CYSTIC FIBROSIS IN CHILDREN

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CYSTIC FIBROSIS IN CHILDREN

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EDITORIAL
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Editorial: Cystic Fibrosis in Children

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Editorial on the Research Topic

Cystic Fibrosis in Children

Cystic fibrosis (CF) is the most common fatal genetic disease among Caucasians, occurring in 1 in 3,000 live births. According to the international registries, ∼150,000 patients have a diagnosis of CF worldwide (1, 2). Based on the improvement in the nutritional and clinical care of the patients and setting CF centers with a multi-disciplined approach, general characteristics and the survival rates of CF patients have been changed remarkably. After the contribution of cystic fibrosis transmembrane conductance regulator protein (CFTR) modulator therapies, these changes will be more prominent in especially high-income countries (3). However, other factors in the diagnosis and treatment of patients in developing countries, even patients receiving CFTR modulators in high-income countries should be taken into account (4). Increased life expectancy, higher costs of new treatment modalities and a better understanding of genetics, call for a more sophisticated approach to these patients.

In contrary to a great amount of data on CF, many discrepancies still exist among countries and even between different centers. New treatment modalities have great potential to change the natural course of the disease, but due to the high economic cost of these treatments, inequality among patients in different socio-economic circumstances may increase. In a recent publication, only 12% of whole CF population in registries eligible for triple modulator therapies, were reported to have access to this modalities (5). An update of guidelines and research is needed for a standardized worldwide approach. For this purpose, we invited researchers to contribute to this collection focusing on main problems in the management of CF, especially emphasizing on the differences in approaches across the world.

This Research Topic gathers a collection 13 original and review articled providing crucial information on different aspects of CF. There is an interesting manuscript on the pathophysiology of CF, investigating the role of IL-22 and neutrophil proteins on the lung damage in the future (Renwick et al.). Early life concentrations of azurocidin and myeloperoxidase were found to have correlation with Brody scores at high resolution CT after 6 years of age. In addition to that, four other neutrophil associated proteins were negatively correlated with Brody scores. Also, first time in the literature, IL-22 levels in early age was correlated with increased lung damage. Identification of early signs of lung damage may help to improve the management of CF patients.

Early diagnosis of CF is still the mainstay of the management of the disease. In order to diagnose early and prevent complications, newborn screening programs have been implemented in many parts of the world. In this collection, different methods of newborn screening was reviewed (Coverstone and Ferkol). The threshold for defining a cut-off level of immunoreactive trypsinogen varies a lot between programmes. Adding genetic panels may additionally help to identify the patients with normal sweat test results. The advantage of screening in patients having mutations eligible for modulator therapies has also mentioned in this review. In animal models, primary prevention of CF was studied, which may lead to clinical trials in fetuses. Achieving primary prevention of CF

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has also been questioned in this article. Sweat testing is the main diagnostic tool for CF, evaluating the children after a positive newborn screening or patients with suggestive clinical findings. This procedure requires standardization and newer easy-to-perform techniques. These advances were discussed in another article in this collection (Gokdemir and Karadag). According to the recent guidelines, pilocarpine iontophoresis is the single accepted method, but accepted several others were also discussed in this review. Wearable sensors for ion exchange technology, skin wipe test for capillary electrophoresis are mentioned as newer methods.

Another series of investigations in this Research Topic, include reports on exercise techniques and different methods of measuring lung function. Oscillometry and multiple breath-washout techniques including lung clearence index (LCI) may give additional information about the lung health in CF. Although the role of LCI in clinical trials has been well-established, its role in daily care is not well-known. In an article from this collection, the authors reviewed the usage of LCI and cardiopulmonary exercise testing in daily care (Hatziagorou et al.). Although spirometry is the well-known method to follow patients, especially in early-stage disease newer methods may be more helpful. LCI is more sensitive to detect impairment of peripheral airways and prevent future damages than spirometry. Also LCI measurements shortly after birth is correlated with respiratory rate later in life and, also predicts the first Pseudomonas colonization. In addition to being expensive and time-consuming especially for severe patients, for LCI there is still a need for validity, age-specific reference values and more data for LCI in clinical follow up of the patients. Exercise and increased muscle strength are also important features for improving the management of CF. Handgrip strength was shown to have an association with lung function (Adair et al.). In this research, exercise program at home as a quality improvement project was shown to result in significant improvement in hand grip strength. In another research in this collection, the authors suggested LCI as a better tool than IOS for patients with CF (Postek et al.).

Early detection of CF-related diabetes (CFRD) is so important, continuous glucose monitoring seems to more useful than oral glucose tolerance test (OGTT) in an article from this collection and the authors suggest continuous monitoring should be included in the guidelines in the near future (Gojsina et al.). There are still limited data on allergic bronchopulmonary aspergillosis in CF. Diagnosis can be difficult in some cases. In addition to these challenges, current and future treatment options like monoclonal antibodies are widely discussed in a review (Sunman et al.).

Other breakthroughs introduced in this collection focus on new treatment modalities including triple therapy and the real life experience that will give insights about the future of the management. In a real-life study among patients above 12 years of age, long-term lumacaftor/ivacaftor treatment improved lung function, nutritional status, and sweat chloride levels especially in younger patients (Bui et al.). In another article, short-term effects of triple combination on glucose tolerance has been reported (Korten et al.). The effect of modulator therapies is still not clear. In this observational study, glucose levels of OGTT was found to be improved and the authors suggest this therapy might prevent CFRD. Also, nasal epithelial cell models seem to be useful in functional profiling of CFTR-directed therapies and enables a better understanding for personalized medicine (Park et al.). Culturing cells from nasal brushings instead of rectal or nasal biopsies also gives a non-invasive nethod for sampling.

During pandemic, appointments were tried to be converted to telemedicine visits and the need for digital health and using telemedicine in the management of CF became so clear (Hendra et al.). In this survey, more than 80% of the patients were satisfied with telemedicine visits but disparities in language and access to internet seems to cause additional problems.

In conclusion, this collection covers different aspects of current approach to CF and adds valuable information to the literature. Great amount of work published from all parts of the world will result in a better understanding of the changing face of CF and hopefully decrease the inequity among countries.

AUTHOR CONTRIBUTIONS

BK prepared the manuscript. EH, AT, and RE reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Functional Profiling of CFTR-Directed Therapeutics Using Pediatric Patient-Derived Nasal Epithelial Cell Models

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Functional profiling of CFTR-directed therapeutics offers the potential to provide significant benefits to young people with cystic fibrosis (CF). However, the development of 2D airway epithelial cell models for individual response tests in CF children remains a central task. The objective of this study was to determine the utility of EpiX^{TM} technology for expansion of nasal epithelial cells for use in electrophysiological CFTR function measurements. An initial harvest of as few as 20,000 cells was sufficient to expand up to 50 million cells that were used to generate air-liquid interface (ALI) cultures for ion transport studies with the Ussing assay. CFTR function was assessed by measuring responses to forskolin and the CFTR potentiator VX-770 (ivacaftor) in ALI cultures generated from passage 3 and 4 cells. Short-circuit current (Isc) measurements of blocked CFTR currents ($\Delta I_{CFTRinh}$) discriminated CFTR function between healthy control (wild type, WT) and patients with intermediate (F508del/R117H-7T: 56% WT) and severe (F508del/F508del: 12% WT) CF disease. For the mixed genotypes, CFTR activity for F508del/c.850dupA was 12% WT, R334W/406-1G>A was 24% WT, and CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb) was 9% WT. The CFTR correctors VX-809 (lumacaftor) and VX-661 (tezacaftor) significantly increased CFTR currents for F508del/R117H to 73 and 67% WT, respectively. Cultures with the large deletion mutation CFTRdele2,3(21 kb) unexpectedly responded to VX-661 treatment (20% WT). Amiloride-sensitive sodium currents were robust and ranged between 20–80 μA/cm^2 depending on the subject. In addition to characterizing the electrophysiological profile of mutant CFTR activity in cultures for five genotypes, our study exemplifies the promising paradigm of bed-to-bench side cooperation and personalized medicine.

Keywords: Cystic fibrosis, mutation-specific CFTR function analysis, CFTRdele2,3(21kb) (c.54-5940_270+10250del21 kb, p.Ser18ArgfsX16), c.850dupA (977insA, p.Met284Asnfs), R334W (c.1000C>T, p.Arg334Trp), 406-1G->A (c.274-1G>A), R117H-7T (c.350G>A, p.Arg117His), F508del (c.1521_1523delCTT, p.Phe508del)

INTRODUCTION

Cystic Fibrosis (CF; OMIM 219700) is an autosomal recessive disorder caused by mutations in the gene coding the cystic fibrosis transmembrane conductance regulator (CFTR) which functions as a chloride and bicarbonate-permeable ion channel protein in the apical cell membranes of various epithelia, including the lungs, pancreas, and sweat glands (1, 2). Patients with CF have salty skin at birth and suffer from various early-onset symptoms resulting from defective ion conductance, such as respiratory mucus buildup, bacterial infections, and pancreatic insufficiency.

CF is a promising case for personalized medicine due to its genomic variety (3). As of January 2020, over 89,000 patients were registered worldwide for CF (http://www.cftr2.org/), and a total of 2,092 CFTR mutations were identified; of these, 352 were described as CF-causing (4). However, the majority of CFTR mutations and resulting variety of genotypes have not been experimentally evaluated in terms of their functional consequences on chloride and bicarbonate transport function. The majority of patients are eligible for a well-characterized treatment regimen developed for carriers with one or two F508del alleles, the most common CF variant (5); however, some remaining 40% of patients who are carriers of rare variants have no defined treatment (6), and may benefit from *ex vivo* patient-specific CFTR functionality testing. In this study, we report the results of a personalized procedure in which patient-derived cells are used to characterize mutant CFTR function and pharmacodynamic response. We examined the electrophysiologic properties of rare genotypes of five pediatric CF patients, whose cells were harvested by a swift nasal swab procedure and then expanded for *in vitro* analysis.

Nasal epithelial cells were collected from CF patients who were between 3 and 20 years old. The isolated nasal cells were subsequently expanded in culture via the novel EpiX^{TM} technology at Propagenix Inc., MD, which has previously been established as a viable method for providing functional cells for the assay (7). The EpiX^{TM} cell expansion method has been shown to successfully conserve physiological function in human bronchial epithelial cells, including CFTR function and modulator responsiveness after multiple passages (7).

The current paradigm of treatment for CF often entails the administration of small-molecule CFTR modulators, which include CFTR *potentiators* – such as ivacaftor (VX-770) – that bind to CFTR and improve its open channel probability, and *correctors* – such as tezacaftor (VX-661) and lumacaftor (VX-809) – that improve CFTR trafficking and localization to the apical plasma membrane. Often, a combination of CFTR corrector and potentiator compounds is prescribed, as in the case for homozygotes of the most common CF variant, F508del (5). In this study, nasal epithelial cultures were studied for their pharmacological responses to the following combination of CFTR modulators: VX-770 (ivacaftor, Kalydeco®) only, VX-770+VX-809 (ivacaftor/lumacaftor; Orkambi®), and VX-770+VX-661 (ivacaftor/tezacaftor; Symdeko®) (8), which were the CF modulators available during the time of this study.

To characterize CFTR function in EpiX^{TM}-expanded nasal cells, we used the Ussing Chamber technique, an established electrophysiological assay for vectorial ion transport measurements in epithelial tissues. Short-circuit current measurements provide a sensitive read-out for the apical membrane chloride conductance and CFTR-mediated chloride current measurements have been established in cultured airway epithelial cells (9, 10) as well as cultures from nasal scrapings (11). Over the past years, functional responses to CFTR-directed therapeutics have emerged as a basis for preclinical studies using primary airway cell cultures (6, 12, 13), often expanded by conditional reprogramming technology in presence of 3T3 feeder cells (14–17) and more recently, by emerging feeder-free technologies (7, 17, 18). CFTR modulator theratyping has been introduced to group and match CFTR-directed medications to CF mutations. The concept is promising and enables the design of efficacious therapeutic intervention in CF patients (5, 19, 20).

In this study, we characterize the CFTR function of five different genotypes: F508del/F508del, CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb), F508del/R117H-7T, R334W/406-1G>A, and F508del/c.850dupA. The variants examined in this study are briefly introduced below and the genomic structure is schematically shown in **Figure 1**.

F508del (c.1521_1523delCTT, p.Phe508del) is the deletion of a CTT sequence from nucleotide positions 1,521–1,523 in exon 10 resulting in a deletion of phenylalanine in amino acid position 508 that affects the first nucleotide binding domain of the CFTR protein (21). It represents 55% of all alleles (99,061/178,104 alleles) recorded in the CFTR2 database, which accounts for CF registries in the US and Europe (4). Interestingly, the spectrum of CFTR mutations varies among different populations, ethnic backgrounds, and geographical locations.

CFTRdele2,3(21 kb) (c.54-5940_270+10250del21kb, p.Ser18ArgfsX16) is an example of a less common CF mutation that is frequently observed in Central and Eastern Europe (1.1–6.4%) and in particular among Slavic populations (22). It represents <1% of all alleles (417/178,104 alleles) recorded in the CFTR2 database, which accounts for CF registries in the US and Europe (4) and is associated with severe CF symptoms (22). The mutation is a large deletion mutation of 21 kb of the region spanning exons 2 and 3 of the CFTR gene including adjacent introns 1, 2, and 3. This deletion leads to a frameshift downstream and a premature stop signal in codon 106, producing a potential product that is 32 amino acids long with no transmembrane or gating domains. The existing literature suggests that CFTRdele2,3(21 kb) produces truncated CFTR mRNA lacking exons 2 and 3, however no electrophysiological studies have been performed to examine the level of CFTR function in CFTRdele2,3(21 kb).

F508del/R117H-7T is a genotype that has been extensively studied. The R117H mutation (c.350G>A, p.Arg117His) is caused by the substitution of guanine to adenine at nucleotide 350 in exon 4, changing arginine to histidine at residue 117 which is located in the extracellular loop that connects the first and second membrane-spanning regions of the first transmembrane domain. In this study, the R117H mutation is *cis* to a 7T polymorphism in the poly T-tract of intron 8 with a reported allele frequency of 125/178,104 alleles (4). The 7T often results in less severe CF phenotype, as opposed to 3T or 5T repeats (23, 24). R117H is clinically treated with ivacaftor.

