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Original Article

The fungal diversity in the lungs of children with cystic fibrosis captured by sputum-induction and bronchoalveolar lavage

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ABSTRACT

Background: The prevalence of fungi in cystic fibrosis (CF) lung infections is poorly understood and studies have focused on adult patients. We investigated the fungal diversity in children with CF using bronchoalveolar lavage (BAL) and induced sputum (IS) samples to capture multiple lung niches.
Methods: Sequencing of the fungal ITS2 region and molecular mycobiota diversity analysis was performed on 25 matched sets of BAL-IS samples from 23 children collected as part of the CF-SpIT study (UKCRN14615; ISRCTNR12473810).
Results: Aspergillus and *Candida* were detected in all samples and were the most abundant and prevalent genera, followed by *Dipodascus, Lecanicillium* and *Simplicillium.* The presumptive CF pathogens *Exophiala, Lomentospora* and *Scedosporium* were identified at variable abundances in 100 %, 64 %, and 24 % of sample sets, respectively. Fungal pathogens observed at high relative abundance (≥40 %) were not accurately diagnosed by routine culture microbiology in over 50 % of the cohort. The fungal communities captured by BAL and IS samples were similar in diversity and composition, with exception to *C. albicans* being significantly increased in IS samples. The respiratory mycobiota varied greatly between individuals, with only 13 of 25 sample sets containing a dominant fungal taxon. In 11/25 BAL sample sets, airway compartmentalisation was observed with diverse mycobiota detected from different lobes of the lung.
Conclusions: The paediatric mycobiota is diverse, complex and inadequately diagnosed by conventional microbiology. Overlapping fungal communities were identified in BAL and IS samples, showing that IS can capture fungal genera associated with the lower airway. Compartmentalisation of the lower airway presents difficulties for consistent mycobiota sampling.

1. Introduction

The prevalence, diversity and clinical epidemiology of fungi in CF lung infections is poorly understood. Historically, fungi have often been disregarded when detected by culture as either contaminants or harmless colonisers of the oral cavity and respiratory tract [1]. In addition, conventional sampling may underestimate the fungal burden in CF, since fungal culture can be highly variable and incapable of detecting the full spectrum of fungal diversity [1]. Mycobiota studies, based on culture-independent analysis of the fungal taxa present, are emerging [2-5] but lag behind bacterial diversity studies.

The majority of fungal lung infection research in CF has focused on adults and we currently know very little about the fungi in the airways of children with CF, with the exceptions that *Aspergillus* and *Candida* abundance may be higher than in healthy individuals [4] and fungal colonisation increases with age [4,6]. Long-term population data for fungal infection in people with cystic fibrosis (pwCF) is limited. The UK CF Registry for example has only been tracking the rate of *Aspergillus*

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lung infection since 2017. In 2022, *Aspergillus* prevalence in the UK CF population was 4.2 % in children age <16 years and 6.4 % in adults; no other fungal pathogens are currently reported in the Registry [7].

In this study we apply molecular mycobiota analysis to respiratory samples we collected in the Cystic Fibrosis Sputum Induction Trial (CF-SpIT) [8,9] to explore the fungal diversity in children with CF. By using both bronchoalveolar lavage (BAL) and induced sputum (IS) samples, we capture and describe fungal communities in the upper and lower airways.

2. Methods

2.1. Study design and participants

CF-SpIT is a prospective internally-controlled interventional trial (UKCRN14615; ISRCTNR12473810) performed at the Children's Hospital for Wales, Cardiff, UK, in children with CF aged between 6 months and 18 years. The median age was 8.5 years (interquartile range: 6.5–12.6). None of the children were receiving antifungal therapy at the time of samplings. Samples from sputum induction and single-lobe, two-lobe, and six-lobe BAL were matched for within-patient comparison, with the aim of testing IS as an infection diagnostic. Results from CF-SpIT using conventional microbiology and microbiota analysis for bacterial diversity are published elsewhere [8,9]. CF-SpIT is subject to Institutional Review by the Cardiff and Vale Research Review Service (CaRRS; Project-ID-11-RPM-5216) and approved by the South Wales Research Ethics Committee (11/WA/0334).

2.2. Respiratory sample collection, microbiological culture and DNA extraction

Sputum induction and BAL procedures were performed as previously described [8]. BAL was performed when clinically indicated. IS was performed within the 24 h before BAL. In strict sequential order, BAL fluid from the right middle lobe was collected and labelled as BAL1, from the left lingula as BAL2, and from the combination of right upper lobe, right lower lobe, left upper lobe and left lower lobe as BAL3. All samples were divided into two aliquots immediately after collection. One aliquot was processed for bacteria and fungi in the microbiology laboratory of the University Hospital of Wales according to the UK CF Trust Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis. The other aliquot was frozen at –80 °C within 30 min of collection for DNA extraction. Aliquots for mycobiota analysis for IS, BAL1, BAL2 and BAL3 were between 0.5–2 ml. DNA extraction was performed as described previously [9].

