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Original Research

Impact of elexacaftor/tezacaftor/ivacaftor on the presence of bacterial and fungal pathogens in the lower respiratory tract of children with cystic fibrosis

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ABSTRACT

Background: Highly effective CFTR modulator therapy, such as elexacaftor/tezacaftor/ivacaftor (ETI), has been linked to significant clinical improvements in people with cystic fibrosis (pwCF). However, its effect on the presence of respiratory pathogens in the lower respiratory tract of younger, mainly non-expectorant children with CF remains poorly understood, especially using lower airway sampling methods. We aimed to investigate changes in lower airway microbiology and clinical outcomes in children with CF aged 6–12 years before and after starting ETI.

Methods: We conducted a prospective observational single-centre study including children with CF commencing ETI who had no prior exposure to CFTR modulators, inhaled antibiotics, or prophylactic antibiotics. Lower airway microbiology was assessed longitudinally using sputum or induced sputum (IS). Pathogen prevalence, sweat chloride concentration, pulmonary function, nutritional status, and rate of exacerbations were evaluated before and up to 12 months after ETI initiation.

Results: Sixteen pwCF were included (median age 8.4 years, 69 % boys, 75% were F508del homozygous). None of the children was expectorant at any point during the study. Following ETI initiation, we observed decreased growth of methicillin-sensitive *Staphylococcus aureus* and *Aspergillus fumigatus*, reduced variability in fungal populations, and an increase in negative bacterial and fungal culture results.

Conclusions: In this exploratory study of young, modulator-naïve children with CF, ETI initiation was associated with changes in the lower airway microbiology composition assessed by IS. These findings highlight potential shifts in lower airway microbiology and the importance of age-appropriate lower airway sampling in future paediatric studies.

1. Introduction

People with cystic fibrosis (pwCF) have entered a new era with the advent of highly effective modulator therapies (HEMTs), such as elexacaftor/tezacaftor/ivacaftor (ETI), which have significantly improved their quality of life [1]. Before the introduction of CFTR modulators, CF therapy was primarily symptomatic, focusing on mucus clearance, antibiotic treatment, and nutritional support. CFTR modulator therapy targets the cystic fibrosis transmembrane conductance regulator (CFTR) protein, enhancing its function. ETI is a triple combination of elexacaftor (ELX), tezacaftor (TEZ), and ivacaftor (IVA): ELX and TEZ improve protein folding and transport to the cell surface, while IVA binds to the CFTR channel to increase its opening probability [1].

CF is characterised by respiratory infections, which lead to bronchiectasis and progressive respiratory impairment [1]. Several clinical trials have demonstrated the benefits of ETI therapy, including improved lung function, reduced sweat chloride concentration, and a lower pulmonary exacerbation (PEx) rate [2,3]. However, the impact of

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ETI on the presence of microbial pathogens in the lower respiratory tract (LRT) of pwCF, particularly in younger children, remains inadequately understood.

Previous studies have examined the impact of HEMT on airways' microbial pathogens composition, primarily focusing on the effects of IVA in pwCF with the G551D variant [4–6]. These studies reported decreased levels of *Pseudomonas aeruginosa* (PA), mucoid PA, and *Aspergillus fumigatus* (Af) in respiratory cultures, along with increased richness and diversity in the airway microbiota. However, they did not achieve eradication of PA from the airways [5–7]. An increasing number of observational studies and registry analyses have also reported changes in airway infection patterns following ETI initiation, including reductions in bacterial density and antibiotic use [8–13]. Nonetheless, most available data derive from adolescent and adult populations, often including individuals previously treated with earlier CFTR modulators, and frequently rely on heterogeneous respiratory sampling methods such as oropharyngeal swabs, spontaneous sputum, or mixed sample types [8–12]. Data on younger, largely non-expectorant children remains limited. To date, few studies have evaluated changes in lower airway microbiology in young children with CF using standardised LRT sampling, particularly in cohorts without prior exposure to CFTR modulators or chronic antibiotic therapy. Understanding ETI-associated microbiological changes in this population is clinically relevant, as early airway infection patterns may influence long-term disease trajectory.

