n = 31$, 41%) and bronchiectasis ($n = 17$, 23%); the remaining participants had either no specific diagnosis ($n = 22$, 29%) or a congenital airway abnormality ($n = 5$, 7%). The most common viruses detected on NPA were HRV ($n = 44$) and HAdV ($n = 15$). In regard to seasonality, HAdV positivity on BAL was more likely to occur in spring or summer than winter or autumn ($p = 0.004$) using chi-square analysis. HRV detections followed a bimodal distribution with peak detections occurring in spring and autumn.

4.1. Comparison of yield on paired NPA and BAL samples

Of the 75 paired NPA and BAL samples, 52 (69.3%) children had one or more virus detected from NPA and 38 (50.7%) had one or more virus from BAL. When considering detection of any virus, 32 (42.7%) NPA-BAL pairs were concordant PCR-positive, 17 (22.7%) pairs were concordant PCR-negative and 26 (34.7%) pairs had discordant PCR results. Of the discordant pairs, most ($n = 20$, 76.9%) were PCR positive on NPA but negative on BAL. Detection rates varied among the different viruses (Table 1). Most marked was the difference between NPA and BAL for HRV. Using positive PCR on BAL as an arbitrary gold standard, we calculated sensitivity, specificity, positive and negative predictive values and likelihood ratios (Table 1). HRV and HAdV were the most common viruses detected from NPA and BAL. Levels of agreement between NPA and BAL varied among the different viruses. The agreement between NPA and BAL for HRV was poor, kappa = 0.398 (95%CI 0.218, 0.578, $p < 0.001$) but good for HAdV at 0.561 (95%CI 0.321, 0.801, $p < 0.001$).

4.2. Relationship between virus positivity and BAL neutrophilia

The median lower airway %neutrophils were significantly higher in children who were NPA-positive for any virus compared to

Table 1
Absolute number of virus detections by PCR from paired NPA/BAL samples ($n = 75$) with diagnostic test parameters (BAL as the arbitrary gold-standard).

NPA/BAL	HRV	HAdV	Other[a]	HBoV	HCoV	WUPyV/KIPyV	Total[b]
NPA+	44	15	5	7	4[c]	4	52
BAL+	24	13	4	4	3[c]	1	38
NPA+/BAL−	22	6	1[d]	5	3	4	20
NPA−/BAL+	2	4	0	2	2	1	6
NPA+/BAL+	22	9	4	2	1	0	32
NPA−/BAL−	29	56	70	66	69	70	17
Sensitivity	92%	69%	–	50%	33%	–	84%
Specificity	57%	90%	99%	93%	96%	–	46%
PPV	0.5	0.6	0.80	0.29	0.25	–	0.62
NPV	0.94	0.93	–	0.97	0.97	–	0.74
LR+	2.12	7.15	–	7.10	7.99	–	1.56
LR−	0.19	0.34	–	0.54	0.70	–	0.35
Kappa	0.40	0.56	0.88	0.32	0.25	0.02	0.30

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio.
[a] RSV, IFA/B, HPIV1-3, hMPV.
[b] Total no. of children with one or more viruses detected.
[c] HCoV-OC43.
[d] HPIV-1.

Table 2
Lower airway cellular differential versus virus status on NPA and BAL.

Lower airway cellular profile	NPA HRV+ ($n = 44$)	NPA HRV− ($n = 31$)	p-Value	NPA AdV+ ($n = 15$)	NPA AdV− ($n = 60$)	p-Value
TCCs ($\times 10^6$/L)	215 (106, 285)	240 (94, 360)	0.894	260 (168, 473)	200 (90, 290)	0.132
Neutr%	16 (6, 55)	13 (4, 32)	0.514	51 (15, 68)	12 (5, 36)	0.017
Macr%	60 (37, 82)	71 (29, 87)	0.605	46 (23, 74)	71 (41, 84)	0.075
Lymph%	6 (4, 13)	6 (3, 15)	0.812	5 (2, 11)	6 (4, 15)	0.183
Eosin%	0 (0, 0)	0 (0, 0)	0.262	0 (0, 2)	0 (0, 0)	0.314
Lower airway cellular profile	**BAL HRV+ ($n = 24$)**	**BAL HRV− ($n = 51$)**	**p-Value**	**BAL AdV+ ($n = 13$)**	**BAL AdV− ($n = 62$)**	**p-Value**
TCCs ($\times 10^6$/L)	210 (108, 413)	230 (95, 350)	0.645	240 (112, 485)	215 (94, 285)	0.534
Neutr%	16 (9, 63)	15 (5, 40)	0.457	44 (14, 70)	12 (5, 37)	0.077
Macr%	65 (27, 82)	68 (39, 84)	0.544	54 (25, 83)	70 (38, 84)	0.497
Lymph%	8 (5, 20)	5 (3, 14)	0.318	3 (2, 7)	7 (5, 15)	0.017
Eosin%	0 (0, 2)	0 (0, 0)	0.162	0 (0, 1)	0 (0, 0)	0.825

Abbreviations: Neutr%, percentage neutrophils; Macro%, percentage macrophages; Lymph%, percentage lymphocytes; Eosin%, percentage eosinophils. Cell counts expressed as median (interquartile range) and rounded to nearest whole number.

NPA-negative children (19 [IQR 7, 65] vs. 11 [IQR 3, 21.5]; $p = 0.045$). Total cell counts (TCCs), %macrophages, %lymphocytes and %eosinophils were similar across groups (data not shown). When evaluating the two most frequent viruses, HRV and HAdV (Table 2), presence of HAdV in the NPA was significantly associated with increased BAL neutrophilia ($p = 0.017$) whereas HRV status on NPA showed no association with %neutrophils ($p = 0.514$).

Similar to the NPA findings, the presence of any virus in the BAL was associated with higher median %neutrophils compared to the virus-negative state (23 [IQR 10, 67] vs. 11 [5,28], respectively; $p = 0.04$). Median lower airway TCC was higher in BAL virus-positive (235×10^6/L [IQR 115, 448]) versus virus-negative (160×10^6/L [58, 255]) groups ($p = 0.068$). No differences in lymphocyte counts was observed when considering total viruses, but for individual viruses, %lymphocytes were significantly lower in HAdV BAL positive compared to HAdV BAL negative participants ($p = 0.017$). Children who were HAdV positive on BAL had higher %neutrophils than those who were HAdV negative on BAL ($p = 0.077$). In comparison, %neutrophils were similar across HRV positive and negative groups on BAL ($p = 0.457$).

4.3. Regression analysis adjusting for virus co-detection and bacterial infection

On multi-variable analysis, HRV in either NPA or BAL showed no association with lower airway %neutrophils. In contrast, HAdV in NPA and/or BAL was significantly associated with increased %neutrophils on BAL (Table 3). Bacterial infection was detected in over half (32; 61.5%) of NPA virus positive participants with much lower rates (6; 26.1%) in virus negative participants ($p = 0.005$). Rates of bacterial infection in BAL virus positive (22; 57.9%) children were also greater than BAL virus negative children (16; 43.2%); however, this difference was not significant ($p = 0.204$). The predominant bacterial organisms were: *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*. Presence of bacterial infection as seen on BAL had the strongest independent influence on lower airway neutrophil counts (beta = 0.256 and 0.311 for NPA and BAL, respectively) as shown in Table 3.

5. Discussion

In this study, we systematically compared upper airway (NPA) to lower airways (BAL) sampling with regards to diagnostic yield for respiratory viruses in children with non-acute respiratory illness. We found that concordance of NPA with BAL is largely dependent on the virus being investigated. NPA has good concordance with BAL for detection of HAdV, but poor concordance for HRV. In comparison to BAL, sensitivity, specificity, PPV and NPV of the most frequently detected viruses on NPA differed according to the virus being investigated. Specificity was high for all viruses, except for HRV. The reverse was observed with regards to sensitivity. Lastly, presence of HAdV on NPA and/or BAL was associated with significantly heightened %neutrophils in the lower airway.

To our knowledge, three major studies have compared upper and lower airway specimens for respiratory virus detection. All were limited by unpaired specimens or focused on specific

Table 3
Multiple linear regression of mean difference in lower airway %neutrophils according to infective status on NPA and BAL.

	Unadjusted[a] mean difference in neutr% (IQR)	*p*-Value	Adjusted[b] mean difference in neutr% (IQR)	*p*-Value[c]	Beta[d]
NPA					
HAdV	20 (3, 37)	0.020	18 (1, 35)	0.042	0.245
HRV	5 (−9, 19)	0.486	−2 (−16, 12)	0.782	−0.033
Bacteria ≥ 10^5 cfu/ml (on BAL)	16 (3, 30)	0.016	15 (1, 28)	0.035	0.256
BAL					
HAdV	15 (−3, 32)	0.097	18 (0.3, 35)	0.046	0.235
HRV	4 (−10, 19)	0.550	0 (−14, 15)	0.950	0.007
Bacteria ≥ 10^5 cfu/ml (on BAL)	16 (3, 30)	0.016	18 (4, 31)	0.01	0.311

[a] Unadjusted mean difference in %neutrophils between HAdV, HRV and bacteria positive and negative groups calculated using independent samples *t*-test.
[b] Adjusted mean difference in %neutrophils using multiple linear regression (adjusted for viral co-detection and bacterial infection).
[c] *p*-Values remain significant after logarithmic transformation of %neutrophils therefore mean differences reported.
[d] Standardized coefficient indicates independent influence of variable on %neutrophils.

diseases or viruses. The first compared NPA to BAL in lung transplant recipients using unpaired, non-contemporaneous NPA and BAL specimens obtained from 72 and 56 participants for NPA and BAL, respectively. A higher yield of hMPV was seen in NPA compared to BAL, with comparable rates of IFAV, HPIV, RSV, HCoV and HRV [6]. Diagnostic accuracy of NPA for BAL was not assessed in this study [6]. The second study used paired specimens, obtained from 21 participants on 92 occasions, and compared induced sputum to NPA for detection of respiratory viruses in children with cystic fibrosis. HRV was the most common virus identified and was present in the same frequency (21.7%) in NPA and sputum. Concordance rates were higher than in the present study (87% for HRV and 92% for other viruses), with higher sensitivity and specificity (70% and 91.7%, respectively) [15]. The third study compared paired nasal wash/tracheal suction specimens to BAL in terms of RSV detection in 6 participants, and found BAL was superior for detection of RSV using antigen detection and culture methods (insensitive methods when compared to PCR) [5].

Studies directly comparing NPA to BAL have focused on atypical pneumonia. The first study evaluated *Pneumocystis jiroveci* pneumonia and the second compared detection methods for mycoplasma pneumonia. Both studies found high concordance between NPA and BAL. However, in contrast to our findings, in both studies, BAL had a higher yield of positive detections than NPA. Interestingly, in the study on mycoplasma pneumonia, BAL positivity was associated with increased lower airway %neutrophils, however, NPA positivity was not. These differing findings may reflect the different modes of transmission and mechanisms of disease progression between viral and bacterial infections of the respiratory tract.

Our study showed that NPA/BAL discordance was most marked for HRV, where 22 (29.3%) children were NPA-positive but BAL-negative. In contrast, HAdV was more likely to also be found in the lower airway, with only 2 NPA-positive/BAL-negative children. Previous studies have found HRV to have greater propensity to infect the nasopharynx and proximal lower airways than the distal airways or alveoli [16]. This may be related to HRV's ability to replicate optimally at cooler temperatures of the upper airway [17], although this finding is inconsistent across HRV types [18]. In vivo studies demonstrate that HRV induces minimal epithelial cytotoxicity with only a small subset of nasal and bronchial mucosal cells becoming infected [16,19,20]. These factors may explain why HRV is an uncommon cause of pneumonia, except in the immune-compromised host [21]. Whilst HRV and HAdV can both cause uncomplicated upper respiratory tract infection [22], disease manifestation differs at the severe end of the spectrum. HRV has been shown to play an important role in asthma exacerbations [23]; HAdV has been implicated in the pathogenesis of obliterative bronchiolitis and bronchiectasis [24]. Thus, differences between HAdV and HRV in their mechanisms of propagation and respiratory disease pathogenesis are likely to explain our observations.

Our cross-sectional findings of elevated rates of HRV (59%) in children who were essentially free of acute RVI symptoms is higher than prior studies showing asymptomatic HRV nasopharyngeal detection ranging from 12% to 33% [25–27]. HRV is frequently identified in patients with viral:bacterial co-infection [28], a fact that may have relevance to our cohort of children with high rates of bacterial infection and chronic cough.

To further understand the implications of our findings in relation to lower airway neutrophilic inflammation and virus detection, the distinction between positive virus detection and active viral disease merits discussion. A PCR-positive NPA in a child without overt symptoms of acute RVI is often believed to represent viral genome shedding from a resolving infection [29] or preceding a new symptomatic episode. However, certain HAdV types can cause prolonged host shedding [30] with intermittent viral excretion over months to years [31,32]. In contrast, HRVs usually do not persist. Garnett et al. postulate that "smoldering HAdV at the site of lung inflammation" may contribute to the pathogenesis of lung diseases such as chronic obstructive pulmonary disease (COPD) in adults [33]. Our finding of low BAL %lymphocytes, together with neutrophilia, in children who were HAdV DNA positive on BAL, supports the notion that active lower airway HAdV replication was likely to be occurring (as opposed to latent HAdV infection of lymphocytes). Whether such persistence of HAdV viral DNA within the respiratory tract of children is benign or contributes to the development of chronic lung disease/s is unknown.

The major strengths of this study are its prospective design, the use of highly sensitive PCR techniques to detect a wide range of respiratory viruses, contemporaneous detection of lower airway bacteria and inclusion of BAL cellularity findings. However, there are a number of limitations. Firstly, this study involved children with chronic respiratory symptoms, predominantly cough. Hence, these findings may not be readily extrapolated to healthy children or to those with acute lower respiratory tract illness. This is particularly pertinent to influenza and RSV, as there were very few children in whom these viruses were detected. Secondly, we used neutrophilic lower airway inflammation as a surrogate marker for active pulmonary disease, as used by other authors [34,35]. We did not, however, test for additional indirect markers of viral infection such as Toll-like receptor 3 (TLR3), interferon gamma induced protein 10 (IP-10) or virus-specific antibodies. Thirdly, we only obtained BAL samples at a single time-point, precluding the ability to make inferences regarding causality. Lastly, although we were fastidious in our BAL technique, contamination of the bronchoscope in its route through the upper airway is possible. The significant number of BAL positive/NPA negative cases, however, negates the

argument that contamination could account for all lower airway detections.

In conclusion, we have shown that while upper airway sampling is the most practical and frequently used method of assessing virus presence, it does not necessarily reflect virus in the lower airways in children with non-acute respiratory illness. However, certain viruses (e.g., HAdV), when detected on NPA, may provide clues to the existence of lower airway neutrophilic inflammation and further research is needed. Studies addressing acute viral respiratory tract illnesses in children are required to ascertain the applicability of our findings to the acute setting.

Author contributions

Dr. Wurzel co-conceptualized the study, was responsible for data analysis and manuscript preparation and was involved in data collection.

Dr. Marchant co-conceptualized the study and assisted in data analysis and manuscript preparation.

Dr. Clark provided intellectual input into the study and critically reviewed the manuscript.

Dr. Mackay and Ms. Wang were responsible for viral processing of specimens and critically reviewed the manuscript.

Prof. Sloots was responsible for viral processing of specimens and critically reviewed the manuscript.

Prof. Upham and Dr. Yerkovich assisted in preparation and critical review of the manuscript.

Assoc. Prof. Masters assisted in data collection and critical review of the manuscript.

Dr. Baker assisted in statistical aspects of the study.

Ms. Anderson-James assisted with data collection and study coordination.

Prof. Chang conceptualized the study and was involved in all aspects of the study.

All authors read and approved the final manuscript as submitted.

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Competing interests

IM has received payment for consultancy services from Firebrick pharma. JU has served on the advisory board for Novartis and has previously received payments for lectures or served on speakers' bureaus for AstraZeneca, GSK, Novartis and Boehringer.

All other authors report no potential conflicts.

Ethical approval

Ethical approval was given by Queensland Children's Health Services (RCH) Human Research Ethics Committee (HREC). Reference number: HREC/03/QRCH/17.

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CHAPTER 4

4.1 INTRODUCTION TO CHAPTER 4

Adenoviruses are known to be associated with bronchiectasis and bronchiolitis obliterans. In Chapters 2 and 3 we have identified the potential role of viruses in chronic respiratory diseases in children. We have also demonstrated a link between neutrophilic lower airways inflammation and presence of adenovirus (as detected by PCR in BAL or NPA).

In Chapter 4 we explored the possible role of adenoviruses in chronic endobronchial suppuration. Specifically, we examined the adenovirus genotypes detected in the lower airways of a large cohort of children with PBB and bronchiectasis. We also explored the potential link between adenovirus and presence of bacterial infection. In doing so, we aimed to further expand our understanding of the role of viral-bacterial co-infection in the pathogenesis of chronic suppurative lung disorders in children.

4.2 Adenovirus Species C Is Associated With Chronic Suppurative Lung Diseases in Children

MAJOR ARTICLE

Adenovirus Species C Is Associated With Chronic Suppurative Lung Diseases in Children

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Background. The role of human adenoviruses (HAdVs) in chronic respiratory disease pathogenesis is recognized. However, no studies have performed molecular sequencing of HAdVs from the lower airways of children with chronic endobronchial suppuration. We thus examined the major HAdV genotypes/species, and relationships to bacterial coinfection, in children with protracted bacterial bronchitis (PBB) and mild bronchiectasis (BE).

Methods. Bronchoalveolar lavage (BAL) samples of 245 children with PBB or mild (cylindrical) BE were included in this prospective cohort study. HAdVs were genotyped (when possible) in those whose BAL had HAdV detected ($HAdV^+$). Presence of bacterial infection (defined as $\geq 10^4$ colony-forming units/mL) was compared between BAL $HAdV^+$ and HAdV negative ($HAdV^-$) groups. Immune function tests were performed including blood lymphocyte subsets in a random subgroup.

Results. Species C HAdVs were identified in 23 of 24 (96%) $HAdV^+$ children; 13 (57%) were HAdV-1 and 10 (43%) were HAdV-2. An $HAdV^+$ BAL was significantly associated with bacterial coinfection with *Haemophilus influenzae*, *Moraxella catarrhalis*, or *Streptococcus pneumoniae* (odds ratio [OR], 3.27; 95% confidence interval, 1.38–7.75; $P = .007$) and negatively associated with *Staphylococcus aureus* infection ($P = .03$). Young age was related to increased rates of $HAdV^+$. Blood CD16 and CD56 natural killer cells were significantly more likely to be elevated in those with HAdV (80%) compared with those without (56.1%) ($P = .027$).

Conclusions. HAdV-C is the major HAdV species detected in the lower airways of children with PBB and BE. Younger age appears to be an important risk factor for $HAdV^+$ of the lower airways and influences the likelihood of bacterial coinfection.

Keywords. bronchiectasis; protracted bacterial bronchitis; respiratory bacteria; respiratory viruses; children.

The burden (prevalence, cost, and importance) of protracted bacterial bronchitis (PBB) and bronchiectasis (BE) are increasingly appreciated [1–4]. PBB is the most common cause of chronic cough in children presenting to pediatric pulmonologists in some series [1, 5]. Over the last 2 decades, the diagnosis of BE in children and adults has increased [6,7]. Both conditions are characterized by chronic wet cough, lower airway endobronchial suppuration with bacterial infection, intense airway neutrophilia, and upregulation of inflammatory and immune markers [5, 8–10]. It is hypothesized, but remains unproven, that PBB and BE represent a clinical continuum based upon degree of severity, sharing common triggers and/or pathophysiology [11].

To date, research into the microbiology of chronic suppurative lung diseases (PBB and BE) in children has largely focused on bacterial pathogens, with a paucity of research into viral contributors. Viral–bacterial coinfection is associated with heightened neutrophilic inflammation of the lower airways of children [12]. Human adenovirus (HAdV) detection is associated with lower

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airway neutrophilic inflammation in children with chronic respiratory symptoms, [13] and is significantly more likely to be present in the lower airways of children with PBB, compared with controls [14]. HAdVs (particularly types 1–5, 7, 14, and 21; members of HAdV species B, C, and E) are known to be associated with future small airways dysfunction and bronchiectasis [15, 16]. However, no studies, to our knowledge, have examined the HAdV genotypes in the lower airways of children with chronic endobronchial suppuration to investigate a possible role in pathogenesis.

Thus, we studied the bronchoalveolar lavage (BAL) of 245 children with PBB and mild BE. We aimed to identify (1) the prevalence of HAdV; (2) the diversity of genotypes/species using sequence analysis, and (3) whether presence of HAdV increased the odds of bacterial coinfection. We hypothesized that, in the lower airways of children with PBB and mild BE, certain HAdV genotypes/species would predominate, and that presence of HAdV increases the risk of bacterial coinfection.

METHODS

Children included in this study were a subset of a larger prospective, longitudinal cohort study on chronic cough in children [12]. Ethical approval was obtained from The Queensland Children's Health Services Human Research Ethics Committee (HREC/03/QRCH/17). Written informed consent was obtained from the parents/guardians.

Children were recruited between March 2008 and September 2013, covering 6 winter seasons. Of 398 children undergoing flexible bronchoscopy and BAL for clinical indication, 245 were diagnosed with PBB or BE, and were therefore eligible for inclusion. Children were divided into 2 groups: BAL positive for HAdV ($HAdV^+$) or BAL negative for HAdV ($HAdV^-$).

Key demographics and cough characteristics were obtained via completion of a standardized clinical questionnaire. Prospective follow-up of participants was undertaken with daily cough diaries and monthly contact via email or telephone.

Basic immune function tests were performed on peripheral blood, including full blood examination, immunoglobulins (IgG, IgA, IgM, IgE), IgG subclasses, specific antibody (IgG) responses to *Haemophilus influenzae* type b and *Clostridium tetani*, and lymphocyte subsets (including $CD16^+$ and $CD56^+$ natural killer [NK] cells). Bronchoscopy and BAL were performed as described previously [13], in accordance with European Respiratory Society guidelines [17]. Quantitative bacteriology and cellularity on BAL specimens were undertaken as outlined in a prior study [8].

Microbiology

Standardized semiquantitative culture of BAL fluid was undertaken using routine laboratory techniques, as described previously [8]. Infection with major respiratory pathogens (*H. influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*) was defined by bacterial load of $\geq 10^4$ colony-forming units (CFU)/mL BAL, using standard culture techniques described elsewhere [8].