2.3. Quantitative PCR (qPCR)

Quantification of total fungal load was performed using a TaqMan® qPCR assay targeting the fungal 18S rRNA gene as described by Liu et al. (2012) [10]. See Supplementary Materials for further details.

2.4. ITS2 region sequencing and bioinformatics analysis using QIIME2

Twenty-five matched sets of BAL1-BAL2-BAL3-IS samples were subjected to ITS2 sequencing analysis. Amplification and sequencing of the ITS2 region was performed by Novogene, generating 250 bp paired-end reads using Illumina sequencing technology. The primers used for amplification of the ITS2 region were ITS3–2024F (5′- GCATCGATGAAGAACGCAGC-3′) and ITS4–2409R (5′- TCCTCCGCTTATTGATATGC-3′) [11]. The sequencing run contained a mock fungal community (Mycobiome Genomic DNA Mix, MSA-1010; ATCC) and 3 DNA extraction controls. Along with the qPCR data, these controls were used to confirm accurate mycobiota profiling and determine subsampling thresholds in downstream analyses (further details in Supplementary Materials).

Bioinformatic analysis was carried out using a virtual machine hosted by the Cloud Infrastructure for Microbial Bioinformatics (CLIMB) consortium [12]. Quality control and Illumina adapter trimming of the raw sequencing reads was performed using FastQC v0.11.5 and Trim Galore! v0.4.3 for paired end reads. Trimmed reads were imported into QIIME2 [13] (q2cli version 2020.11.1), the q2-ITSxpress plugin [14] used to identify and trim the ITS2 region sequences and the DADA2 plugin [15] used to group sequences into amplicon sequence variants (ASVs). Taxonomic classification of fungal ITS2 ASVs was performed using the q2-feature-classifier [16], with the classifier first being trained using the UNITE database v8.2 dynamic dataset [17]. Bioinformatics scripts are available at https://github.com/Beky-Weiser/CFSpIT-ITS2-Microbiota-Analysis.

2.5. Fungal diversity and statistical analyses

The fungal ASV table, fungal taxonomy and sample metadata files were imported into R statistical software v4.0.3 [18] using the package phyloseq v1.34.0 [19]. Low abundance ASVs representing less than 0.01 % of the total number of sequence reads were removed. Subsampling to 6000 sequence reads was performed and coverage for all 100 samples was calculated at ≥99.5 %. See Supplementary Materials for further details.

Further data handling and statistical analyses were carried out in R statistical software and Microsoft Excel. Alpha and beta diversity indices were calculated using the R package vegan (v2.6–2) [20]. Statistical differences in alpha-diversity (Shannon index) and sequence read proportions were evaluated using the Friedman test for non-parametric dependent data with patient as the blocking factor, and *post hoc* Conover pairwise comparisons with Bonferroni-adjusted P values (R package PMCMR v4.4). Beta diversity was analysed using the Bray-Curtis dissimilarity measure and non-metric multidimensional scaling (NMDS) ordination performed to visualise sample distributions in two dimensions. Differences between sample groups were analysed using Permutational ANOVA (PERMANOVA; adonis function in vegan package). Differences between genus relative abundance in sample types was determined by GAMLESS-BEZI as previously described [21]. Indicator species analysis was performed using the R-package indicspecies v1.7.12 [22] to identify ASVs that were significantly associated with sample types. The relationship between Shannon diversity and age was investigated using linear regression and linear mixed models with patient as the random effect (R package nlme). Further details and R code are available at https://github.com/Beky-Weiser/CFSpIT-ITS2-Microbiota-Analysis.

3. Results

3.1. Patient sample sets and mycobiota analysis overview

Mycobiota profiles were obtained for 25 matched BAL1-BAL2-BAL3-IS sets from 23 children with CF aged between 1.1 and 17.7 years. Overall, 668 unique ASVs were identified across the 100 samples, of which 442 could be classified at genus level (130 unique genera) and 321 could be classified at species level (159 unique species). Strikingly, 143 ASVs could not be classified beyond 'kingdom Fungi' and remained unidentified. Detailed sequencing statistics are given in Supplementary Materials.

In relation to CF fungal genera of particular interest, at a presence/absence level mycobiota analysis detected *Aspergillus* and *Candida* in 100 % of sample sets and the other presumptive pathogens *Exophiala, Lomentospora* and *Scedosporium* in 100 %, 64 %, and 24 % of sample sets, respectively (See Supplementary Materials Excel spreadsheet for the ASV table).