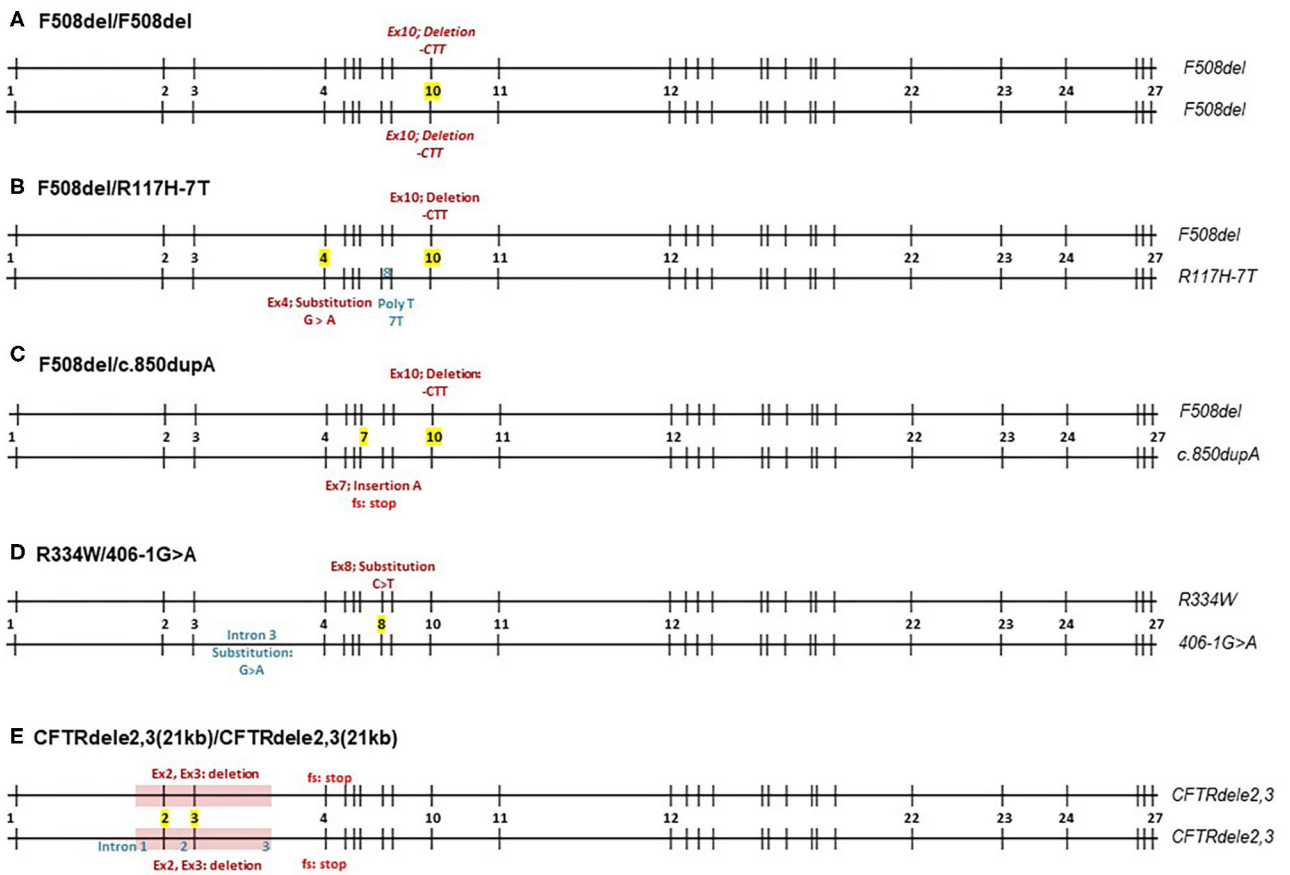

FIGURE 1 | Schematic overview of CFTR mutations by five different CF genotypes. Numbers 1-27 represent exons of the CFTR gene. **(A–E)** F508del homozygote, F508del/R117H-7T, F508del/c.850dupA, R334W/406-1G>A, and CFTRdele2,3(21kb) homozygote.

R334W/406-1G>A. *R334W* (c.1000C>T, p.Arg334Trp) is an uncommon mutation with an allelic frequency of 0.2% [429/178,104; (4)]. It causes a substitution of cytosine to thymine at nucleotide position 1,000 in exon 8. The R334W mutation is located within the sixth membrane-spanning region of the first transmembrane domain and has a high conductivity for bicarbonate (25).

406-1G>A (c.274-1G>A) is a very uncommon mutation with an allelic frequency of 0.02% [40/178,104 alleles; (4)] that has been identified in the Hispanic CF population in the US (26). It belongs to a canonical splice mutation where the guanine is changed to adenine at one nucleotide before cDNA locus 274, in intron 3. This causes an mRNA splicing defect and presumably no proper post-translational processing of CFTR (26).

F508del/c.850dupA. *c.850dupA* (977insA, p.Met284Asnfs) is an extremely uncommon mutation with an allelic frequency of 0.004% [only 8 alleles/178,104; (4)]. It causes an insertion of adenine at nucleotide position 850 in exon 7 that creates a stop codon two triplets downstream due to frameshift. The stop codon presumably leads to termination of CFTR translation at the cytosolic polypeptide chain following the 4th membrane-spanning region of the first transmembrane domain (27).

We report that the genotypes studied here range from intermediate to low CFTR functionality, with F508del/R117H-7T retaining as much as 56% wildtype CFTR function, R334W/406-1G>A retaining 24%, F508del/c.850dupA retaining 12%, F508del/F508del retaining 12%, and CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb) retaining 9%. Among the modulators considered in this study, VX-770+VX-809 (Orkambi®) was most effective in rescuing F508del/R117H-7T and F508del/c.850dup, and less in F508del/F508del and R334W/406-1G>A. Unexpectedly, VX-770+VX-661 (Symdeko®) showed effects on homozygous CFTRdele2,3(21 kb). These findings extend our understanding of the five genotypes studied, some of which are rare and understudied in the standard literature, and exemplify the efficacy of personalized medicine as a paradigm for CF treatment.

MATERIALS AND METHODS

Nasal Swab and Initial Cell Harvest

Nasal cell procurement was performed at UCSF Benioff Children's Hospital Oakland in Oakland, CA on five patients with the following genotypes: *F508del/R117H-7T* (male, 6 year

old, Caucasian, not on CFTR modulators), *R334W/406-1G>A* (female, 3 year old, not on CFTR modulators), *F508del/c.850dupA* (female, 20 year old, Caucasian, on Symdeko), *F508del/F508del* (male, 17 year old, Caucasian, not on CFTR modulators), *CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)* (female, 4 year old, Caucasian, not on CFTR modulators). Mean age was 10 years old (SE = 3.5), 60% (3 of 5 patients) identified as female, and 80% (4 of 5) identified as Caucasian. The procedure was conducted under an IRB approved protocol. Cells were obtained by a simple nasal swab using standard cytology brushes (CYB-1, Medical Packaging Corporation, Inc., sterile). No topical anesthesia was required. Brush tips were cut and placed in 10 mL transport media (**Table S1**).

Expansion by EpiX^{TM} Technology

EpiX^{TM} expansion was conducted by Propagenix Inc., as previously described (7). Cells from nasal brushing samples were resuspended by multiple rounds of centrifugation, which involved trypsinization and neutralization. Total number of viable cells was counted using a hemocytometer. Harvested cells were then seeded in Collagen I-coated culture vessels, and grown in the EpiX^{TM} medium. EpiX^{TM} media was changed every 2–3 days for 26–31 days, yielding a final cell count of 50 million cells per patient's cell line (**Table 1**).

Air Liquid Interface Differentiation and Cell Culture

Expanded cells were plated onto Snapwell Inserts and differentiated at an air-liquid interface (ALI) with the Vertex Differentiation Medium, named V-ALI (**Table S2**) (15) for 10–15 days. Differentiated cells were then sent to UCSF Children's Hospital Oakland Research Institute for ion transport measurements in Ussing chambers. Prior to the electrophysiological studies, some cultures were incubated overnight (16–24 h) with VX-809 or VX-661 (3–30 μM) while others remained untreated, as part of the experimental design for testing combination therapies (8).

Using Chamber Short Circuit Assay

Snapwell inserts were mounted via a slider into modified Ussing chambers (Easy Mount Chamber Systems, Physiologic Instruments, San Diego, CA). Fluid volume was 5 ml on each side. The composition of the basolateral Ussing chamber solution was (in mM): 120 NaCl, 20 $NaHCO_3$, 5 $KHCO_3$, 1.2 NaH_2PO_4, 5.6 glucose, 2.5 $CaCl_2$, and 1.2 $MgCl_2$. In the mucosal solution, all Cl^- salts were exchanged for gluconate salts. Both hemi-chambers were gassed with 5% CO_2 /air and bath temperature was maintained at 35–37°C.

Transepithelial voltage was clamped to zero volts using a standard four-electrode voltage clamp (Physiologic Instruments, San Diego, CA). Short-circuit current was recorded to a computer through an analog-to-digital board (DI710, DataQ Instruments, Akron, OH). At 60 s intervals, transepithelial voltage was clamped to 1 mV for 1 s to calculate transepithelial electrical resistance (TER). Higher resistance represents tighter cultures. Current deflections are shown in Ussing traces to visualize TER of each culture and large current amplitudes indicate lower TER.

After short-circuit currents had stabilized at the beginning of the experiment, each culture was treated with the following protocol: ENaC inhibitor amiloride (100 μM, to mucosa), cAMP agonist forskolin (20 μM, to serosa), CFTR potentiator VX-770 (1 μM, to mucosa), CFTR inhibitors CFTRinh-172 (50 μM, to mucosa), PPQ-102 (50 μM, to mucosa), GlyH-101 (50 μM, to mucosa) (28), and Ca-activated chloride channel (CaCC) activator ATP (500 μM, to mucosa). Approximately 5–15 min elapsed between each addition until stabilization of currents was observed.

Data Analysis and Metrics

As a non-CF control, human bronchial epithelial (HBE) cells were obtained from a patient with idiopathic pulmonary fibrosis. A frozen aliquot of cells at passage 0 was expanded for one passage via the same EpiX^{TM} technology and protocol explained above. Cells were passaged once, then differentiated in a separate differentiation medium for 28 days, followed by an identical Ussing Chamber protocol.

TABLE 1 | Clinical characteristics of CF nasal cell donors and expansion of CF nasal cells by EpiX^{TM} technology.

Genotype	Age (years)	Sex	Sweat NaCl (mM)	FEV-1 %	Number of brushed cells	Days to 50 million cells	Passage number	Population doublings
F508del/ R117H-7T	6	M	45	71	1,216,000^{BC}	29	3	8.7
F508del/ F508del	17	M	103	56	596,000	26	3	10.4
F508del/ c.850dupA	20	F	108	109	20,000	31	4	11.6
R334W/ 406-1G>A	3	F	113	N/A	52,000	29	3	8.7
CFTRdele2,3/ CFTRdele2,3	4	F	>100	N/A	100,000	29	3	10.4

Cells were expanded in EpiX^{TM} media over a period of 26–31 days. Population doublings refers to the total number of times that cells in a population have doubled. Sweat test and lung function measurements (FEV1%) are listed from available patient data prior to cell collection. N/A, not available; BC, sample contained red blood cells.

The standard for 100% normal CFTR function was determined by quantifying the magnitude of the forskolin-stimulated response in non-CF control cells and taking the difference between the forskolin-stimulated current (I_{Fsk}) and the final current that remained after CFTR inhibitors were added ($I_{CFTRinh}$). This approach provides a more appropriate quantification of baseline CFTR activity because it accounts for pre-existing CFTR currents. Total CFTR function in CF cells was similarly assessed for each genotype by measuring the CFTR blocker -sensitive current, which was defined as the total change in current in response to sequential additions of CFTR inhibitors ($I_{CFTRinh}$) after combined stimulation by forskolin plus VX-770 ($I_{Fsk+VX-770}$). Based on these parameters, we calculated % of normal CFTR function, whereby a higher percentage represents greater rescue of CFTR toward a normal phenotype, based on the following equation:

$$\%\ \text{normal CFTR Function} = \left| \frac{I_{Fsk+VX\text{-}770}\text{-}I_{CFTRinh}\ \text{in CF cells}}{I_{Fsk}\text{-}I_{CFTRinh}\ \text{in non-CF cells}} \right| \times 100$$

The use of HBE cells for non-CF control and HNE cells for CF cultures in determining percent WT function was considered as a reasonable approach because CFTR function of bronchial epithelial cells are in a comparable range when compared to nasal epithelial cells (29) (**Figure S7**).

Statistical significance of treatment effects was evaluated by unpaired *t*-tests, or as otherwise stated, using Sigmaplot, version 11.0. A $p < 0.05$ was considered significant.

RESULTS

EpiX™ Expansion of Patient-Derived Nasal Epithelial Cells

Human nasal epithelial (HNE) cells were obtained from five CF patients by a non-invasive brushing procedure and expanded by the EpiX™ technology. Initial cell counts of each individual's nasal brushing sample are listed in **Table 1** and ranged from 2×10^4 to 1.2×10^6 cells. **Figure 2A** illustrates a schematic overview of the nasal cell expansion protocol. Primary nasal cells were grown to passage 1 within 2 weeks and further expanded in EpiX™ medium for ∼25 population doublings. Each patient's isolate reached 25 population doublings within 25–35 days (**Figure 2B**). Population doublings per day are shown in **Figure S1** and remained stable (between 0.5 and 1.5 doublings/day) up to 7–9 passages for each isolate. Expansion of CF nasal cells with the EpiX™ medium yielded ∼50 million cells after 26–31 days in culture. Cells from passages 3 or 4 were grown on Snapwell inserts to generate differentiated air-liquid interface (ALI) cultures that were further analyzed in Ussing chambers. The experimental design for personalized CFTR function measurements in CF patient cultures is shown in **Figure 2C**. Amiloride-sensitive sodium channel (ENaC) and calcium-activated chloride channel (CaCC) activities were included in the protocol and provided as **Supplementary Material** (**Figures S2–S6**).

Preliminary Screening of Inserts With Low Transepithelial Resistance

All experiments were subject to an initial quality control cut-off of a minimum TER of 200 Ωcm^2 before amiloride addition. This procedure allowed for selection against leaky cultures that perform poorly in Ussing chambers. All cultures from non-CF control ($n = 12$), F508del/R117H-7T ($n = 13$), R334W/406-1G>A ($n = 12$), and F508del/F508del ($n = 13$) cells passed. Eight out of 13 F508del/c.850dupA and 9 out of 17 CFTRdele2,3(21 kb) homozygous cultures passed the quality control criterion (**Figure 3**). Overall, 67 out of 80 experiments passed the quality control criterion for subsequent electrophysiologic analysis yielding a pooled success rate of 84%.

CFTR Function and Modulator Responses of Non-CF Cultures

CFTR function and responsiveness to CFTR modulators were assessed after sodium currents were blocked by amiloride. CFTR currents in presence of forskolin across non-CF cultures (17.85 ± 2.21 $\mu A/cm^2$) served as a reference standard for normal CFTR function and was set as 100%. The F508del/R117H-7T genotype served as an internal control standard to compare CFTR function measurement of the other CF genotypes to a mild CFTR function mutation.

Figure 4A shows a typical recording of an Ussing chamber experiment for the activation and block of CFTR-mediated chloride currents in non-CF cultures. **Figure 4B** shows individual responses to forskolin alone (baseline) and corresponding responses to acute stimulation by CFTR potentiator VX-770. **Figure 4C** summarizes chloride current changes in non-CF cultures. The forskolin and forskolin+VX-770-stimulated CFTR currents are plotted upward and corresponding blocked currents are plotted downward. Addition of forskolin alone stimulated CFTR currents by 16.51 ± 1.86 $\mu A/cm^2$ ($n = 12$). The forskolin-stimulated CFTR current was further increased by 15.73 ± 0.70 $\mu A/cm^2$ in response to the CFTR potentiator VX-770. Inhibition of the forskolin+VX-770 stimulated current by CFTR inhibitors yielded a blocked CFTR current (ΔI_{CFTR}) of −34.42 ± 1.77 $\mu A/cm^2$. The blocked forskolin-stimulated CFTR current averaged 17.85 ± 2.21 $\mu A/cm^2$ ($n = 12$) and was used as a reference for the physiological CFTR activity and is referred to as baseline CFTR activity in this study. CFTR activities of bronchial vs. nasal non-CF cultures as well as ENaC and CaCC activities are provided in (**Figure S7**).