Real-time polymerase chain reaction (PCR) [18–21] was used to detect a conventional panel of respiratory viruses in all, and an extended panel in a random subset of, children. Viruses included HAdVs, respiratory syncytial virus, human influenza A virus, human influenza B virus, human parainfluenza viruses 1–3 (HPIV1-3), human metapneumovirus (standard panel), human rhinoviruses, human bocavirus, human coronaviruses (NL63, OC43, 229E, HKU1), WU polyomavirus, and KI polyomavirus (extended panel).

HAdV PCR testing was undertaken on all BAL samples at a clinical laboratory (Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Australia), using a previously described method [22], to identify $HAdV^+$ participants. A random subset of HAdV isolates, based upon sample availability, from 26 $HAdV^+$ on BAL children, underwent nucleotide sequencing at a research laboratory (Queensland Paediatric Infectious Diseases Laboratory, Brisbane, Australia).

HAdV genotyping, based on the hexon gene hypervariable regions 1–6, was conducted as previously described [23]. In brief, 2 μL of nucleic acid extract was amplified in a nested PCR reaction. Round 1 contained primers (0.38 μM; AdHexF1-TICTTTGACATICGIGGIGTICTIGA and AdHexR12-CTGTCIACIGCCTGRTTCCACA), magnesium chloride (4.75 μM), buffer, and MyTaq HS DNA polymerase (Bioline kit, Australia) and was incubated for 1 minute at 94°C followed by 35 cycles of 94°C for 1 minute, 45°C for 1 minute, 72°C for 2 minutes, and a final incubation at 72°C for 1 minute. Round 2 used 2 μL of the Round 1 PCR product as template, the same conditions but different primers (AdHexF2- GGYCCYAGYTTYAARCCCTAYTC and AdHexR2- GGTTCTGTCICCCAGAGARTCIAGCA). Round 2 PCR products were checked by agarose gel electrophoresis, purified, and subjected to nucleotide sequencing (Australian Equine Genetics Research Centre, UQ) results were analyzed to determine the HAdV genotype (Geneious Pro version 6.1).

Relationships between HAdV genotypes, sequenced from 24 $HAdV^+$ study children, were illustrated in a phylogenetic tree (Figure 1) constructed using the neighbor-joining method (with evolutionary history inferred) in MEGA5 [24] after sequence alignment in Geneious version 6.1 [25]. Additional sequences were included from GenBank (labeled with genotype and accession number) to define the species according to a previously described approach [23].

Clinical Definitions

BE was diagnosed based on radiological criteria [26] in children with clinical symptoms consistent with BE [27]. All had evidence of mild (cylindrical) BE on high-resolution computed tomography (CT) scan (reconstructed from a multidetector CT scan). A

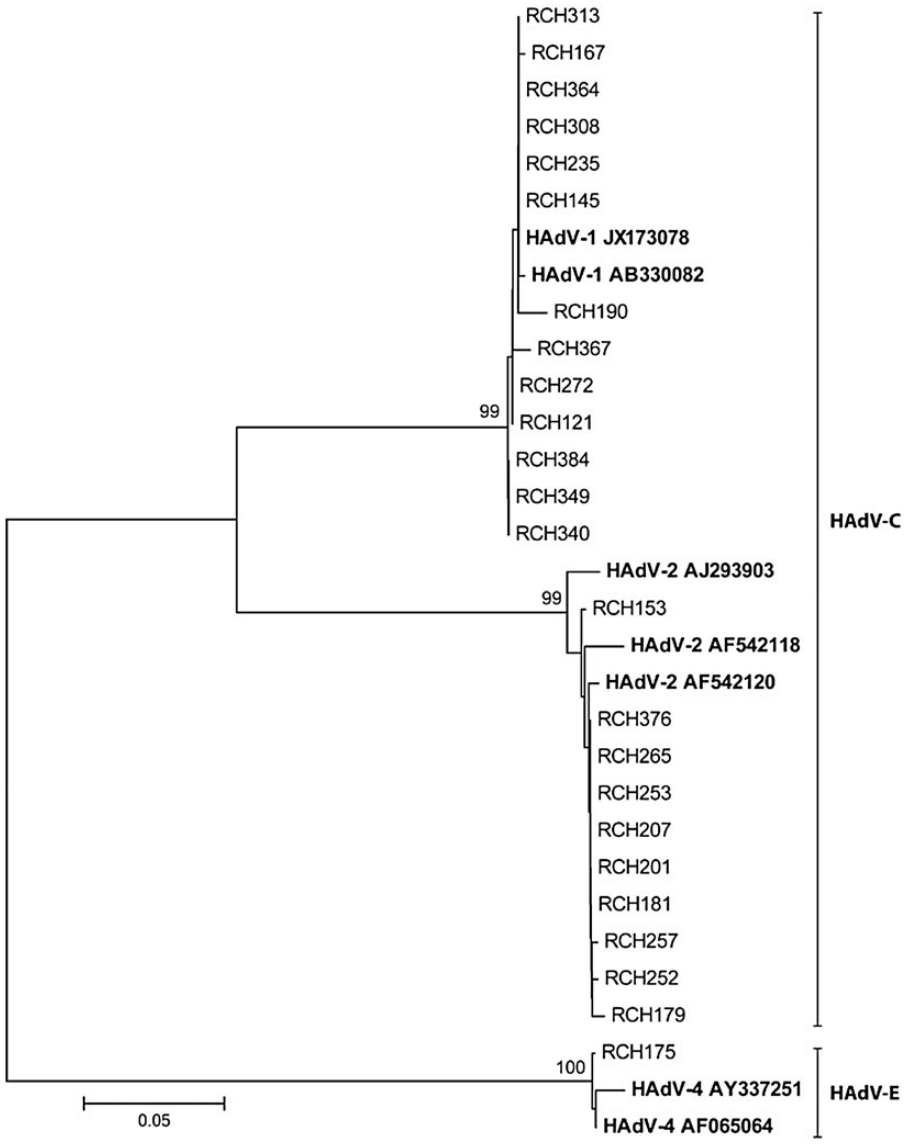

Figure 1. A neighbor-joining method of phylogenetic analysis of human adenovirus (HAdV) genotypes and their assigned species. The 24 nucleotide sequences (approximately 800 nt long) from the hexon gene of HAdV-positive samples from study patients are prefixed with Royal Children's Hospital (RCH). The evolutionary distances were computed using the maximum composite likelihood method [23] and are in the units of the number of base substitutions per site. Additional sequences for speciation are included from GenBank (labeled with genotype and accession number in bold). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the major branches.

diagnosis of PBB was made in children who fulfilled the following criteria: (1) history of chronic (≥4 weeks) wet cough, (2) prospective evidence (supported by cough diaries) of response to 2 weeks of amoxicillin-clavulanate antibiotics, and (3) absence of clinical pointers suggesting an alternative cause for cough [5].

Statistical Analysis

Descriptive statistics were used to summarize baseline patient characteristics. Median and interquartile range (IQR) were reported as data were nonnormally distributed. Univariate analyses were performed using Pearson χ^2 (or Fisher exact test) for categorical variables. Mann-Whitney *U* test was used for 2-group comparisons and Kruskal-Wallis test for >2-group comparisons of continuous variables. Binary logistic regression was used to calculate odds ratios and *P* values. A 2-tailed *P* value of <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 20.0 (IBM SPSS, Armonk, New York).

Table 1. Characteristics of the Study Population

		Age, mo		Sex	
Group	No. (%)	Median	(IQR)	M	F
HAdV$^+$	40	19	(13–25)	29	11
PBB	33 (83)	17	(12–22)	24	9
BE	7 (18)	30	(22–65)	5	2
HAdV$^-$	205	37	(18–69)	126	79
PBB	126 (61)	26	(15–56)	78	48
BE	79 (39)	57	(31–90)	48	31

Abbreviations:+/–, positive/negative detection; BE,bronchiectasis; HAdV, human adenovirus; IQR, interquartile range; PBB,protracted bacterial bronchitis.

Table 2. Univariate Logistic Regression Showing Relationships Between Human Adenovirus Status and Bacterial Infection on Bronchoalveolar Lavage

Bacterial Infection	HAdV$^+$ (n = 40)	HAdV$^-$ (n = 205)	OR (95% CI)	*P* Value
Haemophilus influenzae	27 (68%)	96 (47%)	2.36 (1.15–4.83)	.019
Moraxella catarrhalis	14 (35%)	38 (19%)	2.37 (1.13–4.96)	.022
Streptococcus pneumoniae	14 (35%)	46 (22%)	1.86 (.90–3.85)	.094
Staphylococcus aureus	0 (0%)	21 (10.2%)	. . .	.030[a]

Abbreviations: +/–, positive/negative detection; CI, confidence interval; HAdV, human adenovirus; OR, odds ratio.

[a] Calculated using Fisher exact test.

RESULTS

Study Population

Of the 245 children with PBB or BE, 40 were HAdV$^+$ and 205 HAdV$^-$. The median age of the children in the study was 30 months (IQR, 17–63 months), 23 months (IQR, 14–47 months) in those with PBB, and 57 months (IQR, 30–87 months) in those with BE. The median age was similar between HAdV$^+$ and HAdV$^-$ groups in children with BE ($P = .312$), Table 1. In children with PBB, those in the HAdV$^+$ group were significantly younger than the HAdV$^-$ group ($P = .001$). There were no differences in the sex of the children according to HAdV status within the PBB or BE groups ($P = .248$ and $P = .703$). No seasonal differences in HAdV detection rates were observed ($P = .506$).

CD 16$^+$ and CD56$^+$ NK cells were elevated above the normal range in 20–25 (80%) of HAdV$^+$ and 64 of 114 (56.1%) of HAdV$^-$ children ($P = .027$). When HAdV was excluded, similar rates of NK cell elevation were seen in children with or without standard panel respiratory viruses detected in BAL ($P = .601$). Basic immune function tests were otherwise normal.

HAdV Genotypes

HAdV genotyping was performed on a random subset (based upon sample availability) of 26 HAdV$^+$ on BAL children. Of 24 typeable HAdV isolates, 23 (96%) were identified as HAdV species C; 13 (57%) as HAdV-1, and 10 (43%) as HAdV-2. A single HAdV$^+$ isolate was identified as genotype 4, species E, in a child with low-grade fever and mild upper respiratory tract symptoms at time of bronchoscopy (Figure 1).

Bacterial Codetection With HAdV

The major bacterial pathogens detected in both HAdV$^+$ and HAdV$^-$ groups were *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*. HAdV$^+$ status showed positive association with each major bacterial pathogen (Table 2). *Staphylococcus aureus* was the fourth most common bacterium causing lower airway infection; however, a significant negative association with HAdV$^+$ status was observed ($P = .03$).

Lower airway infection with 1 or more of the 3 major bacteria showed significant positive association with HAdV detection (odds ratio [OR], 3.273 [95% confidence interval {CI}, 1.382–7.748]; $P = .007$). After adjustment for age, using multiple logistic regression, this association was no longer significant (OR, 2.383 [95% CI, .980–5.794]; $P = .055$), whereas the relationship between bacterial infection and age remained significant (OR, 0.987 [95% CI, .980–.994]; $P < .001$].

Although there were slightly more detections of HAdV-1 compared to HAdV-2, no further associations between individual genotypes and bacterial coinfection were observed (data not shown).

The HAdV$^+$ group was more likely to have lower airway infection with multiple (2 or more) major bacterial species (excluding *S. aureus*), compared with the HAdV$^-$ group (OR, 2.471 [95% CI, 1.228–4.969]; $P = .011$). However, on age adjustment, using multiple logistic regression, this association was no longer significant (OR, 1.674; [95% CI, .807–3.475], $P = .166$).

Other Pathogens

Mycoplasma pneumoniae detection rates (using PCR) were similar between HAdV$^-$ and HAdV$^+$ groups (4 in HAdV$^-$ and a 1 in HAdV$^+$ groups; $P = .580$). Similarly, standard panel respiratory viruses were detected at similar rates in HAdV$^-$ and HAdV$^+$ children. There were 3 (1.5%) and 0 detections of influenza virus ($P = 1.00$) and 10 (4.9%) and 2 (5%) detections of HPIV ($P = 1.00$) in the HAdV$^-$ and HAdV$^+$ groups, respectively. Respiratory syncytial virus was detected in 11 (5.4%) and 0 ($P = .22$), and human metapneumovirus was detected in 5 (2.4%) and 0 ($P = .594$) children in the HAdV$^-$ and HAdV$^+$ groups, respectively.

Of 52 (41 HAdV$^-$ and 11 HADV$^+$) participants who had extended viral panel testing of BAL, approximately a quarter of HAdV$^-$ children were human rhinovirus positive on BAL (n = 10 [24.4%]) compared with more than half of HAdV$^+$ children (n = 6 [54.5%]; $P = .054$). Likewise, human bocavirus was detected less commonly in HAdV$^-$, compared to HAdV$^+$ children (1 of 41 [2.4%] vs 3 of 11 [27.3%]; $P = .026$). Human coronavirus (OC43) was detected in 1 HAdV$^-$ participant and no HAdV$^+$ participants ($P = 1.00$).

DISCUSSION

This is the first study to examine the HAdV genotypes detectable in the lower airways of children with chronic endobronchial suppuration (PBB and bronchiectasis). We found HAdV species C (genotypes 1 and 2) to be the major HAdV species in the BAL of these children, irrespective of season. We have also shown that lower airway bacterial infection, with *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*, but not *S. aureus*, are increased in HAdV$^+$ children. Younger age is an independent predictor of infection with HAdV and common respiratory bacteria, and age increases the odds of viral–bacterial coinfection. Our finding of elevated CD16 and CD56 NK cells in the blood of 80% of HAdV$^+$ children provides indirect evidence of a systemic immune response to HAdV in the airways of these children.

The HAdV-C species, comprising genotypes 1, 2, 5, 6, and 57, is one of the most frequent HAdV species known to infect the respiratory tract of children [28]. Infection can result in a range of clinical manifestations, from uncomplicated upper respiratory infection to severe pneumonia. Most primary HAdV-C infections occur within the first 2 years of life [29]. The highest levels of HAdV DNA are detected in adenotonsillar tissue of 2-year-old children undergoing routine adenoidectomy or tonsillectomy, and the amount of HAdV DNA decreases with increasing age [30]. HAdVs demonstrate the ability to establish latent infection within lymphocytes [30], tonsillar tissue [31], and the lung [32] and are capable of evading host innate immune responses via multiple mechanisms [33].

Several studies have demonstrated HAdV's propensity to establish latent and/or persistent infection within the upper and lower respiratory tract [34, 35]. In a recent longitudinal study of children with chronic upper respiratory infections, 13 had repeated HAdV detection; 8 carried an identical HAdV genotype on successive occasions, suggesting chronic rather than repeated infection [34]. Using repeated lower airway sampling, Macek and colleagues demonstrated persistence of HAdV in the lower airways of 9 of 11 children with persistent asthma [35]. In adults, Matsuse and colleagues showed increased presence of latent HAdV DNA in the lung tissue of patients with chronic obstructive pulmonary disease (COPD), compared with those without COPD [32]. No studies to date, however, have genotyped HAdV in lower airways of children with chronic endobronchial suppuration.

A higher prevalence of HAdV in the airways of patients with chronic respiratory diseases has led to speculation that "smoldering HAdV at the site of lung inflammation" [30] contributes to the pathogenesis of chronic lung diseases, including asthma in children [35] and COPD in adults [32, 36]. Marin et al showed that HAdV was detected in 78.4% of nasopharyngeal samples of asthmatic children during symptom-free periods, vs 5% of healthy controls [37]. Furthermore, we have recently shown that HAdV is significantly more likely to be detected in BAL fluid of children with PBB (23%) compared with controls (4%) [14]. It is possible that chronic inflammatory diseases of the respiratory tract predispose to HAdV persistence in the respiratory tract, or that HAdVs induce an inflammatory response that may be a key factor in chronic respiratory disease pathogenesis. If the latter is correct, the association of HAdV with bacterial infection of the lower airways is likely to be a relevant and significant finding.

Childhood lower respiratory infections are known to cause bronchiectasis. Nontypeable *H. influenzae* (NTHi) is the most common bacterium in the lower airways of children with PBB and other chronic lung diseases [5, 38, 39]. Recently, De Schutter and colleagues showed that NTHi is also the commonest cause of (nonresponsive or recurrent) community-acquired pneumonia in children [40]. Similarly, compared with controls, elevated rates of *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* infection are detected in the lower airways of children with PBB [14].

The propensity for codetection of HAdV with bacterial pathogens (eg, NTHi), has been documented in studies on human respiratory diseases [41, 42]. However, the most convincing evidence for microbial synergy arises from an animal study on otitis media. In a chinchilla model of experimental otitis media due to HAdV-1 (species C) and NTHi, Suzuki et al demonstrated a synergistic effect of the 2 pathogens. They found that inflammation was greatest in the presence of both HAdV and NTHi, compared with HAdV or NTHi alone. Furthermore, timing of inoculation was important. HAdV inoculation prior to NTHi inoculation resulted in the greatest tympanic membrane inflammation and mucosal dysfunction [43]. We have previously shown that neutrophilic lower airway inflammation is maximal in the presence of viral–bacterial coinfection of the airways of children [12]. It is indeed plausible that HAdV-C and *H. influenzae* may also play a synergistic role in the initiation and/or exacerbations of chronic suppurative lung diseases in children, and further research is needed.

The negative association of HAdV with *S. aureus* in the lower airways observed in the present study may be due to the increased presence of other bacterial pathogens (eg, *H. influenzae*) in HAdV$^+$ compared with HAdV$^-$ children. Negative associations

between *S. pneumoniae* and *S. aureus* [44–48] and between *H. influenzae* and *S. aureus* [44, 45] are well described in studies of the upper respiratory tract. These interactions are believed to reflect natural competition between colonization with these organisms [48].

Hence, our findings in relation to HAdV in the lower airways of children with PBB and BE are likely to have clinical relevance and may represent an important clue to the underlying pathogenesis of these disease processes. Our hypothesis is based upon several key findings. These include (1) a predominance of HAdV-C in the airways of these children, with a plausible mechanism for disease; (2) the presence of elevated NK cell levels in $HAdV^+$ children (however, not in those with other standard panel respiratory viruses), indicating a systemic immune response to HAdV in the lung; (3) previous research linking HAdV (in the nasopharynx and/or lung) to heightened neutrophilic inflammation and lymphopenia of the lower airways (suggestive of active viral replication) [13]; and (4) the demonstrated association between $HAdV^+$ status and lower airway bacterial infection (the latter being a key feature of PBB and BE). It is therefore unlikely that HAdV is an innocent bystander, as early epidemiologic studies suggested.

Although we have described 2 novel and important findings, several limitations to our study merit discussion. First, although our cross-sectional data highlights important associations, we cannot attribute causality. Repeated lower airway sampling would be needed to establish the temporal sequence of lower airway infection and determine a cause-and-effect relationship. However, subjecting children to repeated general anesthesia for lower airway sampling, solely for research, would be unethical. Second, we did not include HAdV serology in our blood test panel. This ideally requires repeat venipuncture, which was not feasible in our context as many children in the study reside outside the Brisbane area. With regards to methods of HAdV detection, although culture detects infectious virus, PCR is more sensitive and is the current clinical standard [16]. Although a positive PCR for HAdV does not readily distinguish infectious from noninfectious virus, the identification of HAdV DNA indicates that infection has occurred at some stage. Further, HAdV's association with a systemic immune response (ie, elevated NK cells) supports the notion that HAdV may play a clinically relevant role. Third, we detected few conventional respiratory panel viruses (other than HAdV) and only performed extended viral panel analyses on a subset, limiting our ability to explore other possible viral–bacterial and viral–viral interactions. Last, the strong relationship between age and HAdV status, and the increased odds of bacterial coinfection with HAdV in younger children, is likely to be significant. This finding raises the question of whether younger children are predisposed to polymicrobial infection, via immature immune system processes, rather than a true viral–bacterial interaction. Timing of HAdV acquisition, with respect to age, may therefore be important in further evaluating the link between HAdV and future development of PBB and/or BE.

We conclude that HAdV-C genotypes 1 and 2 are the dominant HAdV species infecting the lower airways of young children with chronic endobronchial suppuration. The significant association between HAdV and lower airway bacterial infection suggests a possible role of HAdV-C in the pathogenesis of chronic suppurative lung diseases in young children. Our findings may have implications for targets in the prevention of chronic suppurative lung diseases; however, further research to definitely establish causality would be required.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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CHAPTER 5

5.1 INTRODUCTION TO CHAPTER 5

Thus far, we have demonstrated a relationship between adenovirus (of which most are adenovirus species C) and bacterial infection of the lower airways of children with PBB and bronchiectasis. We have provided further evidence of the role of viral-bacterial co-infection. In Chapter 5, we refocused on PBB as a clinical entity and explored the characteristics of the typical child presenting with this condition.

In this chapter we compared a cohort of children with PBB to a control group. We systematically examined their demographic, clinical, laboratory and bronchoscopic findings. Our specific objectives were to compare rates of viral infection between groups and to further assess the frequency with which wheeze was observed in PBB. The overall aim of this study was to assist clinicians in the more accurate diagnosis of PBB, highlighting the similarities and differences between PBB and other common paediatric respiratory conditions such as asthma.