3.2. Mycobiota analysis was superior to culture for fungal detection and identification

Culture data was available for all 100 samples belonging to the 25 matched BAL1-BAL2-BAL3-IS sets. Although mycobiota analysis identified the presence of fungi in 100 % of samples, routine culture only identified fungi in 20 % (Supplementary Table S1) and fungal growth was indicated broadly as "*Candida*", "*Aspergillus*", "yeast-like growth" or "fungal mycelial-like growth". In addition, culture-based diagnostics failed to identify fungal genera that were at high relative abundance (≥40 %) in mycobiota analysis as follows: *Candida* was identified between 43.5 % and 98.2 % relative abundance in 10 individuals, but only 5 were reported as culture positive; two individuals had 83.4 % and 96.6 % relative abundance of *Aspergillus,* with one case correctly identified, but the other recorded as *Candida* culture positive; and *Exophiala* was seen at 62.9 % and 80.1 % relative abundance in two individuals, and in both instances *Aspergillus/Candida* was reported from culture (Table S1).

3.3. Fungal communities in BAL and IS samples were similar in diversity and composition

The alpha diversity (within-sample diversity) was similar across all sample types (Fig. 1, panel A and B) and BAL1, BAL2, BAL3 and IS samples also overlapped in their fungal community composition (beta diversity; Fig. 1, panel C). The top 25 most abundant ASVs across the dataset represented a similar proportion of total sequence reads for BAL1, BAL2, BAL3 and IS (Fig. 1, panel D) and these proportions were not significantly different.

3.4. Key differences between BAL and IS samples were linked to Candida species

In ranked order, the most abundant and prevalent fungal genera identified across the entire dataset were *Candida, Aspergillus, Dipodascus, Lecanicillium* and *Simplicillium*, and this trend was largely mirrored when sample types were considered separately (Table 1). Notably, *Candida, Aspergillus* and *Simplicillium* were identified in 100 % of samples although their relative abundances were variable (<1 % to >99 %; Supplementary Table S2). *Exophiala, Lomentospora* and *Scedosporium* were also detected in BAL and IS samples, ranking 7th (83 % prevalence), 17th (41 % prevalence) and 89th (11 % prevalence) most abundant across all samples, respectively. The relative abundances of *Aspergillus, Dipodascus, Lecanicillium, Simplicillium, Exophiala, Lomentospora* and *Scedosporium* were not significantly different between the