CFTR Function and Modulator Responses of CF Cultures of Five CF Genotypes

CFTR function and CFTR modulator responses were further tested in CF nasal cultures according to the aforementioned experimental protocol applied to non-treated and corrector-treated CF cultures (VX-809 or VX-661, overnight). CFTR current traces are shown for each genotype in **Figures 5A–E**. Individual responses to forskolin alone (baseline) and corresponding responses to CFTR potentiator VX-770 are shown in **Figures 5F–J**. Average chloride current changes elicited by forskolin, forskolin+VX-770, and CFTR inhibitors

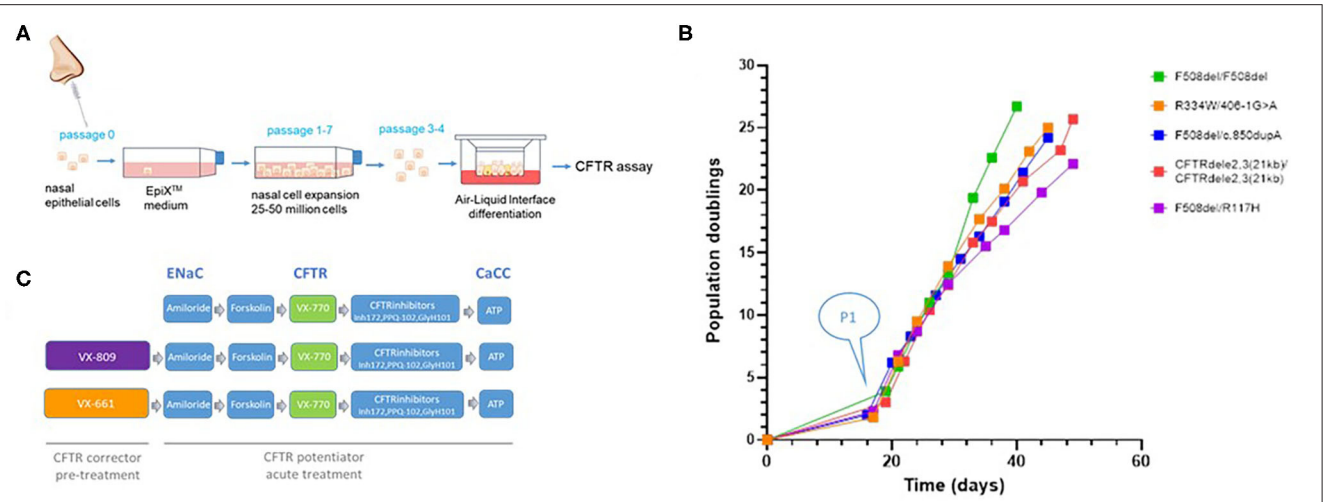

FIGURE 2 | Schematic overview of nasal cell expansion in EpiX™ medium and Ussing chamber protocol. **(A)** Nasal epithelial cells were collected from five CF individuals by nasal brushings. Isolated primary cells were expanded with the EpiX™ method in Keratinocyte-SFM medium with TGF-β inhibitor A83-01 (1 uM), Rho-Kinase inhibitor Y-27632 (5 uM), isoproterenol (3 uM), and low calcium concentrations ($CaCl_2$; 90 uM), as previously described (6). Expanded cells were plated on Snapwell inserts at Passage 3 or 4 and subsequently grown in a serum-free medium at an air-liquid interface for 21–28 days for differentiation. **(B)** Population doublings. The initial "lag phase" illustrates the number of days it took to generate CF airway cultures (passage 1) from the primary cells (P0) that were isolated from the brushed tissue sample. Each CF culture reached ∼25 population doublings within 25–35 days. **(C)** Activities for ENaC, CFTR, and CaCC were assessed in non-treated cultures and cultures treated with CFTR corrector VX-809 or VX-661. CFTR activity was assessed by measuring cAMP-dependent activation by forskolin followed by acute addition of CFTR potentiator VX-770. CFTR inhibitor compounds were used to identify CFTR-mediated chloride currents. Epithelial Na channel activity was quantified by amiloride. Calcium-activated chloride currents (CaCC) were activated by ATP and measured in the presence of CFTR inhibitors.

are summarized as bar charts for untreated, VX-809-treated, or VX-661-treated CF cultures (**Figures 5K–O**).

F508del/R117H-7T

All measured CFTR currents for all cultures in this study are summarized in **Table 2**. CF nasal cultures with the F508del/R117H-7T genotype were examined at passage 3 (PD = 8.7) and demonstrated robust stimulatory responses to both forskolin and VX-770 that were fully blocked by CFTR inhibitors (**Figure 5A**). The blocked forskolin-stimulated CFTR current, averaged 7.23 ± 1.20 μA/cm^2 ($n = 5$) corresponding to 40% WT CFTR currents under baseline conditions (no CFTR potentiator, no CFTR corrector). Maximal activation of mutant CFTR currents in F508del/R117H-7T by forskolin+VX-770 restored ∼56% of WT CFTR activity without prior CFTR corrector treatment (**Figure 5K**) and 73 and 67% after correction with VX809 and VX-661, respectively (**Table 2**). Overall, both CFTR correctors improved CFTR function of the F508del/R117H-7T genotype compared to non-treated cells.

F508del/F508del

CF nasal cultures with the F508del/F508del genotype were examined at passage 3 (PD = 10.4) and demonstrated small but detectable responses to forskolin that were slightly increased by VX-770 (**Figure 5B**, **Table 2**). Corrector treatment showed a tendency of increased current, however, statistical significance for treatment by VX-809 or VX-661 was not observed in our study (**Figure 5L**, **Table 2**).

F508del/c.850dupA

CF nasal cultures with the F508del/c.850dupA genotype were examined at passage 4 (PD = 11.6) and demonstrated a robust response to VX-770 and CFTR inhibitors blocked the stimulated currents (**Figure 5C**). In untreated cultures, currents averaged 12% of WT CFTR. Corrector-treated cultures restored up 18% of WT CFTR function and both VX-809 and VX-661 appeared equally effective (**Figure 5M**, **Table 2**).

R334W/406-1G>A

CF nasal cultures with the R334W/406-1G>A genotype were examined at passage 3 (PD = 8.7). Small currents were activated by forskolin and by VX-770 (**Figure 5D**). Uncorrected cultures showed CFTR currents of 24% of WT CFTR and currents were not significantly increased by correction (**Figure 5N**, **Table 2**).

CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)

CF nasal cultures of homozygous CFTRdele2,3(21 kb) genotype were examined at passage 3 (PD = 10.4) and demonstrated a small but detectable response to forskolin. A small increase by VX-770 was detected in three out of nine experiments. The stimulated currents were blocked by CFTR inhibitors suggesting some functional CFTR activity in this large deletion mutation (**Figure 5E**). Uncorrected cultures showed CFTR currents corresponding to 9% of WT CFTR. VX-661 correction appeared effective with an increase of current to 20% of WT CFTR, while VX-809 treatment showed insignificant effects (**Figure 5O**, **Table 2**). Because the observed effects were unexpected in this large deletion mutation, recordings of all conditions of these cultures are shown in detail in the (**Figure S6**).

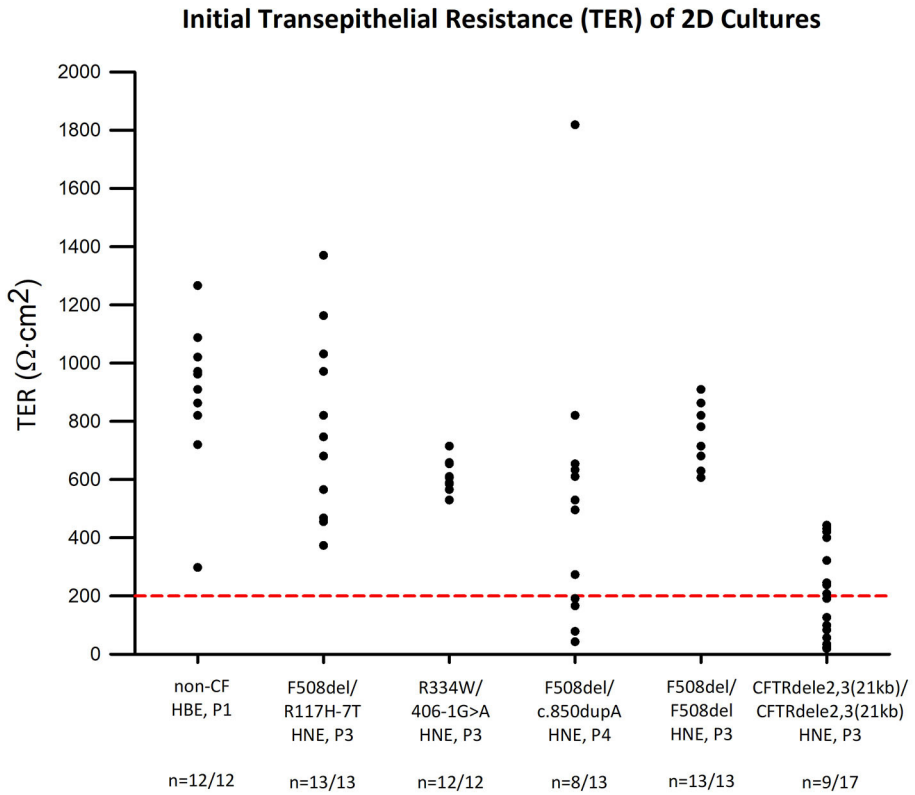

FIGURE 3 | Transepithelial electrical resistance (TER) of CF and non-CF airway cultures. TER values were obtained at the beginning of the Ussing chamber experiment and plotted for each genotype. Red line represents quality control standard set at 200 Ωcm^2. Except for F508del/c.850dupA cells ($n = 8$ of 13) and CFTRdele2,3(21 kb) homozygous cells ($n = 9$ of 17), all other cultures meet the standard and are viable for subsequent analysis.

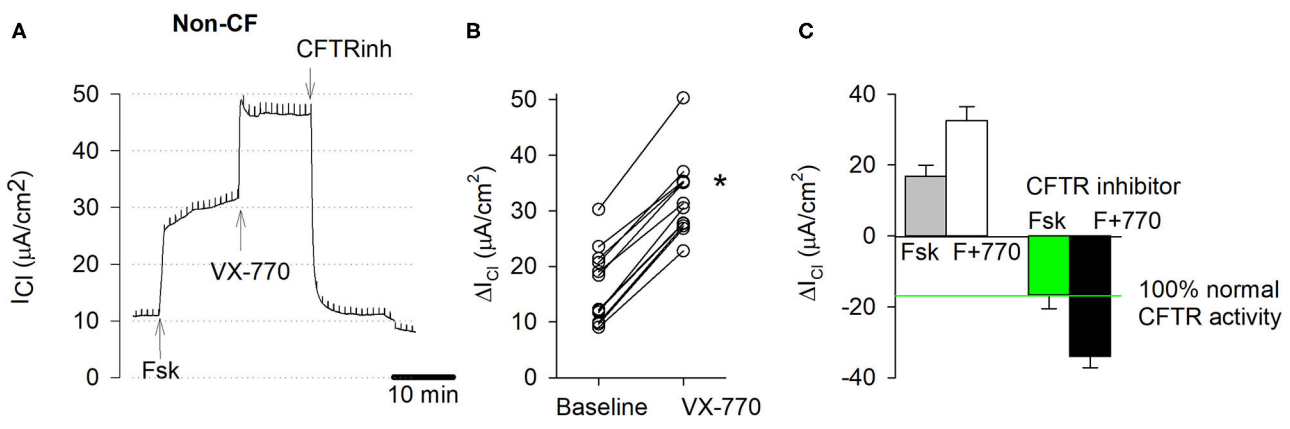

FIGURE 4 | CFTR function measurements in EpiX^{TM}-expanded non-CF cultures. Normal bronchial epithelial cells, one donor, passage 1. **(A)** Original short circuit current trace, untreated. Transepithelial chloride current (I_{Cl}) was recorded in presence of amiloride. Stimulation of wildtype CFTR currents by the cAMP agonist forskolin (20 μM, serosal) and CFTR potentiator VX-770 (1 μM, mucosal) and subsequent inhibition by CFTR inhibitors (CFTRinh-172, PPQ-102, GlyH-101; 50 μM, mucosal). Note, trace does not show initial amiloride and final ATP additions. **(B)** Individual responses. Baseline represents change in cAMP-dependent CFTR current by forskolin alone. VX-770 represents change in current by forskolin+VX-770. * denotes significant increase by VX-770 (paired t-test, $p < 0.05$, $n = 12$). **(C)** CFTR-specific currents represent change in current due to stimulation by forskolin (cAMP) and VX-770 (upward bar) and total inhibition of cAMP-stimulated and cAMP+VX-770 stimulated CFTR currents by CFTR inhibitors (downward bar). Note that 100% normal CFTR activity is defined as the blocked forskolin-stimulated CFTR activity. Bars represent mean ± SE ($n = 12$).

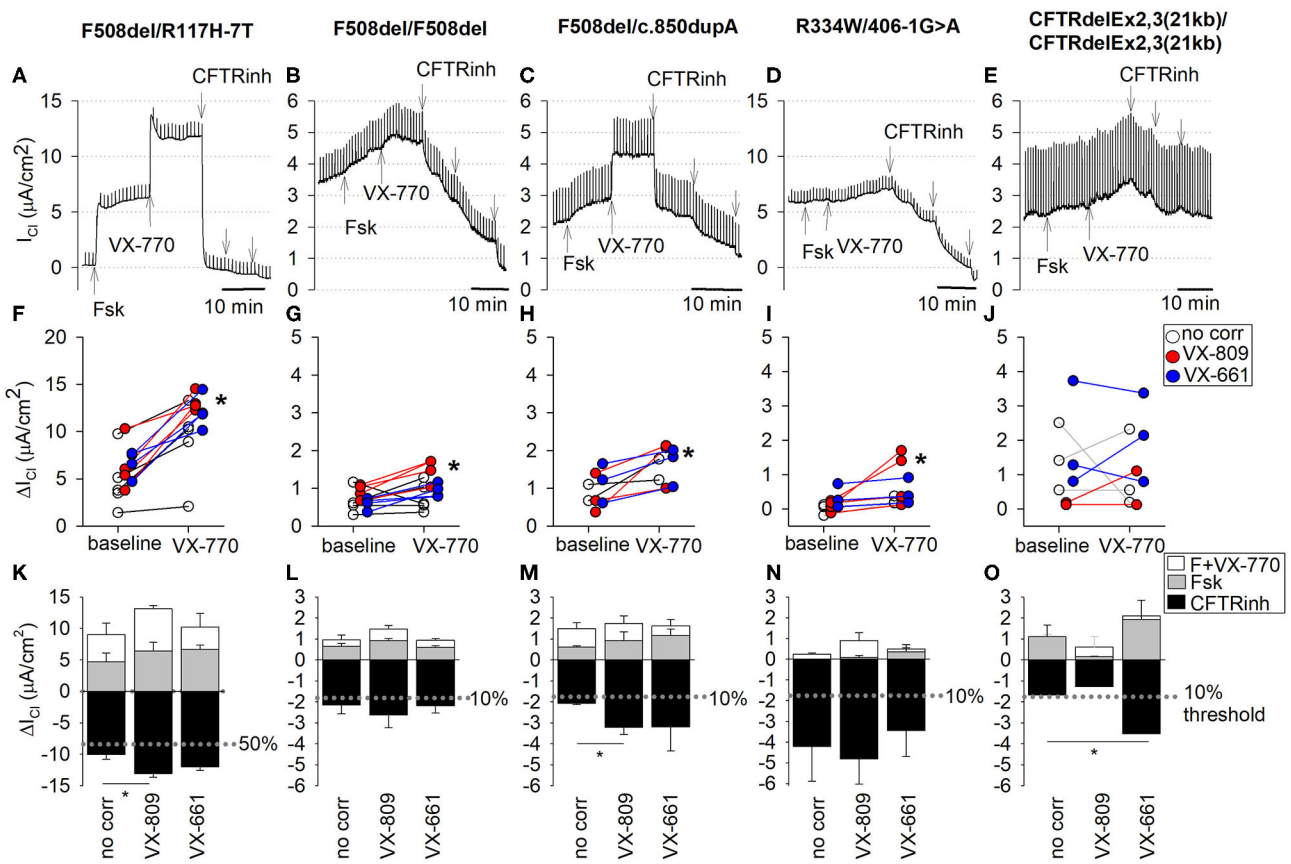

FIGURE 5 | CFTR function measurements in Epix^{TM}-expanded CF patient cultures. CF nasal epithelial cultures, five CF subjects with different genotypes. **(A–E)** *Top*. Detailed recordings of CFTR currents. Traces were from cultures treated with CFTR corrector VX-809. Chloride currents (I_{Cl}) were recorded in presence of amiloride. Note differences in scale, reflective of decreasing CFTR function with CF genotypes. CFTRinh refers to sequential addition of three CFTR inhibitor compounds (CFTRinh-172, PPQ-102, and GlyH-101). *Middle* **(F–J)**: Individual responses to forskolin (baseline) and forskolin plus VX-770 (VX-770). Baseline represents change in cAMP-dependent CFTR current by forskolin alone. VX-770 represents change in current by forskolin+VX-770. *denotes significant increase by VX-770 (paired *t*-test, $p < 0.05$). *Bottom* **(K–O)**: Average stimulated and blocked CFTR currents of non-treated cultures (no corr) and cultures treated with CFTR corrector VX-809 or VX-661. Upward bars present chloride current change (ΔI_{Cl}) after stimulation with forskolin alone (cAMP; gray) or after stimulation with forskolin plus VX-770 (white, VX-770). Downward bars present ΔI_{Cl} after maximal block by CFTR inhibitors (CFTRinh; black). Bars represent mean ± SE. *, significantly different by unpaired *t*-test ($p < 0.05$). **(A,F,K)** F508del/R117H-7T, passage 3 (PD = 8.7). Non-treated: $n = 5$; VX-809 treated: $n = 4$; VX-661 treated: $n = 4$. **(B,G,L)** R334W/406-1G>A, passage 3 (PD = 8.7). Non-treated: $n = 3$; VX-809 treated: $n = 4$; VX-661 treated: $n = 3$. **(C,H,M)** F508del/c.850dupA, passage 4 (PD = 11.6). Non-treated: $n = 2$; VX-809 treated: $n = 3$; VX-661 treated: $n = 3$. **(D,I,N)** F508del homozygote, passage 3 (PD = 10.4). Non-treated: $n = 5$; VX-809 treated: $n = 4$; VX-661 treated: $n = 4$. **(E,J,O)** CFTRdele2,3(21 kb) homozygote, passage 3 (PD = 10.4). Non-treated: $n = 4$; VX-809 treated: $n = 2$; VX-661 treated: $n = 3$.

