Bronchoscopic Findings in Children with Chronic Suppurative Lung Disease

Comparing and contrasting bronchoscopy and lavage results across children with protracted bacterial bronchitis, bronchiectasis and cystic fibrosis from a single tertiary centre.

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Abstract

Background
Chronic suppurative lung disease (CSLD) in children describes a wide variety of respiratory diseases including protracted bacterial bronchitis (PBB), bronchiectasis and cystic fibrosis (CF). Diagnostic flexible bronchoscopy and bronchoalveolar lavage (BAL) are frequently performed in children with CSLD to determine airway inflammation and infection. To date no studies comparing bronchoscopy and BAL findings across these three forms of CSLD in children have been conducted. Therefore, the aim of this study was to compare and contrast the bronchoscopic findings across children with CSLD, in particular PBB, bronchiectasis and CF, from a single tertiary pediatric centre.

Methods
All bronchoscopy reports, including BAL findings, from the Royal Children’s Hospital (relocated and renamed Lady Cilento Children’s Hospital) in Brisbane for bronchoscopies performed between March 2010 and November 2016 were retrospectively reviewed. Bronchoscopic data, including BAL results, and radiological data regarding the presence of bronchiectasis from children (<18 years, post-hoc reduced to <6 years) meeting a-priori defined definitions of PBB (n=126), bronchiectasis (n=138) and CF (n=71) were collected. Our primary outcome was the airway microbiology of the three diagnostic cohorts, measured as proportion of children within a cohort in which specific pathogenic microorganisms were present. Secondary outcomes included airway cytology and aberrant macroscopic findings.

Results
Bronchoscopic results from 335 children (median age: 25 months; interquartile range 15-42 months) were recorded. Children with PBB (87%) and bronchiectasis (80%) had significantly more frequent lower airway bacterial infection compared to children with CF (62%) (P < 0.0001). Children with PBB and bronchiectasis had more frequently positive bacterial cultures of Haemophilus influenzae, Moraxella catarrhalis and Streptococcus pneumoniae, whereas children with CF more commonly grew Pseudomonas aeruginosa and Staphylococcus aureus. Airway neutrophilia was higher in children with CF (P < 0.0001), and amongst all diagnostic groups primarily determined by pathogenic bacterial presence. Malacia had no influence on rates of airway infection or inflammation in PBB and bronchiectasis. Other novel findings included the high prevalence rates of viral pathogens cytomegalovirus (±26%) and Epstein-Barr virus (±18%) in children with PBB and bronchiectasis.

Conclusion
This first single-centre study reviewing the bronchoscopic findings in children with PBB, bronchiectasis and CF revealed that, already during early childhood (<6 years), a distinct microbiological pattern in airway infection exists. Airways of young children with CSLD are frequently infected by microorganisms that are traditionally associated with their underlying disease. This knowledge of diverging early childhood bacterial pathogens, and the observed association between bacterial infection and neutrophilic inflammation, may help guide adequate treatment strategies in young children unable to provide sputum samples.
Research Internship Lady Cilento Children’s Hospital

During the past half year, from November 2016 until April 2017, I completed a research internship at the Centre for Children’s Health Research, related to the Lady Cilento Children’s Hospital in South Brisbane, Australia. The Cough, Asthma and Airways Research Group kindly hosted me during this period, where I worked under direct supervision of Dr. Julie Marchant and Prof. Anne Chang. This research internship was part of my master programme in medicine at the University of Groningen and its main goal was to familiarise myself with medical scientific research. I conducted two projects, which helped me to learn more about designing and conducting medical research, and evidence-based medicine.

My main project was a retrospective review of bronchoscopic data, retrieved from bronchoscopy reports, amongst children with various forms of chronic suppurative lung disease (CSLD). It was a challenging and interesting project, which offered me the opportunity to go through an entire research cycle, commencing with writing a research proposal, collecting and analysing research data to finally reporting the results. To prepare myself for this project, I gained background knowledge of CSLD and bronchoscopies by performing an extensive literature search in the period before I arrived in Australia. During this internship, I retrieved bronchoscopic and radiological data from the hospital’s medical information system and merged this into a large database, applied statistics and wrote this report to present my results. Overall, this project helped me to refresh my knowledge of medical research and improve my English writing skills. As a result of this research, novel findings were discovered, thus we decided to write an additional paper, which will be submitted to ‘Pediatric Pulmonology’ in near future.

My second project was to update a Cochrane-review of Prof. Chang on vitamin A supplementation for cystic fibrosis. This was a whole different project, and the change in topic and research methodology offered me an excellent opportunity to learn more about evidence-based medicine. I gained valuable experience in critically evaluating the quality of research evidence for numerous study designs. This skill will be beneficial for the application of evidence-based medicine during clinical work as a future doctor.

Concurrently to my two main projects I could participate in several other clinical and academic activities. I had the opportunity to join several respiratory outpatient clinics, ward rounds and diagnostic procedures including bronchoscopies. This helped me to understand the major clinical impact of respiratory diseases in children, next to an improved understanding of how data for bronchoscopic research in children is collected. Additionally, I could further improve my clinical skills, specifically diagnosing and treating children with CSLD. I participated in weekly pediatric grand rounds and respiratory department teaching sessions where, during the last meeting, I presented my main project to the respiratory staff. Finally, at the end of the internship, I spent several days in the hospital’s pediatric emergency department, in which I learned a lot about pediatric emergency medicine. Overall, this internship has been an unforgettable experience, working and living abroad helped me develop myself professionally, and personally as well.

It is a pleasure to express my gratitude to all those who made this internship possible and contributed to the unforgettable time I experienced. Firstly, I would like to acknowledge the most important role of my primary supervisors Dr. Julie Marchant and Prof. Anne Chang. They offered me an outstanding opportunity to do my research internship overseas and it was a truly privilege to work together. Their excellent guidance and supervision ensured that I could make the most out of my internship. Additionally, I would like to thank all the people from the Cough, Asthma and Airway’s Research Group who hosted me and made me feel a part of the team.
from the first day on. Special thanks to Jack and Greta, who helped me optimising my database and gaining additional data. Furthermore, I would like to thank Dr. Helen Petsky who helped me starting the review process for the Cochrane review. I would like to thank the entire Respiratory department for hosting me and allowing me to join their excellent teaching sessions. Lastly, I owe my gratitude to Prof. Paul Brand, who helped me with the organisation of this internship and was my faculty supervisor.

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Brisbane, 13th April 2017.
### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
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<td>BE</td>
<td>Bronchiectasis</td>
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<td>CF</td>
<td>Cystic fibrosis</td>
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<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator protein</td>
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<td>cfu</td>
<td>Colony-forming units</td>
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<td>cHRCT</td>
<td>Chest high-resolution computed tomography</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
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<td>CSLD</td>
<td>Chronic suppurative lung disease</td>
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<td>EBV</td>
<td>Epstein–Barr virus</td>
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<td>ERS</td>
<td>European Respiratory Society</td>
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<td>IQR</td>
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<td>PBB</td>
<td>Protracted bacterial bronchitis</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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Introduction

Cough

Cough is one of the most frequent reasons for patients’ doctor attendance worldwide (1,2). While most of these patients likely have acute cough, some coughs are chronic. Assessing the exact prevalence of cough is difficult, since people frequently refrain from seeking medical attendance for this mostly self-limiting disease (3). Cough appears to be common in general pediatric population: in a survey amongst 2717 Dutch children regarding symptoms experienced in the past two weeks, 23.8% reported to have been suffering from cough (4). The global prevalence of chronic cough (>8 weeks) in adults is 9.6%, and showing a strong correlation with smoking (5). In most pediatric guidelines, cough is divided into acute cough (≤4 weeks) and chronic cough (>4 weeks) (6). There is a significant burden for families of children suffering from chronic cough, reflected in repeated medical consultations and increased parental stress levels (7). Additionally, there is a significant economic burden related to upper and lower airway infections in children: nationwide healthcare costs for airway infections in Dutch children (aged 0-19 years) amounted €242,100,000 in 2011 (8).

Wet Cough

Cough is a defense mechanism to clear airway secretions and prevent inhaled foreign material (9). Quality of cough in pediatric practice is determined as dry or wet cough, and clinical validity has been shown (10). Furthermore, cough quality is related to the bronchoscopic quantification of airway secretions: wet cough is always characterised by the presence of increased secretions, compared to dry cough (10). These airway secretions are caused by an increased production or by impaired secretion clearing mechanisms (11). Wet cough is associated with higher rates of bacterial and viral lower airway infection and accompanied by greater neutrophilic airway inflammation compared to dry cough (12). Additionally, wet cough is, compared to dry cough, less likely to resolve spontaneously (13) and dry cough may even develop into wet cough if airway secretions increase (10). The importance of managing the cause of wet cough is highlighted by recent studies, which have suggested an association between persisting wet cough, ongoing bacterial airway infection and chronic suppurative lung disease (CSLD) (13–17).