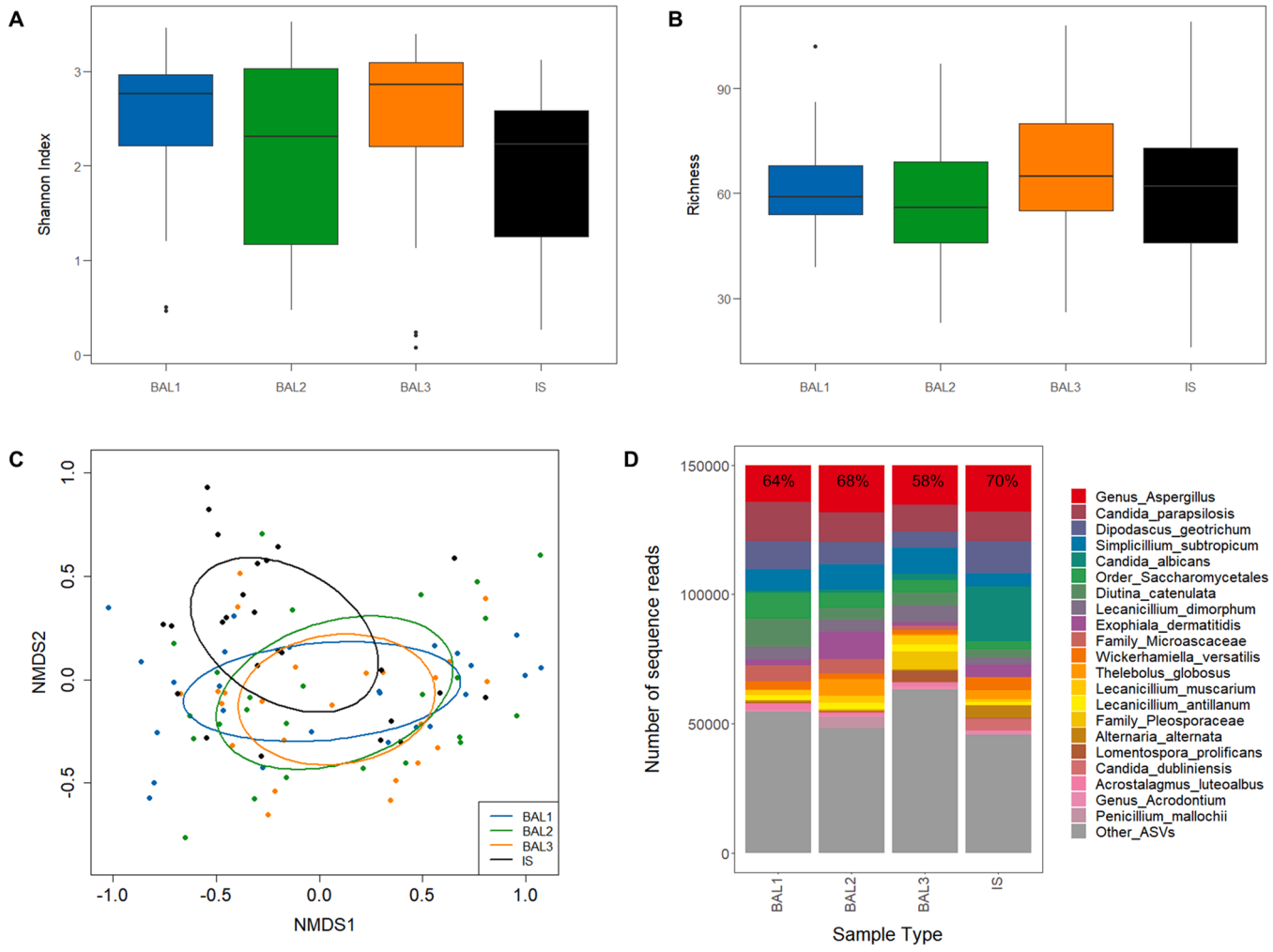

Fig. 1. Fungal communities in BAL and IS samples were similar in diversity and composition. Boxplots (A, B) indicate the spread of the alpha diversity values across sample types. No significant differences were found for the Shannon Index (A) or ASV richness (B). NMDS ordination of Bray-Curtis dissimilarity distances (C) indicated that BAL samples clustered together and IS samples overlapped in composition with BAL samples; there were no significant differences between the groupings. The top 25 most abundant ASVs represented a similar proportion of the total sequence reads in BAL1 (64 %), BAL2 (68 %), BAL3 (58 %) and IS (70 %) samples (D). In (D) the top 25 most abundant ASVs have been consolidated to lowest taxonomic rank and their cumulative percentage indicated at the top of the stacked bar charts, all other ASVs have been consolidated and are shown in grey. The taxa colour key is displayed to the right of the figure.

Table 1
The most abundant and prevalent fungal genera across the different sample types.

Rank abundance	BAL123-IS	BAL1	BAL2	BAL3	IS
1	*Candida* (100)	*Candida* (100)	*Aspergillus* (100)	*Aspergillus* (100)	*Candida* (100)
2	*Aspergillus* (100)	*Aspergillus* (100)	*Candida* (100)	*Lecanicillium* (100)	*Aspergillus* (100)
3	*Dipodascus* (88)	*Dipodascus* (84)	*Simplicillium* (100)	*Candida* (100)	*Dipodascus* (96)
4	*Lecanicillium* (98)	*Diutina* (76)	*Lecanicillium* (100)	*Simplicillium* (100)	*Lecanicillium* (92)
5	*Simplicillium* (100)	*Lecanicillium* (100)	*Exophiala* (84)	*Dipodascus* (88)	*Simplicillium* (100)

ASVs ($n = 668$) were consolidated to genus and genera ranked by total read number (abundance). Percentage prevalence (samples positive for a particular genus) is given in brackets under genus ID.

sample types, but *Candida* had significantly increased relative abundance in IS compared to BAL1 ($p = 0.03$), BAL2 ($p = 0.001$) and BAL3 ($p = 0.006$) samples (Supplementary Table S2).

At species level, the total numbers and proportions of sequence reads assigned to different species within a genus were similar, except for *Candida* (Fig. 2; Supplementary Fig. S1). Eight *Candida* species were identified across the dataset, of which *C. parapsilosis, C. albicans, C. krusei* and *C. dubliniensis* were the most abundant. The total number of reads assigned to *C. parapsilosis* were similar for BAL1, BAL2, BAL3 and IS, but reads were increased for *C. albicans* and *C. dubliniensis* in IS and for *C. krusei* in BAL1. This led to differences in *Candida* species sequence read proportions between BAL and IS samples (Fig. 2). Indicator species analysis (Supplementary Table S3) revealed significant associations between two *C. albicans* ASVs and IS samples (ASV10, $p = 0.02$; ASV14, $p = 0.03$) and one *C. krusei* ASV (ASV29, $p = 0.008$) and BAL1 samples.

3.5. There was large variation between fungal communities in samples from different individuals

A large number and variety of fungal taxa were identified across the 100 respiratory samples and each individual possessed a unique respiratory mycobiota. Examples of different profile types are given in Fig. 3 and the full set is displayed in Supplementary Fig. S2. On average, each sample comprised 62 unique ASVs (range: 16–109) and the 25 most abundant ASVs in each sample averaged a cumulative relative abundance of only 83.6 % (range: 13.0–99.7 %).