Corroboration of Assay Results With Sweat Chloride Measurements

To relate *in vitro* CFTR function data to a clinically relevant measure, we compared our results with *in vivo* sweat chloride measurements from individual patient charts. Sweat chloride was determined after stimulation of sweat using pilocarpine iontophoresis and collected using the Wescor Macroduct coils. The official standards for sweat chloride diagnostics from the CF Foundation are: (1) *Unlikely CF*: ≤29 mM, (2) *Intermediate*: 30 mM $\leq x \leq$ 59 mM, (3) *Likely CF*: ≥ 60 mM (30). The CF individuals with genotypes CFTRdele2,3(21kb) homozygous, F508del homozygous, F508del/c.850dupA, and R334W/406-1G>A all recorded a sweat chloride concentration of >100 mM, indicating a severe CF phenotype. The F508del/R117H individual recorded a concentration of 45 mM, which was within an intermediate range (**Figure 6**). A correlation between *in vitro* CFTR function data (plotted as mean values of blocked CFTR currents obtained from non-treated nasal cultures) and clinical sweat chloride measurements was observed suggesting that the *in vitro* CFTR chloride current assay relates to the clinical sweat chloride measurement ($r = -0.95$, $p = 0.003$, Pearson correlation coefficient).

Subject-Specific Differences in ENaC and CaCC Current Measurements

To control for the expression and activities of epithelial ion channels between the five CF subjects, we compared genotype-specific activities for CFTR, ENaC, and CaCC against cultures from the subject with the F508del/R117H genotype as a reference group (**Figure 7A**). ENaC and CaCC functions

TABLE 2 | Summary of CFTR measurements in EpiX™ expanded CF nasal cultures.

Summary of CFTR Function and Modulator Responses
100% WT CFTR = −17.85±2.21 μA/cm^2 (n=12)

Genotype	Treatment	CFTR current (μA/cm^2, mean ± SE)	Normal CFTR (%)
F508del/R117H-7T	No corrector	−10.04 ± 0.69 (n = 5)	56
F508del/R117H-7T	VX-809	−13.06 ± 0.53 (n = 4)*	73
F508del/R117H-7T	VX-661	−12.01 ± 0.47 (n = 4)	67
F508del/F508del	No corrector	−2.14 ± 0.39 (n = 5)	12
F508del/F508del	VX-809	−2.63 ± 0.52 (n = 4)	15
F508del/F508del	VX-661	−2.19 ± 0.30 (n = 4)	12
F508del/c.850dupA	No corrector	−2.07 ± 0.04 (n = 2)	12
F508del/c.850dupA	VX-809	−3.21 ± 0.35 (n = 3)*	18
F508del/c.850dupA	VX-661	−3.19 ± 1.15 (n = 3)	18
R334W/406-1G>A	No corrector	−4.20 ± 1.38 (n = 3)	24
R334W/406-1G>A	VX-809	−4.81 ± 1.06 (n = 4)	27
R334W/406-1G>A	VX-661	−3.43 ± 1.03 (n = 3)	19
CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)	No corrector	−1.68 ± 0.32 (n = 4)	9
CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)	VX-809	−1.28 ± 0.05 (n = 2)	7
CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)	VX-661	−3.52 ± 0.05 (n = 3)**	20

*All CFTR currents were measured in response to stimulation by forskolin and acute addition of VX-770 in uncorrected, VX-809 corrected or VX-661 corrected cultures. CFTR current refers to blockable current. Significance for CFTR corrector treatment is marked with *($p < 0.05$) and **($p < 0.01$), determined by unpaired t-tests.*

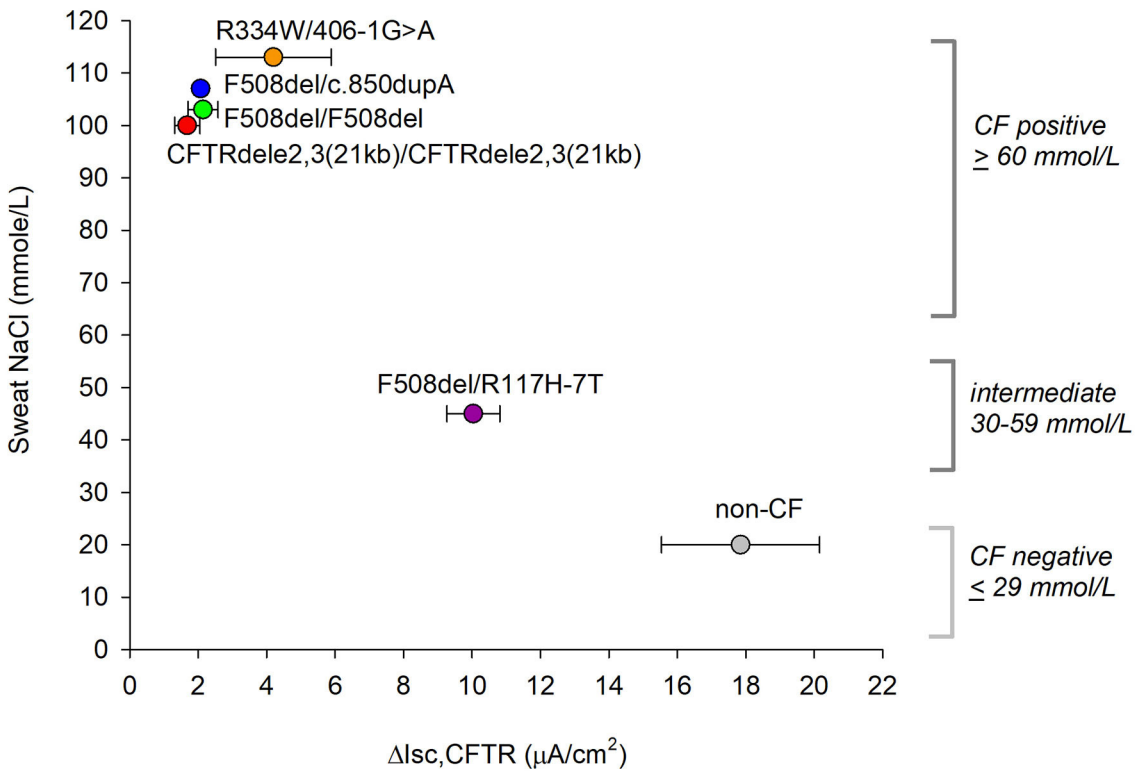

FIGURE 6 | Relation between *ex vivo* CFTR current measurements and clinical sweat chloride concentrations. Official standards for CF diagnostics based on sweat chloride are shown on the right. ΔI_{CFTR} is presented as mean ± SE values of CFTR inhibitor-sensitive current recorded in forskolin plus VX-770 stimulated cells without CFTR corrector treatment. A relation between the two measures appears significant by Pearson correlation with $r = -0.953$, $p = 0.003$, normality passed (Shapiro-Wilk), $p = 0.917$.

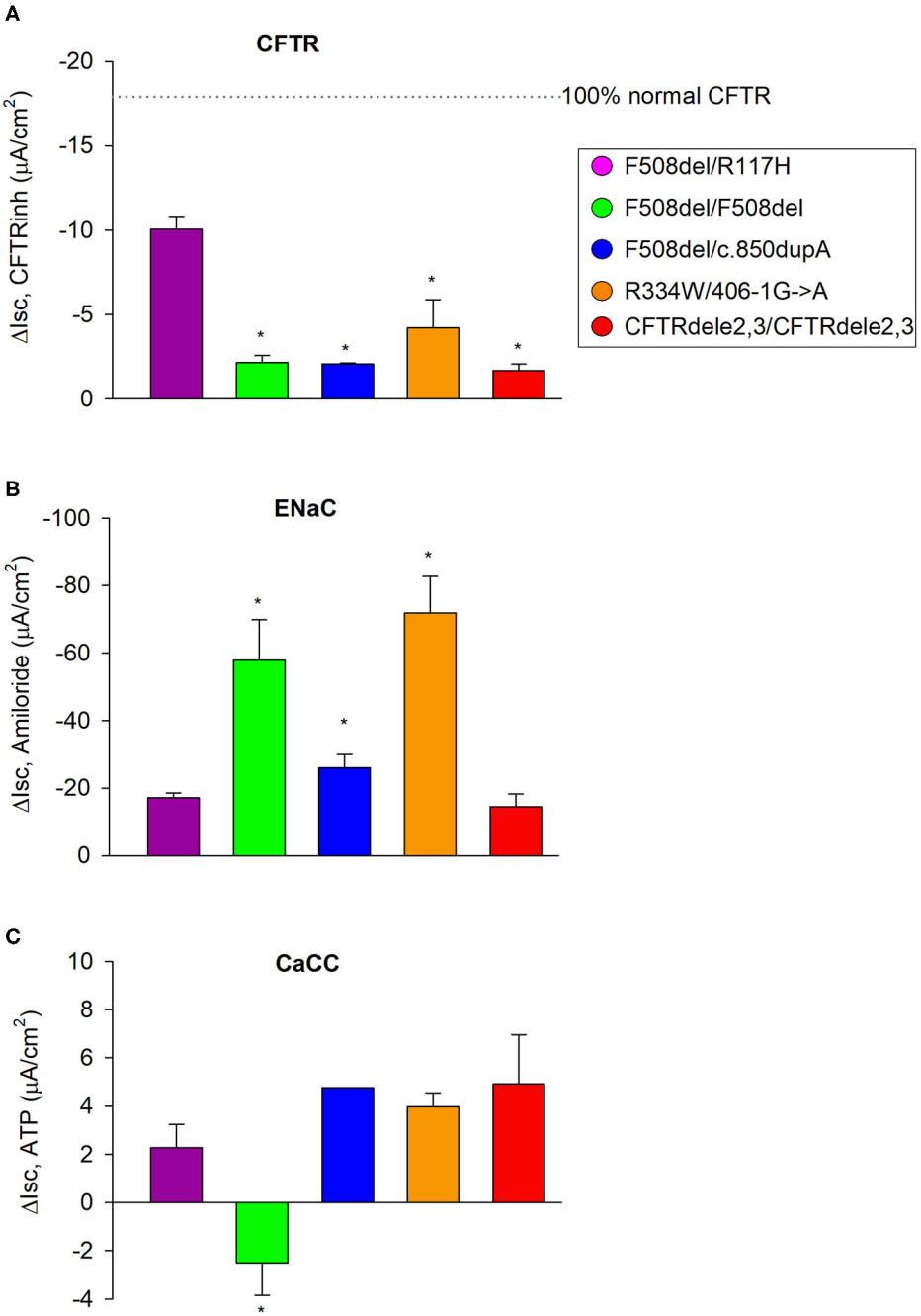

FIGURE 7 | CFTR, ENaC, and CaCC activities of EpiX™ expanded CF patient cultures. **(A)** The F508del/R117H-7H genotype was used as an internal reference to compare CFTR function among genotypes. CFTR function was quantified from the forskolin plus VX-770 stimulated current that was blockable by CFTR inhibitors and in cultures that received no CFTR corrector treatment. **(B)** Corresponding ENaC activity was quantified by measuring the change in current before and after addition of amiloride. Note that ENaC activity was significantly increased in cultures with the F508del/F508del or R334W/406-1G->A genotype. **(C)** Corresponding CaCC activity was quantified by measuring the change in current before addition of ATP and the maximal current stimulated by ATP (peak). Note that ATP induced a decrease in Isc in cultures with the F508del/F508del genotype suggesting that other conductances dominated the secretory response to ATP. All other cultures showed a chloride secretory response to ATP. Bars represent mean ± SE (n = 2–13 experiments per genotype). *denotes significantly different from F508del/R117H control group (Holm-Sidak, $p < 0.05$).

were tested by measuring the inhibition of Isc by amiloride (100 μM, to mucosa) at the beginning of the experiment and peak responses of Isc by ATP (500 μM, to mucosa) at the end of the experiment, respectively. **Figure 7B** summarizes ENaC currents among cultures from the five CF subjects. Isc decreased by −20.9 ± 1.6 μA/cm^2 in F508del/R117H-7T

cultures, -64.6 ± 4.8 μA/cm^2 in F508del homozygous cultures, -21.3 ± 1.6 μA/cm^2 in F508del/c.850dupA cultures, -75.8 ± 3.7 μA/cm^2 in R334W/406-1G>A cultures, and -13.4 ± 2.6 μA/cm^2 in CFTRdele2,3(21 kb) cultures. These data suggest that ENaC activity was significantly increased in cultures with the F508del/F508del genotype (3.1-fold) and R334W/406-1G>A genotype (3.6-fold). Prior treatment with VX-809 or VX-770 did not induce changes in ENaC activity. The effects of CFTR corrector treatment on ENaC and CaCC activities are summarized in **Supplemental Materials** for each genotype (**Figures S2**–**S6**).

ATP caused currents to increase transiently in all genotypes except in cultures with the F508del/F508del genotype where ATP caused a transient downward current (**Figure 7C**). ATP-induced CaCC activation increased currents that peaked at 2.2 ± 0.6 μA/cm^2 in F508del/R117H-7T cells, -1.4 ± 0.7 μA/cm^2 in F508del homozygous cells, 4.9 ± 1.0 μA/cm^2 in F508del/c.850dupA cells, 4.2 ± 0.5 μA/cm^2 in R334W/406-1G>A cells, and 5.2 ± 0.9 μA/cm^2 in CFTRdele2,3(21kb) cells. Comparison against the F508del/R117H-7T control group suggest that CaCC activity is similar among cultures except for the subject with the F508del/F508del that showed an inverse peak response. Assessment of ENaC and CaCC activities in non-CF cultures are shown in **Figure S7**. Non-CF HBE cells expanded by EpiX^{TM} technology were characterized by an amiloride response of -3.3 ± 0.2 μA/cm^2 and an ATP response of 8.4 ± 0.6 μA/cm^2. ENaC activity was low in both bronchial and nasal non-CF cultures when compared to CF cultures. The discrepancy may be explained by variations in the ALI differentiation media and/or by subject-specific differences among non-CF cultures.

DISCUSSION

This study reports the utility of the EpiX^{TM} cell culture technology to expand airway epithelial cells from nasal brushing samples of CF patients for *in vitro* human cell-based models to test individual responses to CFTR-directed therapeutics. Zhang et al. (7) have previously shown that EpiX^{TM} medium supports over trillion-fold of *in vitro* expansion of bronchial epithelial cells under serum-free, feeder-free culture conditions. The expanded cells can be seamlessly integrated into exciting downstream differentiation protocols and put into electrophysiological CFTR function evaluations. The present work builds on this previous work and shows that the technology is applicable to culturing cells obtained from pediatric CF patients using nasal brushings as opposed to rectal or nasal biopsies or bronchoscopy but employing a much less invasive sampling method.

Nasal epithelial cells were collected by a simple nasal brushing procedure in children as young as 3 years and included three children between the age of 3–6 years and two adolescents of 17 and 20 years. The procurement of nasal cells was less invasive compared to an earlier study in adult CF patients (8) which required a topical anesthetic to harvest a sufficient amount of cells for further cell expansion by use of 3T3 feeder-cells and Rho-kinase inhibitor, a technology also known as conditional reprogramming (14). For the present study, we decided to explore the feeder-free EpiX^{TM} technology for nasal cell expansion. Our results showed that as few as 20,000 viable cells were sufficient to enable the proliferation and supply of 50 million patient cells within 1 month. This is a remarkable finding and demonstrates a reduction in starting material by a factor that is 50–100 times less when compared to the conditional reprogramming method. Our study extends the application of the EpiX^{TM} cell culture technology from bronchial epithelial cells to nasal epithelial cells. The derivation of adequate amounts of biomaterials from a small number of basal stem cells can be readily achieved by a simple nasal brushing procedure. The nominal number of viable cells that are required from the nasal brush sample allows for a very brief nasal brushing procedure that is well-tolerated in children without the need for local anesthetics. The EpiX^{TM}-expanded patient cells were grown on cell culture inserts under air-liquid interface conditions and developed typical features of well-differentiated airway cell models including transepithelial resistance, amiloride-blockable sodium currents, CFTR and calcium-activated chloride currents.