Chronic Suppurative Lung Disease

Although suppurative lung disease literally implies any condition that causes the production of pulmonary purulent secretions, the diagnostic entity CSLD is frequently used to cover the spectrum between protracted bacterial bronchitis (PBB) and bronchiectasis (Figure 1) (18). Along this spectrum of disease (15), it is proposed that children with prolonged wet cough initially develop PBB, which if left untreated may progress to CSLD or bronchiectasis (19). In this model, CSLD is considered a separate diagnostic entity, assigned to children showing the clinical characteristics of bronchiectasis without radiological evidence of bronchiectasis being present (18). Hence, as seen in Figure 1, there is overlap between PBB and CSLD, and

Figure 1: Spectrum alongside which disease progression from PBB to bronchiectasis occurs, using the pathobiological model. Adopted from Chang et al. (2016) (18). CSLD, chronic suppurative lung disease; BE, bronchiectasis.
Bronchoscopic Findings in Children with Chronic Suppurative Lung disease

Bronchoscopic Findings in Children with Chronic Suppurative Lung disease

bronchiectasis and CSLD, making comparisons between these groups difficult. In Europe, CSLD is not seen as a separate diagnostic entity, but a description that refers to any chronic disease that results in accumulation of purulent material in the airways, including cystic fibrosis (CF), recurrent aspiration and immunodeficiencies (20). Hence, in this study, in accordance with the European description, CSLD is not considered a separate diagnostic entity, but an overarching term covering various suppurative airway conditions, including PBB, bronchiectasis and CF.

Protracted Bacterial Bronchitis

Protracted bacterial bronchitis was first characterised in 2006 by Brisbane’s Cough, Asthma and Airways Research Group (14), although the condition had been somewhat described in the past as chronic bronchitis of childhood (21). Nowadays, PBB in day-to-day clinical practice is defined as the presence of continuous chronic (>4-weeks duration) wet or productive cough, in the absence of symptoms or signs (i.e. specific cough pointers) suggestive of other causes of wet or productive cough and cough resolved following a 2-4 week course of an appropriate oral antibiotic (22).

The exact prevalence of PBB is unknown, but in prospective cohort studies in the past decade prevalence rates of 6-42% amongst children are seen, and in some pediatric centres it is now found to be the most common cause of chronic wet cough in childhood (18). In all published studies of airway cytology in children with PBB, increased neutrophilic airway counts are found (22), with significantly higher neutrophil counts compared to other causes of chronic childhood cough (14,23). Although outcome measures differ amongst PBB-studies (e.g. quantitative vs. qualitative bacteriology, different number of lobes sampled) (18), many studies conclude that lower airway infection, mainly with Streptococcus pneumonia, Haemophilus influenzae or Moraxella catarrhalis, is present in children with PBB (23–28). Viral airway infection occurs significantly more frequent in children with PBB compared to controls, the most common identified virus is human adenovirus (29).

It is known that a prolonged infectious state is harmful to the lung epithelium and can lead to chronic lung disease (30). According to Coles’ ‘vicious cycle’ hypothesis (Figure 2), in children with chronic airway infection, further impaired airway clearance and ongoing inflammation, can lead to airway damage and eventually long-term to the development of bronchiectasis (31,32). This hypothesis is currently the most accepted explanation on the formation of bronchiectasis, and early adequate therapeutic intervention is needed to prevent this progression in children with PBB and chronic wet cough (33).

Bronchiectasis

Bronchiectasis is a chronic disease in children characterised as irreversible bronchial dilatation due to bronchial wall damage, clinically manifesting as recurrent wet cough due to lower respiratory tract infections (34,35). In bronchiectasis, a neutrophil driven inflammatory response, more intense with bacterial presence, leads to the release of proteolytic enzymes that damage the bronchial wall (31). Oedema and lymph follicle formation, with destruction of elastin, within the bronchial wall result, eventually followed by signs of muscle and cartilage destruction in more advanced disease (35).

Figure 2: The ‘vicious cycle’ hypothesis of bronchiectasis. Adopted from Grimwood (2011) (31).
The authors of a systematic review into the etiologies of non-CF bronchiectasis in children identified an underlying etiology in 63% of the cases, with infectious disease reported as the most common cause (36). Recently published data suggests that children with a chronic or recurrent wet cough, unresponsive to antibiotics, have an increased likelihood of bronchiectasis (adjusted odds ratio: 5.9; 95% confidence interval: 1.2-28.5) and should undergo a chest high-resolution computed tomography (cHRCT) scan to confirm whether or not bronchiectasis are present (34). Commonly detected respiratory pathogens in the airways of patients with bronchiectasis are *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* (35), whereby *P. aeruginosa* infection or colonisation is more likely to occur in older children (31) and associated with more advanced disease (37).

Bronchiectasis has been described in published literature for more than a century (38). The incidence of bronchiectasis has significantly declined in most developed countries during the second half of the last century (19). This is attributed to the general improvement in public health (19) and the more effective treatment of pulmonary infections (39,40), especially with the introduction of broad-spectrum antibiotics (41). Despite this, bronchiectasis remains common amongst people in underdeveloped countries and indigenous populations in developed countries (19). Prevalence rates for bronchiectasis have been reported from 33 per 100,000 children in New Zealand (42) to 1,500 per 100,000 children in the Alaska indigenous population from the Yukon-Kuskokwim Delta region (43).

Diagnosis of bronchiectasis is based on an increased broncho-arterial ratio on a cHRCT scan (>1) (44), although a recent study showed this underestimates the presence and extent of bronchial dilatation in children (45). Bronchiectasis has been classified into subtypes by Reid: cylindrical, varicosal and cystic bronchiectasis (46), which are used as a severity and can be identified on cHRCT (35). Management of bronchiectasis in children focuses on reducing exacerbation frequency and severity, thereby preventing complications and decline in lung function and optimising quality of life (35). Antibiotics are used to treat exacerbations, drug selection and administration method should be based on lower airway cultures if available, exacerbation severity and patient tolerance (34). Early diagnosis and appropriate treatment lead to better outcomes in children with bronchiectasis (47). A recent study by Gaillard and colleagues suggests that, unlike in adults, in childhood bronchiectasis may be reversible as they found total resolution of bronchial wall dilatation in 6 of 22 children following medical therapy (48). Hence, increased knowledge of pulmonary microbiological data in children with bronchiectasis, particularly the younger child who is unable to expectorate, will aid in appropriate therapeutic choices and long-term outcomes.

**Cystic Fibrosis**

Cystic fibrosis is the most common autosomal recessively inherited disorder in the Caucasian population (49), affecting 1 per 4,750 live births in the Netherlands (50) and 1 per 3,650 live births in Australia (51). CF is caused by inherited or spontaneous mutations in a gene on the long arm of chromosome 7 (7q.31.2), encoding for the cystic fibrosis transmembrane conductance regulator protein (CFTR) (52–54). The main function of this protein is cyclic adenosine monophosphate-regulated chloride transport at the apical membranes of epithelial cells (53), dysfunction leads to pathological changes in organs that express CFTR, in particular lungs, pancreas, liver and respiratory tract (55). The most common identified CFTR mutation in Caucasians is the ΔF508 mutation, causing a deletion of phenylalanine at amino acid 508 (53), leading to trafficking defects, as CFTR is not properly folded and therefore not transported to the cell surface (56). The amount of functional CFTR reaching the cell surface varies amongst different mutations in CF, manifestations in patients can consequently be very different (56). The gold standard to diagnose CF is a sweat test (57), in which sweat chloride levels of >60
mmol/L are indicative of CF (58). Performing a second sweat test and/or CFTR mutation analysis is recommended (58).

Signs and symptoms of CF can differ between patients, even siblings, with the same CFTR genotype (56). Lack of functional CFTR leads to a decreased airway surface liquid and an increased density of bronchial mucus, thereby impairing ciliary clearance, resulting in a more suitable environment for bacterial growth (59). Consequently, lungs of patients with CF are frequently colonised by *P. aeruginosa*, *Staphylococcus aureus* and *H. influenzae* (51,60). CF lung disease is majorly characterised by chronic airway infection and inflammation, progressing to bronchiectasis (Figure 3), accompanied by gas trapping, hypoxaemia and hypercarbia (56). Treatment of CF lung disease aims to remove thickened secretions by using airway clearance techniques and nebulisers that decrease mucus viscosity, accompanied by strategies to prevent or eradicate the presence of airway pathogens (61). Chronic colonisation with *P. aeruginosa* is associated with increased morbidity and mortality (62), indicating the importance of adequate treatment of CF lung disease.

![Figure 3: Traditional view of pathophysiology in CF lung-disease. Adopted from Rao & Grigg (2006) (63).](image)

CF is, however, a multi-systemic disease, since CFTR is expressed in numerous organs, resulting in gastro-intestinal symptoms, endocrine disorders and infertility in male patients (56). Of the infants with CF, 85 to 90% has pancreatic insufficiency at birth or develops this during the first year of life, increasing the risk of steatorrhea, fat-soluble-vitamin deficiency, and malnutrition (56). Therefore, treatment in patients with CF is multidisciplinary, frequently including supplementation of pancreatic enzymes and age-appropriate dietetic interventions (61). Due to various interventions, such as newborn screening (64), improved nutritional management (65) and advances of antibacterial therapies (66), average life expectancy in patients with CF has increased from 24 years in 1984 (67) to an expectancy over 40-50 years for children with CF born today (68). Despite advances, CF lung disease remains the most frequent cause of death in patients with CF, pulmonary insufficiency is accounting for at least 80% of the deaths (56). As such research regarding pathogenic bacteria in the lower airways in younger CF-patients remains very important.