The aim of this prospective observational study was to explore changes in lower airway microbiology and selected clinical outcomes in children with CF aged 6–12 years before and after starting ETI. Lower airway infection was assessed longitudinally using sputum or induced sputum (IS), enabling evaluation in a mainly non-expectorant, CFTR modulator–naïve paediatric cohort. Due to the limited sample size, analyses were meant to be exploratory and to generate hypotheses.

2. Methods

2.1. Study design

A prospective single-centre study was conducted at the Paediatric CF Centre of the University Children's Hospital Ljubljana, Slovenia, between October 2022 and March 2024 to assess the impact of ETI treatment on the presence of microbial pathogens in the lower airways of children with CF. All study participants had no prior exposure to ETI therapy. Evaluations were conducted at baseline, 6, and 12 months after the introduction of ETI therapy.

Participants received ETI according to the manufacturer's standard recommendations. Children with CF aged 6 to 12 and weighing less than 30 kg received 37.5 mg IVA, 25 mg TEZ, and 50 mg ELX tablets in the morning, and 75 mg IVA tablets in the evening. Those aged 6 to 12 weighing more than 30 kg received the standard therapy, consisting of two tablets of 75 mg IVA, 50 mg TEZ, and 100 mg ELX in the morning, and 150 mg IVA in the evening.

The study's primary outcome was the change in bacterial and fungal presence in sputum or IS at six and twelve months of ETI treatment. Secondary outcomes included changes in sweat chloride levels, lung function, nutritional status, and PEx from baseline to twelve months.

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (No. 0120-21/2021-10). Written informed consent was obtained from parents or legal guardians of all participants. Considering the children's age (6–12 years), formal written assent was not requested; however, the children were informed about the procedures in an age-appropriate manner. All data were anonymised before analysis and managed in line with institutional and GDPR data protection standards.

2.2. Study eligibility criteria

We included children aged 6 to 12 years with a confirmed diagnosis of CF who began full-dosage ETI treatment, had a baseline sputum or IS sample, and at least one follow-up microbiology result. Exclusion criteria were pretreatment with other CFTR modulators, use of antibiotic prophylaxis, and treatment with inhaled antibiotics.

2.3. Sputum and induced sputum analysis

2.3.1. Sample collection and processing

Sputum or IS was selected as a simple, noninvasive measure to assess the presence of bacterial and fungal pathogens in LRT [14], using culture-based microbiological analysis for bacterial and fungal pathogens with an additional polymerase chain reaction (PCR)-based protocol specifically targeting PA. IS collection was performed in accordance with the ECFS Clinical Trials Network Standard Operating Procedure "Sputum induction for expectorating children and adults" [15]. Each sample was processed immediately for bacterial and fungal culture, and an aliquot of more than 1 ml was stored at 4 °C for nucleic acid (NA) extraction, which was performed within 24 h.

2.3.2. Bacterial and fungal culture

Sputum/IS were plated for bacterial culture onto blood agar, chromogenic agar selective for *Staphylococcus aureus* (SA), colistin nalidixic acid agar, chocolate agar, MacConkey agar, Bcc selective agar for *Burkholderia cepacia* complex, and incubated at 35 °C in ambient air or carbon dioxide depending on the required growth conditions. Plates were evaluated for growth on day 1 and day 2, and the Bcc selective agar was also assessed on day 3. Positive cultures were reported semi-quantitatively. Yeast and filamentous fungal cultures were performed on Sabouraud's agar and chromogenic medium, incubated for 7 days at 25 °C and 30 °C, respectively.

2.3.3. Molecular detection of PA

A 1 ml aliquot of the sample was first homogenized with the MagNA Lyser instrument (Roche) for 60 s at 7000 rpm using MagNA Lyser GreenBeads (Roche). Next, NA was extracted from 200 µl using the MagNA Pure24 (Roche), with an elution volume of 100 µl. PhHV (Roche) was included as an extraction control. The NA of the samples was stored at −80 °C. Detection of PA was performed by real-time PCR targeting *oprL* combined with real-time PCR targeting *ecfX* and *gyrB* [9], with the LightCycler 480 (Roche) using LightCycler Multipleks DNA Master (Roche) following the manufacturer's instructions. The results were interpreted as described previously [16].