The successful establishment of tight ALI cultures from EpiX^{TM}-expanded cells is represented here by the metric of transepithelial resistance, a measure of epithelial barrier function (**Figure 3**). However, impairments in transepithelial resistances have been repeatedly reported for CF cultures including the F508del mutation that were associated with abnormalities in tight junction formation and attributed to the lack of CFTR expression (31). Here, we provide an instance of successfully differentiating nasal epithelial cells that harbor severe CF mutations into electrically tight ALI cultures which is a critical feature for Ussing chamber studies. We found that >80% of the EpiX^{TM}-expanded CF nasal cells maintained epithelial integrity over 3 to 4 passages which is a motivating result. The need for epithelial barrier formation is a caveat for Ussing chamber studies and is in contrast to other fluorescence-based assays; however, short-circuit current measurements are superior in terms of sensitivity and ability to quantify vectorial transport of sodium and chloride ions across epithelial layers.

Our characterization of mutant CFTR function both supports and extends the standard literature on these CF variants. R117H-7T, for instance, is already established as a CF mutation with mild phenotypes when inherited with the longer 7T polymorphism (23, 24), which is supported in our characterization of an intermediate CFTR function. CFTR modulator responses for the R117H/F508del genotype are in a similar range reported by Gentzsch et al. (16).

The mild channel conductance mutation, R334W (25), is similarly characterized here as a functional variant that conserves 24% wildtype function, even when in combination with the 406-1G>A haplotype which is a severe splice mutation (26). In comparison, the two frameshift and non-sense mutations, c.850dupA and CFTRdele2,3(21 kb), are expected to show little to no CFTR function because of the anticipated absence of complete protein synthesis and processing. Our findings from CFTRdele2,3(21 kb) homozygous cultures, however, suggest the presence of a small but detectable residual CFTR on the plasma membrane, since current changes were detected upon acute exposure to CFTR-specific small molecules.

CFTRdele2,3(21 kb) is a large deletion mutation and known to cause severe CF. It was initially classified as a Class I mutation and was recently presented as an example for a class 7 mutation (no mRNA) (32), although it was shown originally that mRNA transcripts of CFTRdele2,3 were similar to wildtype (22). Here, we found that nasal epithelial cell cultures homozygous for the CFTRdele2,3(21 kb) mutation responded to VX-770 and to corrector VX-661 in the Ussing assay, which was unexpected (**Figure 5O**). Based on the disease genomics, we initially hypothesized that homozygous CFTRdele2,3(21 kb) will result in no functional CFTR protein in the plasma membrane. A cluster of acidic residues (at positions 47, 51, 54, and 58) in the amino-terminal cytoplasmic tail is deleted and therefore not available to control CFTR ion channel gating through an intramolecular interaction with the regulatory R-domain (33). However, it is not known to what degree the deleted regulatory cluster will impair the correction of CFTRdele2,3(21 kb) activity. Given that CFTRdele2,3(21 kb) produces a stop codon as early as codon 106 (22), unconventional explanations, such as the use of alternative stop codons (34, 35), may provide further insight into the mechanism of disease of CFTRdele2,3(21 kb). Through this functional characterization of CFTRdele2,3(21 kb), we provided a framework for a better understanding of the disease mechanism of CFTRdele2,3(21 kb), as the cells' response to CFTR-specific compounds suggested some localization of functional CFTR in the apical cell membrane. Alternative explanations for CFTR synthesis in CFTRdele2,3(21 kb), such as the use of downstream start codons, may therefore find support through our findings (34, 35).

This is the first electrophysiological study that examined CFTR activity in ALI cultures from a homozygous CFTRdele2,3(21 kb) patient with the Ussing assay. However, our experimental approach was limited by the inherent low transepithelial resistance of the CFTRdele2,3(21 kb) cultures and the few cultures that developed TER values $>200\ \Omega.cm^2$ (9 out of 17). The current tracings and side-by-side comparison of non-treated, VX-809- and VX-661 treated CFTRdele2,3(21 kb) cultures (**Figures S6F–H**) illustrate a small but detectable improvement of CFTRinh-blocked currents and in particular after correction with VX-661. However, based on the limitations of this study it will be important to perform future studies with a larger number of ALI cultures and to include additional assays that are not limited by low TER values (e.g., single cell patch clamp recordings, iodide influx, forskolin-induced swelling assays, immune-fluorescent staining of CFTR).

Because VX-661 and VX-770 restored a fraction of CFTR function of CFTRdele2,3(21kb), there is a possibility that clinical benefits may be reached. The newly FDA-approved triple combination, Trikafta® – an Elexacaftor (VX-445), Tezacaftor (VX-661) and Ivacaftor (VX-770) combination – is a promising candidate for CFTRdele2,3(21 kb) based on our results we obtained for the dual VX-661 and VX-770 combination. Further studies are warranted that are aimed at determining if VX-445 will also improve function for the homozygous CFTRdele2,3(21 kb) genotype. Moving forward, EpiX™ expanded nasal cells can optimize results even further by (1) testing new approved treatment options (Trikafta®) as well as next generation and investigational compounds [e.g., WX_{corr}-A23, WX_{corr}-B09 (36)], and (2) by including protein or mRNA level analyses to expand from an electrophysiological characterization to an underlying disease mechanisms of each variant. Specifically, analyzing Trikafta® responsiveness would be most important and relevant for future assays, given the drug's suitability and standard of care for the F508del variant (37).

After completion of the study, we noted the following limitations that affected the strength of our data: First, the EpiX™ -expanded non-CF cultures were of bronchial origin while the CF cultures were of nasal origin. This was a result of the limitation of procuring nasal cells from healthy children, which did not pass the risk-to-benefit determination during study planning. Second, some ALI cultures, and in particular CFTRdele2,3(21 kb) had lower resistances than others, which can affect proper voltage clamping (owing to an unfavorable ratio of the epithelial and the fluid resistance) and consequently the short-circuit current measurement; however, the magnitude of the gradient-driven chloride currents was low in amiloride-treated cultures indicating that Isc measurements were performed on CFTRdele2,3(21 kb) cultures that were not leaky (**Figure S6A**). Third, during the course of this study, VX-661 and VX-809 were the only available correctors; the newly approved corrector VX-445 might have provided additional insights and needs to be tested in a future study. Forth, the number of ALI cultures was limited to 2–3 experimental runs per group and a larger study would be needed to establish patient-specific and genotype-specific CFTR functionality and differences in CFTR modulator responses.

Clinically, our study provides an example of bed-to-bench cooperation that can help optimize treatment for all patients with cystic fibrosis. Patients with rare CFTR variants have previously been underrepresented in many large CF pharmacology trials and many have not been eligible for CFTR modulator therapy (38, 39). The development of patient-derived cell models for electrophysiologic CFTR function tests may ultimately provide a theranostic tool and help to decrease health disparities in this patient population. In light of recent advancements in personalized CF therapy (17), such models of institutional co-operation in healthcare research exemplify the feasibility of personalized medicine, especially for diseases such as CF. The improvement of *ex vivo* assays by creating patient-derived culture models can provide a theranostic tool to support the pursuit of personalized medicine and a more efficient relationship between the patient, clinician and the scientist at large.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IRB#2018-048; Institutional Review Board, UCSF

Benioff Children's Hospital Oakland. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BI, JP, and AS performed experiments. NL and EG performed cell procurement. JP and BI wrote the manuscript with critical input from CZ, BP, NL, and WF. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00536/full#supplementary-material

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REVIEW
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Current Approach in the Diagnosis and Management of Allergic Bronchopulmonary Aspergillosis in Children With Cystic Fibrosis

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Allergic bronchopulmonary aspergillosis (ABPA) is a complex pulmonary disorder characterized by a hypersensitivity reaction to *Aspergillus fumigatus*, and almost always seen in patients with cystic fibrosis (CF) and asthma. Fungal hyphae leads to an ongoing inflammation in the airways that may result in bronchiectasis, fibrosis, and eventually loss of lung function. Despite the fact that ABPA is thought to be more prevalent in CF than in asthma, the literature on ABPA in CF is more limited. The diagnosis is challenging and may be delayed because it is made based on a combination of clinical features, and radiologic and immunologic findings. With clinical deterioration of a patient with CF, ABPA is important to be kept in mind because clinical manifestations mimic pulmonary exacerbations of CF. Early diagnosis and appropriate treatment are important in preventing complications related to ABPA. Treatment modalities involve the use of anti-inflammatory agents to suppress the immune hyperreactivity and the use of antifungal agents to reduce fungal burden. Recently, in an effort to treat refractory patients or to reduce adverse effects of steroids, other treatment options such as monoclonal antibodies have started to be used. Intensive research of these new agents in the treatment of children is being conducted to address insufficient data.

Keywords: ABPA, allergic bronchopulmonary aspergillosis, aspergillus, cystic fibrosis, children

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INTRODUCTION

Aspergillus fumigatus (*A. fumigatus*) is the most common ubiquitous airborne fungus, which causes allergic bronchopulmonary aspergillosis (ABPA) (1). Aspergillus spores are found in high concentrations in nature, especially in fertile soil, decaying vegetation, swimming pool water, leaky basements, bedding, and dust from homes (2). Hypersensitivity reactions that occur because of *A. fumigatus* allergens are allergic asthma, hypersensitivity pneumonia, and ABPA (3), which is a localized hypersensitivity reaction to the lung that develops against aspergillus antigens in the colonized bronchial mucus.

The prevalence of ABPA changes according to the population (child/adult), geographic region, or diagnostic criteria that have been used. At the same time, ABPA is believed to be underdiagnosed, especially in developing countries, because its clinical features are much the same as cystic fibrosis (CF). In asthmatic patients the prevalence is reported to be about 1 to 2% and is more common in adults than in children (4, 5). The prevalence is higher in CF patients than in asthmatic patients

and thought to be 8.9% (ranged from 3 to 25%) with a significantly higher occurrence among adults (6, 7).

In this review, immunopathogenesis, clinical features, diagnosis, and current treatment modalities have been tried to be summarized. Although this review is based on the studies and case reports with the pediatric age group, some studies in adults and asthmatics have also been mentioned due to limited number of publications in children. By the way the diagnosis and treatment in children are not much different from adults and the treatment in CF is similar to asthmatics (8).

IMMUNOPATHOGENESIS OF ABPA

The pathogenesis of ABPA involves many immunologic reactions. These are Aspergillus-specific immunoglobulin (Ig)-E–mediated hypersensitivity, IgG-mediated immune complex hypersensitivity, and abnormal cell-mediated immune response (9). These hypersensitivity responses cause mucus impaction in the bronchi and bronchioles, as well as inflammatory cell infiltration in bronchial walls and peribronchial tissues. All of these reactions cause bronchiectasis and bronchocentric non-caseating granulomatosis (10, 11).

Aspergillus conidia, because of its small diameter (2–3 mm), can easily reach the pulmonary alveoli and deposit there. Once they reach the alveoli, spores germinate to produce fungal hyphae and continuously grow in the airways of patients with ABPA. *A. fumigatus* has several virulence factors to escape from the immune system including superoxide dismutases, catalases, mannitol, proteases, ribotoxin, phythiotic acid, phospholipases, gliotoxin, and hemolysin. Most of these proteins are known to be antigenic and are believed to be responsible for the immune response in ABPA. These virulence factors also damage the airway epithelium and cause a larger dose of antigenic factors to pass to the interstitial and vascular compartments. Antigenic cells with human leukocyte antigen (HLA)-DR5 or HLA-DR2 process these antigens together and present them to T lymphocytes in bronchoalveolar lymphoid tissue. In normal hosts, while the organism is eradicated with the T helper (Th)1 response, in patients with ABPA, an extreme Th2 response to the aspergillus antigen is seen, even if the Th1 response is not defective. Protease and antigen released by spores and hyphae cause activation of the innate immune system, and damage in the bronchial epithelium, which causes bronchiectasis and impaired mucociliary clearance. As a result, various chemokines including thymus and activation regulated chemokine (TARC), monocyte chemotactic protein 1, eotaxin, RANTES (regulated on activation, normal T-cell expressed, and secreted), interleukin (IL)-8, and macrophage inflammatory protein 1a are released in the airways. These cytokines activate the Th2 response and this causes the proliferation of CD4+ Th2 lymphocytes, which produce IL-4, IL-5, IL-9, IL-10, IL-13, and eosinophilic growth and survival, mast cell proliferation, IgG and IgE isotype switching occurs (9, 10).

Similarly in patients with CF, due to abnormal mucociliary clearance of secretions and defective innate immune responses, exposure to *A. fumigatus* spores results in accumulation and persistence of fungal spores within the smaller airways (12). Release of antigens, cytokins, and other virulence factors cause airway epithelial damage and antigenic factors are transmitted to the interstitial and vascular compartments (13). The immune response to ABPA in CF patients is also IL-4–mediated T helper cell (Th) type 2–predominant response, which is shown by CFTR mutant mouse expression profiling studies (14, 15).

Finally, there are some opinions about why some patients with CF develop ABPA. One of these is that the predominant CD4+ Th2 cell response can be related to genetic factors and this can explain why some patients with CF develop ABPA while others do not (16). Another conviction is that because patients with ABPA have an exaggerated response to IL-4 and produce a large amount of IgE, IgG, and IgA antibodies against *A. fumigatus* antigens, a gain of function polymorphism in the IL-4 receptor-α chain may be responsible for this situation (17). Lastly, some authors suggest that HLA-DRB1*1501 and HLA-DRB1*1503 confer the highest risk of developing ABPA, whereas HLA-DQ2 (HLA-DQB1*0201 in particular) provides relative protection against the development of ABPA (18–20).

Therefore, a combination of all these factors may determine the outcome of ABPA in patients with CF.

CLINICAL FEATURES

ABPA symptoms are usually non-specific and resemble clinical findings in CF. One-third of patients with CF are asymptomatic and are diagnosed as having ABPA in routine follow-up (7). The most common clinical findings are chronic productive cough and wheezing. Other symptoms are pleuritic chest pain and blood-stained sputum. Expectoration of golden-brownish mucus plugs is a characteristic finding in ABPA and is found in half of all patients (5, 21, 22). The dark mucus plugs are due to the increased production of tenacious mucus in the respiratory tract and consist of inflammatory cells including eosinophils, desquamated epithelial cells, and mucin (23, 24). Hemoptysis can occur due to severe airway inflammation and bronchiectasis (3). Constitutional symptoms such as low-grade fever, myalgia, and weight loss are found in 26% of patients with CF (25, 26). Physical examination is usually not noticeable except for crackles, rhonchi unresponsive to bronchodilator treatment, absence of respiratory sounds distal to dark mucus plugs, and clubbing. In end-stage disease, cor pulmonale findings may be present (4). ABPA should be suspected in a patient with CF who develops wheezing or major reductions of forced expiratory volume in one second (FEV1) without evidence of a CF exacerbation, that do not respond to appropriate antibiotics, standard physiotherapy and which is not explained by another etiology (3).

Stages

The disease can be clinically divided into five stages as shown in **Table 1** (2). Patients may be detected at any stage at the time of diagnosis and the transition from one stage to another may not be in order. It is also important to notify that ABPA serology is most likely to be positive in stage 1 and 3. Early diagnosis and treatment prevent the disease from progressing to stage 5.

TABLE 1 | Clinical and serological characteristics of ABPA stages.