**Bronchoscopy**

In all the three aforementioned forms of CSLD, ongoing inflammation and infection by pathogenic organisms is associated with airway tissue damage (30,31,57,62). Airway inflammation is characterised by an increase of the total cell count and, in case of bacterial infection, an increase in neutrophils in the airway fluid (69). Unfortunately, white blood cell count and serum neutrophil count are not necessarily increased in the presence of airway pathogens in the lungs of children with chronic lung disease (70). Therefore, a clinician needs a lower airway sample, such as sputum, to assess airway cellularity and microbiology. However, in younger children who cannot expectorate, this is challenging (71). Invasive procedures, such as bronchoscopy with bronchoalveolar lavage, are still considered the gold standard in retrieving samples to determine the lower airway cellularity and the composition of the lower airway microbiology (71). Due to both practical and ethical issues, performing a bronchoscopy in children for purely research purposes is unfeasible (72), but it remains an important clinical diagnostic procedure and the use of information gained (whether prospectively or retrospectively) an important research tool.
Procedure
Bronchoscopy is a common diagnostic procedure in pediatric respiratory clinical practice to examine the upper airways, as well as the trachea and bronchi by passing a bronchoscope through the nose (flexible scopes) or mouth (rigid scopes) (Figure 4) (73). This procedure, in pediatric practice in Australia and the Netherlands performed under general anesthesia (74–76), gives the clinician the opportunity to perform a visual examination of the airways and to obtain specimens from the lower airways (73). Common indications for bronchoscopy include stridor, persistent wheeze or cough, atelectasis, recurrent or persistent pneumonia and suspected foreign body inhalation (73,77,78). Flexible scopes are used in most diagnostic procedures, they are smaller than rigid instruments and can therefore be advanced further into the airways (73,79). Nevertheless, rigid scopes still play an important role in preoperative laryngeal and tracheal assessment and interventional airway endoscopy, including foreign body extraction (80). Bronchoscopy is generally considered safe (74), with major complications being rare (<2%), although up to 19% of children develop a self-limiting fever post procedure (81).

Macroscopic Findings
Macroscopic airway abnormalities that can be diagnosed by bronchoscopy include pulmonary anatomic anomalies, foreign bodies, bronchitis associated appearances and airway granulomata and cysts (74). Abnormal anatomic features may include various forms of malacia, aberrant anatomic variations and airway stenoses (74). Airway malacia is divided into laryngo-, tracheo- and bronchomalacia, where respectively the larynx, the trachea and one or more bronchi are affected. Foreign body aspiration, even in cases with little suspicion, should always be evaluated bronchoscopically, since overlooked foreign bodies are associated with major complications, such as persistent granulation tissue, recurrent pneumonia, atelectasis and the formation of bronchiectasis (82). Bronchitis-associated appearances are characterised by purulent secretions with airway oedema and inflammation (18). A broad group of infectious and non-infectious pulmonary conditions leads to the formation of granulomata (83), whereas airway cysts, frequently of laryngeal origin, are mostly congenital or caused by intubation trauma (84,85).

Particularly interesting macroscopic findings in the light of this study are tracheo- and bronchomalacia, since those conditions impair airway clearance (86), leading to chronic cough. Airway malacia, a condition also known as “floppy airways”, is characterised by excessive collapsibility of the airways, in primary malacia due to developmental abnormalities resulting in airway cartilage softening and decreased airway muscle tone (87,88). Symptoms are inversely correlated to the airway diameter (88) and typically improve in maturing children as their airways grow (89,90). Therefore, airway malacia is more common in younger children (88–90). Previous studies revealed that tracheo-bronchomalacia is more common in PBB compared to children in general population (14,28,91). Similarly, prevalence of malacia is increased in bronchiectasis (92,93) and CF (94), but all studies have been conducted in different centres with differing populations. Comparison of data from one centre, with standardised technique and consistent diagnostic definitions, across these patient groups will provide novel prevalence data for this condition.
Bronchoalveolar Lavage

Sampling the lower airways can be done by lower airway biopsies, bronchial wall brushing and by aspirating lower airway secretions or lavage fluid (73). The most common sampling method in paediatrics, bronchoalveolar lavage (BAL), is performed by instilling sterile saline through the suction channel of the bronchoscope into the most affected area of the lung, or otherwise the right middle lobe (73,95). According to the European Respiratory Society (ERS) guidelines, at least 40% of the instilled fluid needs to be withdrawn by gentle suction through the bronchoscope (95), the returned aliquots will not only contain the instilled saline, but will be a diluted reflection of the consistency of the airway surface fluid (73). The BAL fluid represent the lower airway cells and microbiology; a useful diagnostic tool (95).

Reference values for cellularity in BAL fluid in general pediatric population are based on bronchoscopies in children without respiratory tract infection that underwent the investigation for other indications, and bronchoscopies in children that underwent general anesthesia for non-pulmonary related elective surgery (95,96). In general, BAL fluid from healthy children shows variable total cell counts (95), with mainly macrophages (80-90%), some lymphocytes (5-15%), a few neutrophils (<5%) and no eosinophils (<1%) (95–97). Neutrophil counts as a percentage of total cell count are known to be higher in PBB (14,23,24,28,29,98–101), bronchiectasis (98,102–107) and CF (103,107–120) across different studies in different centres. To date no-one has compared the BAL cellularity of the three conditions from the same centre.

Aim of the Study

The various forms of CSLD described are linked to specific microbes that play a role in its disease pathogenesis (121). For example, S. aureus and P. aeruginosa are commonly identified in older children and adults with CF (60), whereas in PBB (18) and bronchiectasis (35), H. influenzae is the most common identified microorganism. Multiple studies, both single- and multicentre, comparing bronchoscopy outcomes between two of the included forms of CSLD have confirmed these findings (93,103,107,122). However, in young children with CF, a high prevalence of H. influenzae has been shown (60,123,124). One study, conducted by van der Gast and colleagues, compared lower airway specimens across PBB, bronchiectasis and CF (121). Interestingly, the authors found in their multi-centre study that children with these various forms of CSLD, in contrast to adults, shared strikingly similar core airway microbiota. This indicates there is a divergence in microbiota at some stage from childhood to adulthood. Further research into the airway microbiology and inflammation in young children with various forms of CSLD is required to identify when this divergence occurs and whether these diseases each begin in a similar or distinct fashion. It is important that future studies use standardised sampling techniques to reduce risk of bias, since distinct methods would yield different results.

To the best of the author’s knowledge, no single-centre studies comparing bronchoscopy and BAL findings across the three aforementioned forms of CSLD in children have been reported. Therefore, the aim of this study is to compare and contrast the bronchoscopic findings of microbiological airway culture in PBB, bronchiectasis and CF during early childhood, and to determine how those airway pathogens relate to airway inflammation, cellular counts and aberrant macroscopic findings, from a single tertiary pediatric centre (i.e. using the same methodological approach). The author hypothesises, based on the study by van der Gast and colleagues (121), that airway microbiology and neutrophilic inflammation of the lower airways are similar in young children with various forms of suppurative lung disease, independent from underlying diagnosis. In addition, the author hypothesises that aberrant macroscopic findings (i.e. tracheo- and/or bronchomalacia) are prevalent amongst children with CSLD and are associated with increased airway inflammation, independent of underlying diagnosis. It is hoped this study may guide future prospective research work in the emerging area of airway microbiology and microbiota in CSLD in children.
Materials and Methods

Study Design
To compare and contrast the bronchoscopic findings across children with CSLD, a retrospective review of the bronchoscopy reports from the Royal Children’s Hospital, now relocated and renamed Lady Cilento Children’s Hospital, in Brisbane, Australia was undertaken. Any child, aged <18 years of age, referred to a pediatric respiratory physician who underwent an elective flexible bronchoscopy including BAL between March 2010 and November 2016 was eligible for inclusion. In total, 2090 reports from 1746 individual children were reviewed, 452 children meeting a-prior definitions of PBB (history of chronic wet cough (>4 weeks), resolution of cough on antibiotic treatment within two weeks, n=146), bronchiectasis (on cHRCT scan proven non-CF bronchiectasis, n=179) or CF (positive sweat test and/or CF gene mutation present, n=127) were included and assigned to categories based on these diagnoses (Figure 5). Eight children meeting pre-defined exclusion-criteria were excluded for reasons including: post liver transplantation, post lung surgery, systemic lupus erythematosus, bilateral bronchial stenosis. The Queensland Children’s Health Services Ethics Committee approved the study.

Figure 5: Study flow diagram. QCHSEC, Queensland Children’s Health Services Ethics Committee; BE, bronchiectasis.
Bronchoscopy and Bronchoalveolar Lavage

Flexible bronchoscopy (Olympus; Tokyo, Japan) was performed in all children under general anesthesia. In this centre, BAL is performed in accordance with the, aforementioned, ERS guidelines (95): sterile saline was instilled in three aliquots of 1 ml/kg (maximum 20 ml) into the most affected area in localised disease or right middle lobe in generalised disease and by gentle suctioning removed, with an appropriate yield greater than 40% of the instilled volume. The first aliquot was used for microbiological assessment, since the first aliquot is known to have a lower cellular yield, compared to subsequent aliquots (95). In most children, microbiological assessment included quantitative aerobic bacterial cultures, specific testing for mycobacterial species using inoculation of Lowenstein-Jensen media, fungal cultures, and polymerase chain reaction (PCR) for mycoplasma and various respiratory viruses. Furthermore, in most children with CF, PCR for detection of Burkholderia species was performed as well. The second and third lavage aliquots were pooled and used for cellular analysis, including total and differential cell counts.