2.4. Sweat test

Sweat production was induced using the iontophoresis of the pilocarpine method, according to the Gibson-Cook technique. Sweat chloride ions were measured before the initiation of ETI therapy and 6 to 12 months afterward. Measurements were provided in mmol/L, with levels of at least 60 mmol/L diagnostic of CF [17].

2.5. Pulmonary function testing

Spirometry was performed following European Respiratory Society standards [18]. All tests were conducted pre-bronchodilation. We used the percent predicted FEV_1 according to sex, height, age, and race. We used the Global Lung Function Initiative Calculation [19] to calculate the reference range. The multiple-breath washout technique was performed using endogenous nitrogen with 100% washout oxygen, utilizing the EcoMedics Exhalyzer D [20].

2.6. Pulmonary exacerbations and pathogen colonisation

We compared the number of PEx and oral and intravenous antibiotics used 12 months before therapy and 12 months after ETI initiation. Microbial colonisation patterns were systematically analysed using respiratory samples collected during the same time intervals. Patients were categorised according to pathogen colonisation status: none, intermittent, or chronic. Chronic colonisation was defined using modified Leeds criteria, consistent with European Cystic Fibrosis Patient Registry standards, which require positive culture results in more than 50% of respiratory specimens (minimum of four samples collected) during the 12-month assessment period [21]. Intermittent colonisation was defined as <50% positive cultures, while the absence of colonisation was recorded when no positive cultures were obtained.

2.7. Statistical analysis

Continuous variables are presented as median (IQR). Paired continuous variables were compared using the Wilcoxon signed-rank test. Binary repeated measures across three time points were analysed using Cochran's Q test. Paired multinomial categorical variables (none/intermittent/chronic) were analysed using the marginal homogeneity test (Stuart-Maxwell equivalent). Analyses were performed using IBM SPSS Statistics (Version 28.0).

Given the small sample size and exploratory design, no correction for multiple testing was applied. Results are hypothesis-generating and interpreted cautiously.

3. Results

In our prospective study, we included 16 children who started ETI treatment, with a median age of 8.4 years; 69% of them were boys. All participants were CFTR modulator–naïve before ETI initiation and did not receive inhaled antibiotics or prophylactic antibiotics during the study period. Baseline demographic and clinical characteristics are summarised in Table 1.

We observed improvements in lung function and nutritional status (Table 2). Sweat chloride decreased from a median of 111.0 mmol/L to 38.4 mmol/L, with a median reduction of −68.5 mmol/L (Z = −3.30; p = 0.001). Nearly one-third of patients achieved normal sweat chloride levels (<30 mmol/L) after treatment initiation. Lung function improved, with $ppFEV_1$ increasing from 89.0% to 94.0% predicted (median change +5.0%; Z = −2.17; p = 0.030), and LCI decreasing from 16.0 to 8.4 (median change −6.0; Z = −2.10; p = 0.036). None of the patients spontaneously produced sputum at any point during the study. Consequently, microbiological testing was performed exclusively on IS samples.

3.1. Bacterial culture and molecular detection of PA

Upon initiation of ETI, we observed a reduction in the presence of methicillin-sensitive SA (MSSA). The prevalence of MSSA decreased from 94% at baseline to 50% at 6 months and 63% at 12 months, with a significant overall change over time (Q = 7.09, p = 0.029) (Table 3, Fig. 1). No similar reduction was noted for other pathogenic bacteria. Following ETI initiation, PA was detected in only one patient in IS at six months, requiring eradication therapy. This patient had a history of intermittent PA colonisation in the year preceding ETI treatment. The detection of PA in IS by PCR was entirely consistent with the IS culture results. The proportion of patients with negative bacterial cultures increased from 6 % at baseline to 31 % after 12 months of ETI therapy.