	Definition	Total IgE	Precipitins	Eosinophilia	IgE- *A. fumigatus*	IgG- *A. fumigatus*	Lung infiltrates
Stage 1 (acute stage)	The patient has all the clinical and radiologic features of ABPA, responds well to oral corticosteroid therapy, and corticosteroids can be discontinued. Patient is considered in remission if improvement continues for six months	+++	+	+	+	+	+
Stage 2 (remission)	At this stage, clinical and radiologic improvement is achieved. Total IgE is at least 25% decreased. Some patients may enter remission spontaneously. Stage 2 can persist indefinitely or the disease may recur	+	±	−	±	±	−
Stage 3 (relapse)	It has all the features of stage 1. If a patient is on routine follow-up, at least a doubling in serum IgE level with new infiltrations on chest radiography is seen	+++	+	+	+	+	+
Stage 4 (steroid-dependent stage)	The patient receives long-term high-dose systemic steroid therapy. When the steroid dose is tried to be reduced and stopped, it relapses	++	±	±	±	±	−
Stage 5 (end-stage lung disease)	Diffuse bronchiectasis, fibrosis, cor pulmonale has developed. Serum total IgE level can be normal or elevated	+	±	−	±	±	−

ABPA, allergic bronchopulmonary aspergillosis; A. fumigatus, Aspergillus fumigatus, IgE, immunoglobulin E, IgG, immunoglobulin G.

DIAGNOSIS

The diagnosis of ABPA in patients with CF is challenging and may be delayed because many of the diagnostic criteria crossover with the clinical manifestations of CF. There is no fully covered individual test that demonstrates the diagnosis of ABPA in patients with CF. The diagnosis is made through a combination of clinical characteristics, and radiologic and immunologic findings (27). There are different sets of diagnostic criteria for the diagnosis of ABPA in patients with CF. The diagnostic criteria are summarized in **Table 2** according to historical date (28–31).

According to Cystic Fibrosis Foundation Consensus Conference recommendations (2003), which is the most common used definition for diagnosis (3):

Classic Case

1. Clinical deterioration, acute or subacute, which is unexplained by another etiology;
2. Serum total IgE concentration of >1,000 IU·mL−1 (unless the patient is receiving systemic corticosteroids);
3. Immediate cutaneous reactivity (skin prick test wheal >3 mm in diameter with surrounding erythema) to Aspergillus or *in vitro* presence of serum IgE antibody to A. fumigatus;
4. Precipitating antibodies to A. fumigatus or serum IgG antibody to A. fumigatus in an *in vitro* test;
5. New or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest computed tomography (CT) (bronchiectasis) that do not respond to appropriate antibiotics and standard physiotherapy.

Minimal Diagnostic Criteria

1. Clinical deterioration, acute or subacute, which is unexplained by another etiology;
2. Serum total IgE concentration of >500 IU·mL−1. If total IgE level is 200–500 and ABPA is suspected, repeat testing in 1–3 months;
3. Immediate cutaneous reactivity to Aspergillus (skin prick test wheal >3 mm in diameter with surrounding erythema while the patient is not being treated with systemic antihistamines) or *in vitro* presence of IgE antibody to A. fumigatus;
4. One of the following: (a) precipitins to A. fumigatus or *in vitro* demonstration of IgG antibody to A. fumigatus; or (b) new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (bronchiectasis) that do not respond to appropriate antibiotics and standard physiotherapy.

The suggestions of Cystic Fibrosis Foundation Consensus Conference for screening ABPA in CF (3):

1. Patients >6 years of age should be considered suspicious for ABPA.
2. Patients should be checked for serum total IgE concentration annually. If the serum total IgE concentration is >500 IU·mL−1, get immediate cutaneous reactivity to A. fumigatus or use an *in vitro* test for IgE antibody to A. fumigatus. If results are positive, consider diagnosis on the basis of minimal criteria;
3. If the serum total IgE concentration is 200–500 IU·mL−1, repeat the test in 1–3 months if there is a suspicion for ABPA.

TABLE 2 | History of acceptable criteria for diagnosing ABPA.

Epidemiologic Study of Cystic Fibrosis recommendations, 1999

Two of the following 3 criteria are required

- Immediate cutaneous reactivity to *A. fumigatus*
- Precipitating antibodies to *A. fumigatus*
- Serum total IgE of >1,000 IU/mL.

In addition, at least 2 of the following are required

- Bronchoconstriction
- Peripheral blood eosinophil count >1,000 mL
- History of pulmonary infiltrates
- Elevated serum anti-*A. fumigatus* IgE or IgG
- *A. fumigatus* in sputum found by smear or culture
- Response to steroids treatment

The UK Cystic Fibrosis Trust recommendations, 2002

- Asthma symptoms
- New chest radiography changes
- Serum total IgE >500 IU/mL or four-fold increase in IgE titers
- Raised specific IgE aspergillus RAST or positive skin prick test to *A. fumigatus*
- Blood eosinophil count >500/mm^3
- Positive Aspergillus culture in sputum or fungal hyphae

Cystic Fibrosis Foundation Consensus Conference recommendations, 2003

Classic case

- Acute or subacute clinical deterioration that is not attributable to another etiology
- A serum total IgE level of >1,000 IU/mL (unless patient is receiving systemic steroids)
- Presence of IgE antibodies to *A. fumigatus in vitro* or immediate cutaneous hypersensitivity to Aspergillus (skin test >3 mm)
- Precipitating antibodies to *A. fumigatus* or serum IgG antibody to *A. fumigatus* by an *in vitro* test
- New or recent abnormalities on chest radiography or computed tomography that do not respond to antibiotics and standard physiotherapy

Minimal diagnostic criteria

- Acute or subacute clinical deterioration that is not attributable to another etiology
- A serum total IgE level of >500 IU/mL (unless patient is receiving systemic steroids)
- Immediate cutaneous hypersensitivity to Aspergillus (skin test >3 mm) or presence of IgE antibodies to *A. Fumigatus*

Plus one of the following

- Precipitins to *A. fumigatus* or IgG antibody to *A. fumigatus in vitro*
- New or recent infiltrates (or mucus plugging) on chest radiography or computed tomography that do not respond to antibiotics and standard physiotherapy

American Academy of Allergy, Asthma & Immunology Committee Report, 2012

Minimum essential criteria

- Asthma or cystic fibrosis
- Immediate cutaneous reactivity on skin-prick testing
- Elevated serum total IgE level (>1,000 ng/mL)

Plus one or both of the following

- Elevated serum IgE and IgG levels to *A. fumigatus* (at least twice asthma controls)
- Proximal (central) bronchiectasis on radiography (inner two-thirds of lung on computed tomography)

International Society for Human and Animal Mycology Working Group, 2013

Predisposing conditions

Asthma or cystic fibrosis

Obligatory criteria (both must be present)

- Aspergillus skin test positivity or elevated IgE levels against *A. fumigatus*
- Elevated total IgE concentration (typically >1,000 IU/mL)

Other criteria (at least 2 must be present)

- Precipitating serum antibodies to *A. fumigatus* or elevated serum Aspergillus IgG by immunoassay
- Radiographic pulmonary opacities consistent with ABPA
- Total eosinophil count of >500 cells/mL in patients who are steroid naive (may be historical)

ABPA, allergic bronchopulmonary aspergillosis; A. fumigatus, Aspergillus fumigatus; IgE, immunoglobulin E; IgG, immunoglobulin; RAST, radioallergosorbent test.

The diagnostic algorithm created with the criteria suggested by the Cystic Fibrosis Foundation is showed in **Figure 1**.

Although there are many diagnostic criteria for ABPA, Maleki et al. showed that there was no significant differences on the reported rate of ABPA prevelance between the Cystic Fibrosis Foundation and International Society for Human and Animal Mycology (ISHAM) diagnostic criteria (32).

Clinical Findings

Acute/subacute clinical worsening defined as cough, increased amount of sputum or changing in color of sputum, wheeze, dyspnea, the onset of new fever, weight loss, exercise-induced asthma and decrease in pulmonary function, that does not respond to appropriate treatment and is not explained with another etiology (3).

Serum Total IgE

This can be used for the detection of fungal sensitization (33). Values of total IgE as high as >500 IU·mL^{-1} (34) or even >1,000 IU·mL^{-1} (35) or >2-fold rise from baseline total IgE have been suggested as diagnostic markers unless the patient is receiving systemic corticosteroids. If the patient is using systemic steroids, retesting is recommended after the completion of steroid treatment (3). Irregular changes in IgE values with clinical symptoms can be a marker for exacerbations and responses to therapy. In patients with ABPA, despite total serum IgE levels often being in concordance with clinical activity and treatment, it is not sufficiently specific for the diagnosis (29, 36, 37).

Aspergillus Skin Test

Another investigation for the detection of sensitization to *A. fumigatus* is the Aspergillus skin test, which shows immediate cutaneous hypersensitivity to *A. fumigatus* (38). However, it shows heterogenity among different centers in terms of procedures, interpretation, and the use of different commercial fungal preparations (39). A skin prick test should be performed for Aspergillus skin testing; if the results are negative it should be confirmed by an intradermal test. Intradermal skin tests are usually preferred for the diagnosis of Aspergillus sensitization because they are more sensitive than the skin prick test (40).

Both type I (immediate) and type III (delayed) skin sensitivity with different Aspergillus antigens can be positive in patients with ABPA. Although type III responses are mainly suppressed by steroid treatment, there is little or no effect of steroids on the type I reactions. For the wheal of immediate skin sensitivity, the authorities have suggested a diameter of surrounding erythema >3 mm to be considered as a positive result (3). Aspergillus skin test is sensitive enough that the lack of a positive skin test reduces the likelihood of ABPA diagnosis; however, the specificity of the test is moderately low, which means it can be positive in patients with CF without ABPA (41). Therefore, a positive Aspergillus skin test must always be followed up with serologic and radiologic testing to confirm ABPA.

Serum Specific IgE to *A. fumigatus*

This is not an appropriate biomarker and lacks sufficient specificity for ABPA because nearly 35% to 50% of patients with CF demonstrate detectable sensitization to *A. fumigatus* despite

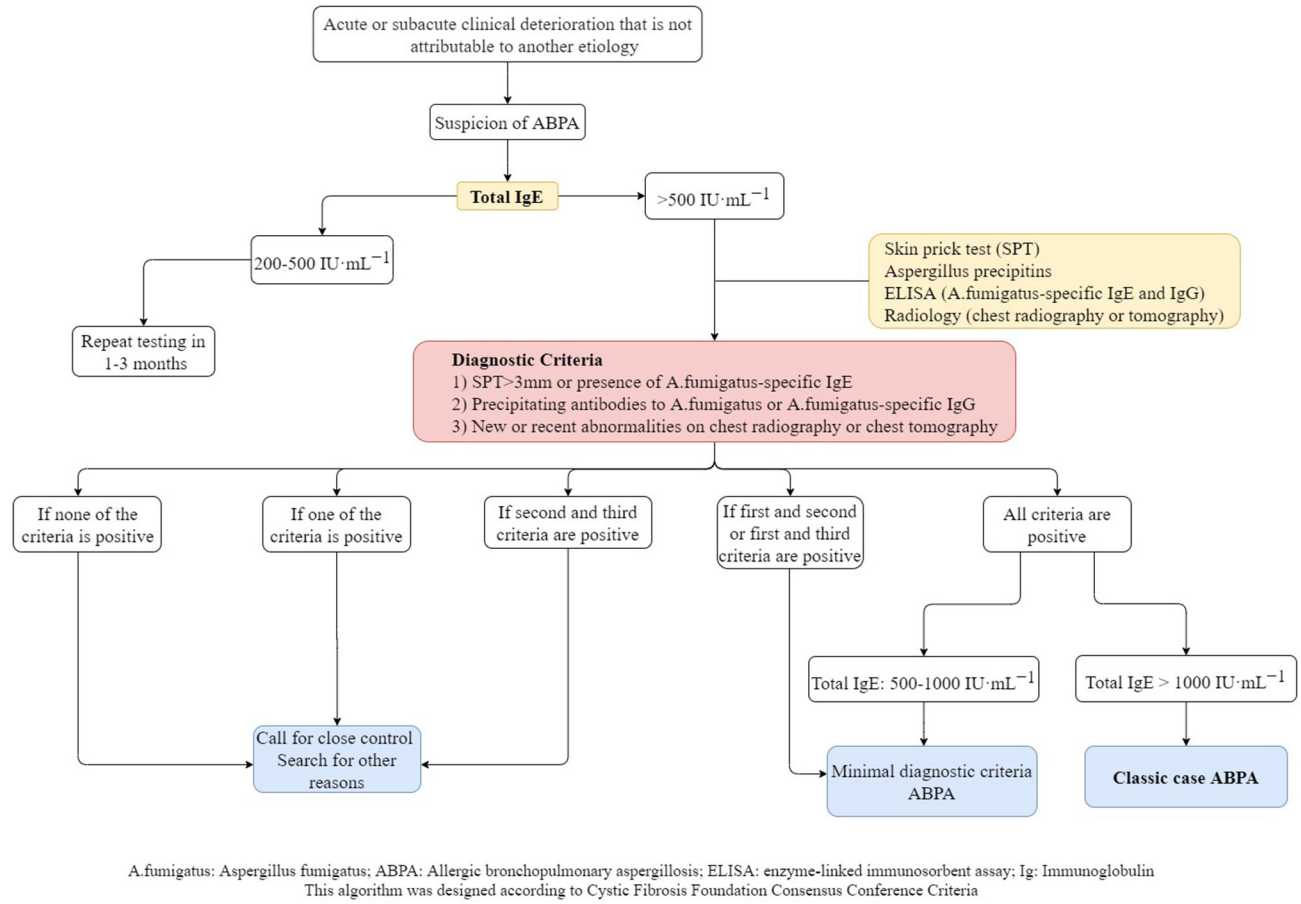

FIGURE 1 | Diagnostic algorithm of ABPA in CF.

not having ABPA. However, elevated specific IgE to *A. fumigatus* is a more sensitive marker than total IgE like the aspergillus skin test for ABPA in CF (38, 39, 42, 43). In 2013, ISHAM accepted 0.35 kUA·L^{-1} as a cut-off value for anti-Aspergillus sensitization and this value was changed and accepted as 0.10 kUA·L^{-1} by the US Food and Drug Administration (FDA) in 2008 and recommended by a 2015 consensus guideline (29, 39). The level of specific IgE to *A. fumigatus* can act as a marker of an exacerbation or remission like total IgE.

Precipitating Antibodies or Serum IgG Antibody to *A. fumigatus*

There are few methods to evaluate the *A. fumigatus* precipitating antibody or specific IgG to the crude antigen. These antibodies, have been found to be a sensitive sign for ABPA in patients with CF. Precipitating antibodies to *A. fumigatus* are usually in the IgG isotype, in particular of the IgG1, IgG2, and IgG4 subclasses, and their increased levels have been reported in patients with CF and ABPA (44, 45).

Traditionally, IgG antibodies against *A. fumigatus* were measured by immunoprecipitation and counterimmunoelectrophoresis (CIE) using double gel diffusion techniques and called *Aspergillus* precipitins (46–48). Because of poor sensitivity and subjective qualitative results of these methods, commercial ImmunoCAP systems using enzyme-linked immunosorbant assays (ELISA) have been developed and this method has increased the detection of cases with ABPA. Recently, this commercial system is the most widely used method for quantifying specific IgG. The results of few studies also suggest that *A. fumigatus* specific IgG measured by the ImmunoCAP system is more sensitive than *Aspergillus* precipitins measured by the double diffusion method (47, 48). The best cutoff value for *A. fumigatus* specific IgG using ImmunoCap system has been reported as 26.9 mgA/L with 88% sensitivity and 100% specificity. Although the sensitivity of *A. fumigatus* specific IgG detected by double gel diffusion technique has been reported low as 27%; the sensitivity of commercial ImmunoCAP systems was 89% in the diagnosis of ABPA (47). However, the cutoff values have been varied in patients with CF within different studies (47–50). The prevalence of serum IgG antibodies to *A. fumigatus* has been reported to increase with age in patients with CF, regardless of ABPA (51). *Aspergillus* precipitins may represent previous exposure, but high levels of precipitins may indicate increased probability of ABPA (31). *A. fumigatus*-specific IgG levels may suggest disease activity, which can be evidenced

by radiographic changes and clinical exacerbations, whereas serum precipitins do not reflect disease activity in most cases (1).