5.2 *Prospective Characterization of Protracted Bacterial Bronchitis in Children*

CHEST

Original Research

SIGNS AND SYMPTOMS OF CHEST DISEASE

Prospective Characterization of Protracted Bacterial Bronchitis in Children

Danielle F. Wurzel, MBBS; Julie M. Marchant, PhD; Stephanie T. Yerkovich, PhD; John W. Upham, PhD; Ian M. Mackay, PhD; I. Brent Masters, PhD; and Anne B. Chang, PhD

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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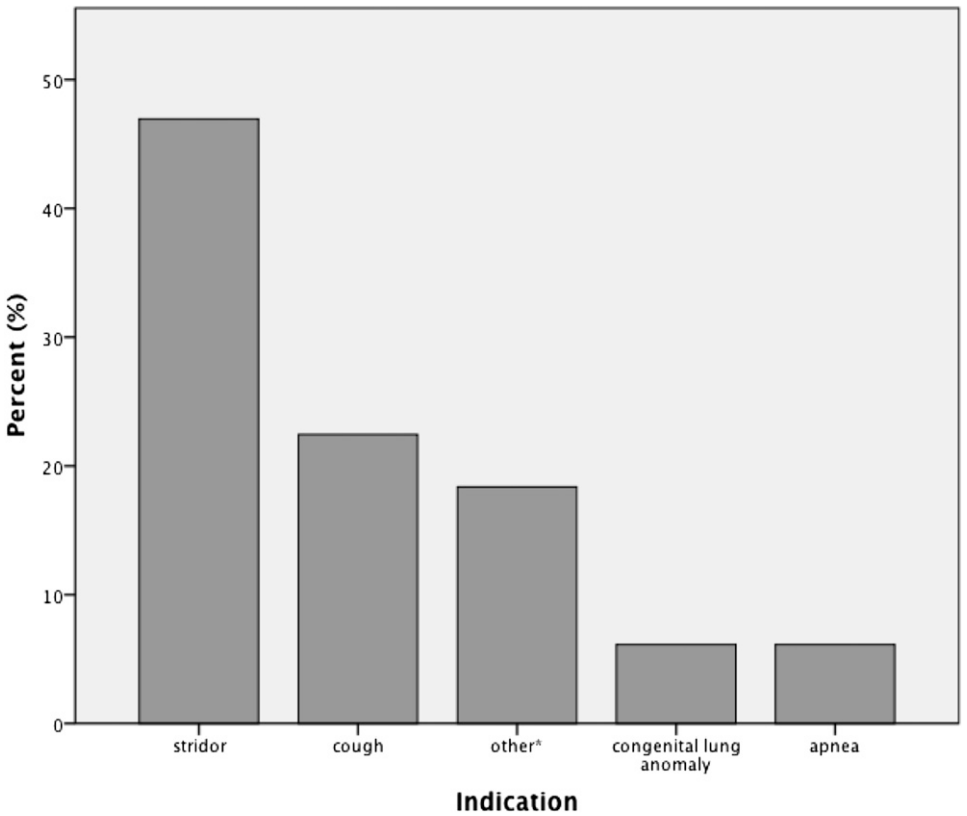

FIGURE 1. Bar graph showing primary indication for bronchoscopy in control subjects. *Includes wheeze, exercise-induced symptoms, imaging abnormality, and hemoptysis.

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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CHAPTER 6

6.1 INTRODUCTION TO CHAPTER 6

In Chapter 5, we further expanded our understanding of PBB by describing the typical child who presents with this condition. These findings will assist the clinician in the more accurate diagnosis of PBB and assist in its differentiation from other common causes of chronic cough. Further research to evaluate the longer-term significance of PBB, with respect to risk of bronchiectasis, was needed. Availability of such data is relevant when counselling parents of children who experience recurrent episodes of PBB.

Chapter 6 explored the significance of PBB with respect to a later diagnosis of bronchiectasis. It evaluated the accuracy of the hypothesis that PBB and bronchiectasis rest upon a common clinical spectrum of disease severity.

In this study, we followed a cohort of children with PBB over a two-year period to determine the proportion later diagnosed with bronchiectasis. We also examined risk factors for bronchiectasis. Findings from this study would aid the clinician in identifying those children with PBB at greatest risk of bronchiectasis. The relevance of these findings is that they would enable closer monitoring of children at risk of bronchiectasis hence leading to earlier diagnosis.

6.2 Protracted Bacterial Bronchitis in Children: Natural History and Risk Factors for Bronchiectasis at 2 years

Protracted Bacterial Bronchitis in Children: Natural History and Risk Factors for Bronchiectasis

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Running title: Risk factors for bronchiectasis in children with PBB

Key words: bacterial infection, bronchiectasis, paediatric lung disease, respiratory infection, viral infection

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The authors declare no conflicts of interest in relation to this manuscript.

ABSTRACT

Importance: Protracted bacterial bronchitis (PBB) and bronchiectasis are distinct diagnostic entities sharing common clinical and laboratory features. It is postulated, but remains unproven, that PBB precedes bronchiectasis in a subset of children.

Objective: In a cohort of children with PBB, our objectives were to: (a) determine the medium-term risk of bronchiectasis and (b) identify risk factors for bronchiectasis and recurrent episodes of PBB.

Design: Between March 2008 and October 2012, children undergoing flexible bronchoscopy and BAL for a clinical indication were recruited to this longitudinal cohort study. All had basic immune function testing. Caregivers completed clinical questionnaires at baseline and cough diaries during periods of illness. CT chest was undertaken if they developed clinical features suggestive of bronchiectasis.

Setting: The study was undertaken in a large, tertiary paediatric hospital in Brisbane, Australia.

Participants: Of 343 children undergoing bronchoscopy, 161 met criteria for PBB including: i) history of chronic (≥ 4 weeks) wet cough, (ii) response to 2 weeks of treatment with amoxicillin-clavulanic acid and (iii) absence of clinical pointers suggesting an alternative cause for cough. In total, 106 children reached the 2-year follow-up time-point (13 withdrew, 42 had incomplete cough diaries).

Main outcome and measures: The primary study outcome was the proportion of children with

PBB, at study entry, diagnosed with bronchiectasis during the follow-up period.

Results: Of 161 children with PBB (66% male), 13 (8.1%) were diagnosed with bronchiectasis. Almost half (43.5%) with PBB had recurrent episodes (>3/year). Major risk factors for bronchiectasis included: *H. influenzae* lower airway infection (in BAL) (p=0.013) and recurrent episodes of PBB (p=0.003). *H. influenzae* infection conferred >7 times higher risk of bronchiectasis [HR 7.55 (95%CI 1.66 - 34.28), p=0.009] compared to absence of *H. influenzae*. The majority of isolates (82%) were non-typeable *H. influenzae*. No risk factors for recurrent PBB were identified.

Conclusion: PBB is antecedent to a diagnosis of bronchiectasis in a subgroup of children. *H. influenzae* lower airway infection and recurrent PBB are significant risk factors. Clinicians should be cognisant of the need to monitor children with PBB over time for evidence of bronchiectasis, particularly those with risk factors.

At a glance:

- Protracted bacterial bronchitis (PBB), a major cause of chronic cough in children, may be associated with later development of bronchiectasis.
- In this study, we have shown that approximately 1 in 12 children with PBB are diagnosed with bronchiectasis at 2-year follow-up.
- Risk factors for bronchiectasis include: lower airway infection with *H. influenzae* and recurrent episodes of protracted bacterial bronchitis.
- Children with PBB should be monitored over time for bronchiectasis and specialist

referral should be considered in those with risk factors.

INTRODUCTION

Protracted bacterial bronchitis (PBB), first described by our group in 2006, is a major cause of chronic cough in children who present to respiratory specialists.[1,2] PBB is now well recognised in Australia,[3] Europe [4] and the US.[5,6] It is characterised by persistent wet cough, response to a 2-week course of appropriate antibiotics and absence of indicators to suggest an alternative cause for cough.[1,7] PBB is more common in young boys and children who have attended childcare.[8] When compared to controls, children with PBB are more likely to have lower airway infection with bacteria, such as *H. influenzae, M. catarrhalis* and *S. pneumoniae,* as well as viruses e.g. adenovirus.[8]

Currently, there are limited published data,[9] and no prospective follow-up studies, evaluating the outcomes of children with PBB. This clinical gap limits the clinician's ability to prognosticate on the likely natural history of PBB in an individual child. Anecdotally, many otherwise healthy children experience recurrent episodes of PBB without long-term consequences. However, in a subgroup of children, recurrent PBB may be an important risk factor for, or herald the onset of, non-cystic fibrosis bronchiectasis, though this remains unproven. Certain characteristics of the lower airway microbiota may also predispose to the development of chronic lung disease.

PBB and bronchiectasis share many common features, spanning from respiratory symptoms (i.e. chronic wet cough) to intense neutrophilic lower airway inflammation and innate immune system activation.[10-12] The lower airway microbiota,[13] including presence of adenovirus type C [14] and predominance of nontypeable *Haemophilus influenzae* (NTHi),[1,8] are also alike. Such similarities underpin the notion that PBB and bronchiectasis occupy distinct poles of a common clinical continuum.[15] To date, the accuracy of this proposed continuum is uncertain, and warrants

evaluation in a prospective cohort study. If it is indeed correct, then risk factors for bronchiectasis, in children with PBB, warrant elucidation in order to further inform clinical practice and potentially determine intervention points.

Hence, in 161 children with PBB and 25 controls, we aimed to determine: (a) the 2-year outcomes with respect to development of bronchiectasis and; (b) the risk factors for diagnosis of bronchiectasis and those for recurrent episodes of PBB.

METHODS

Study participants

Participants were enrolled as part of a larger prospective cohort study aimed at evaluating the long-term outcome of children with chronic cough. Written informed consent was obtained from all parents/guardians and ethics approval granted by The Queensland Children's Health Services (RCH) Human Research Ethics Committee (HREC/03/QRCH/17).

Between March 2008 and October 2012, 343 children were enrolled. Of these, 161 fulfilled criteria for PBB and 25 were recruited as control participants (15 undergoing evaluation for respiratory symptoms other than chronic cough, and 10 healthy controls). Data from the 15 control children undergoing respiratory evaluation have previously been described [8] (see e-supplement). The 10 healthy controls were recruited from colleagues and friends.

All children (excluding the 10 healthy controls) underwent flexible bronchoscopy and bronchoalveolar lavage (BAL) as per clinical indication, and were recruited prior to their bronchoscopy. BALs were processed for cellularity and microbiology. Bacterial infection was

defined as bacterial load of $\geq 10^4$ colony-forming units (cfu)/ml BAL.[8,11] Laboratory tests for suppurative lung diseases were performed, as described previously.[14] *H. influenzae* characterisation was undertaken at a research laboratory (Menzies School of Child Health Research, Darwin) when BAL was available (see e-supplement).

Follow up

Follow-up included monthly contacts (phone-calls or emails) to capture respiratory exacerbations (undertaken by research nurses). Parents completed cough diaries during periods of illness. The majority of children were seen by their pediatric pulmonologist, as part of routine clinical follow-up, 3-4 monthly. At 2-year follow-up, a subset of children (n=106) also underwent clinical assessment (by DW and/or AC) for bronchiectasis.

Given the ethical considerations pertaining to research-related CT chest in children,[16,17] participants underwent CT scans only when clinical features of bronchiectasis were present.[18] Clinicians had similar practices whereby CT chest was undertaken for: (a) chronic wet cough non-responsive to 4 weeks of antibiotic therapy;[19] (b) persistent chest radiographic changes despite appropriate antibiotic therapy or (c) recurrent hospitalisations for acute respiratory events.

Definitions

PBB was defined as: (i) history of chronic (≥ 4 weeks) wet cough, (ii) prospective evidence (supported by cough diaries) of response to 2 weeks of treatment with amoxicillin-clavulanate and (iii) absence of clinical pointers suggesting an alternative cause for cough.[1] Bronchiectasis diagnosis was based on pediatric radiological (CT) criteria,[20] in the context of a child having clinical symptoms of bronchiectasis. Recurrent PBB was defined as >3 episodes of PBB in the

first year after enrolment.

Statistical analyses

Descriptive statistics were utilized to summarize demographic and clinical characteristics. Median and inter-quartile ranges were reported as data were non-normally distributed. Pearson's chi-square (or Fisher's exact test) was used for categorical and Mann-Whitney U for continuous variables. Logistic regression was employed to calculate odds ratios (OR) and 95% confidence intervals (95%CI). A survival analysis (using Cox's proportional hazards regression) examined the relationship between time to diagnosis of, and risk factors for, bronchiectasis. A 2-tailed p-value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (version 21.0, Armonk, NY: IBM Corp.,USA) and STATA (version 12.1, StataCorp, Texas,USA).

RESULTS

Clinical and demographic characteristics of participants

Of the 161 children with PBB, 106 completed the 2-year follow-up period and had clinician assessment for bronchiectasis (Figure 1). The median duration of follow-up was 25 months (IQR 24, 28) in children with PBB and 27 months (IQR 26, 29) in controls undergoing bronchoscopy.

There was no difference between children who completed the 2-year follow-up and those who did not (Table 1), with regards to: length of cough at recruitment (p=0.807), *H. influenzae* status (p=0.518) or proportion with 2+ siblings (p=0.260). However, those completing the 2-year follow-up (n=106) were significantly more likely to have had recurrent PBB (p=0.003). Thus, to reduce the potential for bias, 161 was used as the denominator in subsequent analyses. The 55

additional children who did not complete 2-year follow-up were assumed to be bronchiectasis-free at the 2-year time-point. Comparison of groups at baseline showed that children with PBB had a higher burden of doctor visits in the preceding 12 months, as compared to controls (Table 1). At 2-year follow-up, children with PBB were more likely to be coughing compared to controls (p=0.005) and to have had parent-reported wheeze in the preceding 12 months (p=0.001) (Table 2). Of the 161 children with PBB, 154 had completed cough diaries up until at least the 1-year time-point. Of these, 67 (43.5%) had recurrent PBB (>3 episodes/year).

Bronchiectasis diagnoses on chest CT scan

Multi-detector CT with HRCT reconstruction was performed in 25 of 161 children with PBB. Radiological evidence of bronchiectasis was present in 13 (8.1%). In those with bronchiectasis, CT was performed at median duration of 9 months (IQR 4, 19) post recruitment. The median age of these children was 38 months (IQR 27, 58). Compared to those that did not undergo CT, children with PBB who underwent CT chest, had similar rates of lower airway infection with *H. influenzae (*p=0.142) and *S. pneumoniae* (p=0.135), however, those undergoing CT scan were less likely to have *M. catarrhalis* infection, than those that did not undergo CT (34.2% vs 17%, p=0.029) (this is likely to have been a chance occurrence.) Similar rates of 'recurrent PBB' status were recorded in those children that underwent CT and those that did not (p=0.118).

No children in the control group developed clinical features of bronchiectasis. Of the control children, 3 had CT scan performed for other indications. None had radiological evidence of bronchiectasis.

Risk factors for bronchiectasis and recurrent PBB

Univariate analysis showed that bronchiectasis was significantly more likely to be diagnosed during follow up when the child had recurrent PBB, when *H. influenzae* infection was present (i.e. cultured at a clinically significant level of $\geq 10^4$ cfu/ml in the BAL) or in children with two or more siblings (table 3). There was no significant difference between groups for other factors.

Multivariate logistic regression showed that recurrent PBB status and *H. influenzae* infection were significantly associated with bronchiectasis diagnosis [OR 11.48 (95%CI 2.33-56.50) p=0.003 and OR 7.60 (95%CI 1.53-37.79), p=0.013, respectively], whereas having ≥2 siblings was no longer significant [OR 3.53 (0.98, 12.70), p=0.054]. Survival analysis, using Cox regression, concurred with logistic regression findings. Participants with *H. influenzae* lower airway infection were > 7 times more likely to be diagnosed with bronchiectasis per month of follow-up, compared to those without *H. influenzae,* and recurrent PBB status conferred >9 times greater risk of bronchiectasis diagnosis per month (Table 4).

The percentage of lower airway neutrophils was similar in children with and without a subsequent diagnosis of bronchiectasis (Table 5). Although the percentage of eosinophils in BAL was slightly higher in those who developed bronchiectasis, the median eosinophil count was within the normal range (i.e. <2.5%),[21] and hence considered clinically insignificant. Further, the difference was no longer observed when the major outlier was removed.

In the examination of predictors for recurrent PBB, univariate and multivariate analysis showed no significant difference between children with and without recurrent PBB for the factors examined (sex, age, prior pneumonia, tobacco smoke exposure, maternal tobacco smoking in pregnancy, Indigenous status, number of children in household, childcare attendance and BAL

cellularity and microbiology) (eTable 1). Notably, *H. influenzae* infection in the BAL was not predictive of recurrent PBB.

H. influenzae **typing**

Thirty-four of 55 (62%) *H. influenzae* positive samples were available for further characterisation in the research laboratory (laboratory methods in e-supplement). Of these, *H. influenzae* from the majority of samples (n=28; 82%) were identified as NTHi; encapsulated *H. influenzae* were not identified. *H. influenzae* from the remaining 6 samples were reassigned as *H. haemolyticus* following species-specific PCR.

DISCUSSION

This is the first prospective longitudinal cohort study of children with PBB. In our cohort, based in a major teaching hospital, almost half (43.5%) had recurrent episodes (>3 in the first year after recruitment) and approximately 1 in 12 were diagnosed with bronchiectasis at 2-year follow-up. We have identified 2 significant risk factors for bronchiectasis: recurrent (>3 /year) episodes of PBB and presence of *H. influenzae* infection of the lower airways. Further, *H. influenzae* in the BAL conferred >7 times higher risk of bronchiectasis diagnosis, compared to *H. influenzae* negative state.

Findings from this study, the first to investigate the 2-year outcomes of children with PBB, support the hypothesis that PBB and bronchiectasis likely represent a clinical spectrum. Children with PBB have endobronchial bacterial infection and neutrophilic airway inflammation, factors known to be injurious to the airways.[22] Our findings suggest that, in a subset of children receiving close follow-up by pediatric pulmonologists, recurrent episodes (>3 per year) of PBB precede a diagnosis of bronchiectasis. Further, although a cause-effect relationship cannot be concluded, a relationship between *H. influenzae* infection and later diagnosis of bronchiectasis has been shown. This finding is in accordance with increasing recognition, amongst researchers and clinicians, of the role of *H. influenzae* in chronic respiratory disease pathogenesis.[23]

H. influenzae is the most common bacterial species infecting the lower airways of children with chronic endobronchial suppuration, including PBB,[8] recurrent or non-responsive community-acquired pneumonia [24] and bronchiectasis.[8,10,25] It is also the major bacterial pathogen associated with chronic respiratory disorders in adults e.g. bronchiectasis and COPD.[23,26,27] The exact role of NTHi in bronchiectasis pathogenesis, however, remains unclear. It may represent the primary

insult, or alternatively, a secondary phenomenon whereby damaged and/or dilated airways act as a nidus for *H. influenzae*. Our finding that *H. influenzae* infection (predominantly NTHi on typing in this study) may be a risk factor for chronic respiratory diseases such as bronchiectasis is consistent with current literature.[25,28-31] Nevertheless, there are likely to be other contributory factors.

Our earlier studies identified that children with PBB were significantly more likely, than controls, to have attended childcare, and to have viral infection of the lower airways, particularly with adenovirus type C.[8,14] It is plausible, although beyond the scope of this study, that co-infection with specific microbial pathogens e.g. adenovirus C and *H. influenzae,*[32] initiates the vicious cycle of lower airway infection and inflammation inherent to these conditions.[33] Factors such as young age (and concomitant immune system and respiratory tract immaturity) at initial infection, are likely to play a key role in the aetio-pathogenesis of these disorders.[14]

In this study, bronchiectasis was diagnosed at an early age (median 38 months), similar to our other cohorts of children with bronchiectasis.[10,19,34] Together with findings from our previous studies on PBB,[8,14] this observation raises the question of whether timing of airway infection (with bacteria and/or viruses), with respect to age, influences the likelihood of later diagnosis of bronchiectasis.

In support of previous studies on PBB, our present study showed that PBB is characterised by active neutrophilic inflammation of the lower airways. Although the median percentage of neutrophils in BAL was greater in those with PBB who were subsequently diagnosed with bronchiectasis, compared to those who were not (median 35 vs 25, respectively), this difference

was not statistically significant. This suggested that the degree of airway neutrophilia was not a predictor of subsequent bronchiectasis. Regarding the inter-group disparity in airway eosinophil levels observed between those with and without bronchiectasis, this is unlikely to be of clinical relevance for two reasons. First, eosinophil levels in both groups were within normal reported ranges, and second, when the major outlier (16% eosinophils in a child with features consistent with persistent asthma in addition to PBB) was removed from the 'bronchiectasis present' group, this difference was no longer significant.

Several limitations to this study merit discussion. Firstly, we did not universally perform CT scans on all children at study entry and exit. In view of the potential increased lifetime risk of developing cancer and the relatively common nature of PBB in otherwise well children,[35] we could not justify exposing all children to the radiation dose associated with 2 CT scans.[17] The impact of this is two-fold. First, bronchiectasis may have been missed in some children at recruitment and/or follow-up. Hence, we cannot conclude that PBB progresses to bronchiectasis per se. Rather, our findings indicate that, in children with PBB (who are followed closely by pulmonologists over a 2-year period) a proportion (approximately 8%) will be diagnosed with bronchiectasis on CT chest. Second, we acknowledge the fact that performing CT scan only in a select subgroup introduces the possibility of selection bias in that individual clinicians may have different indications for performing CT chest. To address this, we compared children with PBB undergoing CT chest to those who did not and found no significant differences between groups with respect to bronchiectasis risk factors.

The major strengths of this study are the longitudinal nature of data collection and the inclusion of lower airway inflammatory and microbiological findings. However, as we only performed

bronchoscopy and BAL at a single time-point, the temporal relationship between infection with *H. influenzae* and development of bronchiectasis remains unclear. This does not, however, diminish the utility nor the relevance of our findings. Irrespective of whether *H. influenzae* is a cause (or consequence) of bronchiectasis in young children, its presence in a child with recurrent PBB should alert the pulmonologist to the increased possibility of bronchiectasis, suggesting the need for closer monitoring and/or further investigation. This is a novel finding with direct clinical relevance to those managing children with chronic cough.

Findings from this longitudinal cohort study provide further evidence for the link between PBB and bronchiectasis in young children. We have shown that at least 8% (1 in 12.4 children, median age 23 months at recruitment) with PBB are diagnosed with bronchiectasis at 2-year follow-up. Of those with PBB, at least 43.5% had recurrent episodes in their first year after recruitment. We have identified key risk factors for bronchiectasis i.e. *H. influenzae* lower airway infection and recurrent episodes of PBB.

In view of this study's findings, clinicians should be cognisant of the need to monitor children with PBB over time and consider CT chest in those with risk factors for bronchiectasis. Ongoing research into the potential role of *H. influenzae* in the pathogenesis of bronchiectasis in children is needed in order to inform future preventative and therapeutic strategies.