Table 1
Characteristics of 6-12-year-old patients initiating ELX/TEZ/IVA therapy. Data are presented as median (IQR) or proportion of subjects. Patients did not receive any prophylactic or regular inhalation antibiotic treatment.

	Patients on ELX/TEZ/IVA (n = 16)
Boys/girls (%)	69 %/31 %
Age, years	
Median (IQR)	8.4 (7.3-10.9)
Genotype	
F508del homozygous	12/16 (75 %)
F508del heterozygous	4/16 (25 %)

Table 2
Characteristics of patients on ELX/TEZ/IVA therapy at initiation and after one year of therapy. Data are presented as median (IQR) or proportion of subjects. Significant differences (p < 0.05) are highlighted in bold.

	ELX/TEZ/IVA Start of therapy *(n=16)*	ELX/TEZ/IVA One year on therapy *(n=16)*	P Value
Sweat chloride, mmol/l, median (IQR)	111.0 (100.9 – 122.4)	38.4 (27.6 – 59.0)	**0.001**
Sweat chloride distribution, n/N (%)			
<30 mmol/l	0/16 (0 %)	4/14 (29 %)	**<0.001**
30-59 mmol/l	0/16 (0 %)	8/14 (57 %)	
>60 mmol/l	16/16 (100 %)	2/14 (14 %)	
$ppFEV_1$ (% predicted), median (IQR)	89.0 (73.0 – 95.0)	94.0 (88.0 – 107.0)	**0.030**
LCI, median (IQR)	16.0 (12.0 – 19.0)	8.4 (6.9 – 10.8)	**0.036**
BMI z-score, median (IQR)	−0.66 (−0.95 – 0.12)	−0.39 (−0.79 – 0.37)	0.118

3.2. Fungal culture

The presence of Af declined from 19% to 0% at six and twelve months (Q = 6.00, p = 0.050), accompanied by a decrease in fungal

Table 3
The presence of bacterial and fungal pathogens in induced sputum of patients on ELX/TEZ/IVA therapy at baseline, six months, and twelve months on therapy. Analyses are exploratory and unadjusted for multiple testing.

Pathogen	ELX/TEZ/IVA Baseline n/N (%)	ELX/TEZ/IVA 6 months n/N (%)	ELX/TEZ/IVA 12 months n/N (%)	Overall time effect[b]
Bacteria				
MSSA	15/16 (94 %)	8/16 (50 %)	10/16 (63 %)	0.029
MRSA	1/16 (6 %)	0/16 (0 %)	1/16 (6 %)	0.607
Pseudomonas aeruginosa[a]	0/16 (0 %)	1/16 (6 %)	0/16 (0 %)	0.368
Moraxella catarrhalis	1/16 (6 %)	0/16 (0 %)	1/16 (6 %)	0.607
Haemophilus influenzae	3/16 (19 %)	2/16 (13 %)	2/16 (13 %)	0.846
Streptococcus pneumoniae	0/16 (0 %)	2/16 (13 %)	0/16 (0 %)	0.135
Enterobacter cloacae	1/16 (6 %)	0/16 (0 %)	0/16 (0 %)	0.368
Stenotrophomonas maltophilia	0/16 (0 %)	0/16 (0 %)	1/16 (6 %)	0.368
Negative bacterial culture	1/16 (6 %)	5/16 (31 %)	5/16 (31 %)	0.135
Fungi				
Candida albicans	9/16 (56 %)	7/15 (47 %)	7/15 (47 %)	0.779
Candida dubliniensis	2/16 (13 %)	3/15 (20 %)	2/15 (13 %)	0.607
Candida krusei	1/16 (6 %)	0/15 (0 %)	0/15 (0 %)	0.368
Aspergillus fumigatus	3/16 (19 %)	0/15 (0 %)	0/15 (0 %)	0.050
Aspergillus niger	1/16 (6 %)	0/15 (0 %)	0/15 (0 %)	0.368
Negative fungal culture	4/16 (25 %)	5/15 (33 %)	9/15 (60 %)	0.016

[a] Concordant culture and PCR results.
[b] Overall time effect assessed using Cochran's Q test.