The top 25 ASVs across the dataset were consolidated to their lowest taxonomic rank for further analysis. Twenty-seven out of one hundred samples belonging to 13 sample sets had a dominant fungal taxon (defined as >50 % relative abundance) with the remaining 73/100 samples considered to have diverse mycobiota profiles. A higher percentage of IS samples (10/25; 40 %) had a dominant fungal taxon than BAL samples (17/75; 24 %). Dominant fungal taxa included *Aspergillus* (6 samples from 4 sample sets), *C. parapsilosis* (6 samples from 2 sample sets), *C. albicans* (3 samples from 3 sample sets), *E. dermatitidis* (3 samples from 2 sample sets), *Microascaceae* (2 samples from 1 sample set), *Thelebolus globosus* (2 samples from 2 sample sets) and *Pleosporaceae, Alternaria alternata, Lomentospora prolificans, C. dubliniensis* and *Penicillium mallochii* (1 sample each from 5 different sample sets) (Supplementary Fig. S2).

Increased relative abundances of *Aspergillus, Exophiala* and *C. parapsilosis* were seen in both BAL and IS samples with increasing age (Supplementary Fig. S3, Panel B). A trend for decreasing alpha diversity (Shannon diversity) with increasing age was observed for both BAL and IS samples, although this correlation was not significant in the cohort examined (Supplementary Fig. S3, Panel A).

3.6. Compartmentalisation of the lower airway mycobiota can occur

BAL1, BAL2 and BAL3 directly sample the lower respiratory tract but fungal communities were not always uniform across BAL samples from the same patient (Supplementary Fig. S2). To investigate this further, Bray-Curtis dissimilarity distances between BAL samples within- and between-patients were compared. The median values of the within-(0.76) and between-patient (0.88) comparisons were similar and the interquartile ranges overlapped (Fig. 4), indicating that within-patient comparisons could be as dissimilar as between-patient comparisons; 11/25 sample sets fell above the 25 % percentile of the between-patient comparison dataset (Fig. 4).

At a presence/absence level only 66 % (range: 20–100 %) of the top 10 and 44 % (range: 16–92 %) of the top 25 ASVs were shared across all 3 BAL samples within a set, suggesting that the lower airway mycobiota can be compartmentalised with different fungal communities present in different lobes of the lung.

4. Discussion

The current understanding of the epidemiology of fungal lung infections in adults with CF is limited, with even less known in children with CF. In this study, we used respiratory samples collected as part of the CF-SpIT trial [8,9] to characterise the fungal communities in the airways of children with CF between 1.1 and 17.7 years of age.

A diverse range of fungi were identified and there was large variation between different individuals. The fungal communities in BAL and IS samples overlapped in diversity and composition suggesting that IS is capable of capturing the upper and lower airway mycobiota. A key difference between the two sampling methods was the increased abundance of *C. albicans* in IS samples. This increased *Candida* signature may be linked to the nature of IS sampling, where samples pass through the upper respiratory tract and oral cavity before collection. *C. albicans* is commonly cultured from oropharyngeal samples and has been found in the oral mycobiota of pwCF at higher prevalence than people without CF [23]. Whether microbes such as *C. albicans* and other *Candida* spp. that traditionally inhabit the oral cavity are of clinical relevance is still under investigation [2] and they have the potential to impact the respiratory system either directly (by migration) or indirectly (through metabolite production) [24]. The identification of *C. parapsilosis* in both BAL and IS samples was particularly interesting as although this species is not commonly associated with respiratory colonisation, it has been found as the most prevalent *Candida* spp. in sputum samples from adults with CF [2] and recently identified as causing pulmonary infection that presented as lung nodules in an individual with CF [25]. It is therefore possible that *C. parapsilosis* could be an opportunistic respiratory pathogen.

Aspergillus and *Candida* species were the most prevalent fungal genera in our cohort, with both genera detected in all respiratory samples from all individuals at variable relative abundances (<1 % to >99 %), and Multiple species of each genus identified (Fig. 2). In addition, at a presence/absence level, the emerging fungal pathogens *Exophiala, Lomentospora* and *Scedosporium* were detected in 100 %, 64 % 24 % of sample sets, respectively. The molecular-detection based prevalences we observed were far higher than matched fungal culture results, and are higher than reported prevalences from other culture-based studies in both adults [6,26,27] and children with CF [6,28,29]. Culture-independent mycobiota studies in adults have shown prevalence rates are underestimated by conventional fungal culture [2] and in this study we demonstrate the same situation in children with CF.

