Lessons from the lower airway microbiome in early CF

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Lung disease in cystic fibrosis (CF) is characterised by chronic airway infection and inflammation.[1] Advances in sequencing technology have expanded our understanding of airway infection in CF, with complex polymicrobial bacterial communities frequently detected along with typical CF bacterial pathogens such as *Pseudomonas aeruginosa*.[2] Investigations of CF airway microbiota rely on interrogation of airway samples, most commonly expectorated sputum. Young children with CF rarely expectorate sputum spontaneously, leaving clinicians with the option of oropharyngeal swabs which may not reflect lower airway bacteriology or bronchoalveolar lavage fluid (BALF) collection which requires an invasive procedure with anaesthesia.[3]

Microbial diversity, a measure that takes into account the number of different bacterial taxa and the relative amount of each taxa detected within a community, has been associated with disease status in CF with lower diversity associated with lower lung function, the presence of pathogenic bacteria and increased airway inflammation.[4–6] Microbial analyses of CF samples from younger, healthier patients often show highly diverse bacterial communities,[7] whereas lung explants from patients with end-stage lung disease reveal extremely low diversity with typically only one or two pathogenic bacteria detectable.[8] The transition from a diverse community to one dominated by CF pathogens likely begins early, but difficulty with airway sampling hinders our ability to study changes in young children.

Lung disease begins early in CF with airway infection, inflammation and bronchiectasis, evident as early as 3 months.[9] Airway inflammation is tightly linked to development of bronchiectasis, as the presence of BALF neutrophil elastase (NE) in early infancy is strongly associated with bronchiectasis in young children. Airway inflammation is also increased in the presence of known CF pathogens such as *P. aeruginosa* and *Staphylococcus aureus*, with less inflammation seen with bacteria detected by molecular approaches but not on conventional routine culture.[6 10 11] The relationship between microbiota and inflammation in early lung disease is not well understood however.

In *Thorax*, Frayman and colleagues present results from a study of lower airway microbiota detected from stored BALF samples collected during a single-centre longitudinal study of young children with CF performed almost two decades ago.[12] Bacterial communities were analysed using 16S rRNA gene sequencing, a technique not available at the time of the study. Inflammatory markers and standard microbiology results measured at the time of the study and lung function at age 6 years were used to determine the association between early lower airway microbiota, inflammation and pulmonary function. Infants were enrolled in the study at the time of a CF diagnosis via newborn screening or after presenting with meconium ileus in the neonatal period. Bronchoscopy with bronchoalveolar lavage (BAL) was performed at enrolment, annually and at the time of hospitalisation for a pulmonary exacerbation. BALF samples with sufficient bacterial load for PCR amplification (n=95) were obtained from 48 subjects, including longitudinal samples from 27 subjects. Inflammatory markers, interleukin (IL)-8 and NE were measured from BALF. Clinical data including respiratory symptoms and antibiotic use were captured at quarterly clinic visits and at the time bronchoscopies were performed. The best FEV_1 and FVC at age 6 years were captured from the medical record.

Several key findings related to lower airway microbiota in young children with CF are highlighted in this study. Similar to what has been seen in older CF cohorts, microbial diversity decreased with age in those children with serial samples, even in the absence of respiratory symptoms or antibiotic use. Dominance (defined as ≥60% of all sequences within the community) of a CF pathogen-associated genera *Pseudomonas*, *Staphylococcus*, *Burkholderia*, *Haemophilus* or *Stenotrophomonas* was associated with increased airway inflammation. Conversely, *Streptococcus*-dominant samples (n=6) had inflammatory profiles similar to samples without a dominant genus. Lower bacterial diversity was associated with increased NE, although an association with IL-8 was not seen. Consistent with other studies, increased lower airway inflammation was observed in children with active respiratory symptoms at the time of bronchoscopy compared with those who were asymptomatic. Importantly, despite the increased inflammation in those children with dominant CF genera, the authors did not find an association between microbial diversity in early childhood and lung function at age 6 years.