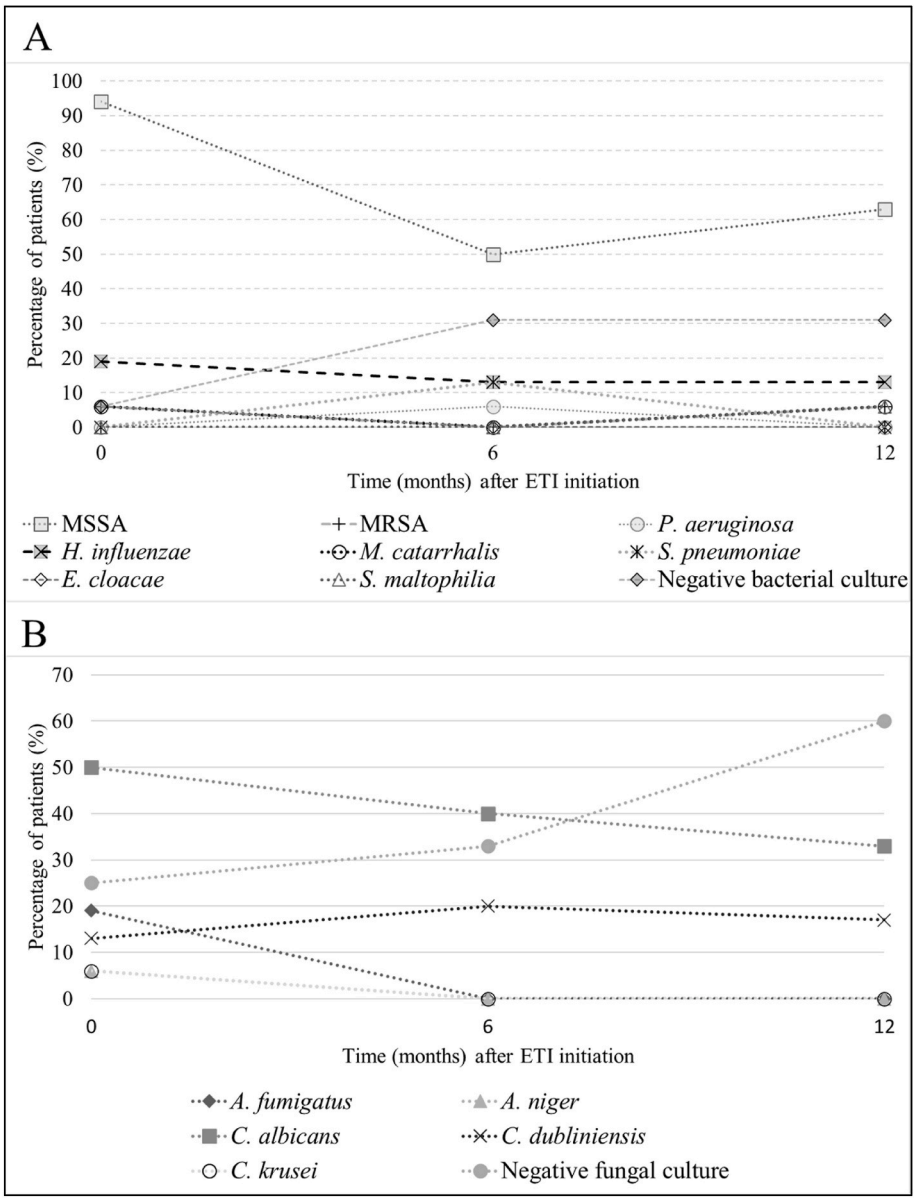

Fig. 1. Effect of elexacaftor/tezacaftor/ivacaftor on the presence of (A) bacterial and (B) fungal pathogens in induced sputum samples.

diversity. Five fungal species were identified at baseline in the study group, but after 6 and 12 months of therapy, only *Candida albicans* and *Candida dubliniensis* persisted (Table 3, Fig. 1). Fungal culture negativity increased from 25 % at baseline to 60 % after 12 months of therapy.