Data Collection and Outcome Measures

Data, including patient characteristics, indication for bronchoscopy and aberrant macroscopic findings, were retrospectively retrieved from bronchoscopy reports. Aberrant macroscopic findings recorded and analysed were laryngo-, tracheo- and bronchomalacia, whereby tracheobronchial airway diameter reduction of more than 50% of the cross-sectional area was considered significant. In the case cHRCT-scans were performed, radiological reports and images were reviewed to determine the presence of bronchiectasis (broncho-arterial ratio: >1). BAL microbiological and cytological data, were retrieved from the hospitals laboratory information system (Auslab, Citadel Health; Melbourne, Australia). The primary outcomes for this study were the airway microbiology amongst the three cohorts, measured as proportion of children in a cohort with specific lower airway infection. Lower airway infection was defined as a positive BAL culture (at a density ≥10^4 colony-forming units (cfu)/ml (18)) growing recognised respiratory pathogens, including S. pneumoniae, β-hemolytic streptococci, haemophilus species, M. catarrhalis, S. aureus, Enterobacteriae, P. aeruginosa (≥10^3 cfu/ml) and Strentomonas Maltophilia (≥10^1 cfu/ml) (102). Lower respiratory infection was also considered present if any fungi and/or one or more viruses, mycoplasma or mycobacteria were detected by PCR. Other outcome measures were airway cytology (total cell count, percentage macrophages, percentage lymphocytes, percentage neutrophils and percentage eosinophils) and aberrant macroscopic findings.

Missing and Inconsistent Data

For each child, in case multiple bronchoscopies were performed, data from the first bronchoscopy was included in the study. Exceptions were made for children in which a cHRCT scan was performed around the time of a bronchoscopy (up to one year before or after the bronchoscopy), then data from that bronchoscopy was included, to give a reliable assessment on the presence of bronchiectasis at the time of bronchoscopy. In children who underwent more than one cHRCT scan, the first scan within a year of a bronchoscopy was included. In the case bronchiectasis was diagnosed on cHRCT scan more than a year post-bronchoscopy, or a cHRCT scan revealing no bronchiectasis was performed more than a year before the bronchoscopy, radiological findings were excluded from analysis (n=9). Cellularity outcomes from bronchoscopies with no total and/or differential cell count data were available were also excluded from analyses (n=49). Eighteen children with CF had a bronchoscopy undertaken as per study protocol for another study conducted in this centre (clinicaltrials.gov identifier: NCT01270074), hence their BAL results were excluded from analyses to prevent bias, since those children were more likely to be well than CF-children who underwent a bronchoscopy for clinical indications. For one child with PBB, BAL results were missing.

Bronchoscopic Findings in Children with Chronic Suppurative Lung disease | 13
**Statistical Analyses**

IBM SPSS Statistics Version 23.0 (IBM Corp.; Armonk, NY, USA) was used to carry out statistical analyses. As data were non-normally distributed and unable to be transformed into a normal distribution (Figure S1, supplementary material in the appendix), continuous outcomes were reported as medians and inter-quartile ranges (IQR). Based on the large difference in median age between the cohorts (PBB-CF: 21 vs. 56 months), post-hoc the decision was made to include only children younger than six years of age in analyses, to create more comparable cohorts. Descriptive statistics are provided for all cohorts. Statistical comparison between cohorts for categorical variables was performed using the chi-square test, with continuity correction for 2x2 analyses, or Fisher’s exact test if there were any expected values <5. Continuous variables were compared between two cohorts using Mann-Whitney’s U-test and using Kruskal-Wallis test for comparison of >2 cohorts. Furthermore, to explore the factors influencing neutrophil percentage amongst the three different cohorts, variables were, based on the strength of their univariate association with neutrophil percentage, selected (P < 0.2) and included in multiple linear regression analyses with backward elimination. For all analyses, two-sided tests were used with P-values <0.05 considered statistically significant.
Results

Study Population

Characteristics
The median age of the 335 children (207 male, 128 female) under the age of six at the time of bronchoscopy was 25 months (IQR 15, 42). Children with PBB were significantly younger than children with bronchiectasis ($P < 0.0001$) and CF ($P = 0.012$), no significant difference in age between the bronchiectasis- and CF-cohorts was observed ($P = 0.269$). With regards to sex, no significant differences between the three cohorts was observed ($P = 0.969$), nor between the bronchiectasis- and CF-cohorts ($P = 0.923$). Amongst all cohorts, 228 children (69%) underwent a cHRCT-scan, which revealed radiologically proven bronchiectasis in 189 children (83%). No comparison between the cohorts was made, because bronchiectasis on cHRCT scan was used as an inclusion criterion for the bronchiectasis-cohort (Table 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PBB, n = 126, n (%)</th>
<th>BE, n = 138, n (%)</th>
<th>CF, n = 71, n (%)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M:F (% male)</td>
<td>78:48 (62)</td>
<td>86:52 (62)</td>
<td>43:28 (61)</td>
<td>0.969</td>
</tr>
<tr>
<td>Median age at bronchoscopy, mo (IQR)</td>
<td>18 (12, 28)</td>
<td>31 (20, 47)</td>
<td>36 (11, 45)</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>cHRCT scan included</td>
<td>39 (31)</td>
<td>138 (100)</td>
<td>51 (72)</td>
<td>- b</td>
</tr>
<tr>
<td>Bronchiectasis (radiologically proven)</td>
<td>0 (0) (n=39)</td>
<td>138 (100) (n=138)</td>
<td>26 (51) (n=51)</td>
<td>- b</td>
</tr>
<tr>
<td>Bronchoscopy, cHRCT scan on same day</td>
<td>29 (74) (n=39)</td>
<td>114 (83) (n=138)</td>
<td>33 (65) (n=51)</td>
<td>- b</td>
</tr>
</tbody>
</table>

BE. Bronchiectasis; mo, months; IQR, inter-quartile range. Medians, IQR and percentages rounded to the nearest whole number. the number of samples tested for a specific outcome noted in heading or directly behind result if distinct.

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

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

Bronchoscopy Indications
Most frequent indications to perform a bronchoscopy were ‘recurrent or chronic cough’ (85%) and ‘stridor or noisy breathing’ (11%) (Table 2). Children who had ‘other indication’ recorded as their bronchoscopy indication, most frequent underwent the procedure due to lobar collapse or atelectasis (n=5, including the two children with PBB and bronchiectasis) and to examine possible (ongoing) Pseudomonas aeruginosa infection or colonisation (n=3). Indications per cohort are provided in Table 2.

<table>
<thead>
<tr>
<th>Indication</th>
<th>PBB, n = 126, n (%)</th>
<th>BE, n = 138, n (%)</th>
<th>CF, n = 71, n (%)</th>
<th>Total, n = 335, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent pneumonia and/or LRTI</td>
<td>3 (2)</td>
<td>8 (6)</td>
<td>0 (0)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>Recurrent or chronic cough</td>
<td>112 (89)</td>
<td>132 (96)</td>
<td>41 (58)</td>
<td>285 (85)</td>
</tr>
<tr>
<td>Stridor and/or noisy breathing</td>
<td>28 (22)</td>
<td>8 (6)</td>
<td>0 (0)</td>
<td>36 (11)</td>
</tr>
<tr>
<td>Study protocol</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>18 (25)*</td>
<td>18 (5)*</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>12 (17)</td>
<td>14 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>144b</td>
<td>149b</td>
<td>71</td>
<td>364b</td>
</tr>
</tbody>
</table>

BE. Bronchiectasis; LRTI, Lower Respiratory Tract Infection. Percentages rounded to the nearest whole number.

*Performed as per protocol for another study (clinicaltrials.gov identifier: NCT01270074)

*bTotal numbers exceed the total number of children included, since for some bronchoscopies multiple indications were recorded.
Airway Microbiology

Of the 316 BAL results from children who underwent a bronchoscopy due to clinical indications, 314 (99%) had growth of at least one organism detected, including upper airway flora such as α- and non-hemolytic streptococci, coagulase negative staphylococci and Neisseria and Corynebacteria species. Two hundred and sixty-eight (85%) results revealed lower airway infection by at least one pathogenic bacteria or virus. In total, 253 (80%) BAL results cultured one or more bacterial species, 122 of 314 (39%) results that underwent PCR for viral detection, were positive for one or more virus and 100 of 266 (32%) tested results revealed fungal growth. Bacterial-viral co-infection was present in 107 of 314 results that underwent both bacterial culture and PCR for viral detection. Numbers per cohort are provided in Table 3 and as Venn diagrams in supplementary material in the appendix (Figure S2).

Table 3: Lower airway infection amongst the three diagnostic cohorts.