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CONFLICTS OF INTEREST

None

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Table 1: Baseline demographic and clinical characteristics of study participants

	PBB followed-up (N=106)	PBB total (N=161)	Controls (N=25)	P-value[1]
Sex, M:F	74:32	106:55	12:13	0.085
Age - Mo	23 (14, 53)	22 (13, 50)	44 (7, 97)	0.061
Prior pneumonia – X-ray confirmed[2]	24 (23%)	35 (22%)	2 (8%)	0.109
Household tobacco smoke exposure	36 (34%)	55 (34%)	6 (24%)	0.662
Aboriginal or Torres Strait Islander	3 (3%)	10 (6%)	0	0.363
Current cough	89 (84%)	134 (83%)	2 (8%)	**<0.001**
Length of current cough – wks, median (IQR)	26 (7, 52)	26 (6, 52)	0	**<0.001**
Current antibiotics	16 (15%)	19 (12%)	1/15 (7%)	0.695
>5 doctor visits past yr for cough	95 (90%)	140 (87%)	3/15 (20%)	**<0.001**

Abbreviations: PBB followed-up = Subset of patients with PBB who completed 2-year follow-up;

PBB total = all patients with PBB irrespective of whether 2-year follow-up was completed.

[1] Comparison of 'PBB total' to controls; [2] Parent-reported

Table 2: Clinical characteristics of study participants at 2-year follow-up

Characteristic	PBB followed-up (N=106)	Controls (N=25)	P-value
Current cough	47 (44%)	3 (12%)	**0.005**
Current antibiotics	20/98 (20%)	0	**0.012**
Recurrent OM (>5 episodes)	58/97 (60%)	7/20 (35%)	**0.042**
>5 doctor visits past yr for cough	43/97 (44%)	1/20 (5%)	**0.001**
Asthma diagnosed ever	57/95 (60%)	4/20 (20%)	**0.001**
Eczema diagnosed ever	29/92 (32%)	3/19 (16%)	0.265
Wheeze ever[1]	76/95 (80%)	7/19 (37%)	**<0.001**
Wheeze past 12 months	54/93 (58%)	3/19 (16%)	**0.001**
Family history of atopy[2]	12/88 (14%)	1/20 (5%)	0.455
Recurrent PBB[3]	67 (63%)	0	**<0.001**
CT chest (any indication)	20	3	0.564

Abbreviations: PBB followed-up = Subset of patients with PBB who completed 2-year follow-up; PBB total = all patients with PBB irrespective of whether 2-year follow-up was completed.

[1] Parent-reported ('has your child ever had a high-pitched whistling or wheezing sound')

[2] Asthma, eczema and/or hay fever in first-degree relative/s

[3] In 12-month period after enrolment

Table 3: Uni- and multi-variate analysis of risk factors for bronchiectasis (BE) in children with PBB

Risk factor	Group 1 BE present (N=13)	Group 2 BE absent (N=148)	Odds ratio (95% CI)	P-value
Univariate analysis				
Sex, M:F	8:5	98:50	0.816 (0.25, 2.63)	0.733
Recruitment age – mths, median (IQR)	29 (10, 45)	22 (13, 50)	0.99 (0.97, 1.01)	0.521
Prior pneumonia	3 (23%)	32 (22%)	1.09 (0.28, 4.19)	0.903
Recurrent PBB (>3 ep/year)[1]	11 (85%)	56 (38%)	9.04 (1.93, 42.27)	**0.005**
Household tobacco smoke exposure	3 (23%)	52 (35%)	0.55 (0.14, 2.08)	0.377
Maternal smoking in pregnancy	3 (23%)	18/85 (21%)	1.40 (0.34, 5.81)	0.647
Aboriginal or Torres Strait Islander	1 (8%)	9 (6%)	1.29 (0.15, 11.03)	0.818
No. children in household, median (IQR)	3 (2, 4)	2 (2, 3)	1.28 (0.91, 1.80)	0.156
≥2 siblings	8 (62%)	47 (32%)	3.44 (1.07, 11.08)	**0.039**
≥1 sibling	13 (100%)	123 (83%)	-	
Childcare attendance, ever	8/9 (89%)	71/82 (87%)	1.24 (0.14, 10.90)	0.847
BAL organism				
Adenovirus positive (on PCR)[2]	3/12 (25%)	26/138 (19%)	1.44 (0.36, 5.68)	0.606
H. influenzae	11 (85%)	72 (49%)	5.81 (1.24, 27.10)	**0.025**
M. catarrhalis	4 (31%)	43 (29%)	1.09 (0.32, 3.71)	0.896
S. pneumoniae	3 (23%)	41 (28%)	0.78 (0.21, 2.99)	0.720

S. aureus	1 (8%)	12 (8%)	0.94 (0.11, 7.90)	0.958
Multivariate analysis				
H. influenzae			7.60 (1.53, 37.79)	**0.013**
≥2 siblings			3.53 (0.98, 12.70)	0.054
Recurrent PBB (>3 ep/year)			11.48 (2.33, 56.50)	**0.003**

[1] As determined at 1-year time-point in study. 7 children had not reached the 1-year time-point, for the purposes of the multi-variate analysis these children were assumed to have non-recurrent PBB.

[2] As compared to previously published rate of 4% (adenovirus positivity) in BAL samples of control children.[8]

Table 4: Survival analysis – PBB to bronchiectasis (Months) (n=161)

	HR (95% CI)	P-value
Univariable		
H. influenzae[1]	5.81 (1.28 – 26.37)	**0.022**
≥2 siblings	3.18 (1.04 – 9.73)	**0.042**
Recurrent PBB (>3 episodes/yr)	7.65 (1.67 – 34.92)	**0.009**
Multivariable		
H. influenzae[1]	7.55 (1.66 – 34.28)	**0.009**
Recurrent PBB (>3 episodes/yr)	9.77 (2.13 – 44.80)	**0.003**

[1] Lower airway infection with *H. influenzae* defined as $\geq 10^4$ cfu/ml growth on BAL fluid culture

Table 5: Cellularity from BAL of children with and without bronchiectasis (BE)

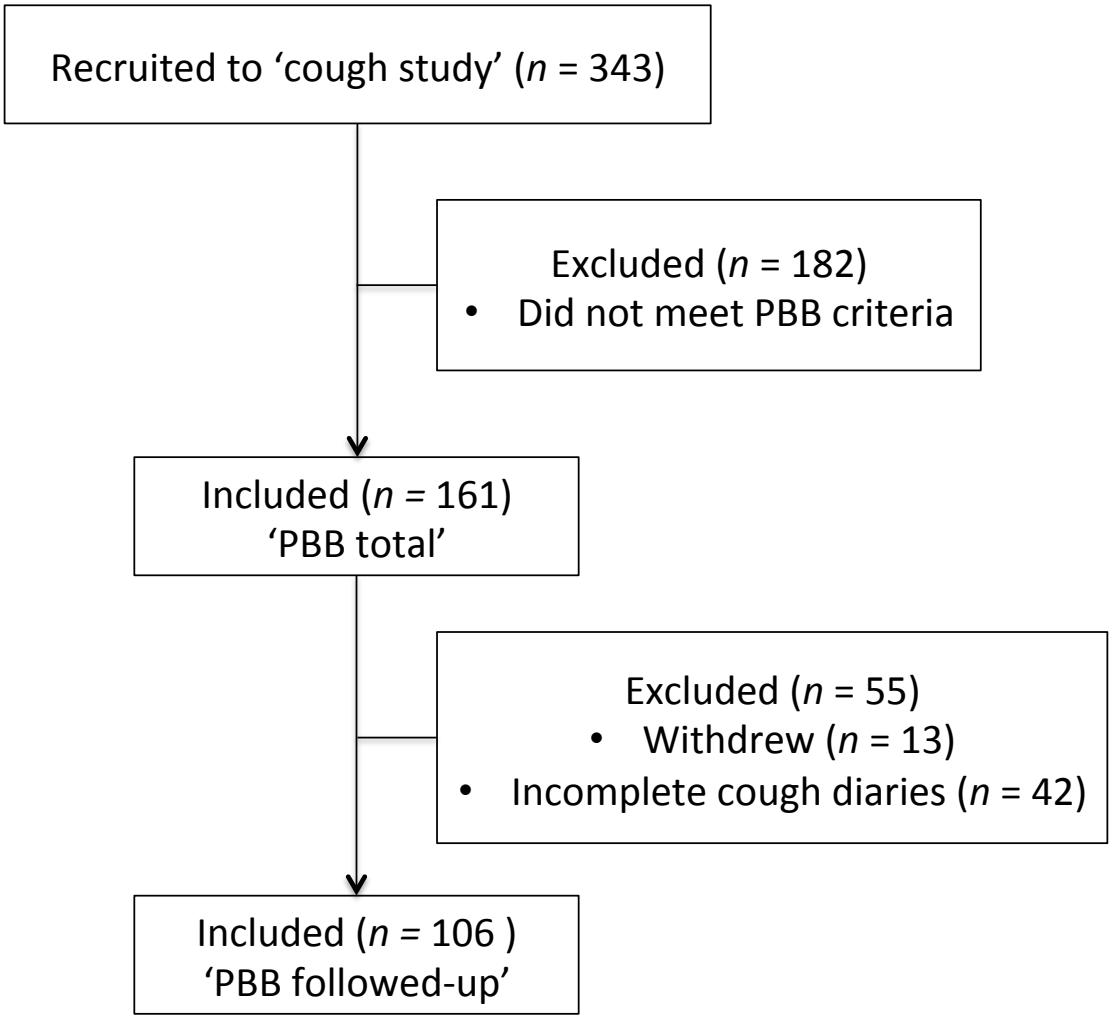
Cellularity, median (IQR)	BE present (N=12)[1]	BE absent (N=138)[1]	P-value
BAL TCC x 10^6/L	240 (210, 560)	240 (120, 420)	0.29
BAL neutr%,	35 (12, 75)	25 (10, 55)	0.35
BAL macro%,	56 (25, 72)	61 (35, 80)	0.47
BAL lymph%,	6 (4, 9)	9 (4, 14)	0.51
BAL eosin%,	1 (0, 2)	0 (0, 0)	**0.001**[2]

[1] Cellularity was unavailable, due to insufficient sample, from 11 participants (10 'BE absent' children and 1 'BE present' child).

[2] When outlier (16% eosinophils) is removed from 'BE present' group, the difference between groups, for eosinophil percentage, was no longer significant.

FIGURE LEGENDS

Figure 1: CONSORT diagram



CHAPTER 7

7.1 INTRODUCTION TO CHAPTER 7

Previous chapters have focused on original research relating to chronic wet cough and PBB in children. Chapter 7 is comprised of 2 manuscripts that reviewed the current literature relating to cough treatments. Chapter 7.1 was a Cochrane meta-analysis examining the efficacy of short-course antibiotics (treatment duration of 4 weeks or less), in the treatment of bronchiectasis in children and adults. Chapter 7.2 was a review of available literature pertaining to cough remedies in children. Both manuscripts are highly applicable to clinical practice, with the latter having direct relevance to clinicians working in general practice.

7.2 Cochrane systematic review: short courses of antibiotics for children and adults with bronchiectasis

Short courses of antibiotics for children and adults with bronchiectasis (Review)

Wurzel D, Marchant JM, Yerkovich ST, Upham JW, Masters IB, Chang AB

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2011, Issue 6

http://www.thecochranelibrary.com

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[Intervention Review]

Short courses of antibiotics for children and adults with bronchiectasis

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ABSTRACT

Background

Bronchiectasis is an important cause of respiratory morbidity in both developing and developed countries. Antibiotics are considered standard therapy in the treatment of this condition but it is unknown whether short courses (four weeks or less) are efficacious.

Objectives

To determine whether short courses of antibiotics (i.e. less than or equal to four weeks) for treatment of acute and stable state bronchiectasis, in adults and children, are efficacious when compared to placebo or usual care.

Search methods

The Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*), MEDLINE, EMBASE, OLDMEDLINE, CINAHL, AMED and PsycINFO and handsearching of respiratory journals and meeting abstracts were performed by the Cochrane Airways Group up to February 2011.

Selection criteria

Only randomised controlled trials were considered. Adults and children with bronchiectasis (defined clinically or radiologically) were included. Patients with cystic fibrosis were excluded.

Data collection and analysis

Two review authors independently reviewed the titles, abstracts and citations to assess eligibility for inclusion. Only one study fulfilled the inclusion criteria and thus meta-analysis could not be performed.

Main results

The single eligible study showed a small benefit, when compared to placebo, of four weeks of inhaled antibiotic therapy in adults with bronchiectasis and pseudomonas in their sputum. There were no studies in children and no studies on oral or intravenous antibiotics.

Authors' conclusions

There is insufficient evidence in the current literature to make reasonable conclusions about the efficacy of short course antibiotics in the management of adults and children with bronchiectasis. Until further evidence is available, adherence to current treatment guidelines is recommended.

PLAIN LANGUAGE SUMMARY

Short courses of antibiotics for children and adults with bronchiectasis

There is a paucity of evidence to conclude whether short courses of antibiotics (i.e. less than or equal to four weeks) are equivalent or superior to placebo in the treatment of stable or exacerbation state bronchiectasis. One single study showed some benefit of short-course inhaled antibiotics over placebo, in terms of microbiological response and subjective improvement in medical condition, although this was balanced against an increase in adverse effects and antimicrobial resistance in the treatment group. Given the potential benefits of shorter duration antibiotic therapy in bronchiectasis, further RCTs are clearly needed to answer this important question.

BACKGROUND

Non cystic-fibrosis bronchiectasis, previously termed an 'orphan disease' is increasingly recognised as a major cause of respiratory morbidity both in developing countries as well as affluent countries, and particularly within Indigenous populations (Chang 2008; Edwards 2003; Singleton 2000).

There are many known aetiological associations with bronchiectasis. Severe lower respiratory tract infection has previously been identified as the most common preceding medical condition in children diagnosed with bronchiectasis (Chang 2008b; Eastham 2004; Karakoc 2009; Singleton 2000), and continues to be a major cause of bronchiectasis worldwide (Callahan 2002; Edwards 2003; Karakoc 2009). However, diseases that affect the pulmonary system (such as immunodeficiency) have become increasingly identified as important causes of bronchiectasis within developed countries (Li 2005; Shoemark 2007).

In adults, the major aetiological associations include post-infection pneumonia or TB, primary ciliary dyskinesia, allergic bronchopulmonary aspergillosis and immunodeficiencies (Shoemark 2007). Primary lung diseases such as chronic obstructive pulmonary disease (COPD) (O'Brien 2000; Patel 2004), sarcoidosis (Lewis 2002), and bronchiolitis obliterans (Chang 1998) have been associated with bronchiectasis as a secondary manifestation. In children, immunodeficiency, aspiration, primary ciliary dyskinesia and congenital airway anomalies are important considerations (Li 2005). Despite intensive investigations, an underlying cause is often not found in many children and adults with bronchiectasis (Shoemark 2007).

Description of the condition

Bronchiectasis is a disease that primarily affects the airways in the initial disease phase. The postulated pathophysiology includes a 'vicious circle' of infection, inflammation and impairment of mucociliary clearance mechanisms eventually leading to airway destruction and bronchial dilatation (Cole 1986).

Adult patients usually present with cough, daily sputum production, dyspnoea, rhinosinusitis, haemoptysis and recurrent pleurisy (King 2004). Children usually present with chronic wet cough or recurrent pneumonia (Singleton 2000). They may have clinical signs such as crackles and wheeze on chest auscultation and sometimes peripheral clubbing (King 2004). With appropriate treatment (usually antibiotics and airway clearance), the chronic cough can totally resolve (Kapur 2009), as can radiological evidence of bronchiectasis (Gaillard 2003). High-resolution Computed Tomography (HRCT) of the chest is considered the gold-standard in diagnosis of bronchiectasis. However, controversy exists as to the normal cut-off of broncho-arterial ratio (particularly in children) (Chang 2008b). Hence, bronchiectasis is sometimes defined clinically in children (Chang 2008b; Singleton 2000). Recent studies suggest that volumetric scans acquired using multi-detector CT are substantially more sensitive and accurate than conventional HRCT for assessing bronchiectasis (Hill 2009).

A combination of airway clearance techniques and antibiotic therapy, with or without other therapies such as anti-inflammatory agents and bronchodilators, are current recommended treatment for non-cystic fibrosis bronchiectasis (Chang 2008b). Specific treatments targeted to the underlying aetiology (such as intravenous immunoglobulin for common variable immunodeficiency) is also important in the management of bronchiectasis.

Early recognition and treatment of bronchiectasis may improve long term outcomes (Kapur 2010).

Description of the intervention

Antibiotics are the mainstay of therapy in the management of bronchiectasis (Prasad 2007). There are various methods to deliver antibiotics to the pulmonary system (oral, intravenous or inhaled). The type of antibiotics given may be targeted to the patient's known airway organism(s) or used empirically.

How the intervention might work

Infection and inflammation are key components in the aetiopathogenesis of bronchiectasis (Cole 1986). Bronchiectatic airways facilitate chronic bacterial colonisation and predispose them to recurrent infections (Loebinger 2007). Chronic bacterial infection elicits a systemic inflammatory response with local release of inflammatory cytokines (including TNF-α and IL-8) causing migration of inflammatory cells such as neutrophils and lymphocytes (Gaga 1998; Shum 2000). Neutrophils release proteolytic enzymes e.g. neutrophil elastase (Amitani 1995) and reactive oxygen species (Anderson 1996), into the airway lumen which cause epithelial damage and stimulate mucous production (Adler 1990). Antibiotics can potentially halt the bacterial infection and subsequently limit ongoing neutrophilic inflammation.

An existing Cochrane Review found that prolonged courses of antibiotics for bronchiectasis, i.e. greater than four weeks in length, are effective in reducing sputum volume and purulence but have a limited impact on the natural history of the condition (Evans 2007). We have reviewed the literature to determine the impact of shorter courses of antibiotics during stable state and exacerbation state in bronchiectasis.

Why it is important to do this review

Most adults and children with bronchiectasis are given frequent courses of antibiotics, the optimal duration of which is unknown. The published Cochrane Review on antibiotics for bronchiectasis is limited to prolonged courses greater than four weeks in duration (Evans 2007).

In clinical practice, short courses of antibiotics during stable and exacerbation states of bronchiectasis often result in improvement in symptoms. Objective measures of airway inflammation also improve, as some studies have shown, for example use of short-course inhaled gentamicin resulted in improvement in airway hypersecretion and inflammation (Lin 1997).

There is currently a paucity of evidence on the optimal duration of antibiotics in stable and exacerbation states. However, there is a trend towards use of shorter courses (i.e. less than two weeks) amongst clinicians. The risks of antibiotic side effects and resistance are a significant concern when using longer courses of antibiotics. A review of the literature to determine the evidence for use of shorter courses of antibiotics in adults and children in stable and exacerbation states of bronchiectasis is important to assist in guiding clinical practice.

OBJECTIVES

To evaluate the efficacy of short courses (i.e. four weeks or less) of antibiotics in children and adults with bronchiectasis;

(a) during stable state bronchiectasis; and

(b) for reducing the severity and frequency of acute respiratory exacerbations.

METHODS

Criteria for considering studies for this review

Types of studies

Any randomised controlled trial comparing outcomes with use of antibiotics (intravenous, oral or inhaled) versus placebo or usual care as the control, for periods of less than, or equal to, four weeks, in non-cystic fibrosis bronchiectasis. Participants are allowed to have adjunctive therapies (such as airway clearance) as long as they have equal chance of having these adjunctive therapies.

Types of participants

Adults and children with bronchiectasis (defined clinically or radiologically) not related to cystic fibrosis.

Types of interventions

Any short (four weeks or less) course of antibiotics given by intravenous, oral or nebulised routes.

Types of outcome measures

Primary outcomes

The primary outcome measures were change in:

1. Symptom score (e.g. bronchiectasis severity control, cough-specific/respiratory-specific quality of life (QoL) or generic health-specific QoL)

2. Lung function indices (airway resistance or airway calibre measurements)
3. Adverse events

Secondary outcomes

1. Sputum or airway markers (weight, purulence, colour (Bronkotest), inflammatory profiles)
2. Microbiological data (density, resistance patterns)
3. Exacerbation data (length, time to next exacerbation)

Search methods for identification of studies

Electronic searches

Randomised controlled trials were identified using the Cochrane Airways Group Specialised Register of trials, which is derived from systematic searches of bibliographic databases including the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, OLDMEDLINE, CINAHL, AMED and PsycINFO, and handsearching of respiratory journals and meeting abstracts (please see the Airways Group search methods for further details). We searched all records in the Specialised Register coded as 'bronchiectasis' using the following terms:

antibiotic* OR *cillin OR *tetracycline OR *mycin OR macrolide* OR quinolone* OR *oxacin OR trimethoprim OR *sulpha OR *ceph or anti-bacteri* or "anti bacteri* OR anti-microbial* OR anti*
The search was conducted in February 2011.

Searching other resources

We handsearched all the papers and reviews identified for further references and contacted authors to request their identification of any unpublished or missed trials. We contacted researchers directly as required to establish whether other unpublished or ongoing studies were available for assessment. We handsearched clinical trials web sites (www.clinicalstudyresults.org; www.clinicaltrials.gov; www.fda.gov).

Data collection and analysis

Selection of studies

Following electronic literature searches, AC and DW independently reviewed the searches to identify potentially relevant trials for full review. We searched bibliographies and texts to identify additional studies. From the full text using specific criteria, AC and DW independently selected trials for inclusion. There was complete agreement between review authors.

Data extraction and management

We extracted information from each study for the following characteristics:

1. Design (description of randomisation, blinding, allocation, number of study centres and location, withdrawals)
2. Participants (N, mean age, age range of the study, baseline lung function, duration of antibiotics < 4 weeks versus no antibiotics)
3. Intervention (type and duration of antibiotic, appropriateness of antibiotic choice, dosing schedules of groups)
4. Outcomes (type of outcomes and results of outcomes)

We requested further information from the trial authors where required. This occurred with Barker 2000 as explained in the included studies section of the Results.
Studies were translated to English where possible.

Assessment of risk of bias in included studies

We assessed trial bias protection in the following domains and study quality according to whether studies met the following prespecified quality criteria (as met, unmet or unclear) using the risk of bias (RoB) table in Review Manager 5.

1. Sequence generation
2. Allocation concealment
3. Blinding of participants and investigators
4. Loss to follow-up

Measures of treatment effect

We extracted data for each of the outcomes (where data were available) from the trial publication that fulfilled the inclusion criteria.
We performed an initial qualitative comparison of all the individually analysed studies taking into account differences in study populations, inclusion/exclusion criteria, interventions, outcome assessment and estimated effect size, to examine whether pooling of results (meta-analysis) was reasonable.