3.3. Pulmonary exacerbations and pathogen colonisation

Although study subjects experienced fewer PEx and reduced antibiotic use in the year following ETI initiation compared to the preceding year, these reductions did not achieve statistical significance (Table 4). Similarly, we observed an increased proportion of patients free of colonisation with specific CF respiratory pathogens (including MSSA, PA, and Af) one year after ETI was introduced. However, these improvements failed to reach statistical significance (Table 4). Before ETI initiation, respiratory specimens consisted primarily of sputum from expectorant patients and throat swabs from non-expectorant individuals. Following ETI, IS became the predominant specimen type collected.

4. Discussion

Beyond the established clinical improvements—including significant improvements in lung function and nutritional status—the results of our study suggest potential shifts in the microbial profile of the lower airways in younger pwCF following ETI initiation. Specifically, we observed decreased growth of MSSA and Af, reduced variability in fungal populations, and an increase in negative bacterial and fungal culture results. These findings may reflect shifts in airway microbiota, potentially contributing to the overall health improvements in pwCF.

A prospective study that also examined the effect of ETI therapy on microbial pathogen growth in the sputum of 236 pwCF with at least one F508del variant found that PA sputum culture positivity declined after the first month of ETI therapy and remained stable, with no significant change for SA [7]. However, the study reported a decrease in the mean sputum density of MSSA, PA, *Stenotrophomonas maltophilia*, *Achromobacter* spp., and *Burkholderia* spp. after one month of therapy [7]. Another observational study included 282 pwCF aged over 12 years who either spontaneously expectorated sputum, had nasopharyngeal aspirates, or underwent bronchoalveolar lavage, and showed a decrease in

Table 4
Pulmonary exacerbations and pathogen colonisation in the twelve months before and after ELX/TEZ/IVA therapy initiation. Data are presented as median (IQR) or proportion of subjects.

	Pre ELX/TEZ/IVA *(n=16)*	Post ELX/TEZ/IVA *(n=16)*	P Value
Number of exacerbations, median (IQR)	2 (0 - 2)	1 (0-2)	0.101
Total days on antibiotics, median (IQR)	20 (0 - 39)	14 (0 - 26)	0.140
Total days on i.v. antibiotics, median (IQR)	0 (0 - 0)	0 (0-0)	0.276
Pseudomonas aeruginosa			
No	13/16 (81 %)	15/16 (94 %)	
Intermittent	3/16 (19 %)	1/16 (6 %)	0.157
Chronic	0/16 (0 %)	0/16 (0 %)	
MSSA			
No	0/16 (0 %)	3/16 (19 %)	
Intermittent	2/16 (13 %)	3/16 (19 %)	0.052
Chronic	14/16 (87 %)	10/16 (62 %)	
MRSA			
No	15/16 (94 %)	14/16 (88 %)	
Intermittent	0/16 (0 %)	1/16 (6 %)	0.739
Chronic	1/16 (6 %)	1/16 (6 %)	
Haemophilus influenzae			
No	8/16 (50 %)	9/16 (56 %)	
Intermittent	5/16 (31 %)	7/16 (44 %)	0.157
Chronic	3/16 (19 %)	0/16 (0 %)	
Aspergillus fumigatus			
No	11/16 (69 %)	13/16 (81 %)	
Intermittent	5/16 (31 %)	3/16 (19 %)	0.414
Chronic	0/16 (0 %)	0/16 (0 %)	

PA, SA, *Aspergillus* spp., and *Stenotrophomonas maltophilia* following ETI therapy [9]. In this referenced study, 81 pwCF had not trialled other CFTR modulators before participating [9], contrasting with our study, where none of our pwCF had trialled another CFTR modulator. A statistically significant decrease in PA and SA was observed 21 months after ETI initiation in a German CF registry study of 1092 pwC aged 12 or older, where both throat swabs and sputum samples were collected [8]. In our study, we collected IS samples from all participants at baseline, 6 months, and 12 months after ETI. None of our patients was sputum expectorant. As with other studies using either spontaneously expectorated or IS samples, both methods are demonstrated to be superior to throat swabs, offering better bacterial yields, especially in polymicrobial infections [14,22]. As observed in other studies, sputum induction was well tolerated and safe in our cohort.