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>PBB n=125, n (%)</th>
<th>BE n=138, n (%)</th>
<th>CF n=53, n (%)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt; PBB-BE</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt; BE-CF</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt; all cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>No significant pathogenic infection&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10 (8)</td>
<td>16 (12)</td>
<td>10 (19)</td>
<td>0.442</td>
<td>0.282</td>
<td>0.113</td>
</tr>
<tr>
<td>Pathogenic bacterial infection&lt;sup&gt;e&lt;/sup&gt;</td>
<td>109 (87)</td>
<td>111 (80)</td>
<td>33 (62)</td>
<td>0.189</td>
<td>0.015</td>
<td>0.001</td>
</tr>
<tr>
<td>H. influenzae&lt;sup&gt;f&lt;/sup&gt;</td>
<td>81 (65)</td>
<td>91 (66)</td>
<td>9 (17)</td>
<td>0.948</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>H. influenzae (BLP)</td>
<td>17 (14)</td>
<td>22 (16)</td>
<td>2 (4)</td>
<td>0.701</td>
<td>0.041</td>
<td>0.076</td>
</tr>
<tr>
<td>H. influenzae (BLN)</td>
<td>65 (52)</td>
<td>70 (51)</td>
<td>7 (13)</td>
<td>0.934</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>M. catarrhalis (BLP)</td>
<td>41 (33)</td>
<td>27 (20)</td>
<td>1 (2)</td>
<td>0.023</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>31 (25)</td>
<td>33 (24)</td>
<td>3 (6)</td>
<td>0.978</td>
<td>0.007</td>
<td>0.009</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10 (8)</td>
<td>9 (7)</td>
<td>13 (25)</td>
<td>0.836</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>H. Parainfluenza &amp;&lt;sup&gt;g&lt;/sup&gt;</td>
<td>15 (12)</td>
<td>5 (4)</td>
<td>1 (2)</td>
<td>0.021</td>
<td>1.000&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.008</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>4 (3)</td>
<td>5 (4)</td>
<td>15 (28)</td>
<td>1.000&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>5 (9)</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.010&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>PBB n=124, n (%)</th>
<th>BE n=138, n (%)</th>
<th>CF n=52, n (%)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt; PBB-BE</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt; BE-CF</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt; all cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any viral species</td>
<td>58 (47)</td>
<td>57 (41)</td>
<td>7 (14)</td>
<td>0.444</td>
<td>0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>32 (26) (n=122)</td>
<td>23 (17) (n=136)</td>
<td>2 (4)</td>
<td>0.094</td>
<td>0.034</td>
<td>0.002</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>10 (8) (n=122)</td>
<td>12 (9) (n=136)</td>
<td>1 (2)</td>
<td>1.000</td>
<td>0.117&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.248</td>
</tr>
<tr>
<td>RSV</td>
<td>9 (7) (n=122)</td>
<td>4 (3) (n=136)</td>
<td>2 (4)</td>
<td>0.180</td>
<td>0.669&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.237</td>
</tr>
<tr>
<td>CMV</td>
<td>12 (26) (n=47)</td>
<td>13 (27) (n=49)</td>
<td>0 (0) (n=19)</td>
<td>1.000</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>EBV</td>
<td>7 (17) (n=41)</td>
<td>8 (19) (n=43)</td>
<td>0 (0) (n=19)</td>
<td>1.000</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bacterial-viral co-infection</td>
<td>55 (44)</td>
<td>48 (35)</td>
<td>4 (8)</td>
<td>0.145</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>PBB n=94, n (%)</th>
<th>BE n=119, n (%)</th>
<th>CF n=53, n (%)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt; PBB-BE</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt; BE-CF</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt; all cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any fungal growth</td>
<td>29 (31)</td>
<td>42 (35)</td>
<td>29 (55)</td>
<td>0.592</td>
<td>0.026</td>
<td>0.013</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>PBB n=118, n (%)</th>
<th>BE n=133, n (%)</th>
<th>CF n=52, n (%)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt; PBB-BE</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt; BE-CF</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt; all cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any mycobacterial infection</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

<sup>a</sup>P-value tests whether the PBB- and the BE-cohorts have the same prevalence of an outcome. P-values <0.05 denote statistical significance and are noted in bold.

<sup>b</sup>P-value tests whether the BE- and the CF-cohorts have the same prevalence of an outcome. P-values <0.05 denote statistical significance and are noted in bold.

<sup>c</sup>P-value tests whether all three cohorts have the same prevalence of an outcome. P-values <0.05 denote statistical significance and are noted in bold.

<sup>d</sup>No significant pathogenic organisms identified (includes testing for bacteria, viruses, fungi and mycobacteria).

<sup>e</sup>Significant pathogenic bacterial growth defined as growth ≥10⁵ cfu/ml (or ≥10⁴ cfu/ml for P. aeruginosa and/or S. maltophilia).

<sup>f</sup>Two children (PBB and BE) grew significantly H. influenzae (BLP) as well as H. influenzae (BLN).

<sup>g</sup>Fisher’s exact test, P-values <0.05 denote statistical significance and are noted in bold.

<sup>h</sup>Due to an expected value <1, no statistics were applied.

<sup>i</sup>Fisher’s exact test between PBB- and CF-cohort, P-values <0.05 denote statistical significance and are noted in bold.

<sup>j</sup>Fisher’s exact test between BE- and PBB-cohort, P-values <0.05 denote statistical significance and are noted in bold.

16 | Research Clerkship Report J.J.V. de Vries
Bacteria
Amongst the three different cohorts, significant differences in the presence of pathogenic bacterial organisms were found, with significantly less lower airway infection in the CF-cohort (33 (62%) positive cultures) \( (P = 0.001) \). Furthermore, pathogenic bacterial presence differed significantly between the bronchiectasis- and the CF-cohort \( (P = 0.015) \), which have statistically similarly aged cohorts (see Table 3).

In the PBB- and the bronchiectasis-cohort, \( H.\ influenzae \) was the most common cultured pathogen, respectively present in 81 of 125 (65%) and 91 of 138 (66%) results, of which the majority was \( \beta \)-lactamase negative. Other common cultured organisms in these cohorts were \( M.\ Catarrhalis \) (PBB: 41 of 125 (33%); bronchiectasis: 27 of 138 (20%)) and \( S.\ pneumoniae \) (PBB: 31 of 125 (25%); bronchiectasis: 33 of 138 (24%)). \( M.\ Catarrhalis \)-presence differed significantly between the cohorts \( (P = 0.023) \), however, this might be confounded by the difference in age between the cohorts, since children with \( M.\ Catarrhalis \)-presence were significantly younger \( (P = 0.010) \). Additionally, \( H.\ Parainfluenza \) was more common in children with PBB than in children with bronchiectasis \( (P = 0.021) \).

In CF, in contrast to children with PBB and bronchiectasis, \( H.\ influenzae \) was the third most cultured pathogen, present in 9 of 53 (17%) children. Well-known CF-pathogens \( (57) \), \( P.\ aeruginosa \) and \( S.\ aureus \), were more common, respectively grown in 15 (28%) and 13 (25%) of 53 BAL fluid cultures. \( S.\ maltophilia \), also frequently involved in chronic pulmonary infection in CF \( (57) \), was cultured in 5 of 53 (9%) cases.

Figure 6 summarises the differences in pathogenic bacterial growth between the three diagnostic groups.

Figure 6: Bar graph showing prevalence of most common bacteria identified \( \text{growth} \geq 10^4 \text{cfu/ml} \), specified per cohort (PBB, bronchiectasis (BE) and CF). For \( P.\ aeruginosa \) any growth \( \geq 10^4 \text{cfu/ml} \) is considered significant.
**Viruses**

Respiratory viral infections, detected by PCR, were seen more significantly in the PBB-cohort (58 of 124 (47%)) and the bronchiectasis-cohort (57 of 138 (41%)), compared to the CF-cohort (7 of 52 (14%)) \( (P < 0.0001) \). Relatively, the most common virus detected in PBB and bronchiectasis was cytomegalovirus (CMV) (PBB: 12 of 46 (26%); bronchiectasis: 13 of 49 (27%)), followed by adenovirus (PBB: 32 of 122 (26%); bronchiectasis: 23 of 136 (17%)). This differed significantly from the CF-cohort, where no CMV was identified and only 2 (4%) PCR-tests for adenovirus were positive \( (P = 0.002) \). Between the PBB- and bronchiectasis-cohorts, no significant differences in the presence of respiratory viruses were found. Overall, the numbers of children who had PCR performed on their lavage aliquots to detect CMV and Epstein–Barr virus (EBV) were reduced, compared to PCR for other respiratory viruses due to recent practice standard changes within this centre.

**Bacterial-viral co-infection**

Of all children who underwent viral PCR-testing, bacterial-viral co-infection was detected in 55 of 124 (44%) children with PBB, 48 of 138 (35%) children with bronchiectasis and 4 of 52 (8%) children with CF \( (P < 0.0001) \). These numbers might underestimate the actual prevalence, since, as mentioned before, recent BAL samples were not regularly tested for CMV and EBV. Most common bacterial-viral combinations identified were *H. influenzae* and adenovirus (38 of 107 (36%)), *H. influenzae* and CMV (17 of 107 (16%)) and *M. Catarrhalis* and adenovirus (15 of 107 (14%)).

**Other microorganisms**

Fungal growth was detected in 100 of 266 (38%) children who had fungi reported in their microbiology results and fungal growth was relatively more common in CF \( (P = 0.013) \). For CF, fungal subspecies were present, with *Aspergillus* as the most common fungus identified \( (n=9) \). No mycobacteria, Burkholderia species or mycoplasma were present, although those organisms were detected in older children \( (\geq 6 \text{ years}) \), mainly with CF.

**Airway Cellularity**

Airway cellularity data of 291 complete BAL results were included in analyses (Figure 5). Median total cell count differed significantly across the different cohorts \( (P = 0.039) \), with the highest median total cell count found in CF, followed by bronchiectasis and PBB. There was no significant difference between the total cell counts for the, statistically similarly aged, bronchiectasis- and CF-cohorts \( (P = 0.187) \). Neutrophilia, defined as >6.5% neutrophils (14), was identified in 252 (87%) children. Eosinophilia, defined as >1% eosinophils (14), was identified in 47 (16%) children. Cellularity data including differential data is presented in Table 4.