Unit of analysis issues

We sought to obtain data that were reported with patients (rather than events) as the unit of analysis for the primary outcomes.

Dealing with missing data

The proportion of randomised patients who provided data for the main outcomes was reported and we had planned to compare this with the number of patients with events in each outcome category.

Assessment of heterogeneity

We had planned to describe and explore heterogeneity between the study results, and to use the 95% confidence interval, estimated using a random-effects model, whenever there were concerns about statistical heterogeneity.

Assessment of reporting biases

If combining data was possible, we had planned to assess publication bias using a funnel plot. We planned to identify and report on any selective reporting in the included trials.

Data synthesis

We combined data using Review Manager 5, with a view to using a fixed-effect mean difference (calculated as a weighted mean difference) for continuous data variables. If different scales were combined, we had intended to use the standardised mean difference.

For the dichotomous outcome variables of each individual study, we had planned to calculate odds ratios using a modified intention-to-treat analysis (i.e. failure assumed if participant drops out of study). This analysis assumes that children or adults not available for outcome assessment have not improved (and probably represents a conservative estimate of effect).

For the primary outcomes we intended to calculate a number needed to treat (benefit or harm) when possible for the different levels of risk as represented by control group event rates over a specified time period using the pooled Odds Ratio and its confidence interval using an online calculator, Visual Rx (Cates 2003). A summary of findings (SoF) table would be constructed for the primary outcomes.

Subgroup analysis and investigation of heterogeneity

We had intended to perform an a priori subgroup analysis for:

1. Children versus adults (adult studies will be considered as those which recruited participants from 18 years upwards)
2. Type of antibiotics (oral, intravenous, inhaled)
3. Type of control arm (placebo/no treatment or control antibiotic i.e. prolonged duration antibiotics)
4. State during enrolment (stable state or exacerbation)

Sensitivity analysis

Sensitivity analyses were also planned to assess the impact of the potentially important factors on the overall outcomes:

1. variation in the inclusion criteria;
2. differences in the medications used in the intervention and comparison groups;
3. analysis using random-effects model;
4. analysis by "treatment received"; and
5. analysis by "intention-to-treat".

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies.

Results of the search

From the searches, the Cochrane Airways Group specialised register/search identified 187 potentially relevant titles. After assessing the titles/abstracts, we obtained19 papers for consideration for inclusion in the review. We reviewed 11 full text articles and 8 further abstracts. We excluded 18 studies (see Excluded studies). We found two potentially eligible studies, however they appeared to have used the same patient population, leaving only one eligible study in adults. We identified no studies in children.

Included studies

The included studies were Barker 2000 and Couch 2001a, which described the same study populations. Couch 2001a was an "early review of data" for a chest supplement on aerosolised therapeutics as discussed by Fiel 2001 in his editorial. For this reason, we used the final data in Barker's paper in this Cochrane Review.

The sole study was a multicentre parallel RCT that examined the effect of nebulised tobramycin (Tobramycin Solution for Inhalation) versus placebo, in adults with CT confirmed bronchiectasis and *Pseudomonas aeruginosa* infection. There were 16 study sites, 74 adults enrolled with completion rate of 81% (60/74). Further details are described in the Characteristics of included studies.

Excluded studies

Eighteen studies were excluded as they did not fulfil criteria for the review. Fifteen of the eighteen studies excluded had no suitable comparator/placebo group, one study did not specify bronchiectasis as an inclusion criterion, one study had treatment periods greater than four weeks, two studies shared a common study population, resulting in the exclusion of one.

Bilton 2006 and Shrewsbury 2004 examined short courses of antibiotics in bronchiectasis and compared oral ciprofloxacin (in treatment doses) plus placebo to oral ciprofloxacin plus inhaled tobramycin. Although these studies almost fulfilled criteria for inclusion, there was no "antibiotic-free" placebo for comparison, resulting in their exclusion from this review.

Couch 2001a was excluded as it was an early review of the same data used in the included study Barker 2000. Attempts to contact the corresponding author were unsuccessful, but this was confirmed in the editorial Fiel 2001 which accompanied the paper.

Risk of bias in included studies

Allocation concealment was unclear in this study (Barker 2000). Block randomisation was utilised to balance group sizes. The study was double-blinded, with both investigators and participants being blinded to study group allocation to reduce selection bias. Six patients were withdrawn from the placebo group due to need for an antibiotic other than the study drug.

Allocation

Block randomisation of patients was used. Eligible patients were randomly assigned in blocks of two to parallel groups at each study centre to receive either inhaled tobramycin or placebo.

Blinding

The study was double-blinded i.e. both participants and investigators were blinded to the study drug assignment until completion of the follow-up visit and data were collected from all study sites. The placebo used (quinine sulphate) was chosen because of its similar taste to tobramycin. It was unclear whether investigators were blinded to the study hypothesis.

Incomplete outcome data

The number of participants withdrawn (due to adverse events or use of antibiotic other than the study drug) and those lost to follow-up were reported for both inhaled tobramycin and placebo groups.

Selective reporting

There was no suggestion that selective reporting had occurred.

Effects of interventions

The included trial involved 74 patients with 60 completing the study. In the absence of additional suitable studies, there were insufficient data to perform a meta-analysis. The outcomes from the single study (also presented in the forest plot) were:

Primary Outcomes

1. Symptom score (e.g. bronchiectasis severity control, cough-specific/respiratory-specific quality of life (QoL) or generic health-specific QoL)

The study did not specifically measure any symptoms to determine a symptom score. Instead, the investigator's subjective assessment of a change in the patient's general medical condition ("improved" or "not improved") was recorded at week six: 23 of 37 (62%) patients in the tobramycin-treated group significantly improved compared to 14 of 35 (40%) in the placebo group that improved (Analysis 1.1; OR 0.37; 95% CI 0.14 to 0.95).

2. Lung function indices (airway resistance or airway calibre measurements)

Percent change in forced expiratory volume in one second (FEV_1) percent predicted and in Forced Vital Capacity (FVC) percent predicted from baseline to week four were not statistically significant between the tobramycin and placebo groups (-2.2% versus 1.5% respectively, P = 0.41) for FEV_1 % predicted and FVC % predicted (-2.8% versus 2.2%, P = 0.19). Airway reactivity (percent change in FEV1 from pre- to post-study drug administration) was not significantly different from zero percent for either the tobramycin group (mean = -1%, Week 0, -3% Week 4) or placebo group (mean = -3% Week 0, -1% Week 4).

3. Adverse events

Thirty-one of 37 (84%) patients in each treatment group reported at least one adverse event. Respiratory system adverse events were reported by 26 (70%) tobramycin patients and by 19 (51%) placebo patients. There was no statistical significance, but results favoured the placebo group (OR 2.24; 95% CI 0.86 to 5.82; Analysis 2.1). Five patients in the tobramycin group and one patient in the placebo group were hospitalised and treated for an exacerbation of their pulmonary disease (OR 5.63; 95% CI 0.62 to 50.73; Analysis 2.2).

Secondary outcomes

1. Microbiological response (i.e. change in *P. aeruginosa* density from baseline to week 4 and week 6 and antibiotic resistance)

At the end of the trial, significantly more subjects in the tobramycin group (13 of 37) had *P. aeruginosa* eradicated from their sputum compared to the placebo group (0 of 37); (OR 0.03; 95% CI 0.01 to 0.14; Analysis 3.1.) Furthermore, a further 12 patients in the tobramycin group had reduced *P. aeruginosa* carriage (PA cfu/g decreased by at least 2 $\log_{10}$ at week 4), compared to two patients with reduced carriage in the placebo group.

However, at follow-up two weeks post cessation of antibiotics, the mean reduction in the tobramycin group was smaller than in previous weeks, suggesting some regrowth of organisms after ceasing the antibiotic.

Four of 36 patients in the tobramycin group and one of 32 in the placebo group, who began the study with susceptible *P. aeruginosa*, had resistant *P. aeruginosa* at their last visit (P = 0.36). Three of the four patients in the tobramycin group who developed resistant *P. aeruginosa* showed no microbiological response. All four patients

were considered to have not improved when their general medical condition was assessed.

DISCUSSION

This review is limited to a sole study in adults. The data suggest that short term inhaled antibiotics show some benefit in the treatment of patients with bronchiectasis and *P. aeruginosa*. The study followed the patients for only two weeks post-treatment to assess longevity of response. Despite its weaknesses, this single study supports previous Cochrane Reviews on the use of longer term antibiotics in bronchiectasis (Evans 2007), in showing subjective improvement in general medical condition as assessed by an investigator.

This review has highlighted the fact that, although antibiotics are the mainstay of treatment in adults and children with bronchiectasis, there remains a paucity of data, particularly high quality data, to support this practice. In today's evidence based medical era and with bronchiectasis becoming increasingly recognised as a major cause of respiratory morbidity, there will be an increasing need to provide evidence-based answers to these common questions.

A small number of patients in the study antibiotic arm developed "resistant" pseudomonas. This highlights an important complication of antibiotic therapy, particularly when antibiotic use in any one patient is prolonged or recurrent. Previous research by Hillier 2007 has shown that antibiotic resistance increases with increased duration of antibiotic therapy. These findings are related to urinary tract infections but are likely to be applicable to infections elsewhere in the body including the lung. This issue, combined with the likely improved quality of life for patients receiving shorter courses of antibiotics, highlights the need for further studies to determine the optimal duration of antibiotic therapy in bronchiectasis.

The included study followed patients for only two weeks post-therapy. Given that antibiotics are known to indirectly limit neutrophilic inflammation in the airways and ideally eradicate the bacterial infection, it is not surprising that patients showed improvement over this follow-up period. It is unknown if two weeks is an optimal timeframe to assess bacterial eradication adding further to the limitations of this study. To assess longevity of symptom response and microbiological eradication, a longer timeframe of follow-up would have been required.

Evans 2007's Cochrane Review on prolonged antibiotics found a significant benefit of prolonged antibiotics (i.e. four or more weeks) in terms of response rates. They found no significant difference between placebo and prolonged antibiotics in terms of exacerbation rates and lung function. This would concur with Barker 2000, which found a subjective improvement in patients' general medical condition with inhaled tobramycin therapy as compared to placebo, with no significant differences in lung function between treatment groups.

The single included study (Barker 2000) showed some benefit of four weeks of inhaled antibiotic therapy compared with placebo in patients with bronchiectasis and *P. aeruginosa* infection in terms of microbiological response (at the expense of increased resistance) and improvement in general medical condition. The lack of other suitable studies for this Cochrane Review precluded a meta-analysis, and as a result we could not draw any firm conclusions on the topic.

AUTHORS' CONCLUSIONS

Implications for practice

When considering implications for practice, one must acknowledge the fact that this review was based on one study of adult patients and the intervention was "inhaled" antibiotics. This study showed a small benefit in microbiological response and overall subjective improvement in general medical condition, with no effect on lung function and an increase in adverse events in the intervention group. The study failed to address primary outcome measures with symptom scores or objective outcomes. Therefore, although this one study is suggestive of benefit of short courses of antibiotics for bronchiectasis, one would conclude, for current practice implications, that there is insufficient evidence available in the current literature to make any reasonable conclusions. However until further evidence is available, clinicians should adhere to guidelines (Chang 2010; Pasteur 2010) that include data from non RCTs.

Implications for research

Well-designed, double-blinded, parallel, randomised controlled trials are required to assess the role of short courses of antibiotics in the treatment of bronchiectasis. These studies should include validated outcome measures such as improvements in symptom score, QOL and lung function, balanced against adverse effects as primary outcome measures. Trials including oral, inhaled and intravenous antibiotic administration methods are needed. Future RCTs should be undertaken in paediatric as well as adult patients. Well-designed studies should include sufficient longitudinal follow-up of patients to assess longevity of intervention response and therefore applicability of evidence to practice. Such well-designed research would have the potential to improve the quality of life for individuals with bronchiectasis.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Barker 2000

Methods	The study was a randomised placebo-controlled, double-blinded trial with antibiotics administered for 4 weeks. Eligible patients were randomly assigned in blocks of two to parallel groups at each of 16 study sites across the United States to receive either Tobramycin solution for inhalation or placebo. This was administered twice daily for 4 weeks in bronchiectasis patients whose sputum contained *Pseudomonas aeruginosa*. Patients were then observed for 2 weeks after administration of their last dose. Of patients enrolled, they were divided equally between the two treatment groups. Patients were withdrawn if they required any additional antibiotics at any stage during study participation Patients were screened 2 weeks prior to their initial dose of the study drug, were dosed for 4 weeks, and then observed for 2 weeks after their last dose. Thus the total duration of the study was 8 weeks. At each visit a sputum sample was obtained and the density of the *P. aeruginosa* in sputum was measured. Pulmonary function testing (FEV1 and FVC) was performed at baseline and at the final treatment visit (week 4). Tobramycin levels were measured. Adherence was measured at week 4 by counting the number of vials of study drug used. A subjective clinical assessment of the patient's general medical condition was made, by a study investigator, at the follow-up visit on week 6 There were 6 patients who withdrew from the tobramycin group (3 for adverse events, 2 for use of antibiotics other than study drug, and 1 was lost to follow-up.) 8 withdrew from the placebo group (2 for adverse events, and 6 for use of antibiotics other than the study drug.)
Participants	125 patients were screened and 74 patients (45 female), mean age 66.6 (tobramycin group) and 63.2 (placebo group) were enrolled. Patients were block randomised to receive either 300 mg of inhaled tobramycin or placebo twice daily for 4 weeks. 37 received inhaled tobramycin and 37 received placebo Inclusion: Bronchiectasis diagnosed by conventional or high-resolution CT and sputum containing at least 10^4 cfu/mL *P. aeruginosa*. Exclusion: cystic fibrosis, allergic bronchopulmonary aspergillosis, acute pulmonary process requiring medical intervention as indicated by a new infiltrate on a chest radiograph, significant recent haemoptysis, or had received antibiotics within 2 weeks of the screening visit
Interventions	Nebulised tobramycin solution for inhalation (300 mg tobramycin) twice daily or placebo for 4 weeks
Outcomes	Primary end point was (1) Change in *P. aeruginosa* density from baseline to week 4. Additional endpoints included: (1) change in *P. aeruginosa* density from baseline values to week 2 and to week 6; (2) an investigator's subjective assessment of a change in the patient's general medical condition ("improved" or "not improved") was made and recorded at week 6, (3) the percent change in FEV1 percent predicted and in FVC percent predicted from week 0 to week 4 and (4) Safety endpoints included the incidence of adverse events, change in serum chemistry and haematology measurements, and airway reactivity

Barker 2000 (*Continued*)

	Each patient's microbiological response was categorised according to whether *P. aeruginosa* was eradicated, reduced by treatment, or did not respond to treatment. *P. aeruginosa* was considered eradicated if it was not detected at week 6 or if it was not detected at week 4 and the patient was unable to produce sputum at week 6. A patient's response was defined as reduced by treatment if *P. aeruginosa* was recovered from the week 6 sputum and reduced by at least 2 log 10 cfu/g at week 4 compared with baseline. A patient had no microbiological response if *P. aeruginosa* did not decrease 2 log 10 cfu/g at week 4 or if the patient withdrew from the study
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Allocation concealment (selection bias)	Unclear risk	Unclear
Blinding of participants and investigators.	Low risk	Patients and investigators were blinded to the study drug assignment
Incomplete outcome data assessed?	Low risk	Number of dropouts and loss to follow-up included.
Free of selective reporting?	Low risk	No suggestion that selective reporting may have occurred.
Free of other bias?	Low risk	No other bias identified
Sequence generation.	Low risk	Sequence generation was referred to, however details were not provided

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Bilton 2006	No suitable placebo/comparator group. (Comparator was not adequate to satisfy inclusion criteria, i.e. no "antibiotic-free/placebo" or long-term antibiotic group for comparison.)
Chrysanthopoulos 1989	No suitable placebo/comparator group.
Couch 2001a	Overlap of study participants with included study.
Douglas 1957	No suitable placebo/comparator group.
Howie 1976	Study participants had unknown bronchiectasis status.

(*Continued*)

Lam 1989	No suitable placebo/comparator group.
Lin 1996	No suitable placebo/comparator group.
Matsumoto 1986	No suitable placebo/comparator group. Not an RCT.
May 1972	No suitable placebo/comparator group.
Mehta 1991	No suitable placebo/comparator group.
Messens 1973	Translated from French. No suitable placebo/comparator group
Mijuskovic 1972	No suitable placebo/comparator group.
Nagy 1968	No suitable placebo/comparator group. (Compared antibiotic treatment to surgery.)
Oki 1993	No suitable placebo/comparator group. (Examined long term antibiotic treatment, no short course treatment arm.)
Santiveri 1995	No suitable placebo/comparator group.
Shrewsbury 2004	No suitable placebo/comparator group. (Comparator was not adequate to satisfy inclusion criteria, i.e. no "antibiotic-free/placebo" or long-term antibiotic group for comparison.)
Tagaya 2002	No suitable placebo/comparator group.
Twiss 2008	Longer-course antibiotics (i.e. 2 to 3 months) compared to placebo. No short course group

DATA AND ANALYSES

Comparison 1. Change in general medical condition

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Improvement in general medical condition	1		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only

Comparison 2. Respiratory system adverse events

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Respiratory system adverse events	1		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2 Hospitalised for respiratory events	1		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only

Comparison 3. Microbiological response

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Microbiological response	1		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only

Comparison 4. Development of pseudomonas resistance (when initially sensitive)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Pseudomonas resistance	1		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only

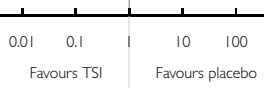
Analysis 1.1. Comparison 1 Change in general medical condition, Outcome 1 Improvement in general medical condition.

Review: Short courses of antibiotics for children and adults with bronchiectasis

Comparison: 1 Change in general medical condition

Outcome: 1 Improvement in general medical condition

Study or subgroup	TSI n/N	Placebo n/N	Odds Ratio M-H,Fixed,95% CI	Odds Ratio M-H,Fixed,95% CI
Barker 2000	14/37	23/37		0.37 [0.14, 0.95]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 14 (TSI), 23 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				



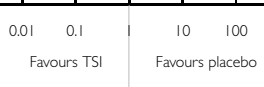
Analysis 2.1. Comparison 2 Respiratory system adverse events, Outcome 1 Respiratory system adverse events.

Review: Short courses of antibiotics for children and adults with bronchiectasis

Comparison: 2 Respiratory system adverse events

Outcome: 1 Respiratory system adverse events

Study or subgroup	TSI n/N	Placebo n/N	Odds Ratio M-H,Fixed,95% CI	Odds Ratio M-H,Fixed,95% CI
Barker 2000	26/37	19/37		2.24 [0.86, 5.82]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 26 (TSI), 19 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				



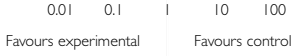
Analysis 2.2. Comparison 2 Respiratory system adverse events, Outcome 2 Hospitalised for respiratory events.

Review: Short courses of antibiotics for children and adults with bronchiectasis

Comparison: 2 Respiratory system adverse events

Outcome: 2 Hospitalised for respiratory events

Study or subgroup	Tobramycin n/N	Placebo n/N	Odds Ratio M-H,Fixed,95% CI	Odds Ratio M-H,Fixed,95% CI
Barker 2000	5/37	1/37		5.63 [0.62, 50.73]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 5 (Tobramycin), 1 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				



Analysis 3.1. Comparison 3 Microbiological response, Outcome 1 Microbiological response.

Review: Short courses of antibiotics for children and adults with bronchiectasis

Comparison: 3 Microbiological response

Outcome: 1 Microbiological response

Study or subgroup	TSI n/N	Placebo n/N	Odds Ratio M-H,Fixed,95% CI	Odds Ratio M-H,Fixed,95% CI
Barker 2000	12/37	33/35		0.03 [0.01, 0.14]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 12 (TSI), 33 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				

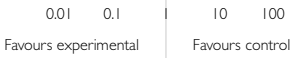
Analysis 4.1. Comparison 4 Development of pseudomonas resistance (when initially sensitive), Outcome 1 Pseudomonas resistance.

Review: Short courses of antibiotics for children and adults with bronchiectasis

Comparison: 4 Development of pseudomonas resistance (when initially sensitive)

Outcome: 1 Pseudomonas resistance

Study or subgroup	TSI n/N	Placebo n/N	Odds Ratio M-H,Fixed,95% CI	Odds Ratio M-H,Fixed,95% CI
Barker 2000	4/36	1/32		3.88 [0.41, 36.63]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 4 (TSI), 1 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				



HISTORY

Protocol first published: Issue 9, 2010

Review first published: Issue 6, 2011

CONTRIBUTIONS OF AUTHORS

DW and AC wrote the protocol, based on previous protocols on cough in children. DW and AC selected articles from the search and together with JM, wrote the manuscript. BM, JU and SY contributed by reviewing the manuscript.

DECLARATIONS OF INTEREST

No conflicts of interest.

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- NHMRC, Australia.

Practitioner Fellowship for AC

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The duration of short course antibiotics was originally specified in the protocol as less than four weeks, however, in the actual review this was amended to four weeks or less to enable inclusion of Barker 2000.

INDEX TERMS

Medical Subject Headings (MeSH)

Anti-Bacterial Agents [administration & dosage; *therapeutic use]; Bronchiectasis [*drug therapy]; Drug Administration Schedule; Randomized Controlled Trials as Topic

MeSH check words

Adult; Child; Humans

7.3 Drug treatments of childhood cough

ARTICLE

Drug treatments of childhood coughs

SUMMARY

Appropriate management of cough in children depends upon accurate assessment. The diagnosis is often unclear at the initial presentation.

Acute cough is frequently caused by a viral infection, and often no specific therapy is indicated. Urgent treatment may be needed if history suggests a more serious disorder such as a foreign body or pneumonia.

When treating children with chronic cough, paediatric-specific algorithms should be used. Empirical use of medicines without looking for a specific cause should be avoided.

In the absence of an alternative specific cause of cough, chronic wet cough (lasting at least four weeks) is most frequently due to protracted bacterial bronchitis. Antibiotics are indicated.

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Key words
asthma, bronchiectasis, bronchiolitis, bronchitis, croup, pertussis, pneumonia

Aust Prescr 2014;37:115–9

Introduction

Cough is the most common symptom presented to GPs and pharmacists in Australia. An Australian study found that 'one in three (28.7%) respiratory episodes were associated with a doctor's visit, and one in four (23%) necessitated time off school or work'.[1] When a child first presents with cough, determining the precise diagnosis is not always possible.