Peripheral Blood Eosinophilia

Peripheral blood eosinophil counts of >1,000 cell/ μL were previously considered as a major criteria for the diagnosis of ABPA (52, 53). However, now it is known to be of limited value in diagnosing patients with CF and ABPA because high eosinophil counts may be present due to chronic *Pseudomonas aeruginosa* infection (3) and many other disorders other than ABPA (54, 55). In a recent study on children with CF reported that 75% of ABPA patients had eosinophil count >400 cells/μL and 40% of them having counts >1,000 cells/μL, while none of the patients in the *A. fumigatus* sensitized and non-sensitized groups had eosinophilia and these findings suggested that eosinophil count could be a specific biomarker for ABPA in children (56).

New Serologic Tests

Specific IgE Antibodies Against Recombinant A. fumigatus Allergens

Recombinant allergens are raw extracts from *A. fumigatus* which uses for immunological assays of ABPA. There are recognized 23 specific allergens of *A. fumigatus* but five of them (rAsp f1, f2, f3, f4, and f6) are commercially available (57). However, those allergens can cross-react with other fungal antigens. Due to rAsp f1 and f2 have minimal cross-reactivity with other fungal antigens, they are considered as the specific allergens for *A. fumigatus* (58). Specific IgE against a combination of rAsp f1 or f3 found to be the most sensitive (97%) and specific IgE against a combination of rAsp f4 or f6 had highest specificity (99%) for diagnosing ABPA in patients with asthma (57). Some authors suggested combining serum total IgE with specific IgE to recombinant *A. fumigatus* allergen (rAspf) to differentiate ABPA from sensitization (59). A recent study reported that IgE against rAsp f1 and f2 were found to be the most useful in differentiating ABPA from *A. fumigatus* sensitization in patients with asthma (60).

Thymus and Activation-Regulated Chemokine (TARC)

TARC is produced as a result of the antifungal immune response. The serum levels of TARC are found to be increased in patients with CF and ABPA (61). TARC was found to be a more sensitive and specific marker of ABPA when it was compared with other serum markers. TARC levels are increased even before the development of clinical features of ABPA and before total IgE increment (59). TARC stays elevated for a prolonged period of time; therefore, the changes in TARC levels can be a sign of exacerbations and remissions of ABPA (59, 61). However, it has not been added to classic case definitions.

Cellular Allergen Stimulation Test (CAST)

CAST measures cysteinyl-leukotrienes, which are produced *in vitro* by allergen-stimulated basophils. CAST is used for the diagnosis of allergic and pseudoallergic reactions. CAST has a high sensitivity (100%) but a low specificity (74%) for ABPA. The combination of a positive CAST, serum total IgE >500 IU·mL^{-1}, and positive IgE antibodies against rAsp f4 and f6 was found only in those with ABPA, giving rise to 100% specificity (62).

Further studies with a larger number of patients are required to investigate CAST and TARC before they become routine investigations for ABPA.

Basophil Activation Test (BAT)

BAT is an *in vitro* flow cytometry-based cellular assay that measures the activation of basophils with Ig-E mediated mechanism and using stimulation with *A. fumigatus* extract and CD63, CD193, and CD203c as activation surface markers which they found to have high diagnostic accuracy for ABPA. This test should be performed within the four hour of blood collection to increase viability and functionality of basophils; because basophil reactivity decreases over time (63). Different studies suggest that BAT is a useful, reliable diagnostic tool especially for ABPA in patients with CF (64, 65) and it is useful in distinguishing ABPA from *Aspergillus* colonization and sensitization in patients with CF (66, 67) but not in patients with asthma (68). However, BAT needs a flow cytometer and the requirement of performing this test immediately after the collection of blood sample limit its usability.

Culture of Sputum for *A. fumigatus*

Sputum culture is a supportive marker for ABPA (43, 69), whereas others have considered it as only a minor criterion because *A. fumigatus* hyphae in sputum smears or *A. fumigatus* in sputum cultures may not be detected in ABPA or can also be seen in other pulmonary diseases (70, 71). In ABPA, rates of culture positivity were reported as 40% to 60% in different studies (72). Also, *A. fumigatus* culture-negative patients with ABPA were found to have *A. fumigatus* DNA in their sputum (73). Aspergillus polymerase chain reaction (PCR) is more sensitive than culture in ABPA diagnosis and it may be used to monitor the efficacy of antifungal therapy (72). Both real-time Aspergillus PCR and galactomannan in respiratory samples have also been used for enhanced recognition of ABPA in CF with traditional immunological tests (serum total IgE, *A. fumigatus*-specific IgE and IgG) (74).

Pulmonary Function Tests

In mild or early stages of ABPA, partially reversible airflow obstruction is a common pulmonary function test finding. Prolonged airflow obstruction and decreased lung volumes in total lung capacity (TLC), vital capacity (VC), and FEV1 suggest interstitial changes in progressive disease (75, 76). The diffusing capacity of lung for carbon monoxide (DLCO) may be decreased during an exacerbation and it remains low at the end stage of ABPA (5). Despite pulmonary function tests not being diagnostic for ABPA, they are useful during follow-up to monitor improvement.

Bronchoscopy

Bronchoscopic evaluation and histology are not necessary for the diagnosis of ABPA and bronchoscopy may be performed in patients with ABPA when the diagnosis is unclear. Bronchoalveolar lavage (BAL) shows elevated levels of IgA,

IgG, IgM, and IgE, as well as elevated eosinophil counts. However, the sensitivity of staining BAL washes or sputum samples for Aspergillus is poor. Detecting Aspergillus species in BAL is not specific for active disease of ABPA because it may reflect colonization (75, 77).

Interpretation of diagnostic findings of ABPA are summarized in **Table 3**.

Radiologic Manifestations of ABPA

Both chest radiography and chest CT are useful for the diagnosis of ABPA. Radiologic findings are summarized in **Table 4**.

Chest radiography has 50% sensitivity for the diagnosis of ABPA. It can be normal in the early stages but temporary or permanent parenchymal opacities can also be seen. Parenchymal infiltrate and bronchiectasis are mostly in the upper lobes; however, all lobes may be affected (40). Central bronchiectasis is one of the hallmarks of ABPA, and bronchiectasis affecting more than three lobes is highly suggestive of diagnosis (78, 79). A massive homogeneous shadow without fissure displacement usually located in the upper and middle lobes that frequently shifts from one side to another is the most common abnormality seen on a chest radiography with ABPA. The shadow may be patchy, triangular, oblong, or lobar (6). "Ring sign" indicating bronchial inflammation with or without plugs can be a sign of bronchial wall thickening or bronchiectasis (80). "Tramline" shadows and "finger-in-glove" opacities are temporary findings that indicate bronchial wall edema and thickening, whereas once the mucus plug is expectorated, it can remain as permanent parallel line shadows (5).

High-resolution CT (HRCT) of the lungs is more sensitive for detecting bronchiectasis distribution and other abnormalities that cannot be detected in chest radiography (40). The findings of ABPA on chest HRCT include centrilobular nodules, central bronchiectasis often with mucoid impaction "tree-in-bud" pattern, mosaic attenuation, fibrosis, and cavitation (81). Bronchiectasis seen in ABPA is typically central (bronchiectasis that involves two-thirds of the central part of the lung parenchyma) but peripheral bronchiectasis is not infrequent. Central or cystic varicose bronchiectasis, infiltrations that completely resolve with corticosteroid treatment, and mucus plugs are common in CF and ABPA, high-attenuation mucus (HAM) has been reported in 28% of patients with ABPA on HRCT. Mucoid impaction causes "toothpaste" shadows or "gloved-finger" shadows (75, 81, 82). HAM is the name given to mucus that appears denser than skeletal muscles (radiodensity of >70 Hounsfield units). Mucus plugs may cause segmental, lobar or total atelectasis. HAM and bronchiectasis are indicators of serious disease and recurrent exacerbations. Also, the presence of bronchiectasis makes it difficult to enter remission (83). In the later stages, pneumothorax might be seen in patients who develop

TABLE 3 | Interpretation of diagnostic findings of ABPA.

Investigation	Result	Interpretation
Serum total IgE levels	Normal	Exclude ABPA
	>500–1,000 IU/mL	Consider ABPA
Aspergillus skin test	Type 1 reaction	ABPA characteristic
	Type 3 reaction	ABPA characteristic, Immune complex hypersensitivity reaction, suggest fungal hypersensitivity
Serum specific IgE to *A. fumigatus*	Elevated IgE levels	Consider ABPA or Aspergillus hypersensitivity
Serum precipitins (IgG) against *A. fumigatus*	IgG antibodies present	Supportive, not diagnostic
Peripheral eosinophilia	Elevated	Supportive, not diagnostic
Sputum culture	Presence of *A. fumigatus*	Supportive of ABPA Seen in ≤ 50% patients
Aspergillus PCR	Presence of Aspergillus DNA	Supportive, not diagnostic Consider also Aspergillus hypersensitivity, Aspergillus bronchitis, colonization
Pulmonary function tests	Typical obstructive findings	No role in diagnosis Can assess severity, improvement
Bronchoscopy	Elevated eosinophil count and levels of IgA, IgG, IgM, and IgE	Unclear for ABPA diagnosis Not necessary for ABPA diagnosis

ABPA, allergic bronchopulmonary aspergillosis; A. fumigatus, Aspergillus fumigatus; Ig, immunoglobulin; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction.

TABLE 4 | Radiologic findings in ABPA.

Chest radiography	Computed tomography
Transient changes	**Common**
Common	Central bronchiectasis
Patchy areas of consolidation	Mucus plugging with bronchoceles
Radiologic infiltrates, due to mucoid impaction in dilated bronchi;	Consolidation
• Toothpaste shadows	Non-homogeneous patchy opacities
• Gloved finger shadows	Centrilobular nodules with tree-in-bud opacities
Collapse; segmental or lobar	Bronchial wall thickening
Uncommon	Areas of atelectasis
Bronchial wall thickening;	Cavitation
• Tramline shadows	Mosaic perfusion with air trapping on expiration
Air-fluid levels from dilated central bronchi filled with fluid	**Uncommon**
Perihilar infiltrates simulating adenopathy	High-attenuation mucus (most helpful finding in differential diagnosis)
Massive consolidation: unilateral or bilateral	Pleural involvement
Small nodules	Randomly scattered nodular opacities
Pleural effusions	
Permanent changes	
Common	
Parallel-line shadows representing bronchial widening	
Ring-shadows 1–2 cm in diameter representing dilated bronchi en face	
Pulmonary fibrosis: fibrotic scarred upper lobes with cavitation	
Uncommon	
Pleural thickening	
Mycetoma formation	
Linear scars	

ABPA, allergic bronchopulmonary aspergillosis.

pulmonary fibrosis (84). Pleural thickening is seen in ABPA and in advanced CF (85, 86). Radiographic findings in ABPA are demonstrated in **Figure 2**.

Chest HRCT findings in ABPA may correlate with the immunologic severity. Agarwal et al. reported that the presence of HAM plugs was more consistent with serologic severity and recurrent relapses (75, 83). ABPA can be classified radiologically based on clinical and HRCT findings (83) (**Table 5**).

Magnetic resonance imaging (MRI) has not been traditionally used for the evaluation of lung parenchyma due to the low proton density of the tissue and high susceptibility of artifacts. Recently with the major technological advancements; MRI has been used in the diagnosis of respiratory pathologies including ABPA. In the diagnosis of ABPA; MRI demonstrated high specificity and positive predictive value; but less sensitivity and negative predictive value compared with the HRCT scan in children (87). The match of HAM on MRI seems to be inverted mucus impaction, which is characterized by a high signal intensity on T1-weighted images and low signal intensity on T2-weighted images (87, 88). However; MRI is not being used as a routine clinical practice of patients with ABPA and there is a need for new clinical investigations to use MRI in the diagnosis of ABPA.

The differences and similarities of clinical features and diagnostic criteria of ABPA in patients with CF or asthma are summarized in **Table 6**.

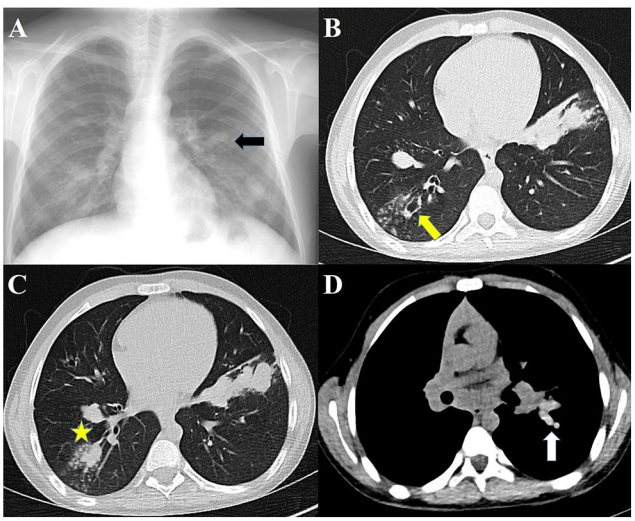

FIGURE 2 | Radiographs of patients with ABPA. **(A)** Chest radiograph showing finger-in-glove sign, **(B)** HRCT showing central bronchiectasis, **(C)** HRCT showing mucus plugging in dilated bronchi, **(D)** HRCT showing high-attenuation mucus.

TABLE 5 | Radiologic classification of ABPA based on clinical and HRCT findings.

ABPA-S (Serologic ABPA)	ABPA without any radiologic findings on HRCT of the thorax
ABPA-B (ABPA- Bronchiectasis)	ABPA including bronchiectasis on chest HRCT
ABPA-HAM (ABPA-High-attenuation mucus)	ABPA including HAM on chest HRCT
ABPA-CPF (ABPA- Chronic pleuropulmonary fibrosis)	ABPA with two or more radiologic features suggestive of fibrosis (including fibrocavitary lesions, pulmonary fibrosis, pleural thickening) without the presence of mucoid impaction (or HAM)

ABPA, allergic bronchopulmonary aspergillosis, HAM, high-attenuation mucus.

TREATMENT

The principles in the treatment of ABPA include the use of anti-inflammatory agents to suppress the immune hyperreactivity and the use of antifungal agents to attenuate immune hyperresponsiveness by reducing the fungal burden in the airways (89). There are several goals of treatment such as treating the acute stage of ABPA, controlling symptoms of CF, preventing or treating pulmonary exacerbations of ABPA, and reducing progression to end-stage disease. Inadequate and delayed treatment can lead to complications such as pulmonary fibrosis, bronchiectasis, and loss of lung function (90). At the same time, treatment options should also have minimal or no adverse reactions.

Treatment of ABPA in CF is not much different from ABPA in asthma, and involves the use of corticosteroids, antifungal agents, and monoclonal antibodies, mainly prednisolone, itraconazole, and omalizumab, respectively. The doses, side effects and important comments of administration of the drugs used in the treatment of ABPA are summarized in the **Table 7**.

TABLE 6 | Similarities and differences of ABPA in patients with cystic fibrosis or asthma.

	ABPA in CF	ABPA in Asthma
Childhood onset	Common	Uncommon
Sex	Male/Female=1	Male/Female=1
Clinic	Pulmonary exacerbation of CF	Worsening of asthmatic symptoms
Mucus Production	Increased, brown-black	New, brown-black
Clubbing	Common	Rare
Eosinophilia	Uncommon	Common
Total IgE >1,000 IU/mL	+	+
Specific serologic test	rAsp f6 specific IgE	Combination of rAsp f4 and f6 specific IgE
Aspergillus skin prick test	+	+
Concomitant bacterial infections	Common	Uncommon
Bronchiectasis	Central, but generally extensive	Central
Transient pulmonary opacities	+	+
High attenuation mucus plugs on chest CT	+	+

ABPA, allergic bronchopulmonary aspergillosis; CF, cystic fibrosis; IgE, immunoglobulin E; rAsp, recombinant Aspergillus fumigatus antigens.

TABLE 7 | Summary of drugs for children with ABPA in CF.