**Table 4: Different cohorts versus BAL cellularity data including differential cell count**

<table>
<thead>
<tr>
<th></th>
<th>PBB, n= 119</th>
<th>BE, n=129</th>
<th>CF, n=43</th>
<th>( P)-value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count (x10^6/L)</td>
<td>260 (120, 450)</td>
<td>270 (150, 645)</td>
<td>430 (175, 1070)</td>
<td>0.039</td>
</tr>
<tr>
<td>Macrophage %</td>
<td>50 (22, 70)</td>
<td>54 (23, 78)</td>
<td>25 (12, 51)</td>
<td>0.002</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>8 (5, 14)</td>
<td>9 (4, 17)</td>
<td>3 (1, 6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>36 (18, 68)</td>
<td>22 (8, 64)</td>
<td>68 (42, 83)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>&gt;b</td>
</tr>
</tbody>
</table>

BE, Bronchiectasis;

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

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

\(^b\) Since median eosinophil percentages and IQR equalled 0, no statistics were applied.
Children with PBB and bronchiectasis had significantly lower neutrophil percentages compared to children with CF ($P < 0.0001$) (Figure 7) and, inversely related, higher percentages macrophages (Table 4). Surprisingly, children with PBB had significant higher neutrophil percentages than children with bronchiectasis ($P = 0.043$). To examine whether this was confounded by the age difference between these cohorts, univariate regression analysis was undertaken, showing a very weak association between age and neutrophil percentages ($R^2 = 0.017$, $P = 0.041$). Subgroup analyses were undertaken, but revealed no significant association between age at bronchoscopy and neutrophil percentages within the PBB- and bronchiectasis-cohorts, as illustrated in Figure S3 in the supplementary material in the appendix.

![Boxplot illustrating median and IQR of neutrophil percentage in BAL fluid for diagnostic cohorts (PBB, BE and CF).](image)

**Figure 7:** Boxplot illustrating median and IQR of neutrophil percentage in BAL fluid for diagnostic cohorts (PBB, BE and CF). $P$-value tests whether all the cohorts have the same median. $P$-values $< 0.05$ denote statistical significance and are noted in bold. BE, bronchiectasis.

Regarding the state of infection, independent from underlying diagnosis, median neutrophil percentages was the highest in the bacterial-viral co-infection group, followed by bacterial infection, no infection and viral infection groups, whereby only the bacterial-viral co-infection group differed significantly from the no infection group ($P = 0.045$) and viral infection group ($P = 0.017$).

To examine whether other factors were influencing neutrophil percentages, multiple linear regression analyses with backward elimination were undertaken. For the entire study population, only total cell count remained in the final model ($R^2 = 0.338$, $P < 0.0001$). Within the different cohorts, next to total cell count, the number of different pathogenic bacteria cultured appeared to be a significant predictor in PBB ($R^2 = 0.322$, $P < 0.0001$) and, with addition of the presence of adenovirus, in CF ($R^2 = 0.399$, $P < 0.0001$). Additionally, univariate analyses revealed that children with CF who underwent their bronchoscopy as per study protocol for another study (clinicaltrials.gov identifier: NCT01270074), had significantly decreased neutrophil percentages ($\pm 27\%$), compared to children with CF who underwent their bronchoscopy for clinical indications ($R^2 = 0.173$, $P = 0.001$). In bronchiectasis, total cell count and the presence of EBV were significant predictors for increased neutrophil percentages ($R^2 = 0.438$, $P < 0.0001$). Adding positive test results for adenovirus and/or *H. influenzae* in BAL fluid as variables (122), lead to a very minor, non-significant, improved prediction model in PBB ($R^2 = 0.329$) and bronchiectasis ($R^2 = 0.456$).
Aberrant Macroscopic Findings

Tracheo- and/or bronchomalacia was detected in 160 (48%) of the 330 children in whom bronchoscopy reports reported on malacia, and, as expected, were children with malacia significantly younger ($P < 0.0001$). Malacia was more prevalent in children with PBB (88 of 124 (71%)) than children with bronchiectasis (63 of 135 (47%)) ($P < 0.0001$). Malacia was significantly less frequently diagnosed in children with CF ($P < 0.0001$), only 9 of 71 (13%) children had malacia. Laryngomalacia was detected in 14 (11%) children with PBB, versus 5 (4%) children with bronchiectasis ($P = 0.002$), no children with CF had laryngomalacia.

To prevent confounding, children with CF were, due to their small numbers, excluded from analyses on malacia (analyses including CF presented in Table S1 in supplementary material in the appendix). There appeared to be no difference regarding respiratory infection rates and microbiology between children with and without malacia; *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* were the most frequent identified microorganisms. Except for a significant lower macrophage percentage in the non-malacia group ($P = 0.026$), no differences in cellularity parameters were observed. Details on BAL results from children with and without malacia are shown in Table 5 and in boxplots in the supplementary material in the appendix (Figure S4).

Table 5: Malacia within and between PBB and bronchiectasis-cohorts; characteristics, BAL cellularity data and microbiological data.

<table>
<thead>
<tr>
<th>PBBMalacia$^a$ n = 88, n (%)</th>
<th>PBBNon-Malacia n=36, n (%)</th>
<th>P-value$^b$</th>
<th>BEMalacia$^c$ n = 63, n (%)</th>
<th>BENon-Malacia n = 72, n (%)</th>
<th>P-value$^c$</th>
<th>P-value$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, M:F (%) male</strong></td>
<td>53:35 (60)</td>
<td>24:12 (67)</td>
<td>0.641</td>
<td>41:22 (65)</td>
<td>45:27 (63)</td>
<td>0.895</td>
</tr>
<tr>
<td><strong>Median age, mo (IQR)</strong></td>
<td>17 (11, 25)</td>
<td>24 (16, 48)</td>
<td><strong>0.004</strong></td>
<td>27 (17, 47)</td>
<td>34 (23, 47)</td>
<td>0.263</td>
</tr>
<tr>
<td><strong>Stridor/noisy breathing an indication</strong></td>
<td>20 (23)</td>
<td>7 (19)</td>
<td>0.871</td>
<td>5 (8)</td>
<td>3 (4)</td>
<td>0.472$^e$</td>
</tr>
<tr>
<td><strong>Laryngomalacia present</strong></td>
<td>11 (13)</td>
<td>3 (8)</td>
<td>0.756$^e$</td>
<td>3 (5)</td>
<td>2 (3)</td>
<td>0.664$^e$</td>
</tr>
<tr>
<td><strong>Tracheomalacia present$^f$</strong></td>
<td>32 (36)</td>
<td>0 (0)</td>
<td>- $^f$</td>
<td>28 (44)</td>
<td>0 (0)</td>
<td>- $^f$</td>
</tr>
<tr>
<td><strong>Bronchomalacia present$^f$</strong></td>
<td>20 (23)</td>
<td>0 (0)</td>
<td>- $^f$</td>
<td>7 (11)</td>
<td>0 (0)</td>
<td>- $^f$</td>
</tr>
<tr>
<td><strong>Tracheobronchomalacia present</strong></td>
<td>36 (41)</td>
<td>0 (0)</td>
<td>- $^f$</td>
<td>28 (44)</td>
<td>0 (0)</td>
<td>- $^f$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PBBMalacia$^a$ n = 81$^b$</th>
<th>PBBNon-Malacia n=56$^b$</th>
<th>P-value$^b$</th>
<th>BEMalacia$^c$ n = 58$^b$</th>
<th>BENon-Malacia n = 68$^b$</th>
<th>P-value$^c$</th>
<th>P-value$^d$</th>
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<tr>
<td><strong>Total cell count (x10$^9$/L)</strong></td>
<td>270 (120, 445)</td>
<td>255 (107, 521)</td>
<td>0.890</td>
<td>230 (138, 580)</td>
<td>340 (154, 680)</td>
<td>0.219</td>
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<tr>
<td><strong>Macrophage %</strong></td>
<td>50 (21,71)</td>
<td>53 (20, 69)</td>
<td>0.934</td>
<td>71 (31, 83)</td>
<td>44 (15, 71)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Lymphocyte %</strong></td>
<td>8 (5, 14)</td>
<td>8 (4, 16)</td>
<td>0.991</td>
<td>9 (5, 14)</td>
<td>10 (3, 18)</td>
<td>0.814</td>
</tr>
<tr>
<td><strong>Neutrophil %</strong></td>
<td>37(19, 67)</td>
<td>33 (16, 72)</td>
<td>0.832</td>
<td>13 (6, 45)</td>
<td>38 (10, 75)</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td><strong>No significant infection</strong></td>
<td>7 (8)</td>
<td>2 (6)</td>
<td>1.000$^e$</td>
<td>8 (13)</td>
<td>7 (10)</td>
<td>0.784</td>
</tr>
<tr>
<td><strong>Pathogenic bacterial infection$^f$</strong></td>
<td>78 (90)</td>
<td>30 (83)</td>
<td>0.369$^e$</td>
<td>49 (78)</td>
<td>60 (83)</td>
<td>0.550</td>
</tr>
<tr>
<td><strong>Any viral infection</strong></td>
<td>42 (48)</td>
<td>16 (46)</td>
<td>0.955</td>
<td>27 (43)</td>
<td>29 (40)</td>
<td>0.898</td>
</tr>
<tr>
<td><strong>Bacterial-viral co-infection</strong></td>
<td>41 (47)</td>
<td>14 (40)</td>
<td>0.607</td>
<td>22 (35)</td>
<td>25 (35)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Any fungal growth</strong></td>
<td>21 (33)</td>
<td>8 (28) (n=29)</td>
<td>0.757</td>
<td>18 (34) (n=53)</td>
<td>23 (37) (n=63)</td>
<td>0.928</td>
</tr>
</tbody>
</table>

BE. Bronchiectasis; mo, months; IQR, inter-quartile range.

Medians, IQR and percentages rounded to the nearest whole number. Number of samples tested for a specific outcome noted in heading or behind result if distinct.

$^a$ Includes children with tracheo-, broncho- or tracheobronchomalacia; for analyses combined as one group.