Acute cough

Acute cough in a child may represent a variety of pathologies, from self-resolving viral-induced acute respiratory infection to acute severe respiratory disease or an acute presentation of an underlying chronic disorder. Appropriate management depends on accurate assessment. Patient history should include:[2]

- cough duration (acute <2 weeks, sub-acute 2–4 weeks, chronic >4 weeks)
- characteristics of cough (whooping cough, wet vs dry cough)
- questions about choking episodes and previous respiratory illness
- associated wheeze
- other symptoms such as weight loss, appetite or rash
- immunisation history.

In the differential diagnosis, it is important to consider inhaled foreign body, pneumonia and other treatable infections like pertussis and underlying lung disease such as bronchiectasis.

Uncomplicated acute upper respiratory infections

It is commonly said that young children have up to 6–12 acute respiratory infections per year. However, a Melbourne-based community study involving 600 families showed fewer episodes and an age-dependent trend (see Table).[1] The mean duration of episodes was 6.3 days (range 1–70 days) and younger children were more likely to have a longer duration of cough (6.8 days in youngest age group and 5.5 days in oldest group).

Management

Supportive therapy is the mainstay of treatment for viral acute respiratory infections. Paracetamol and ibuprofen are useful for related symptoms. Over-the-counter cough and cold medicines are not recommended due to a lack of proven efficacy and the possibility that they may present a safety risk.[3] The Therapeutic Goods Administration now recommends that they should not be used in children under 6 years and only in children aged 6–11 years on advice from a doctor.[4]

Honey,[5,6] and menthol-based rubs[7] may reduce the impact of nocturnal cough. It is reasonable to recommend one teaspoon of honey before bedtime for children aged over one year. Honey should be avoided in children under one year due to the risk of botulism.

Table **Australian rates of uncomplicated acute upper respiratory infections in children and young adults** [1]

Age (years)	**Mean number of episodes a year**
0–1	3.8
2–3	3.3
4–5	2.8
6–10	2.2
11–20	2

Antibiotics should be avoided for the treatment of acute cough associated with mild upper respiratory tract infection, as the cough is most likely viral in origin. A recent Cochrane review reported that in cases of confirmed or suspected exposure to influenza in healthy children, oseltamivir shortens the time to first alleviation of symptoms by 29 hours (95% confidence interval 12–47 hours, p=0.001).[8] No effect however was seen in children with asthma. Oseltamivir may reduce the risk of otitis media in children aged 1–5 years, especially if commenced within the first 12 hours, but is associated with a significantly increased risk of vomiting.[9] Laboratory-based polymerase chain reaction (PCR) techniques enable rapid influenza diagnosis.[10]

Management of acute cough should include counselling and advice on:

- the expected duration of cough (typically 5–7 days, but up to 3 weeks)
- when to come back and see the GP and when to seek urgent medical review (for example suspected foreign body, tachypnoea, dyspnoea, vomiting, inability to feed, persistent fever, lethargy)
- avoidance of passive smoke exposure.

Specific causes of acute cough

A number of specific diseases need to be considered in a child presenting with acute cough. Many of these have specific symptoms and signs.

Croup

The acute or sub-acute onset of a barking 'brassy' cough, hoarse voice, stridor with or without evidence of upper airway obstruction, is characteristic of croup. It often begins with a viral upper respiratory tract infection (for example rhinorrhoea, sore throat with or without fever) and typically affects children aged 1–6 years. Children outside this age range or with severe or recurrent stridor or other symptoms require careful evaluation for an underlying airway lesion. Children with bacterial causes of stridor such as tracheitis or epiglottitis usually appear more toxic.

Prednisolone 1–2 mg/kg orally for two consecutive days is effective for croup. Dexamethasone 0.15 mg/kg orally is an appropriate alternative therapy. In severe croup, when a child has ongoing stridor at rest, increasing fatigue and marked tachycardia with or without signs of impending hypoxaemia (for example, lethargy and increased irritability), urgent transfer to an emergency facility is recommended. Potentially distressing interventions, such as throat examination, should be avoided, as these may worsen respiratory obstruction.

Pneumonia

Children with pneumonia often have cough, fever and tachypnoea, but occasionally present with fever and upper abdominal pain. Signs of severity include grunt and intercostal recession. Wheeze is usually absent in bacterial pneumonia.

A chest X-ray does not need to be performed routinely in all children with suspected pneumonia. However, it should be considered in any child with an atypical presentation (recurrent pneumonia, prolonged fever, signs of pleural effusion) or severe pneumonia requiring hospital admission.[11]

Recommendations for antimicrobial therapy vary according to the age of the child, context, presence of underlying disease (risk factors), presence of hypoxaemia, non-respiratory symptoms (such as vomiting), length and severity of symptoms and the presence of complications. Guidelines for antimicrobial therapy should be consulted.[11-13] For a child with subacute onset and prominent cough (with or without headache or sore throat), or who is not improving, mycoplasma pneumonia should be suspected.[13]

Indications for hospitalisation for community-acquired pneumonia include:

- very young children (less than 6 months) with suspected bacterial pneumonia[12]
- clinical evidence of moderate to severe pneumonia, including hypoxaemia and signs of respiratory distress[12]
- significant comorbidities or factors which predispose to more severe disease e.g. immunodeficiency, congenital heart disease, bronchiectasis[11]
- pneumonia suspected or confirmed to be secondary to a pathogen with increased virulence e.g. community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA)[12]
- dehydration or inability to tolerate oral therapies[11]
- significant parental concern or anxiety[11]
- family unable to provide appropriate care or adhere to management plan[12]
- toxic-looking child e.g. pale or cyanotic, lethargic or inconsolably irritable
- complicated pneumonia e.g. empyema
- poor response after 48 hours of oral antibiotics.

All children with suspected pneumonia should be followed up regularly to ensure complete resolution of their symptoms. A repeat chest X-ray is not routinely performed following simple pneumonia unless there are persisting symptoms.[11]

Bronchiolitis

Children under two years presenting acutely with cough, tachypnoea (with or without poor feeding) and often with a history of a viral prodrome may

have viral bronchiolitis. Clinical examination reveals hyperinflation with widespread wheeze and crackles on chest auscultation. Respiratory syncytial virus is the most common infection associated with bronchiolitis.

Any infant with apnoeas, hypoxia (oxygen saturations ≤92%), dehydration or poor feeding requires hospital admission for supplemental oxygen with or without hydration therapy. Children frequently worsen in the first 72 hours before showing improvement. The cough can persist for 2–3 weeks after other symptoms resolve. There is no evidence for the routine use of antibiotics, steroids or asthma drugs in viral bronchiolitis.

Pertussis

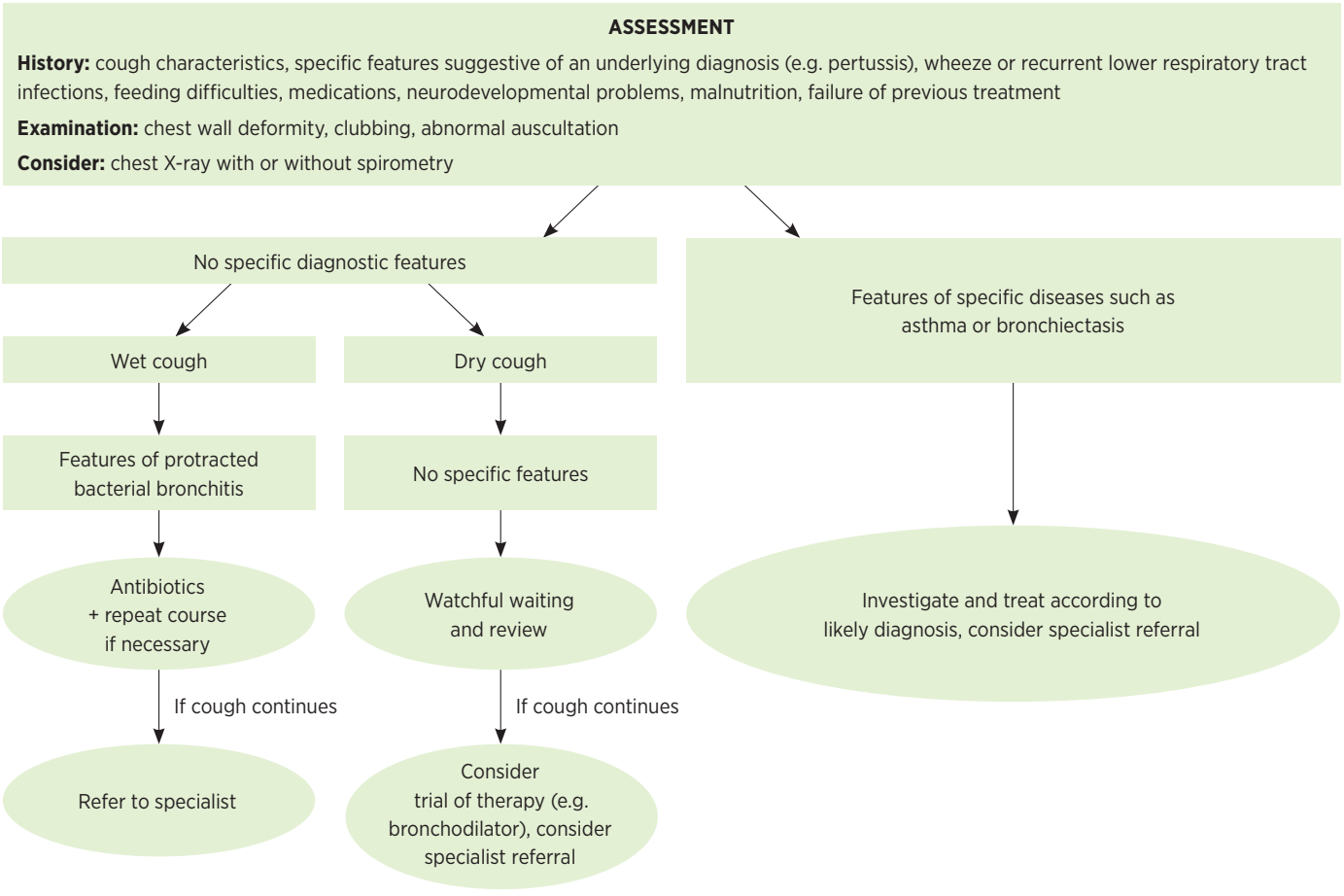
Pertussis (whooping cough) typically presents with cough lasting two or more weeks with cough paroxysms, inspiratory whoop or post-tussive vomiting. Confirmation with a PCR-positive nasopharyngeal aspirate or swab is recommended. If there is a high clinical suspicion, start antibiotics before receiving the test results. Clarithromycin (7.5 mg/kg up to 500 mg orally, 12-hourly for 7 days) or erythromycin (10 mg/kg up to 250 mg orally, 6-hourly for 7 days) is recommended.[13] Treat early to improve symptoms (within 1–2 weeks of start of symptoms) and reduce the infectious period. Patients are seldom infectious after having a cough for longer than three weeks and antibiotics are not recommended at this point.

Chronic cough

The common causes of chronic cough in children differ from those in adults[14] so adult-type management approaches directed at asthma, rhinitis and gastro-oesophageal reflux disease do not apply. In a multicentre study involving 346 new referrals to respiratory paediatricians for chronic cough, the most common diagnoses included protracted bacterial bronchitis (41%), asthma (15.9%) and bronchiectasis (9%). In 13.9% of children, cough resolved without a specific diagnosis.[15]

A detailed respiratory history and examination as well as use of a chronic cough algorithm (see Fig.)[16] assist in the assessment and diagnosis of chronic cough.

Fig. **Simplified paediatric chronic cough algorithm**



Adapted from reference 16

The cough algorithm also significantly improves quality of life and reduces duration of cough.[16] This approach is based on determining the cause of the cough (through systematic history taking and a thorough examination), in addition to spirometry (in a child >5 years of age) and chest X-ray. Indications for referral to a specialist are listed in the Box.

Box Common indications for specialist referral in chronic childhood cough

Chronic cough (>4 weeks) of unclear aetiology (with or without failure to thrive)
Suspected airway malformation e.g. tracheo-oesophageal fistula, vascular ring
Cough and feeding difficulties (suspected aspiration disease)
Clinical features of chronic lung disease e.g. clubbing
Persisting auscultatory findings e.g. crepitations
Recurrent pneumonias
Abnormalities on chest X-ray or spirometry
Failure to respond to treatment e.g. in asthma

Protracted bacterial bronchitis

Protracted bacterial bronchitis is the most common cause of chronic wet cough in Australian children.[14,16] It is defined as:

- cough lasting more than four weeks
- response to two weeks of antibiotic therapy
- absence of specific pointers indicating an alternative cause.

A history of a preceding viral infection is common. Protracted bacterial bronchitis is more common in boys than girls and in those aged 1–3 years.

Lower airway bacterial infection is frequently found on bronchoalveolar lavage sampling and is usually accompanied by elevated neutrophils suggestive of active airway inflammation. The major bacterial organisms found are *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*. After exclusion of other causes of chronic cough, a two-week course of amoxycillin-clavulanate is recommended. Children should receive follow-up after 2–3 weeks to ensure complete resolution of cough. A chest X-ray should be performed in any child with clinical suspicion of an alternative cause of chronic cough or if their cough persists despite antibiotic therapy.

Bronchiectasis

Bronchiectasis is another important cause of wet cough to consider, and should be suspected in any child with the following:

- chronic wet cough lasting longer than eight weeks
- two or more episodes of chronic wet cough (lasting ≥4 weeks) per year responding to antibiotics
- chest radiographic changes lasting more than six weeks despite appropriate antibiotic therapy.[17]

Antibiotic therapy is usually started at the onset of wet cough in children known to have bronchiectasis. Antibiotic selection is based upon lower airway culture, local antibiotic susceptibility patterns and clinical severity. If symptoms do not respond promptly or adequately to oral antibiotic therapy patients should be hospitalised for intravenous antibiotics. Regular physiotherapy, physical exercise, avoidance of triggers (for example tobacco smoke) and routine vaccinations are recommended.[17] Aboriginal and Torres Strait Islander children are at increased risk of bronchiectasis and doctors should be aware that cough may be under-reported by those from remote communities.[18]

Asthma and chronic cough

While asthma can cause chronic cough, isolated chronic cough without any other symptoms in children is rarely due to asthma.[19,20] Other symptoms usually present in asthma are wheeze, dyspnoea, chest tightness or exercise limitation. Risk factors such as eczema, hay fever, allergies or a family history of asthma in a first-degree relative are often present.

Spirometry and measurements of airway responsiveness (for example exercise challenge) in children aged over five years can help to diagnose asthma. The presence of atopy does not distinguish asthma from other causes of chronic cough. Previous response to asthma therapies may be helpful, however response on a single occasion does not necessarily mean that the child has asthma. Guidelines for the management of asthma are available from the National Asthma Council of Australia (www.nationalasthma.org.au/handbook).

Conclusion

Accurate diagnosis of cough in children depends upon a thorough clinical history and examination to guide appropriate prescribing. The nature of the cough and its chronicity provide important diagnostic clues as to a specific cause of cough. Cough guidelines and algorithms further enhance diagnostic accuracy and may help to ensure more effective prescribing of cough therapies in children. ◅

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Q:

SELF-TEST QUESTIONS

True or false?

1. Aboriginal and Torres Strait Islander children have an increased risk of bronchiectasis.
2. There is evidence for use of steroids in viral bronchiolitis.

Answers on page 143

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CHAPTER 8

8.1 DISCUSSION

Over the past two decades, the significance of chronic wet cough has been increasingly appreciated. Using a systematic approach to evaluate the most common cause of chronic cough in children, the relationship between wet cough, bacterial infection and neutrophilic airway inflammation was identified and the clinical entity of protracted bacterial bronchitis (PBB) was first described in 2006 [2]. Early research into PBB was subsequently supported by studies by other research groups, both locally and internationally [75-77]. Defining the clinical entity of PBB has been pivotal in our understanding of chronic wet cough in children. It has also facilitated further studies on the most common aetiologies of chronic cough in children [16, 27].

Prior to this PhD thesis, several research gaps existed in the area of PBB and chronic wet cough in children. These included:

1) the significance of wet cough as a symptom

2) the role of viruses in PBB

3) the clinical profile of children with PBB and

4) the validity and accuracy of the postulated link between PBB and bronchiectasis.

Addressing these questions in this PhD has resulted in a body of research that has direct relevance to the clinician, whilst also providing insight into areas that warrant further study. Our findings in relation to wet cough progress our understanding of the ‘grass roots’ symptom (and sign) of PBB.

My PhD work has demonstrated that children with wet cough are significantly more likely to harbour lower airways infection with bacteria, viruses and viral-bacterial co-infection, when compared to children without wet cough [130] (Chapter 2). We have shown that viral-bacterial co-infection is associated with maximal neutrophilic lower airway inflammation, suggesting that microbial synergy may also play a role in the pathogenesis of chronic bronchitis in children [130]. In further exploring the potential role of viruses,

chapter 3 showed that adenoviruses and rhinoviruses are the most common viruses detected in the lower airways of children with chronic respiratory symptoms (most of whom had chronic cough). Further, adenovirus detection (via PCR) on NPA or BAL is associated with significantly increased lower airway neutrophilic inflammation, when compared to adenovirus negativity [131]. This finding was independent of the presence of bacterial infection, suggesting that adenovirus is an independent predictor of neutrophilic lower airway inflammation in children. Further supporting the potential relevance of this finding was the fact that adenovirus was significantly more likely to be detected in the BAL of children with PBB compared to controls (23% vs 4% respectively) [132](Chapter 5) suggesting a possible role in disease pathogenesis.

As adenovirus had not previously been described in PBB, the logical next step was to examine the genotypes of adenovirus in the lower airways of children with PBB and bronchiectasis (Chapter 4). We showed that adenovirus species C (genotypes 1 and 2) is the major adenovirus species detected in the lower airways of children with PBB and bronchiectasis [133]. Adenovirus C is known to establish latent infection within lymphocytes and is believed to remain dormant within tonsillar and adenoidal tissues of young children [102, 134] and to persist in the lung [135]. We also found adenovirus to be positively associated with lower airway bacterial infection with *H. influenzae* and *M. catarrhalis* [133]. These findings provide further evidence for a role of (viral-bacterial) microbial synergy in the development of PBB and/or bronchiectasis.

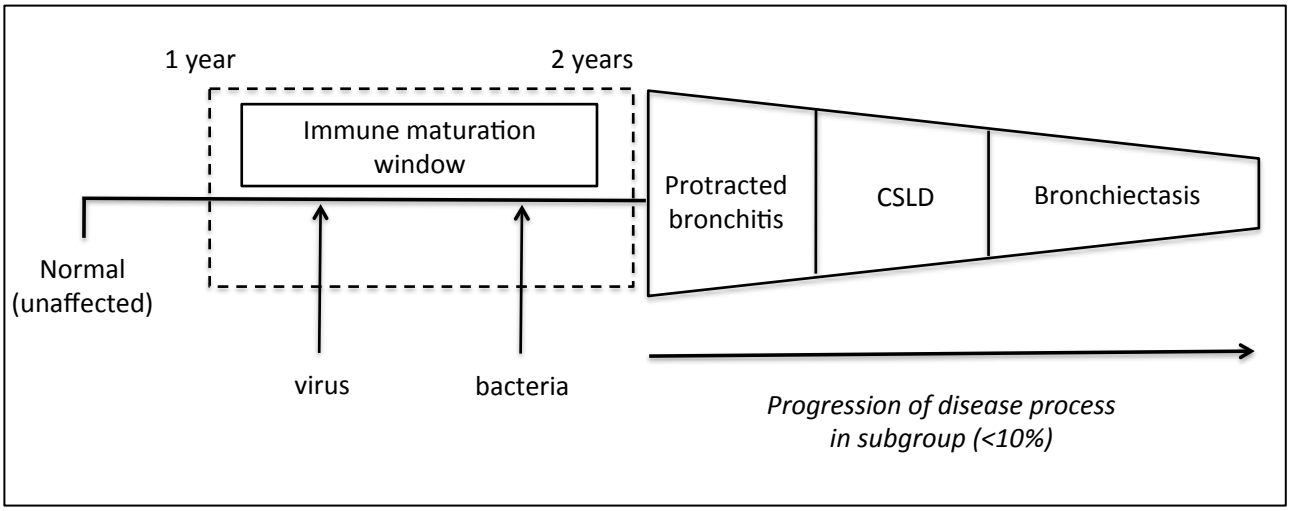
In further delineating the clinical entity of PBB, chapter 5 describes the typical child who presents with PBB. We have shown that young boys, in their second year of life, who have attended childcare and have a history of protracted wet cough and parent-reported wheeze, typify the average child with PBB [132]. We have also shown that basic immune function in these children is intact. However, natural killer (NK) cell levels are often elevated [132]. This latter finding is in keeping with the observation that viruses are significantly more likely to be detected in the lower airways of children with PBB compared to controls, a common factor in NK cell elevation. However, the postulated link between PBB and bronchiectasis is perhaps the most significant theme addressed in this PhD thesis in chapter 6.

We prospectively followed a group of young children with PBB over a two-year period and showed that a significant proportion (8.1%) were subsequently diagnosed with

bronchiectasis on HRCT chest [136] (chapter 6). Predictors of bronchiectasis included: recurrent episodes of PBB (defined as >3 episodes in the first year after recruitment) and lower airway infection with *H. influenzae* (most commonly *non-typeable H. influenzae*) [136]. These findings are first to lend support to the proposed clinical spectrum linking PBB and bronchiectasis, suggesting that this theory [51] is likely to be accurate and robust.

I have thus far summarised the major novel findings arising from the original research encapsulated within this PhD. In addition, a review of the broader medical literature on major cough treatments available for children was summarised in chapter 7. This was followed by a Cochrane review examining the efficacy of short-course antibiotic therapy for the treatment of bronchiectasis. The relevance of this chapter extends beyond the paediatric community and has direct utility for general practice.

The findings of the original research studies contribute to previous work on this topic and allow us to come closer to a hypothesis on the complete pathophysiological process underlying chronic wet cough and PBB in children. The following schematic diagram depicts this proposed theory and I have further elaborated in the text below. The theory expands on Cole's vicious circle hypothesis [81] and Chang's proposed clinical spectrum of PBB and bronchiectasis [51]. I have intentionally omitted discussion of extrinsic factors, such as antibiotic therapy, that are likely to attenuate the infection/inflammation process, as it was beyond the scope of our findings.