It is important to consider whether fungi detected in CF airways are

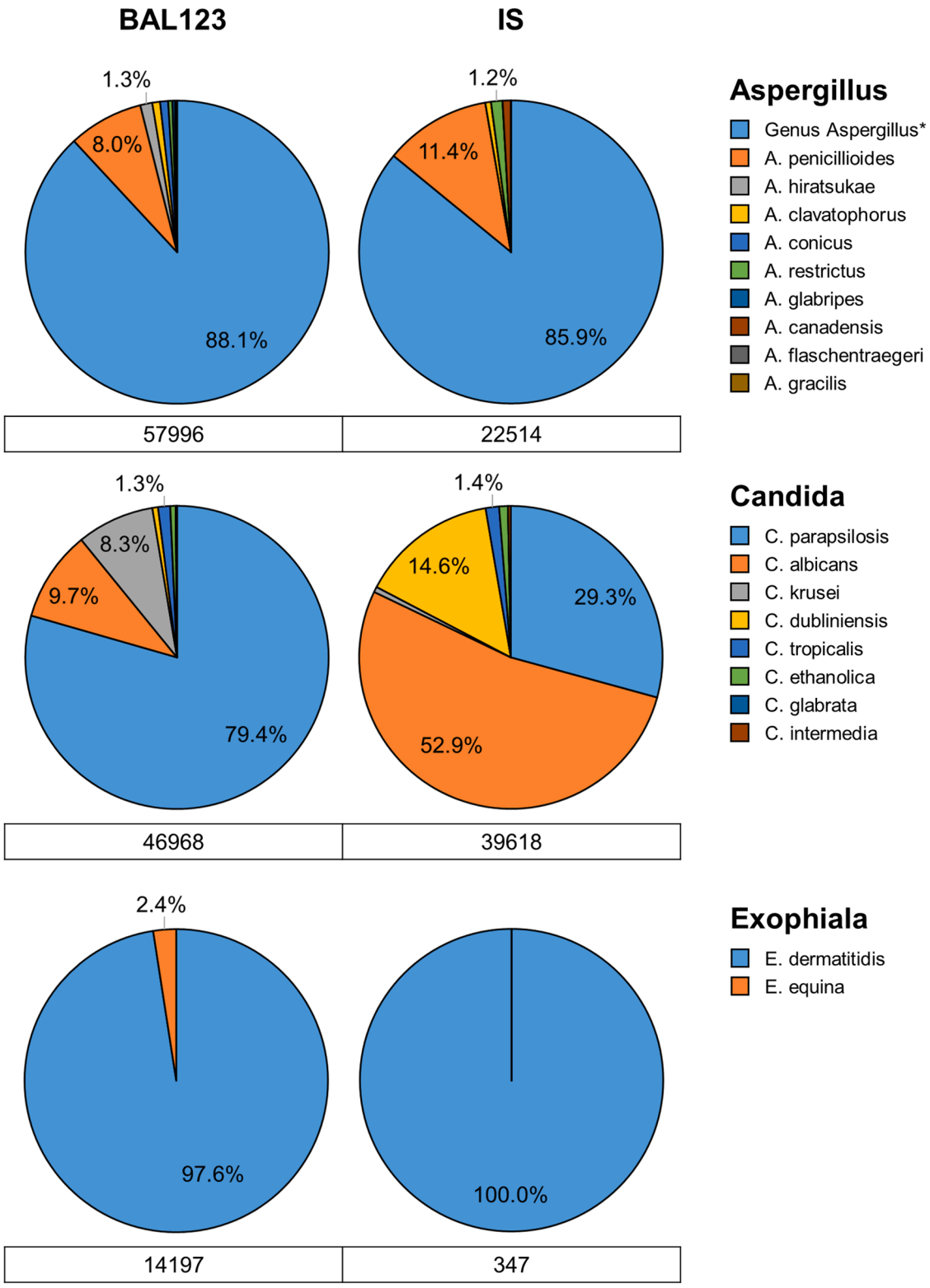

Fig. 2. Distribution of sequence reads at species level in BAL and IS samples. The two most abundant and prevalent genera (*Candida* and *Aspergillus*) and presumptive CF pathogen *Exophiala* are shown. Reads from BAL1, BAL2 and BAL3 samples are consolidated into one group as 'BAL123'. Pie charts display the proportion of sequence reads assigned to different species within a genus, proportions ≥1 % are displayed. Total sequence reads for each genus are below each pie chart. *Genus_Aspergillus comprised 21 ASVs, 4 of which (56 % of the Genus_Aspergillus sequence reads) were further classified to *A. fumigatus* and were present in 98 % of samples (see Supplementary Materials).