$^b$ P-value tests whether outcomes of the PBB-malacia group differ from the PBB-non-malacia group. P-values <0.05 denote statistical significance, noted in bold.

$^c$ P-value tests whether outcomes of the BE-malacia group differ from the BE-non-malacia group. P-values <0.05 denote statistical significance, noted in bold.

$^d$ P-value tests for PBB- and BE-malacia groups combined vs. PBB- and BE-non-malacia groups combined. P-values <0.05 denote statistical significance, noted in bold.

$^e$ Fisher’s exact test, P-values <0.05 denote statistical significance and are noted in bold.

$^f$ No children with tracheobronchomalacia included in these numbers.

$^g$ Since the presence of tracheo- and/or bronchomalacia was used to define current groups, no statistics could be applied.

$^h$ Data presented as medians (IQR). Only BAL results with full cellularity and differential data included in analyses.

$^i$ Significant pathogenic bacterial growth defined as growth ≥10$^5$ cfu/ml (or ≥10$^6$ cfu/ml for *P. aeruginosa* and/or *S. maltophilia*).
Discussion

Summary
Results from this first, single-centre study comparing bronchoscopic findings in CSLD, indicate that, already during early childhood (<6 years), a distinct pattern of lower airway infection by various microorganisms exists between PBB, bronchiectasis and CF. Airways of young children with CSLD are frequently infected by microorganisms that are traditionally associated with their underlying disease: *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* in children with PBB (18) and bronchiectasis (35), and *P. aeruginosa* and *S. aureus* in children with CF (60). Previously unreported, high prevalence rates of CMV and EBV infection in children with PBB and bronchiectasis were present. Airway neutrophilia is higher in children with CF and amongst all diagnostic cohorts most determined by pathogenic bacterial presence. Malacia appeared to not influence airway infection and inflammation in PBB and bronchiectasis. These results suggest, although all diagnostic groups are considered CSLD, even young children with these conditions have distinct cellular differences and microbiological airway pathogens, as expressed by the significant differences in growth for almost all airway pathogens cultured.

Airway Microbiology Outcomes
Although van der Gast and colleagues recently revealed that the microbiome in young children with PBB, bronchiectasis and CF share similar core microbiota (121), our study in comparison (using culturable bacteria) showed significant variation among the different cohorts regarding pathogenic bacterial presence. Our results showed similar pathogenic bacterial patterns to several previous studies from other centres on PBB (25–27) and bronchiectasis (104,106,125), with *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* being most common organisms seen. The high prevalence of CMV (>26%) and EBV (>17%) in PBB and bronchiectasis, has not been reported before and further research is needed to determine clinical significance. Except for the prevalence of *M. catarrhalis*, which is known to be more prevalent in young children (126), no major microbiological differences between the PBB- and bronchiectasis-cohorts were revealed. In contrast, young children with CF showed a significantly different pattern, mainly culturing *P. aeruginosa* and *S. aureus*, which accords with several previous age-comparable studies (115,123,127–130). Additionally, viral respiratory infections were significantly less frequent present in children with CF, also compared to rates determined by PCR found in previous studies in CF (107,112). Although individual lower respiratory infection data for the various forms of CSLD has been described previously, results from this first single-centre study, comparing the three conditions within the same centre, confirm the significant microbiological difference between PBB and bronchiectasis, and CF in the very young child (<6 years).

We defined lower airway infection as a positive culture at a density ≥10^4 cfu/ml (18), and although the most robust cut-off is unknown, this is in accordance with the original diagnostic criteria of PBB (18). In accordance with previous studies from this centre (12,102), we considered *S. pneumoniae*, β-hemolytic streptococci, haemophilus species, *M. catarrhalis*, *S. aureus*, Enterobacteriaceae, *P. aeruginosa* and *S. Maltophilia* as lower airway pathogens. Children with PBB had, in accordance with previous studies (27,29), high (>80%) lower airway infection rates. The bronchiectasis-cohort described in this study had prevalence rates similar to those of the PBB-cohort, contrasting the lower rates found by, in age and threshold comparable, prospective studies (104,106,131,132). Children with CF had higher infection rates compared to other prospective studies using the same threshold (108,118,128), nevertheless were rates still significantly lower than rates within our PBB- and bronchiectasis-cohorts. The higher prevalence rates in the bronchiectasis- and CF-cohort, compared to other studies, might be explained by the retrospective design of the present study, especially for CF, since in those studies results from an annual BAL programme were included. Hence, our results are indicative
of lower airway microbiology and infection rates for children with CSLD who undergo a bronchoscopy for clinical indications and hence reflect diseased state. The exact mechanisms explaining the occurrence of differences in microbiological patterns between the various diagnoses of CSLD are laying beyond the scope of this study, due to its design no causative conclusions can be drawn. Prospective, well-designed studies are needed to clarify underlying mechanisms.

**Airway Cellularity Outcomes**

Pulmonary bacterial infection is known to initiate an inflammatory reaction characterised by neutrophil migration into the airways (133), which plays a key role in the ‘vicious cycle’ hypothesis of bronchiectasis (31). In the present study, as described before (22,31,60,102), neutrophilia (>6.5% neutrophils) was observed in the vast majority of children with various forms of CSLD. In accordance with previous studies, children with bronchiectasis had significantly decreased neutrophil percentages compared to CF (103,107) and PBB (98). Airway eosinophilia (>1% eosinophils), frequently (34%) reported in a prospective study on bronchoscopies in Australian indigenous children with bronchiectasis (104), was hardly observed amongst all cohorts. Since the spectrum between PBB and bronchiectasis reflects lung disease severity (18), one would not expect relatively decreased neutrophil percentages in children with bronchiectasis, and one may assume this could be related to such clinical measures. Despite performed regression analyses, no explanation for the differences in neutrophil percentages between the various cohorts could be revealed. Studies assessing clinical measures, such as cough on day of bronchoscopy, timing of the bronchoscopy and current antibiotic treatment, are necessary to explain this further.

Previously literature has suggested a relationship between higher neutrophil percentages and younger age (134), but this was not present in our cohort. In contrast, in our study, higher neutrophil percentages, in CF cohort only, were weakly associated with older age. Neutrophil percentages in airway fluid appeared to be mainly determined by the presence of pathogenic bacteria, and showed association with the presence of EBV in bronchiectasis and adenovirus in CF. Association between pathogenic respiratory viruses and increased inflammatory markers in PBB (29,122), bronchiectasis (135) and CF (107,112), has been described before. The previously proposed synergistic role of bacterial-viral co-infection by *H. influenzae* and adenovirus in initiating or exacerbating CSLD (122) was not reflected in the results (i.e. increased inflammation) of the present study. However, we found an association between EBV infection in bronchiectasis and increased neutrophil percentages, a particular new finding. Similarly, the high prevalence of CMV in PBB and bronchiectasis is a novel and unexpected finding and further research is necessary to determine its clinical significance. The pathogenic role of these viruses, alone or in the proposed synergistic role, in CSLD is poorly understood and remains therefore an emerging area for further research.

**Aberrant Macroscopic Outcomes**

In all cohorts, the prevalence of large airway malacia in children with CSLD who underwent a bronchoscopy exceeded the reported prevalence of 1 per 2100 newborns reported in general pediatric population (75). Airway malacia prevalence rates (PBB: 71%; bronchiectasis: 47%; CF 13%) were in concordance with previous studies on PBB (28,91), bronchiectasis (93) and CF (94). Our results reflect the first examination on the prevalence rates of malacia amongst children with various forms of CSLD within one centre, revealing that malacia is significantly more common in children with PBB compared to children with bronchiectasis, and more common in both cohorts compared to CF. In support of the results found by Wang and colleagues (99), no differences in airway infection rates and airway neutrophil percentages between children with and without malacia were observed. These findings are in contrast to a previous proposed hypothesis that intense airway inflammation leads to secondary development
of airway malacia (89,136), especially given airway inflammation was more severe in children with CF in our cohort, who had lower rates of malacia. Airway malacia and airway inflammation, seen the high rates in CSLD, are undoubtedly associated conditions, but further research is needed to determine any causative relations.

**Limitations of the Present Study**

The major limitation of the present study comes with its retrospective design, impairing the ability to assess current disease severity at the time of bronchoscopy. Clinical parameters, such as information on the presence of respiratory symptoms and their duration, the frequency and severity of exacerbations, pulmonary function and previous and current antibiotic use were not assessed, limiting the ability to draw clear conclusions on the clinical significance of our findings. Nevertheless, we reported clinically relevant differences between children with various forms of CSLD that have not been described before. Further prospective research into the bronchoscopic findings amongst children with CSLD is needed to verify the results of this study and to determine clinical implications.

Another important limitation was the lack of an appropriate healthy control group. Within this study, results were only compared between various forms of CSLD, whereas comparing bronchoscopic findings with healthy controls would help to assess clinical significant differences. However, since performing bronchoscopy including lavage in healthy children is unfeasible due to ethical issues, data from healthy children is not available in this retrospective study.

A further limitation may be airway sampling as, in accordance with the ERS guidelines (95), in our centre the most affected lobe or lobes are sampled, or the right middle lobe in generalised disease. Gilchrist and colleagues concluded that pathogens present elsewhere in the airways might not be identified, consequently underestimating the lower airway infection prevalence rate (137).

Lastly, PCR to detect rhinovirus, a common upper airway pathogen, is not regularly performed in this centre, while rhinovirus is known to have a high prevalence amongst children with CSLD (29,104,107,122). Therefore, it is possible that the prevalence of viral infection and thereby the prevalence of bacterial-viral co-infection is underestimated in the present study.