Compared to our findings, a study involving 124 pwCF older than 12 years (average age 28,41 years) assessed the positivity rates of PA, MSSA, and MRSA in respiratory samples before and 12 months after ETI therapy initiation. This study found no reduction in MSSA and MRSA but a significant decrease in PA in respiratory isolates [23]. Sputum was mainly used for pre-ETI cultures, whereas throat samples were more common post-ETI in their study. The higher average age of their cohort compared to ours, the increased use of throat swabs after ETI, and the fact that 71 % of their pwCF had previously trialled at least one modulator before starting ETI could explain the differences in our results. The CF PROMISE study, which examined microbiological changes in a similar age group (6-11 years), observed no decrease in SA in cultures after ETI therapy; however, sputum culture density decreased at 12 months [24]. Although the study included 125 pwCF, sputum samples were successfully collected from a smaller group, 53 of 125 (42.4%) participants at visit 1 (81% induced), and 23 of 119 (19.3%) at visit 4 (82.6% induced). Additionally, more than half of their cohort had previously been exposed to other CFTR modulators, with 11.3% of patients on chronic inhaled antibiotics and 28.2% on chronic azithromycin. In contrast, all our subjects were CFTR modulator naïve, and none were on inhaled or prophylactic antibiotics, which helped minimise confounding factors that could influence our results, thereby adding significant value to our findings.

In our cohort, we observed trends towards fewer PEx and reduced antibiotic use following ETI initiation compared to the previous year. However, unlike larger studies [2,3], these reductions did not reach statistical significance, likely due to our limited sample size rather than an absence of effect. Larger registry-based studies have demonstrated significant reductions in exacerbations and antibiotic use following ETI initiation, and our findings align with these reports. A recently published UK-based study LONGITUDE included 5187 pwCF aged 6 years or more on ETI therapy, using UK Cystic Fibrosis Registry data, and showed a 67.4% reduction in the annualised rate of PEx [25]. Similar results were observed in the German CF Registry study [26] and the US Cystic Fibrosis Foundation Patient Registry (CFFPR) study [11]. The German registry study included 2645 pwCF and observed a statistically significant 75.9 % decline in PEx after 1 year of ETI therapy [26]. The annualised mean number of PEx over the two years of ETI therapy also decreased by 79 % in the CFFPR study, the largest to date involving 16, 116 pwCF [11].

After one year of ETI therapy, we observed an increased proportion of patients free of colonisation with CF respiratory pathogens (including MSSA, PA, and Af). However, these improvements also did not reach statistical significance. Nevertheless, similar findings were observed in other larger studies; for instance, a notable increase in the percentage of pwCF without colonisation of PA, *Aspergillus* spp., and *Stenotrophomonas maltophilia* was observed after ETI therapy in the larger study by Bendixen et al., which followed pwCF over 8 years [9]. Another study involving 198 adult pwCF reported a statistically significant decrease in colonisation with PA, MSSA, MRSA, *Stenotrophomonas maltophilia*, *Achromobacter* spp., and nontuberculous mycobacteria after one year of ETI therapy [27].

Changes in the presence of bacterial and fungal pathogens in the IS of our pwCF after initiation of ETI therapy could be linked to altered lung pathophysiology. HEMT enhances mucociliary clearance, improving the removal of pathogenic bacteria in the lungs [28]. Better mucociliary clearance was also associated with improvement in FEV_1 [28]. The airway surface layer in pwCF has reduced pH due to a defective CFTR ion channel, which fails to transport bicarbonate ions (HCO3-) effectively. The airway surface area with a lower pH has decreased antimicrobial properties [29]. On the other hand, HEMT increases the pH of the airway surface layer, making the environment less appropriate for pathogenic bacteria [30] Additionally, research shows that HEMT decreases the growth of laboratory and clinical Af biofilms by disrupting cell wall permeability and causing metabolic instability [31].