8.1 Schematic diagram of proposed pathophysiological process uniting PBB and bronchiectasis (figure adapted from Chang's proposed clinical spectrum of PBB and bronchiectasis [51]).

A primary insult to the lung occurs in a window of susceptibility. This window of susceptibility is defined by an immature immune system as well as structural immaturity of the lung. The insult is an extrinsic initiating event, such as adenovirus C infection. This event impairs host respiratory defences, including mucociliary clearance and immune surveillance mechanisms. This impairment in lung defences increases host susceptibility to secondary bacterial infection. Infection with respiratory bacteria e.g. *non-typeable H influenzae* occurs, or, bacterial load increases if infection is already present. Microbial synergy between pathogens heightens mucosal inflammation and dysfunction further promoting persistence of both virus and bacteria in the airways. Viral-bacterial co-infection underlies and drives the vicious cycle of airway inflammation and infection. For a period of time (weeks/months), airway epithelial injury occurs at the same rate as repair. As lung growth and immune system maturation occur, airway clearance improves and epithelial repair exceeds injury and the vicious cycle abates. This 'reversible airway epithelial injury' occurs in the majority of children with PBB.

In a subset of susceptible individuals (approximately 8.1%[136]), however, a threshold of 'irreversible airway epithelial injury' whereby injury has exceeded repair, is reached. This airway damage acts as a nidus for ongoing infection and inflammation. As the rate of airway injury continues to exceed repair, the discrepancy between the two increases, and the trajectory from PBB to bronchiectasis begins.

8.2 LIMITATIONS OF THIS THESIS

The limitations of this thesis are outlined in the manuscripts contained within each chapter. I will summarise some of the major limitations common to the majority of studies in this thesis.

With the exception of the longitudinal follow-up component of Chapter 6, studies were cross-sectional in nature. Hence, interpretation of lower airway findings, obtained from single time-point sampling, is limited to the observation of association. Cause-effect relationships cannot be assumed. Nevertheless, there is substantial value in the analysis of lower airway data from such a large cohort of young children. Due to the high numbers of included participants, our findings provide invaluable clues to the state of the lower airways in children with (and without) chronic cough. The inability to perform longitudinal

studies involving BAL in children is based upon ethical constraints that are inherent to studies in children.

A second limitation that merits discussion is the duration of follow-up of children with PBB. In Chapter 6, the clinical outcomes of a cohort of children with PBB followed for a 2-year period were described. Indeed, this relatively short period of follow-up allowed detection of a number of children with bronchiectasis. However, it is likely that this observed value would be higher with a longer period of follow-up e.g. 5 years. Furthermore, only a subset of children with PBB underwent CT scan of their chest.

Due to the ethical issues pertaining to the performing of CT chest primarily for research purposes, children in this study did not have routine CT scan at study entry. Only those with clinical suspicion of bronchiectasis were subjected to CT scan. The consequences of this are two-fold. First, as not all children had CT at baseline, we cannot assume that those subsequently diagnosed with bronchiectasis entered the study free of bronchiectasis. Hence, progression from PBB to bronchiectasis per se cannot be assumed. Second, as not all children had CT at follow-up, bronchiectasis may have been unrecognised in a subset that did in fact develop it, hence under-estimating the true rate of this disease in the cohort.

8.3 FUTURE PROJECTS AND FURTHER RESEARCH

The findings from the research arising from this PhD thesis provide the foundation for future research in the area of chronic wet cough and PBB. Potential studies are outlined below. Concepts are presented in preliminary format.

BIRTH-COHORT STUDY

The exact influence of early life respiratory tract infections upon the trajectory of lung health is unknown. Findings from our research suggest that viral-bacterial co-infection is likely to be relevant to the pathogenesis of chronic suppurative lung diseases in children. In addition to organism type/s, it is possible that the timing and sequence of early respiratory tract infections is of clinical significance and impacts upon the risk of development of bronchiectasis.

A birth cohort study would enable documentation of early viral and bacterial respiratory tract infections. Frequent, time-intervalled sampling of respiratory tract secretions when a child is in the well-state, with additional samples during periods of illness, would enable determination of both the timing and sequence of viral and bacterial respiratory tract infections and their relationship to clinical symptoms.

NPA would be used for detection of an extended panel of common respiratory viruses via PCR. Further speciation and genotyping of adenoviruses and rhinoviruses would establish the duration of infection with individual virus types and identify chronic carriage, if any. Cough swab or induced sputum would be used to obtain respiratory specimens for bacteriology.

Documentation of clinical symptoms and PBB episodes, undertaken over time, with regular follow-up with a specialist respiratory physician, would identify those children requiring further investigation for bronchiectasis. The frequency of PBB episodes in relation to presence and timing of viral and bacterial infection would be analysed. Risk factors for recurrent PBB and bronchiectasis (as separate outcome measures) would be determined using longitudinally collected data.

CHRONIC COUGH AND OBSTRUCTIVE SLEEP APNOEA

The observation in clinical practice that chronic cough is often associated with obstructive sleep apnoea (OSA) symptoms and that the chronic cough often resolves after effective intervention for OSA, has been the focus of recent research [137, 138]. Several theories in relation to the cause of cough in children with OSA have been proposed. These range from aspiration of upper airway secretions during sleep (resulting in bacterial bronchitis) to mechanical irritation of the epiglottis by enlarged tonsillar tissue [139]. Indeed an association between asthma and sleep-disordered breathing has also been observed in both adults [140] and children [140-143].

In this PhD we have also collected data on OSA symptoms in a large number of children who have undergone bronchoscopy for investigation of chronic cough. Hence, a cross-sectional study, further examining the relationship between chronic cough and OSA, with exploration of clinical and laboratory factors influencing this association, would be highly clinically relevant. Further, it is possible that PBB is the cause of chronic cough in children

with large adenoids and tonsils and analysis of already collected data would enable further evaluation of this postulated link.

8.4 CONCLUSION

The novel findings of this PhD thesis complement current knowledge on chronic wet cough and PBB in children in several ways. We have shown that viruses, in addition to bacteria, are likely to play a role in wet cough and PBB. We have also provided further depth to our understanding of PBB as a diagnostic entity by delineating the major clinical features of this condition. We have shown that adenovirus C is the major adenovirus species in the airways of children with PBB and bronchiectasis and, in demonstrating an association with bacterial co-infection, we have provided evidence for the possible role of microbial synergy. Lastly, we have evaluated the accuracy of the proposed clinical spectrum of PBB and bronchiectasis and shown that this is likely to be robust. We have identified key risk factors for bronchiectasis and highlighted important areas for future research.

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APPENDIX 1

SUPPLEMENTARY MATERIAL FOR CHAPTERS 5 AND 6

CHEST Online Supplement

Prospective Characterization of Protracted Bacterial Bronchitis in Children

Danielle F. Wurzel, MBBS; Julie M. Marchant, PhD; Stephanie T. Yerkovich, PhD; John W. Upham, PhD; Ian M. Mackay, PhD; I. Brent Masters, PhD; and Anne B. Chang, PhD

e-Appendix 1.

Bronchoscopy and BAL were undertaken as per European Respiratory Society guidelines.[1] The first aliquot was utilized for microbiology (bacteria and viruses), the second for total cell counts and cellular differentials.[2, 3] Bacterial infection was defined as growth of $\geq 10^4$ colony-forming units (cfu)/mL of any of the following pathogens: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenza* or *Staphylococcus aureus* using standard culture methods previously described.[3]

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E-Supplement – Chapter 6

Protracted Bacterial Bronchitis in Children: Natural History and Risk Factors for Bronchiectasis

Indications for flexible bronchoscopy in controls

Indications for bronchoscopy in the 15 control participants undergoing respiratory evaluation included: stridor in 6 (40%), apnoeas in 3 (20%), dyspnoea in 3 (20%), haemoptysis in 1 (7%), congenital lung lesion in 1 (7%) and imaging abnormality in 1 (7%).

H. influenzae typing

H. influenzae characterisation was based upon colony morphology and hemin and nicotinamide adenine dinucleotide (X and V factor) dependence.[36] Non-typeable *H. influenzae* (NTHi) were identified by failure to react with capsular antisera (Phadebact® Haemophilus coagglutination test). A *fucP*-based real time PCR assay was used for differentiation of NTHi from *H. haemolyticus* [37].

eTable 1: Univariate analysis of predictors of recurrent PBB (>3 episodes/yr)[1]

Characteristic	Recurrent PBB (N=67)	Non-recurrent PBB (N=94)	Odds ratio (95% CI)	P-value
Sex, M:F, %male	48:18, 73%	57:36, 61%	1.767 (0.90, 3.49)	0.101
Recruitment age, mths, median (IQR)	24 (16, 53)	20 (12, 49)	1.003 (0.994, 1.012)	0.508

Prior x-ray confirmed pneumonia	16 (24%)	19 (20%)	1.24 (0.583, 2.63)	0.578
Household tobacco smoke exposure	22 (33%)	33 (36%)	0.89 (0.46, 1.73)	0.728
Maternal smoking in pregnancy	13/60 (22%)	8/36 (22%)	0.97 (0.36, 2.62)	0.949
Aboriginal or Torres Strait Islander	1 (2%)	9 (10%)	0.143 (0.018, 1.158)	0.068
No. children in household, median (IQR)	2 (2, 3)	2 (2, 3)	1.005 (0.784, 1.288)	0.971
≥ 3 children	22 (33%)	33 (35%)	0.904 (0.466, 1.753)	0.765
Childcare attendance, ever	37/41 (90%)	42/50 (84%)	1.762 (0.490, 6.331)	0.385
TCC x 10^6/L	260 (100, 420)	200 (130, 415)	1.000 (0.999, 1.000)	0.476
BAL neutr%, median (IQR)	25 (8, 55)	27 (11, 56)	0.997 (0.986, 1.010)	0.680
BAL macro%, median (IQR)	63 (30, 79)	60 (35, 80)	1.002 (0.990, 1.014)	0.734
BAL lymph%, median (IQR)	6 (5, 12)	9 (4, 15)	0.997 (0.964, 1.032)	0.869
BAL eosin%, median (IQR)	0 (0, 0)	0 (0, 0)	1.164 (0.926, 1.463)	0.194
HAdV on BAL	10/60 (17%)	19/90 (21%)	0.747 (0.320, 1.743)	0.500
H. influenzae*	30 (45%)	53 (56%)	0.627 (0.334, 1.179)	0.147
M. catarrhalis*	16 (24%)	31 (33%)	0.638 (0.314, 1.293)	0.212

S. pneumoniae*	18 (27%)	26 (218%)	0.961 (0.475, 1.943)	0.911
S. aureus*	6 (9%)	7 (7%)	1.222 (0.392, 3.817)	0.729

[1]Multi-variate analysis did not identify any significant predictors of recurrent PBB.

APPENDIX 2

PUBLICATIONS NOT INCLUDED IN THIS THESIS

Chang AB, Yerkovich ST, Gibson PG, Anderson-James S, Petsky HL, Carroll ML, Masters IB, Marchant JM, **Wurzel DF**, Upham JW. *Pulmonary innate immunity in children with protracted bacterial bronchitis.* The Journal of Pediatrics. 2012;161(4): 621-5.

Wurzel DF, Masters IB, Isles AF. *A Case For Early Bronchoscopic Airway Assessment After Disc Battery Ingestion.* Pediatric Pulmonology 2014 Mar;49(3):E72-4. doi: 10.1002/ppul.22858.

Pulmonary Innate Immunity in Children with Protracted Bacterial Bronchitis

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Objective To determine bronchoalveolar lavage (BAL) levels of 3 innate immunity components (human β-defensin-2 [hBD2], mannose-binding lectin [MBL], and surfactant protein-A [SP-A]), the relationship with airway neutrophilia and infection, and cytokine production of stimulated BAL cells in children with current protracted bacterial bronchitis (PBB), children with resolved PBB (PBB well), and controls.

Study design BAL of 102 children (mean age 2.8 years) fulfilling predefined criteria of current PBB (n = 61), PBB well (n = 20), and controls (n = 21) was cultured (quantitative bacteriology) and viruses examined by polymerase chain reaction. hBD2, MBL, and SP-A were measured, and cytokine production by lipopolysaccharide-stimulated BAL cells was determined.

Results Median hBD2 and MBL levels were significantly higher in the current PBB group (hBD2 = 164.4, IQR 0-435.5 pg/mL; MBL = 1.7, 0.4-4 ng/mL) than in the PBB well group (hBD2 = 0, IQR 0-85.2; MBL = 0.6, IQR 0.03-2.9) and controls (hBD2 = 3.6, IQR 0-126; MBL = 0.4, IQR 0.02-79). hBD2 was significantly higher in children with airway infection (n = 54; median 76.9, IQR 0-397.3) compared with those without (n = 48; 0, IQR 0-236.3), $P = .04$. SP-A levels and cytokine production of stimulated BAL cells were similar between groups.

Conclusion In children's airways, hBD2, but not MBL and SP-A, relates to inflammation and infection. In children with PBB, mechanisms involving airway hBD2 and MBL are augmented. These pulmonary innate immunity components and the ability of BAL cells to respond to stimuli are unlikely to be deficient. *(J Pediatr 2012;161:621-25)*.

Protracted bacterial bronchitis (PBB) is a relatively new and significant diagnostic clinical entity characterized by chronic wet cough and resolution of cough within 2 weeks of treatment with appropriate antibiotics.[1,2] PBB, increasingly recognized as an important pediatric condition worldwide,[2-6] is a common cause of chronic cough in children.[2] Not only is PBB easily treatable (thus improving health-related quality of life[7,8]) but, if left untreated, it may progress to chronic suppurative lung disease and radiological bronchiectasis.[3,7]

The lower airways of children with PBB are characterized by bacterial infection and airway neutrophilia.[1,7] The latter suggests that pulmonary innate immunity likely plays a pivotal role.[9,10] However, there are few prospective studies on PBB; only 2[9,10] have evaluated mechanistic links. Innate immune defense involves recognition and clearing responses. In the recognition arm, specific pattern recognition receptors are key components and include Toll-like receptors (TLRs) and lectin-like molecules such as, surfactant protein-A (SP-A) and mannose-binding lectin (MBL).[11] Pulmonary collectins (eg, SP-A, MBL) play roles in pulmonary innate immunity and regulate inflammatory responses.[12,13] SP-A also has antimicrobial properties.[14] Serum MBL deficiency is associated with bronchiectasis but there is only 1 article on MBL in children's bronchoalveolar lavage (BAL). Fidler et al[15] reported MBL in the BAL of children with lung infection. The MBL levels were not associated with bacteria or systemic inflammation but correlated with neutrophil elastase.[15] However, quantitative bacteria culture was not defined, polymerase chain reaction for viruses was undertaken in only 4 children, and no other innate immunity components were examined.[15]

The clearing mechanism of the innate immune system includes antimicrobials such as the defensin family. In the lungs, epithelial production of β-defensin subtypes 1-3 are likely important.[14,16] In the middle ear, human β-defensin-2 (hBD2) has bactericidal activity against *Streptococcus pneumoniae*, *Moraxella catarrhalis*,

BAL	Bronchoalveolar lavage
hBD2	Human β-defensin-2
IL	Interleukin
LPS	Lipopolysaccharide
MBL	Mannose-binding lectin
PBB	Protracted bacterial bronchitis
SP-A	Surfactant protein-A
TLR	Toll-like receptor

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and nontypeable *Haemophilus influenzae*.[17] These bacteria are the same dominant pathogens in the lower airways of children with PBB[7] and bronchiectasis.[18] Yet there is only 1 small study (n = 20) that has measured airway human β-defensin in children.[16] In children, human β-defensin is relevant to several conditions including inflammatory gut disease like Crohn's disease.[17]

In a cohort of 102 children, we determined whether BAL levels of hBD2, SP-A, and MBL: (1) differed between children with current PBB, children with resolved PBB (PBB well), and controls; and (2) were related with airway neutrophilia and endobronchial infection. We further examined if the cytokine production of lipopolysaccharide (LPS)-stimulated BAL cells were different in these groups. We tested the hypothesis that current PBB would be associated with deficient levels of hBD2, SP-A, and MBL.

Methods

Children undergoing flexible bronchoscopy for clinical reasons were eligible for enrollment. Informed consent was obtained and the study was approved by the Ethics Committees of the Royal Children's Hospital (No. 2003/017) and the University of Queensland School of Medicine (No. 2008000064). Enrolled children had a standardized medical history taken that focused on the respiratory system including cough quality (wet/dry)[19] and key demographics that contribute to frequency of respiratory infections (day care attendance, smoke exposure, number of siblings in household). Post bronchoscopy, children with a wet cough were treated with amoxillin-clauvanate (45 mg/kg/d in 2 doses).[1] A validated cough diary card[20] was used to document response to antibiotics, defined as absence of cough or >75% reduction in score (for ≥3 days) within 2 weeks of antibiotic use post bronchoscopy.

Flexible bronchoscopy was performed under general anesthesia and BAL was obtained using European Respiratory Society guidelines as previously described[1]; 3 aliquots of sterile normal saline were instilled. The first aliquot was used for microbiology examination; the second and third aliquots were pooled for cytology, fluid phase measurements, and cell cultures. Microbiological assessment (previously described[1,21]) included quantitative aerobic cultures of bacteria and positive bacterial culture and was defined as growth of $\geq 10^5$ colony-forming units/mL.[9,10] Viral assessment on BAL to detect respiratory syncytial virus, adenovirus, parainfluenza virus types 1-3, influenza, *Mycoplasma pneumoniae*, and human meta-pneumovirus[21] were performed using polymerase chain reaction.[21]

For this study, only children who fulfilled the clinical definitions of PBB, PBB well, and controls were included. Children were classified before innate immunity studies were performed. We defined children as PBB if there was a history of chronic (>4 weeks) wet cough and response to antibiotic treatment with resolution of the cough within 2 weeks in the absence of signs and symptoms of other disease.[2] PBB well were children who previously had a wet cough that had responded to antibiotics and were treated by a pediatric pulmonologist but were no longer coughing when the bronchoscopy was undertaken. Controls were children having bronchoscopy for the assessment of the airways (eg, stridor) with no history of chronic cough and no acute respiratory infection in the preceding 2 weeks. In all children, immune deficiencies and cystic fibrosis were excluded through serum immunoglobulins (IgG, IgM, IgA), neonatal screening for controls, and sweat test for PBB and PBB well. Children with recurrent pneumonia (>2), bronchiectasis (on high-resolution computed tomography scan), and neurological abnormalities were also excluded.

Measurement of hBD2, MBL, and SP-A

The cellular and supernatant components of the BAL fluid were separated within 3 hours of collection, with the cellular portion used immediately to assess cytokine production and the supernatant stored at −20°C for later batch analysis. hBD2 (Phoenix Pharmaceuticals, Burlingame, California), MBL (Bioporto Diagnostics, Gentofte, Denmark), and SP-A (Biovendor, Modrice, Czech Republic) were measured using commercial kits according to the manufacturers' directions. The limits of detection were 3.9 pg/mL (hBD2), 200 pg/mL (MBL), and 500 pg/mL (SP-A).

Measurement of Cytokine Production by LPS-Stimulated BAL Cells

Where available, BAL cells were resuspended in Roswell Park Memorial Institute with 10% fetal calf serum at 2×10^4 cells/well in round-bottom culture plates. Cells were stimulated with 1 ng/mL LPS (Alexis Biochemicals, Lausen, Switzerland) for 24 hours at 37°C. Cytokine (interleukin [IL]-6, IL-8, IL-10, and tumor necrosis factor-α) production was measured in culture supernatants using in-house enzyme-linked immunosorbent assays with commercially available paired antibodies and recombinant cytokines (Becton Dickinson, Franklin Lakes, New Jersey). The limit of detection was 15 pg/m.

Statistical Analyses

Data were examined for type of distribution using normality plots. Data that had a normal distribution were described using mean and SD values; medians and IQRs were used for nonparametric data. Chi-squared tests were used for comparison of categorical data, Mann-Whitney for 2-group comparisons, Kruskal-Wallis used for >2-group comparisons, and ANOVA for age comparison between groups. Spearman correlation (r_s) was used to examine correlations among variables. The "enter" method was used for multiple regression analyses. SPSS (SPSS Inc, Chicago, Illinois) was used, and a 2-tailed P value of <.05 was considered significant.

Results

Of the 164 children enrolled, 62 were excluded because they did not fulfil the criteria of current PBB, PBB well, or controls. The demographics of the cohort of the 102 children (Table) showed that the groups significantly differed in age

Table. Baseline data

Demographics	Current PBB group (n = 61)	PBB well group (n = 20)	Control group (n = 21)	*P*
Age, y (mean, SD)	2.5 (2.3)	4.2 (3.0)	2.2 (2.8)	**.018**
Sex, M:F	47:14	10:10	11:10	**.025**
Length of current cough, wk*	28 (8-57)	0	0	**.0001**
Household tobacco smoke exposure, n (%)	20 (32.8%)	6 (30%)	7 (33.3%)	.97
No. of children in household (median, range)	2 (1-8)	2 (1-10)	2 (1-6)	.13
BAL data (median, IQR)				
Total cell count ($\times 10^6$/L)	305 (157-498)	131 (78.5-277.5)	100 (45-139)	.0001
% Macrophage	45.5 (26.0-68.5)	87.0 (80.5-93.0)	91.0 (81-95)	.0001
% Neutrophil	41.5 (18.7-69.0)	5.0 (2.5-7.8)	2.0 (1.0-8.0)	.0001
% Eosinophil	0 (0-8.0)	0 (0-0.0)	0 (0-0.0)	.021
% Lymphocyte	9.0 (4.0-12.0)	5.5 (4.0-12.0)	5.0 (3.0-9.0)	.171

Bolded values are $P < .05$.
*From day of bronchoscopy.

and sex. The PBB group had the highest number of boys, and children in the PBB well group were older. There was no significant difference in exposure to tobacco smoke or number of children in the household.

Analysis by Clinical Group

Figure 1 depicts the significantly higher median hBD2 level in the PBB group compared with the PBB well and the control groups. There was no significant difference between the PBB well group and controls. For MBL, the PBB group also had significantly higher levels than controls but was not significantly different from the PBB well group (Figure 2). There was no difference in SP-A between groups (Figure 3, Kruskil-Wallis $P = .505$). Similarly, there was no difference in the LPS-stimulated cytokine response by BAL cells between the groups (Figure 4; available at www.jpeds.com). There was no correlation between any cytokine and the proportion of either macrophages or neutrophils within the cellular component (data not shown).