**Conclusion**

In conclusion, results from this first single-centre study comparing and contrasting the bronchoscopic findings across children with CSLD revealed that a distinct pattern of airway infection by various microorganisms between PBB, bronchiectasis and CF already exists during early childhood (<6 years). Additionally, increased neutrophilic response in children with CF compared to children with PBB and bronchiectasis was shown. Some novel findings include the prevalence of viral pathogens EBV and CMV and consequent inflammatory responses. Pathogenic bacterial presence was, amongst all cohorts, a significant predictor of neutrophilic inflammation, thereby implying the importance of adequate treatment strategies to decrease ongoing airway injury. Interestingly, lower airway inflammation in children with CSLD was not affected by the presence of airway malacia. Overall, results from this study provide valuable reference data regarding pulmonary microbiological and cellular outcomes in young, often non-expectorating, children with CSLD. Large, well-designed prospective studies to verify our results and determine the clinical significance of novel findings from this retrospective study are needed.
Bibliography


Dutch Summary / Samenvatting

Achtergrond
De overkoepelende term chronische suppuratieve longziekten (CSLD) omvat een breed scala aan longziekten, inclusief persisterende bacteriële bronchitis (PBB), bronchiëctasieën en cystic fibrosis (CF). Diagnostische flexibele bronchoscopie met bronchoalevolaire lavage (BAL) wordt vaak toegepast in kinderen met CSLD om pulmonaire infectie en inflammatie vast te stellen. Tot op heden zijn er geen studies gedaan waarin de uitkomsten van bronchoscopie en BAL tussen de drie eerdergenoemde vormen van CSLD in kinderen zijn vergeleken. Het doel van de huidige studie is dan ook om de bronchoscopische bevindingen in kinderen met CSLD, specifiek PBB, bronchiëctasieën en CF, binnen één derdelijns kinderziekenhuis te vergelijken.

Methode
Alle verslagen van bronchoscopieën uitgevoerd tussen maart 2010 en november 2016 in het Royal Children’s Hospital (tegenwoordig Lady Cilento Children’s Hospital) in Brisbane, Australië, werden retrospectief onderzocht. Bronchoscopie gegevens, inclusief BAL-resultaten, van kinderen (<18 jaar, post-hoc gereduceerd tot <6 jaar) die voldeden aan a-priori opgestelde definities van PBB (n=126), bronchiëctasieën (n=138) en CF (n=71) werden verzameld, alsmede ook radiologische beelden en verslagen om de aanwezigheid van bronchiëctasieën te bepalen. Primaire uitkomstmaat was de samenstelling van de pulmonaire microbiologie in de drie cohorten, gemeten als de proportie kinderen binnen een cohort waarin specifieke pathogene micro-organismen in de luchtwegen aanwezig waren. Secundaire uitkomstmaten betroffen pulmonaire cytologie en afwijkende macroscopische bevindingen.

Resultaten
Bronchoscopie gegevens van 335 kinderen (mediane leeftijd: 25 maanden; interkwartielafstand: 15-42 maanden) werden vastgelegd. Kinderen met PBB (87%) en bronchiëctasieën (80%) hadden significant vaker bacteriële lagere luchtweginfecties dan kinderen met CF (62%) (P < 0,0001). Kinderen met PBB en bronchiëctasieën hadden voornamelijk lagere luchtweginfecties met Haemophilus influenzae, Moraxella catarrhalis en Streptococcus pneumoniae, terwijl in kinderen met CF Pseudomonas aeruginosa en Staphylococcus aureus vaker luchtweginfecties veroorzaakten. Pulmonaire neutrofiele leukocytose was uitgesprokener in kinderen met CF (P < 0,0001) en werd in alle diagnostische cohorten voornamelijk bepaald door de aanwezigheid van pathogene bacteriën in de luchtwegen. Luchtwegmalacie bleek zowel de pulmonaire infectie prevalentie, als de pulmonaire inflammatie parameters niet significant te beïnvloeden. De hoge prevalenties van luchtweginfecties door cytomegalovirus (±26%) en Epstein-Barrvirus (±18%) in kinderen met PBB en bronchiëctasieën zijn niet eerder beschreven.

Conclusie
Deze studie, het eerste single-centre onderzoek naar bronchoscopie resultaten in kinderen met PBB, bronchiëctasieën en CF laat zien dat, al op jonge leeftijd (<6 jaar), een verschillend patroon qua respiratoire pathogene micro-organismen bestaat. Luchtwegen van jongen kinderen met CSLD zijn frequent geïnfecteerd met micro-organismen die geassocieerd zijn met het onderliggende lijden. De gevonden verschillen in luchtweg microbiologie in de vroege kinderleeftijd, en de geobserveerde associatie tussen de bacteriële infectie en neutrofiele luchtwegontsteking kunnen bijdragen aan adequate behandelstrategieën voor jonge kinderen die geen sputum opgeven.
**Appendix**

- **Figure S1:** Histograms illustrating data distribution for the age at bronchoscopy. Log- or ln- transformation did not realise normal distribution, Kolmogorov-Smirnov test (0.114, \(P < 0.0001\)) and Shapiro-Wilk test (0.936, \(P < 0.0001\)) for all cohorts combined were both significant. BE, bronchiectasis; mo, months.

- **Figure S2:** Venn diagrams illustrating number of children with specific airway pathogen present. Numbers in the overlapping portions of the Venn diagram indicate the amount of children with both or all conditions present. Percentages adjusted to the number of BAL results tested for a specific outcome. Circle sizes are not corresponding with relative proportions of microorganisms present. BE, bronchiectasis.
Figure S3: Scatter plot including univariate regression lines illustrating association between age at the time of bronchoscopy and neutrophil percentage in BAL fluid per cohort. *P*-value test whether regression model is better predictor than mean neutrophil percentage of the corresponding cohort, *P*-values <0.05 denote statistical significance and are noted in bold. BE, bronchiectasis; mo, months.

Figure S4: Boxplot illustrating median and IQR of neutrophil percentage in BAL fluid for children with and without malacia within diagnostic cohorts (PBB and BE) and for both cohorts combined. *P*-value tests whether all the subcohorts have the same median. *P*-values <0.05 denote statistical significance and are noted in bold. BE, bronchiectasis.
Table S1: Comparison of groups with and without malacia (all cohorts including children with CF included); characteristics, BAL cellularity data and microbiological data.

<table>
<thead>
<tr>
<th></th>
<th>Malacia present*, n = 160, n (%)</th>
<th>Malacia absent, n = 170, n (%)</th>
<th>P-valueb</th>
</tr>
</thead>
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<tr>
<td>Sex, M:F (% male)</td>
<td>98:62 (61)</td>
<td>108:62 (64)</td>
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</tr>
<tr>
<td>Median age, mo (IQR)</td>
<td>20 (13, 34)</td>
<td>34 (18, 46)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Bronchiectasis (radiologically proven)</td>
<td>66 (71) (n = 93)</td>
<td>95 (72) (n = 132)</td>
<td>0.989</td>
</tr>
<tr>
<td>Stridor/noisy breathing an indication</td>
<td>25 (16)</td>
<td>10 (6)</td>
<td>0.007</td>
</tr>
<tr>
<td>Laryngomalacia present</td>
<td>15 (9)</td>
<td>5 (3)</td>
<td>0.027</td>
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<th>Malacia present*, n = 170, n (%)</th>
<th>Malacia absent, n = 170, n (%)</th>
<th>P-valueb</th>
</tr>
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<tr>
<td>Total cell count (x10⁶/L)</td>
<td>242 (124, 468)</td>
<td>340 (151, 660)</td>
<td>0.039</td>
</tr>
<tr>
<td>Macrophage %</td>
<td>56 (30, 76)</td>
<td>42 (16, 66)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>8 (5, 14)</td>
<td>6 (3, 15)</td>
<td>0.092</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>30 (11, 56)</td>
<td>43 (14, 77)</td>
<td>0.004</td>
</tr>
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</table>

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<th>Malacia absent, n = 156, n (%)</th>
<th>P-valueb</th>
</tr>
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<td>No significant infection</td>
<td>16 (10)</td>
<td>18 (12)</td>
<td>0.871</td>
</tr>
<tr>
<td>Pathogenic bacterial infectiond</td>
<td>130 (84)</td>
<td>120 (77)</td>
<td>0.161</td>
</tr>
<tr>
<td>Any viral infection</td>
<td>70 (45) (n = 155)</td>
<td>51 (33) (n = 154)</td>
<td><strong>0.040</strong></td>
</tr>
<tr>
<td>Bacterial-viral co-infection</td>
<td>64 (41) (n = 155)</td>
<td>42 (27) (n = 154)</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>Any fungal growth</td>
<td>43 (36) (n = 121)</td>
<td>56 (40) (n = 140)</td>
<td>0.540</td>
</tr>
</tbody>
</table>

Mo, months; IQR, inter-quartile range. Medians, IQR and percentages rounded to the nearest whole number. Number of samples tested for a specific outcome noted in heading or behind result if distinct.

*Includes children with tracheo-, broncho- or tracheobronchomalacia; for analyses combined as one group.

bP-value tests whether outcomes of the malacia group differ from the non-malacia group. P-values <0.05 denote statistical significance, noted in bold.

cData presented as medians (IQR). Only BAL results with full cellularity and differential data included in analyses.

dSignificant pathogenic bacterial growth defined as growth ≥10⁶ cfu/ml (or ≥10⁵ cfu/ml for P. aeruginosa and/or S. maltophilia).