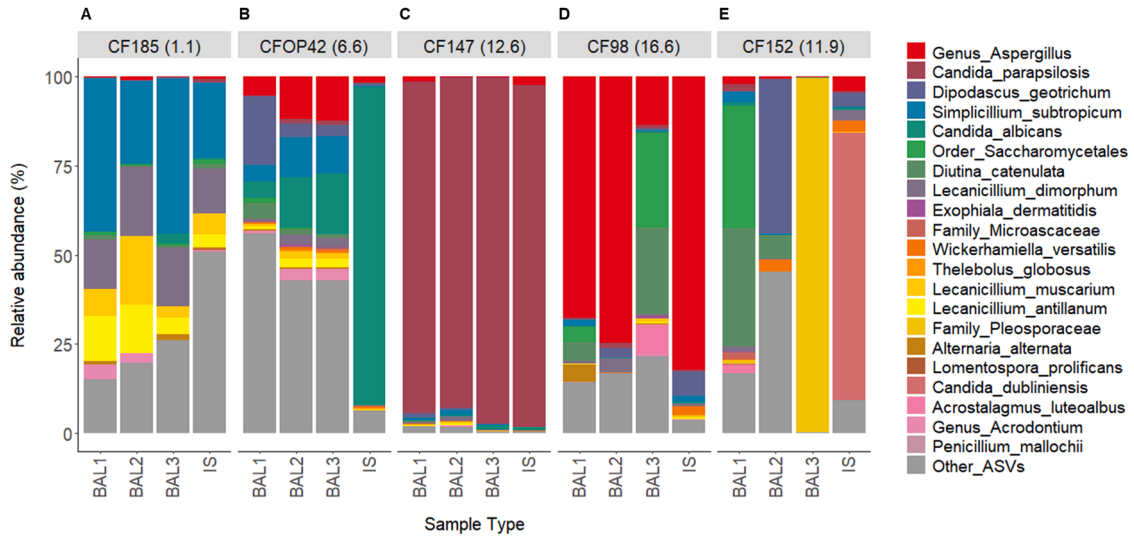

Fig. 3. BAL1, BAL2, BAL3 and IS samples sets illustrating different mycobiota profile types. Stacked bar charts of mycobiota profiles are shown for 5 sample sets to provide examples of samples with a diverse mycobiota (A – all samples, B – BAL samples), samples dominated by a single taxon (B – IS sample, C – all samples), sample sets that are largely similar (A, C), sample sets where one sample is dissimilar from the others (B – IS sample, D – BAL3 sample) and sample sets where all samples are dissimilar (E). The top 25 most abundant ASVs consolidated to lowest taxonomic rank (coloured, not grey) and all other ASVs consolidated (coloured grey) are shown. The taxa colour key is displayed to the right of the figure.

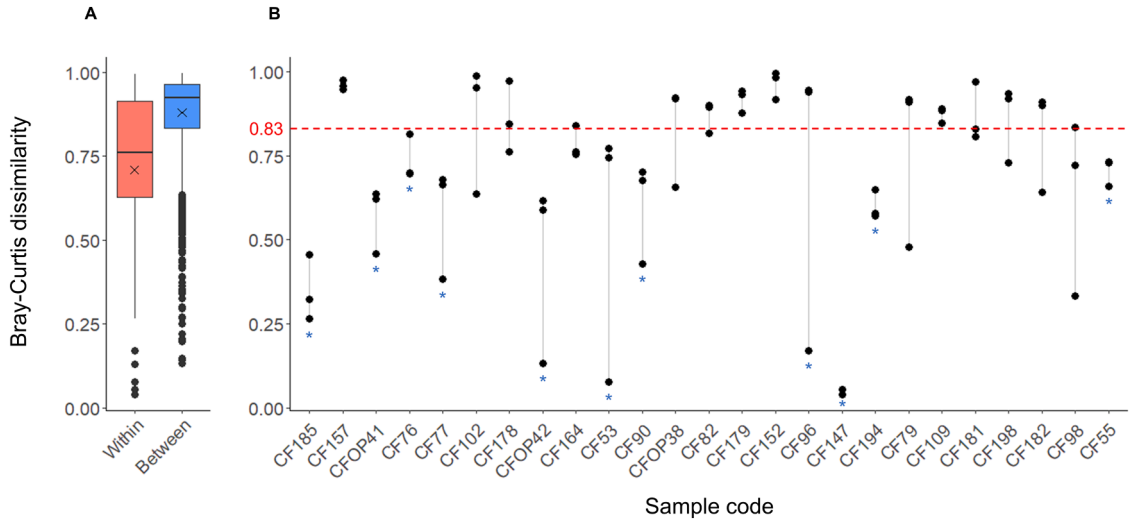

Fig. 4. Comparison of within-patient and between-patient mycobiota diversity of BAL samples. Bray-Curtis dissimilarity distances for within-patient and between-patient comparisons are shown as follows. (A) Boxplots of BAL within-patient comparisons (median = 0.76 (IQR 0.63–0.91) and between-patient comparisons (median = 0.93 (IQR 0.83–0.96). The mean values are shown as a cross inside the boxplot (within = 0.71; between = 0.88). (B) Bray-Curtis dissimilarity distances between BAL samples taken from individual patients are visualised using strip charts; three values are plotted per patient (BAL1-BAL2, BAL1-BAL3 and BAL2-BAL3 distances), points are joined to highlight the spread. The first quartile of the between-patient data (0.83) has been highlighted by a red dotted line and 14 sets of within-patient comparisons falling below this line highlighted with a blue asterisk. The remaining 11 sets are considered highly dissimilar. Sample sets are arranged in order of increasing patient age. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transient or persistent colonisers, as fungi may be regularly acquired via inhalation or potentially via seeding from the oral cavity [5]. A recent mycobiota study in CF adults with longitudinal samples suggested that fungal communities were predominately composed of transient members, although there were exceptions where fungal pathogens were repeatedly isolated from the same patient [5]. In our study, two patients provided sample sets of two separate occasions and whilst their profiles were not identical, fungal taxa were shared (Supplementary Fig. S2; CFOP38 and CF194 shared *C. parapsilosis* and *S. subtropicum*, CF96 and CF198 shared *C. parapsilosis* and *Aspergillus*).