Analysis by Presence/Absence of Bacteria, Viruses, and Other Potential Factors

Considering the whole cohort, hBD2 was significantly higher in the group with BAL presence of either bacteria or virus (n = 54, median 76.9 pg/mL, IQR 0-397.3) compared with the absence of both bacteria and virus antigens (n = 48, median 0, IQR 0-236.3) ($P = .04$). There was no significant difference in MBL or SP-A between groups ($P = .17$ and .61, respectively). However, when the children were grouped by the presence of positive bacteria culture (n = 43) compared with the absent (n = 59), there was no significant difference between groups for hBD2, MBL, or SP-A ($P = .393$, .051, and .393, respectively). Likewise, there was no significant difference in hBD2, MBL, or SP-A when the groups were divided by presence/absence of virus (present in 18, absent in 84) ($P = .196$, .61, and .664, respectively).

There were modest but significant correlations between hBD2 with MBL ($r_s = 0.22$, $P = .027$), BAL% neutrophils ($r_s = 0.43$, $P = .0001$), BAL% macrophages ($r_s = 0.32$, $P = .0001$), and blood% neutrophils ($r_s = 0.27$, $P = .010$) but not with SP-A ($P = .25$), blood% monocytes ($P = .79$), age ($P = .78$), and sex ($P = .21$). MBL also significantly correlated with BAL% neutrophils ($r_s = 0.44$, $P = .0001$) and BAL% macrophages ($r_s = 0.4$, $P = .0001$). There was no significant correlation between MBL and any of the other factors (blood% neutrophils, SP-A, blood% monocytes, age, and sex; $P = .25$-.57). SP-A did not correlate to any factor ($P = .25$-.99).

In multivariate regression analysis, hBD2 significantly related to BAL% neutrophils (β coefficient 5, 95% CI, 1.3-8.9, $P = .012$) and MBL (β coefficient = 16.5, 95% CI, 7.2-25.9, $P = .001$) but did not relate to BAL% macrophages ($P = .26$), blood neutrophils ($P = .13$), age ($P = .8$), or sex ($P = .7$). With MBL as the dependent variable, the only significant factor in multivariate regression analysis was hBD2 (β coefficient 0.01, 95% CI, 0-0.013, $P = .0001$). There was no significant association in any of the other factors (BAL % neutrophils, BAL% macrophages, age, and sex, *P* range .23-.94). Because lower airway bacteria infection causes airway neutrophilia and both are present in those with PBB,[1] we excluded both these factors in our regression analyses.

Discussion

In a cohort of 102 children, we found that hBD2 and MBL levels in BAL were significantly higher in children with PBB

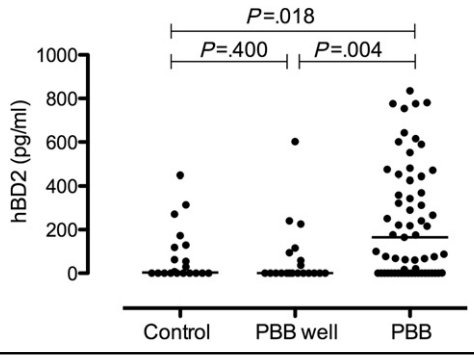

Figure 1. hBD2 levels in the BAL of children grouped into current PBB, PBB well children, and controls. Median hBD2 levels were significantly higher in the PBB group compared with the PBB well and the control groups. There was no difference between PBB well and controls.

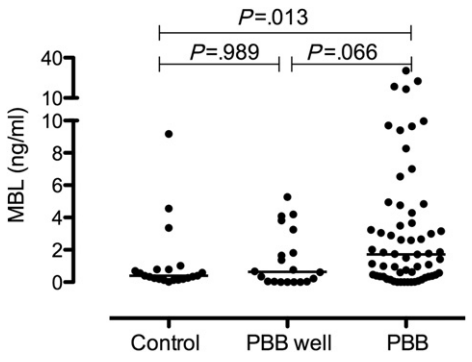

Figure 2. MBL levels in the BAL of children grouped into current PBB, PBB well, and controls. The median MBL level in the PBB group was significantly higher than that of controls but was not significantly different from the PBB well group.

compared with children who have recovered from a PBB episode (PBB well) and controls. We have also found that hBD2 levels were associated with airway infection and are related to airway neutrophilia and MBL levels. However, SP-A levels in the BAL and cytokine production of stimulated BAL cells were similar between the groups.

Discoveries of the complex innate immune system and its contributions to health and disease are rapidly expanding. Augmentation of the innate system may have a therapeutic role,[22] yet there are relatively little BAL data relating to innate immunity in children, outside of cystic fibrosis. In this study, we examined key components of the human pulmonary innate immunity defense system with respect to PBB, an increasingly recognized clinical entity.[1-7] Innate immunity may be particularly important in light of the recognition of the likely association between PBB, especially when recurrent, and chronic suppurative lung disease and bronchiectasis.[3,7]

hBD2 is 1 of 6 β-defensins identified in humans.[23] Only hBD2-4 can be induced and produced by airway epithelial cells. As hBD2 has been shown to have bactericidal activity against *S pneumoniae*, *M catarrhalis*, and nontypeable *H influenzae* in otitis media,[17] we considered it to be a key innate immunity component relevant to PBB and bronchiectasis because these bacteria are major lower airways pathogens

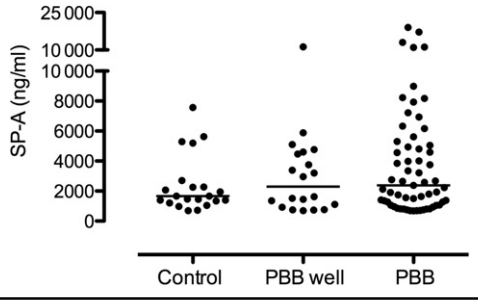

Figure 3. SP-A levels in the BAL of children grouped into current PBB, PBB well, and control groups. Median SP-A levels did not differ between groups.

in these conditions.[7,18] We also examined MBL given its relevance to bronchiectasis and lung infection.[15] If children with PBB have a deficiency in these selected innate immunity mechanisms, reduced levels in BAL fluid would have been found. Instead, we found increased hBD2 and MBL levels in the BAL from children with PBB compared with controls. Although the PBB well group formed an intermediate group, their values were similar to controls and different from the current PBB group. This suggests that children with PBB are unlikely to have a deficiency in the hBD2 and MBL components of the complex pulmonary innate immunity system. We also found that the cytokine production of stimulated BAL cells was similar in children with current PBB, children with resolved PBB (PBB well), and controls, supporting our data that these aspects of the pulmonary innate system are not deficient in children with PBB.

However, it remains unknown if children with recurrent PBB differ from those without recurrent PBB as some children with PBB develop recurrence while others do not.[7] It is also possible that children with PBB have an initial suboptimal innate immune response that becomes excessive later in the course of the illness, in a similar mechanism to influenza-infected BAL cells. Didierlaurent et al showed that influenza-infected ex vivo BAL cells have an initial reduced neutrophil recruitment that later resulted in heightened bacterial load during secondary respiratory infection and increased neutrophilia.[24] They also showed that this was related to TLR signals.[24] Children with PBB in our cohort have sustained bacterial infection for many weeks and thus it is possible a differential response may be found in the early phase of the infection. Indeed, in our earlier study, we found that mRNA expression of preprotachykinin I, TLR-2, and TLR-4 in BAL cells of children who did not present to respiratory clinics (hence likely to be less severe) and had bacterial infection in their airways were lower than those of controls.[10]

In our current cohort, hBD2, but not MBL, varied significantly in relation to airway neutrophilia and airway infection (defined by presence of bacteria or viruses). This suggests that the epithelial–neutrophil interaction is actively engaged in PBB. hBD2 also varied significantly in relation to MBL. Reasons for this include independent stimulation of both MBL and hBD2 responses. It is also biologically plausible that MBL, which functions in part as a pattern recognition receptor and further stimulates pathogen clearance, likely involves hBD2, an important antimicrobial peptide in the lungs. A highly significant association does not prove causality, so this is merely speculative as we were unable to find any published reports that have measured both MBL and hBD2 in BAL.

In adults, increased hBD2 levels in the BAL have been associated with inflammatory lung disease and infection.[23] However, pediatric data are important in light of the known difference in mucosal immunity and other immunity of the airways of children compared with adults.[25] Our findings on hBD2 are in contrast to the sole published study in children[16] that has measured BAL hBD2. The study by

Chen et al[16] did not find any relationship between hBD2 levels and airway inflammation or infection, in contrast to our study's findings. The most likely explanation relates to sample size; n = 102 in our study and n = 20 in the earlier study.[16] Our data on SP-A are consistent with a study that described no change in BAL SP-A levels in children with acute lung injury.[26]

Although we have investigated a previously unexplored area in children, we have not examined many other important components of the innate system, especially mechanisms involved in resolution of inflammation, which might explain the persistence of symptoms and neutrophilic inflammation in children with PBB. There are also many other possible contributors to PBB such as impaired airway clearance related to tracheobronchomalacia[4] (from mechanical interference) and other causes such as mucus hypersecretion, deficient efferocytosis, and epithelial cell dysfunction, among others.

We conclude that innate immune pathways involving hBD2 and MBL are augmented in the lower airways of children with PBB. Components of the pulmonary innate system represented by BAL levels of hBD2, MBL, and SP-A and cytokine production of BAL cells are unlikely to be deficient in children with established PBB. We also conclude that in the airways, hBD2 is strongly associated with MBL, neutrophilia, and infection. ■

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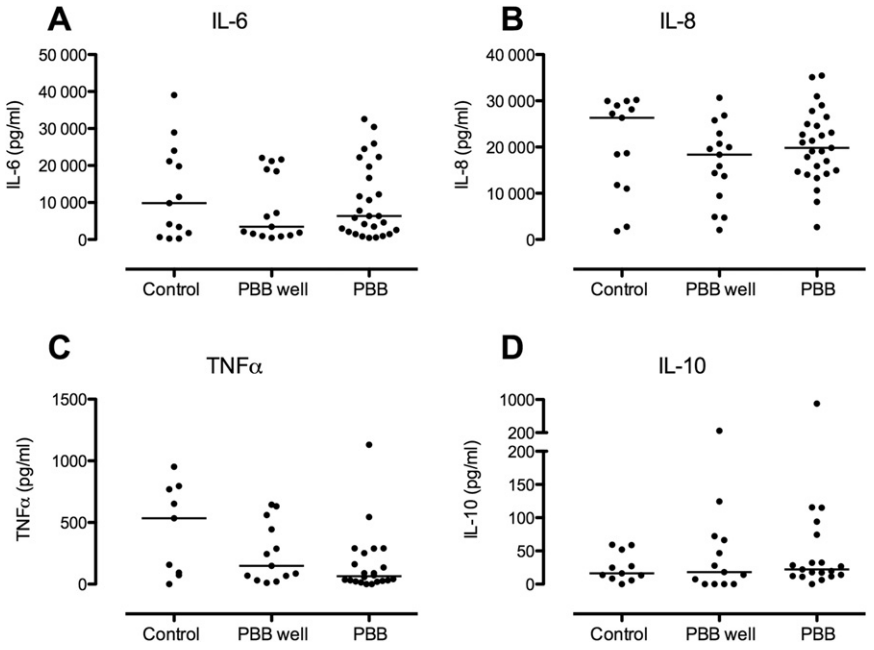

Figure 4. LPS-stimulated **A,** IL-6, **B,** IL-8, **C,** tumor necrosis factor-α, and **D,** IL-10 cytokine production from BAL cell showed no difference between current PBB (n = 27), PBB well (n = 15), and control (n = 13) groups.

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Case Report

A Case for Early Bronchoscopic Airway Assessment After Disc Battery Ingestion

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Summary. Disc battery ingestion in children is becoming increasingly common with the proliferation of small battery-powered electronic devices. In the case of esophageal impaction, the likelihood and severity of complications are proportionate to the time between ingestion and removal. Tracheo-esophageal fistulae (TOF) are a recognized complication and can be life-threatening. We describe an interesting case of disc battery ingestion with delayed recognition of a TOF. We document the tracheal mucosal healing process of a large airway defect and describe the role of bronchoscopy in guiding the timing of surgical intervention. This case highlights the important role of early bronchoscopic assessment in management of these patients. **Pediatr Pulmonol. 2014; 49:E72–E74.** © 2013 Wiley Periodicals, Inc.

Key words: disc battery ingestion; button battery ingestion; acquired tracheo-esophageal fistula; paediatric bronchoscopy.

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INTRODUCTION

Disc battery ingestion by young children can have devastating or even fatal consequences.[1] When impaction in the esophagus occurs, the time between ingestion and removal is critical. When batteries $\geq$20 mm in size are ingested, they are likely to lodge in the esophagus, typically at sites of physiological narrowing, for example, the thoracic inlet. Tissue necrosis occurs predominantly via hydrolysis.[1] We report on a case of delayed recognition of lithium disc battery ingestion and impaction, which resulted in severe esophageal necrosis with tracheo-esophageal fistula (TOF) formation. We describe the role of bronchoscopy and advocate early airway assessment in the case of deep esophageal ulceration; initially, for TOF diagnosis, and subsequently for monitoring tracheal mucosal healing during esophageal rest.

CASE REPORT

A 13-month-old child presented with his parents to a regional general practitioner with a history of sudden onset of cough and irritability. The child had rhinorrhea

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Conflict of interest: None.

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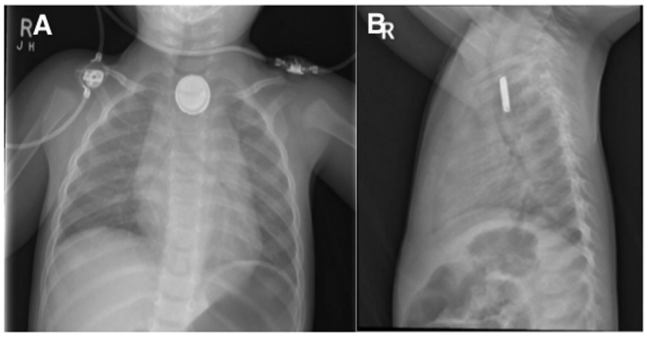

Fig. 1. A: Frontal CXR demonstrating rounded, midline opacification in the inter-clavicular region measuring roughly 2 cm in diameter with lateral bowing of the trachea to the right. The "double rim" or "halo" appearance of the opacity in the AP view confirms the foreign body to be a disc battery.[1] B: Lateral CXR demonstrating anterior orientation of negative node of battery (narrower side).

but an otherwise normal physical examination. He was presumed to have an early viral upper respiratory tract infection and was discharged home.

The family returned the following morning after the child had been unusually irritable overnight and was not tolerating oral intake. He appeared mottled and pale, and was referred to the local Emergency Department where a chest X-ray was performed (Fig. 1). Further history taking revealed the child had experienced a choking episode at 4 pm the day prior.

On examination in the Emergency Department, he was febrile at 38.0°C, tachycardic at 175 bpm, and tachypnoeic with respiratory rate of 42. He had intercostal recession, moist cough, and was drooling. His oxygen saturations were initially 88% on room air, improving with suctioning of his oral secretions. Attempts to examine him in the supine position were unsuccessful due to increased irritability.

The rounded opacity on his chest film was initially thought to be a coin lodged in his upper esophagus. After discussion with pediatric gastroenterologists at a tertiary pediatric hospital, the patient was transferred urgently for endoscopic removal. The patient arrived 23 hr after the choking episode. Three hours later, flexible endoscopy revealed a button battery impacted in the wall of the esophagus, 2.5 cm distal to the cricopharyngeus muscle. Circumferential esophageal sloughing and necrosis was noted at the impaction site. The battery was removed intact and identified as a lithium 3 V disc battery. The depth of the injury initially appeared to be confined to the esophageal mucosa.

The child remained unwell in hospital, with fevers, rhinorrhea, and moist cough. A nasopharyngeal aspirate was positive for respiratory syncytial virus. He continued to produce large amounts of frothy secretions for several days, requiring frequent oropharyngeal suctioning and supplemental oxygen.

On day 7, a contrast study demonstrated flow of contrast into the trachea and mainstem bronchi, indicative of a TOF. The child proceeded to flexible bronchoscopy. A large posterior tracheal wall defect, 3 cm proximal to the carina, was visualized. Air bubbles could be seen passing through the defect (Fig. 2A). Ventilation during anesthesia was challenging due to gas leak from the large tracheal defect and gastric hyperinflation. Following the bronchoscopy, the child was admitted to the intensive care unit, antibiotic cover was broadened for possible mediastinitis, and surgical consultation obtained.

The surgical opinion was that primary repair of the fistula would involve a high degree of risk due to the child's compromised respiratory state; the large size of the fistula and the degree of tissue necrosis. Esophageal diversion to protect the airways was considered the safest initial option.

The esophagus, approached via a low-neck incision, was divided and diverted proximally as an esophagostomy. Via a laparotomy, the gastro-esophageal junction was also divided and a gastrostomy fashioned for enteral

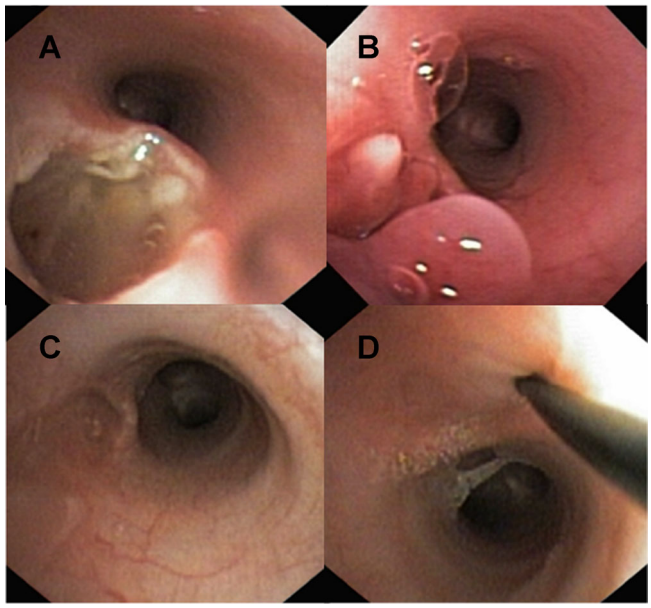

Fig. 2. A: Acute phase TOF: posterior tracheal wall defect with evidence of patency and free flow of secretions and air from esophagus to trachea. B: Acute phase TOF: fronds of (pulsatile) granulation tissue protruding from the TOF site into the trachea. Pulsatility was likely due to proximity to vessels. C: Chronic phase TOF: by 10 weeks there had been significant interval healing with tracheal mucosalization. Although not visible in this image, during esophageal insufflation a small residual fistula was observed at the lower aspect of the earlier large defect. D: Chronic phase TOF: at 6 months, healing was complete however there was a small residual fistula in the lateral groove of trachea, allowing passage of 0.9 mm guide wire.

ABBREVIATION:
TOF tracheo-esophageal fistula

feeding. The child improved quickly and was discharged after establishment of gastrostomy feeds on day 22.

A repeat bronchoscopy, 3 weeks post-discharge, showed interval healing with "pulsatile" fronds of granulation tissue, suspicious of vascular proximity to the TOF site (Fig. 2B). A contrast magnetic resonance angiogram showed no evidence of vasculitis or aneurysm. Serial bronchoscopies were performed to monitor healing (Fig. 2C and D) of the TOF. At 6 months post-injury, despite complete resolution of inflammatory granulation tissue, a pinhole fistula remained necessitating surgical closure.

A right thoracotomy was performed, the fistula was divided, and the trachea reinforced with a pedicled intercostal muscle flap. The remaining isolated segment of intra-thoracic esophagus, badly strictured at the fistula site, was sacrificed. Three months later, esophageal reconstruction was undertaken using a retrosternally tunnelled greater curve gastric tube, and the esophagostomy was closed. When full oral nutrition was maintained, gastrostomy closure was performed. At most recent review, the patient was tolerating an oral diet and achieving normal growth and developmental milestones.

DISCUSSION

Disc battery ingestion poses a special hazard to young children, as the initial ingestion may be unwitnessed and the child often presents with non-specific symptoms.[1] This case illustrates how delays in diagnosis and treatment can occur. Although the choking episode was witnessed, the significance was not initially appreciated. The full extent of the injury was determined only after performing a flexible bronchoscopy a week after first presentation.

In the case of esophageal impaction, tissue necrosis has been documented to occur within 2–2.5 hr of battery lodgement.[1] The risk of severe injury after disc battery ingestion is also influenced by the age of the child and the battery size, residual charge, and orientation within the esophagus.[1] This case involved a large (>20 mm) 3-volt lithium manganese battery.

When a battery lodges in the esophagus, tissue injury is caused predominantly by generation of an electrolytic current that hydrolyses tissue fluids and produces hydroxide at the battery's negative terminal, the narrower side of the battery.[2,3] If the negative terminal is positioned anteriorly in the esophagus, as it was in this case, erosion through the esophagus into the trachea may occur. If the negative terminal is positioned posteriorly, erosion into the mediastinum and aorta may occur.[4] Bronchoscopy plays an important role in assessing the presence, location, and extent of potential airway injury.

Prior literature emphasizes the role of timely bronchoscopic airway examination in disc battery impaction with "meticulous endoscopic examination of the esophagus and trachea after battery removal" for early diagnosis of complications.[5] Bronchoscopy is generally considered superior to esophagoscopy for diagnosing TOF, as eosophagoscopy may not discover a small fistula hidden behind a mucosal fold.[6] Early bronchoscopic airway assessment may facilitate more timely surgical intervention and improved symptomatic management.

Our case serves to illustrate several important points. Firstly, it reinforces previously published studies highlighting the significance of the orientation of the impacted battery within the esophagus;[2,3] when the negative node is facing anteriorly, the risk of TOF is increased. Secondly, it demonstrates the extent of tissue injury that can occur from delayed disc battery removal, indicating the need for a high level of suspicion for TOF in children with respiratory symptoms who have ingested a battery. Lastly, it emphasizes the important role of bronchoscopic airway assessment, in the diagnosis and management of TOF after disc battery ingestion.

CONCLUSION

We recommend bronchoscopic airway assessment at the time of endoscopic battery removal, particularly if removal is delayed, the negative pole of the battery is anteriorly orientated within the esophagus and/or respiratory symptoms are present.

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