Two prior publications reported microbiota results from lower airway samples from infants and young children with CF. Laguna and colleagues examined 12 BALF samples from eight asymptomatic infants with CF.[13] 16S rRNA gene sequencing detected 223 taxa across all samples, with *Streptococcus*, *Burkholderia*, *Prevotella*, *Haemophilus*, *Porphyromonas* and *Veillonella* most commonly detected. *P. aeruginosa* was cultured from only one BALF sample. In this cohort, diversity, bacterial load and the presence of *Burkholderia* were not associated with neutrophilic inflammation, while increased abundance of *Streptococcus* was correlated with higher infant lung function. Renwick *et al*[14] examined 13 BALF samples from children with CF (ages 1–8 years) and compared results with oropharyngeal swabs in CF and BALF from children without CF. They found that CF airway samples were significantly less diverse than control samples, with distinct taxa detected from each group, suggesting that disruption of bacterial communities occurs at a young age in CF. The detection of *P. aeruginosa* by culture was associated with lower diversity. Although small numbers, these studies suggested that early airway microbiota differs from that detected during later disease and from healthy children.

As the largest longitudinal study of BALF microbiota in infants and young children with CF, the study from Frayman and colleagues represents an important contribution to the existing knowledge of early airway infection. However, there are several limitations. BALF samples were collected and stored for almost two decades prior to sequencing which may have altered sequencing results. An additional 53 BALF samples collected during the study were not sequenced due to failed PCR amplification, possibly due to low bacterial load or degradation with

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time. It is reassuring that the molecular evaluation of the samples with successful sequencing matched well with standard culture results, suggesting that although the BAL samples were previously obtained and banked, the results are reasonably representative of the airway microbiology. Given that lower airway samples are difficult to obtain in young children, the use of banked specimens offers a key opportunity for future studies. In addition, CF care has changed significantly since the original study, and therefore findings may not be representative of current birth cohorts. Changes in CF care that could directly impact microbial diversity include the availability of inhaled antibiotics for infection with *P. aeruginosa*, the increased use of mucolytics (dornase alfa, hypertonic saline), and as cystic fibrosis transmembrane conductance regulator modulators become increasingly available to younger children, bacterial diversity may be impacted, as changes in standard microbiology cultures have been seen with their use.[15]

Children participating in this study were not routinely prescribed prophylactic antibiotics. However, approximately half of the children were receiving antibiotic therapy and had respiratory symptoms at the time of their BAL. The frequent antibiotic use may have affected the microbiota results and highlights the extensive use of antibiotics in young children with CF, particularly in the setting of respiratory illnesses, and the difficulty in being able to distinguish lower airway microbiota changes due to disease process versus antibiotic pressure. Although there was no association between microbial diversity and antibiotic use, there was reduced diversity seen in the BAL from symptomatic children. Notably, bacterial communities fluctuated with return of diversity in children with previously detected dominant CF genera.

Microbial diversity was not associated with lung function at age 6 years in contrast to findings in older children and adults.[4–7] Although FEV_1 was not associated with diversity, FEV_1 may not be sensitive to early airway injury in children.[16] Recent studies have demonstrated that lung clearance index (LCI) may be a more sensitive marker of early lung disease in CF, as individuals with normal FEV_1 values can have elevated (abnormal) LCI values.[17] As the authors note, normal spirometry does not exclude the possibility of bronchiectasis, and therefore the use of CT scans to determine the degree of bronchiectasis may have been a more sensitive marker for underlying lung disease.

Despite limitations, the study by Frayman and colleagues provides the broadest view to date of the lower airway microbiota in young children with CF. The association between a dominant CF pathogen and increased inflammation suggests that emergence of a CF pathogen within the community is associated with disease progression. One hopeful aspect of this study was that the bacterial communities appeared to fluctuate with increases in diversity seen in specific individuals, suggesting the possibility that an initial loss of diversity is not irreversible. This has also been seen in adults with CF in whom bacterial communities appear to regain diversity following treatment of pulmonary exacerbations.[18] Much remains to be learned about the early CF microbiota. Future studies are clearly needed to examine longitudinal changes and the impact of antibiotics. In addition, recent publications suggest that the gastrointestinal microbiota may also be important in CF, with influences on nutritional status, systemic inflammation and airway pathogen acquisition.[19 20] Understanding the impact of antibiotics, novel therapies, environmental exposures and nutrition on CF microbiota may lead to improved clinical outcomes. Although this study adds to our understanding of the lower airway microbiota, many questions remain, and further studies in young children with CF are needed to form a more comprehensive understanding of its impact on later structural lung disease and disease progression.

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