As the remainder of our dataset was cross-sectional, we were unable to further investigate the transience of fungal taxa, but we can speculate about the implications of differences in fungal diversity. Numerous studies have linked a decrease in bacterial diversity to a decline in lung function in CF [30] and there is evidence to suggest a similar link to fungal diversity [31]. The majority of samples in our study had a diverse mycobiota but 27/100 samples belonging to 13 samples sets had a dominant fungal taxon. All 27 samples were from children over 6 years of age and there were sample sets where multiple matched samples had the same dominant fungal taxon. In these cases, the dominant fungi identified below family level were *C. parapsilosis* (CF96, CF147), *Aspergillus* (CF98) and *E. dermatitidis* (CF181), all of which have been found to dominate sputum mycobiota profiles in CF adults [2]. We propose that the discovery of these presumptive fungal pathogens at

high levels in multiple airway compartments for our paediatric CF cohort represents more than transient colonisation, and could indicate the beginning of a decline in overall fungal diversity.

As *A. fumigatus* is the most commonly detected filamentous fungal species in respiratory samples from pwCF [6], it was surprising that none of the ASVs identified as *Aspergillus* were further classified to *A. fumigatus* by the QIIME2-UNITE database pipeline. Further analysis (detailed in Supplementary materials) determined that 56 % of all reads identified as 'Genus_Aspergillus' did indeed belong to *A. fumigatus* and these reads were present in 98 % of samples, highlighting current limitations in fungal classification for mycobiota analysis. The clinical significance of the nine other *Aspergillus* species found in our dataset (Fig. 2) is unknown, as these are less commonly associated with human infection, although those belonging to the sections *Fumigati* (*A. hiratsukae*) and *Restricti* (*A. penicilloides, A. clavatophorus, A. conicus, A. restrictus, A. glabripes, A. canadensis* and *A. gracilis*) have been isolated from the respiratory tract and can be linked to respiratory problems and allergies [32,33]. In addition to fungi previously associated with CF, we discovered the predominantly environmental fungi *Simplicillium, Lecanicillium* and *Dipodascus* in 100 %, 98 % and 88 % of all samples, respectively. Although at high prevalence, these genera had an average relative abundance of <10 % and reached maximum relative abundances of 47–54 %. Human infections with these genera are rare, and the clinical impact of these genera in the CF respiratory mycobiota is unknown, but their detection herein warrants further investigation.

We previously investigated all of the samples in this study by culture-independent microbiota analysis to determine the bacterial diversity [9]. Similarly, we found that the bacterial communities captured by BAL and IS samples overlapped in composition, and that in certain patients the bacterial microbiota was compartmentalised in the lower airways [9]. Compartmentalisation has been found in adults and children with CF in relation to bacterial infection, but to our knowledge this is the first study reporting that different lobes of the CF lung can harbour different fungal communities, which could present difficulties for consistent mycobiota sampling.

An important first step in our understanding the CF lung mycobiota is identifying which fungi are present. We have shown that the airway mycobiota is diverse and complex, and that presumptive CF fungal pathogens may be detected in children, with their dominance in samples potentially increasing with age. The clinical implications of fungal detection are still not understood and would require longitudinal sampling and correlations to clinical parameters to comprehensively investigate. In addition, although it was not within the scope of this study, with both bacterial and fungal diversity datasets now available for CF-SpIT samples we can begin to investigate inter-kingdom networks in children with CF, which will ultimately broaden our ecological understanding of the CF lung mycobiota [34]. Finally, the overlapping and correlating mycobiota signals obtained from BAL and IS, also indicate that IS sampling can be taken forward as a straightforward means to capture CF fungal epidemiology in future studies.

CRediT authorship contribution statement

Rebecca Weiser: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Katherine Ronchetti:** Methodology, Investigation, Project administration. **Jo-Dee Tame:** Methodology, Investigation, Project administration. **Sven Hoehn:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation. **Tomasz P. Jurkowski:** Methodology, Software, Validation, Formal analysis, Resources, Supervision, Project administration. **Eshwar Mahenthiralingam:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Julian T. Forton:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors do not have any conflicts of interest to disclose in relation to this study.

Data availability

Raw sequence data have been submitted to the European Nucleotide Archive under project number PRJEB34389.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2024.07.011.

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