The present study offers several notable strengths. First, we systematically collected high-quality lower airway samples via IS in a young, predominantly non-expectorant population. Many previous larger studies have relied on oropharyngeal swabs or mixed sampling strategies, which may underestimate or misclassify lower airway infection [8–12]. In contrast, IS provides a more reliable representation of lower airway microbiology and allows for more precise longitudinal assessment of pathogen presence [14]. Second, our exclusive focus on paediatric patients addresses a significant gap in the literature, as research specifically targeting pwCF under 12 years remains limited. Third, none of our participants had been given prior CFTR modulators, inhaled antibiotics, or prophylactic antibiotic therapy, reducing key confounders and enabling a clearer evaluation of ETI-related microbiological changes. Furthermore, our investigation extends beyond conventional microbiological assessments by evaluating the impact of HEMT on multiple clinically relevant parameters, including pulmonary function, nutritional status, chloride sweat test levels, and the frequency of PEx. This multifaceted approach provides a more holistic understanding of HEMT's effects on children with CF. Additionally, our findings emphasise the importance of vigilant monitoring, even during HEMT treatment, as patients continue to experience PEx and PA

colonisation, requiring appropriate antibiotic intervention.

5. Limitations

Despite its strengths, our study has several important limitations. The single-centre design and relatively small sample size warrant caution when interpreting our findings. The small sample size reduces statistical power and raises the likelihood of Type II error, especially for secondary outcomes such as PEx and individual pathogen prevalence. Consequently, non-significant results should be viewed with caution. Given the small sample size, sex-stratified analyses were underpowered. However, no clear sex-related differences were observed. Additionally, multiple statistical comparisons were performed without formal adjustment for multiple testing. Given the exploratory nature of the study and the limited cohort size, statistical analyses aimed to be descriptive and hypothesis-generating rather than definitive. Therefore, focus has been placed on overall trends rather than isolated statistically significant findings. A relatively short observation period is another limitation. A longer follow-up would provide more comprehensive insights into the long-term impact of ETI on airway microbial populations in pwCF. Future multicentre studies involving larger cohorts are essential to generate more robust evidence on the effects of HEMT on airway microbiota, particularly in younger pwCF, where data remain limited. Our study did not investigate the immunological response, which is a key factor in the progression of CF disease and PEx. Future research should include analyses of peripheral neutrophil counts and proinflammatory cytokine profiles to better understand the broader immunomodulatory effects of HEMT and their relationship with clinical outcomes, especially PEx. Such a detailed immunological assessment would provide valuable insights into the mechanisms underlying the clinical benefits observed with HEMT.

6. Conclusion

Our results offer real-world, hypothesis-generating data on the impact of ETI on lower airway microbiology in younger children with CF. Using IS in a modulator-naïve paediatric population provides a unique perspective that complements existing literature and emphasises the importance of age-appropriate sampling strategies in future research. Our findings show several potentially clinically meaningful changes, including a decrease in MSSA and Af and a higher number of patients with negative bacterial and fungal cultures. The indicated shifts in the presence of bacterial and fungal pathogens in the lower airways may be a key mechanism behind HEMT's beneficial effects. As HEMT becomes available for progressively younger patients, including the recent expansion of ETI approval to children under 6 years, future research should explore how early therapeutic intervention influences airway microbiology and long-term clinical outcomes in this younger group.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, Grammarly software was used for grammar correction and language editing. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the publication's content. No figures or images were created or altered with generative AI.

CRediT authorship contribution statement

Gaja Setnikar Kimovec: Data curation, Investigation, Methodology, Writing – original draft. **Katja Seme:** Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Tadeja Matos:** Formal analysis, Methodology, Writing – review & editing. **Vesna Cvitković Špik:** Formal analysis, Methodology, Writing – review & editing. **Uroš Krivec:** Writing – review & editing. **Marina Praprotnik:** Writing – review & editing. **Jasna Rodman Berlot:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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