Drugs	Dose	Adverse effects	Comments
Corticosteroid			Systemic corticosteroids remain the mainstay of treatment
Prednisolone	Recommendation: Initial dose: 0.5–2.0 mg·kg^{-1}·day^{-1} (max 60 mg) for 1–2 weeks, then 0.5–2.0 mg·kg^{-1}·day^{-1} every other day for 1–2 weeks, then taper in next 2–3 months Alternative option: 0–2. weeks: 1 mg·kg^{-1}·day^{-1} (max daily dose 50 mg) 2–4. weeks: 0.5 mg·kg^{-1}·day^{-1} 4–6. weeks: 0.5 mg·kg^{-1} 3 times weekly 6–8. weeks: 0.25 mg·kg^{-1} 3 times weekly 8–10. weeks: 0.1 mg·kg^{-1} 3 times weekly	Growth retardation, diabetes, hypertension, cataracts, acne, osteoporosis, increased appetite, weight gain, striae, susceptibility to infections, increased intracranial pressure, ulcer disease	Orally used prednisolone is the most recommended corticosteroid treatment model
Pulse steroid	10–20 mg·kg^{-1}·day^{-1} intravenous for 3 days every 3–4 weeks for 6–12 months	Hot flashes, epigastric pain, headache, be aware of circulatory collapse following rapid administration of large doses of methylprednisolone	Long-term follow-up data are not available and this published experience was uncontrolled
Antifungal			Antifungal therapy has been used as an adjunct in the treatment of ABPA
Itraconazole	Recommendation: 5 mg·kg^{-1}·day^{-1} once or twice a day (max 400 mg·day^{-1}), for 3–6 months	Nausea, vomiting, hypokalemia, hepatotoxicity	First-line antifungal agent Liver function tests should be obtained at baseline, 1 month, and for every 3 months thereafter, or if there is a suspicion of liver dysfunction
Voriconazole	Dosage based on an uncontrolled, open label, retrospective review of children with CF and ABPA: • <12 years: 6 mg·kg^{-1} (max 200 mg) BD orally for 1 day, then 4 mg·kg^{-1} (max 100 mg) BD >12 years and <40 kg: 200 mg BD orally for 1 day, then 100 mg BD; • >12 years and >40 kg: 400 BD orally for 1 day, then 200 mg BD for a median of 22 weeks Dosage based on prescribing recommendation for invasive aspergillosis: >12 years: 6 mg·kg^{-1} BD for 1 day, 4 mg·kg^{-1} BD intravenous or 200 mg BD orally (<40 kg orally maintenance dose: 100–150 mg BD)	Visual changes, photosensitivity, hepatotoxicity	The safety in children under the age of 12 has not been established. Dosage is based on observational study, no RCT Second line antifungal agent for patients who have not responded to or cannot tolerate itraconazole
Posaconazole	One dosage option based on a prospective, non-randomized, open-label observational study of children with CF and aspergillus- related lung disease: • >35 kg: 400 mg BD (liquid suspension) or 300 mg daily (tablets with a loading dose of BD on day 1) • >25 kg and <35 kg: 300 mg (liquid suspension) BD • < 25 kg 18–24 mg·kg^{-1}·day^{-1} BD for 12 weeks Another dosage option based on a case study of a children with CF and ABPA: 200 mg orally thrice per day	Abdominal pain, nausea-vomiting, diarrhea, rash, fever, headache Hepatotoxicity QTc interval prolongation	The tolerability and efficacy in children under the age of 13 has not been established May be third line antifungal agent for patients who did not tolerate itraconazole and voriconazole Delayed-release tablets and oral suspension are not interchangeable due to the differences in the dosing of each formulation
Isavuconazole	Dosage based on case series of hemato-oncologic children (3–18 years) with invasive aspergillosis or mucormycosis and European Congress of Clinical Microbiology and Infectious Diseases 2018: 2–17 years: 10 mg·kg^{-1} (max 200 mg) every 8 h for the first 48 h, then 200 mg once daily (oral or intravenous) for a median of 75 days	Nausea, vomiting	Efficacy and safety have not been tested in children (<18 years) and the dosage and schedule have not been established. There is no published use in children with CF and ABPA and needs more studies, may be a rescue treatment
Human monoclonal antibody			
Omalizumab	Dosage based on case reports in CF children with ABPA: 300–375 mg SC every 4 weeks for 6–18 months Dosage based on prescribing recommendation for allergic asthma: 75 mg to 375 mg (determined by total Ig E and body weight) SC every 2–4 weeks	Mild rash, joint pain, bone fractures, nausea, dizziness, cold symptoms such as stuffy nose, sneezing, cough, sore throat	No RCTs evaluating the efficacy and safety profile of omalizumab in children with CF Approved for patients with severe asthma aged 6 years and older Early initiation of omalizumab may be an alternative therapy in patients with CF and ABPA in those who fail to respond to systemic corticosteroids or have severe adverse effects of prednisolone

(Continued)

TABLE 7 | Continued

Drugs	Dose	Adverse effects	Comments
Mepolizumab	Dosage based on a multinational, nonrandomized, open-label study of 6–11-year-old children with severe asthma: • <40 kg: 40 mg SC every 4 weeks • ≥40 kg: 100 mg SC every 4 weeks for 52 weeks Dosage based on prescribing recommendation for allergic asthma: • 6–11 years: 40 mg SC every 4 weeks • >12 years: 100 mg SC every 4 weeks	Headache, feeling tired, pain, swelling, redness, burning, or itching where the medicine was injected	There are case reports in adult patients with ABPA in CF There is no study for children with ABPA in CF Approved for patients with severe asthma aged 6 years and older
Benralizumab	Dosage based on two phase-3 studies of 12–75-year-old patients with severe asthma: 12 years and >40 kg: 30 mg SC every 4 or 8 weeks (first three doses every 4 weeks) for 48 weeks Dosage based on prescribing recommendation for allergic asthma: >12 years: 30 mg SC every 4 weeks for the first 3 doses, and then once every 8 weeks	Headache, sore throat, fever, hypersensitivity reactions, injection site reactions (pain, redness, itching, or a small lump)	There are case reports in adult patients with ABPA in CF Approved for the treatment of severe asthma for 12 years and older
Dupilumab	Dosage based on two phase-3 studies of >12-year-old patients with severe asthma: >12 years: 200–300 mg SC (loading dose 400–600 mg) every 2 weeks for 52 weeks Dosage based on prescribing recommendation for severe atopic dermatitis: >12 years: initial dose of 600 mg SC (two 300 mg injections), followed by 300 mg given every other week	Injection site reactions (erythema, edema), conjunctivitis, eye irritation, headache, herpes simplex viral infections	There are case reports in adult patients with ABPA in CF Approved for the treatment of moderate-to-severe atopic dermatitis and severe asthma for 12 years and older

ABPA, allergic bronchopulmonary aspergillosis; BD, bis in die (twice a day); CF, cystic fibrosis; max, maximum; RCT, randomized controlled trial; SC, subcutaneously.

Corticosteroids

Oral Corticosteroids

Systemic corticosteroids are currently the most effective agents in the treatment of ABPA. Use of corticosteroids is based on clinical experience because randomized trials do not exist and are unlikely to be performed due to ethical concerns. Prednisolone is the most widely used corticosteroid, and the dosage and duration of treatment have been investigated in several trials.

The Cystic Fibrosis Foundation Consensus Conference recommends 0.5–2.0 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ prednisone equivalent (maximum 60 $mg{\cdot}day^{-1}$) for 1–2 weeks, then 0.5–2.0 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ prednisone equivalent every other day for 1–2 weeks, then tapering on the basis of clinical and immunologic improvement. An attempt should be made to begin to taper off corticosteroids in 2–3 months (3).

In patients with asthma with ABPA, Agarwal et al. compared two steroid regimens in a randomized controlled trial (RCT). The medium-dose regimen (0.5 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 1–2 weeks, then on alternate days for 6–8 weeks, taper by 5–10 mg every 2 weeks, and discontinue after 3–5 months) was equally efficacious as a high-dose regimen (0.75 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 6 weeks, 0.5 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 6 weeks, taper by 5 mg every 6 weeks to complete a total duration of 6–12 months) in terms of the improvement in lung function, time to first exacerbation, the number of subjects with exacerbation at 1 year, and the occurrence of corticosteroid-dependent ABPA at 2 years (91).

A third dose regimen of prednisolone different from the other two above (0.5 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 4 weeks, 0.25 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 4 weeks, 0.125 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 4 weeks, then taper by 5 mg every 2 weeks and discontinue after 4 months) was associated with 100% early composite response at 6 weeks (clinical, immunologic, and radiologic improvement) in three studies of patients with asthma with ABPA (92–94). Therefore, the third regimen may offer the right balance between early treatment response and toxicity.

The main goal in these studies was to investigate the treatment protocol with fewer adverse effects while providing similar efficacy. In order to reduce the adverse effects of steroid therapy, the addition of an antifungal agent to the treatment was investigated. Very recently, a study was performed to see the effectiveness of combining short-term prednisone (2 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for 3 days, taper every 5 days to 1, 0.5, and 0.25 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ and discontinued after 18 days in total) and long-term itraconazole (10 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for capsules and 5 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ for suspension for at least 12 months) in treatment of patients with CF and ABPA. It was shown that a combination of itraconazole with short-term prednisone improved long-term pulmonary outcome in patients with ABPA without undesired glucocorticoid adverse effects (95). In a recent survey, although the majority of consultants were found to use both corticosteroids and itraconazole to treat a first diagnosis of ABPA, only one-third was reported to use prednisolone alone (96).

Pulse Steroid Therapy

The role of intravenous (iv) corticosteroids in ABPA, especially "pulse" steroid therapy is still being investigated. Pulse steroid therapy consists of iv methylprednisolone infused daily for three

consecutive days every month. Intravenous pulse steroid therapy in ABPA has been used in patients who have adverse effects with daily corticosteroids or do not respond to standard doses of oral steroid therapy, usually associated with prolonged use of steroids. However, there are no controlled trials comparing oral steroids with iv steroids. In several reports, pulse methylprednisolone was successfully used in oral steroid-dependent patients with CF and ABPA (10–20 $mg \cdot kg^{-1} \cdot day^{-1}$ for three consecutive days every month) (97, 98). In another report of an 11-year-old child with CF who was unresponsive to oral steroids, the use of iv pulse methylprednisolone made an improvement in clinical stabilization and better control of ABPA (20 $mg \cdot kg^{-1}$ for 3 days followed by 10 $mg \cdot kg^{-1}$ for 3 days) (99). In most of the studies, iv pulse steroid therapy was well tolerated and patients were able to stop the pulse therapy after 6–12 months with disease control (100).

Inhaled Corticosteroids (ICS)

ICS are known to have significantly fewer adverse effects compared with oral corticosteroids. Several case reports and small case series suggest some benefit of ICS in the management of ABPA without CF, but a study in 32 patients of ABPA found no benefit of using low doses of ICS (400 μg of beclomethasone per day) compared with placebo (101). In another study conducted retrospectively, 21 adult patients with asthma and serologic ABPA who refused conventional treatment received higher doses of ICS (1,600 μg of budesonide per day). The authors found that ICS were ineffective in controlling the immunologic activity because the total IgE levels continued to increase (102). As a result, ICS alone have no role as first-line therapy in ABPA.

Antifungal Therapy

Antifungal azoles are the most frequently combined agents with steroids. It is frequently used in steroid-resistant cases or for the purpose of reducing steroid dose and duration (103). Antifungal agents are thought to decrease the fungal burden in the respiratory tract, hence reducing the antigenic stimulus responsible for the inflammation, improving symptoms, and possibly slowing progression (104). Thus, antifungal drugs can act as steroid-sparing agents.

Itraconazole

The most widely used azole in the management of ABPA is itraconazole. Although azoles are considered to be fungostatic drugs, itraconazole seems to be efficacious in the treatment because fungal burden in ABPA is thought to be lower than in other invasive disorders of fungi (27). Studies on treatment for ABPA in CF are outdated and contain few patients. Recently, two RCTs evaluated the role of itraconazole, but only adult patients with asthma with ABPA were included. In a study involving 55 patients who were using oral corticosteroids regularly, the subjects were randomized to receive itraconazole and placebo for 16 weeks. It was shown that the rate of response to therapy was significantly higher in the itraconazole group than in the placebo group (105). The other study included 29 patients with ABPA randomized to receive itraconazole or placebo for 16 weeks. In this study, itraconazole was found to be effective in normalizing eosinophilic airway inflammation, reducing systemic immune activation, and reducing severe exacerbations (106).

In an RCT, 131 adult patients with asthma with ABPA were randomized to receive either oral itraconazole or prednisolone. All subjects treated with prednisolone showed a composite response after 6 weeks of treatment, whereas 12% of subjects in the itraconazole group did not exhibit a composite response. All subjects who failed to respond to itraconazole were treated with prednisolone, and showed a composite response after 6 weeks of treatment. This study suggests that oral corticosteroids are more effective than itraconazole (100 vs. 88%) in the treatment of acute-stage ABPA (93). The Cystic Fibrosis Foundation Consensus Conference recommends that the initial dose should be 5 $mg \cdot kg^{-1} \cdot day^{-1}$, which may be given once or twice daily (maximum 200 $mg \cdot dose^{-1}$). The daily dosage should not exceed 400 $mg \cdot day^{-1}$ unless low serum itraconazole levels are obtained. The duration of therapy should be short (3–6 months) because of the emerging risk of azole-resistant Aspergillus species. In addition, itraconazole cannot be recommended for initial therapy in patients with CF and ABPA. However, it should be added to therapy if there is a slow or poor response to corticosteroids, for relapse of ABPA, in corticosteroid toxicity, and corticosteroid-dependent ABPA. For patients receiving itraconazole, liver function tests should be obtained before therapy. Routine liver function testing after 1 month and every 3–6 months thereafter should be considered. There are several medications that are known to interact with itraconazole. Therefore, determining serum concentrations of other drugs and/or itraconazole may be required. Itraconazole concentrations should also be determined when there is a lack of clinical response or if there is concern about adequate drug absorption or patient compliance. Besides, itraconazole is associated with gastrointestinal symptoms, congestive heart failure, and rash (3).

Newer Azoles

Few studies have evaluated the newer azoles (voriconazole, posaconazole and isavuconazole) for their efficacy in ABPA. In the largest study, Chishimba et al. retrospectively analyzed the efficacy and safety of voriconazole and posaconazole in 20 adult patients with asthma with ABPA. Overall, clinical improvement with voriconazole or posaconazole therapy was seen in about 70–75%, so both drugs were found to be alternative treatments to itraconazole (107). Monotherapy of voriconazole vs. prednisolone in patients with asthma with ABPA was evaluated in an RCT (92). Fifty subjects were randomized to receive either prednisolone or voriconazole. In this study, voriconazole monotherapy had similar efficacy to prednisolone. According to this study, voriconazole appeared to be as effective as corticosteroids in acute-stage ABPA. Fewer studies have been conducted on voriconazole therapy in patients with CF and ABPA. Glackin et al. reported that serum total IgE level was decreased with voriconazole therapy in patients with CF (108). In the other study, an uncontrolled, retrospective study in 21 patients with ABPA in

CF showed an improvement in lung function with voriconazole therapy (109). Unique adverse reactions among patients receiving voriconazole include transient vision changes, visual hallucinations, and photosensitivity. However, voriconazole is a reasonable alternative to itraconazole because it is better tolerated in some patients and is well-absorbed.

In a retrospective study that compared posaconazole with other azoles in the treatment of ABPA in 32 adult patients with CF, it was found that there was a significant reduction in specific IgE to Aspergillus with posaconazole compared with itraconazole and voriconazole (110). Recently, Patel et al. reported a prospective single-center, non-randomized, open-label observational study over a 53-months period evaluating the safety, tolerability, and efficacy of posaconazole in pediatric patients with CF. A total of 23 episodes of Aspergillus-related lung disease were treated. Posaconazole was well-tolerated in children with CF and an improvement in FEV1 and serologic parameters in response to posaconazole was noted in this study (111). It is also associated with gastrointestinal symptoms depending on the formulation, and there are sparse data supporting its use for ABPA.

Isavuconazole is also a new azole that is approved for primary therapy of invasive aspergillosis. However, the use of isavuconazole in ABPA is less well-studied. The first report of the use of isavuconazole for the treatment of ABPA presented an adult patient with asthma who was successfully treated with isavuconazole after unsuccessful treatment with corticosteroids, itraconazole, and voriconazole (112). The patient tolerated isavuconazole well, had marked symptomatic improvement, and demonstrated a normal FEV1/FVC ratio for the first time in 7 years after being diagnosed as having ABPA. Treatment with isavuconazole is generally safe and well-tolerated but there are no studies on isavuconazole treatment of ABPA either in the pediatric population or patients with CF. However, a non-randomized open-label multicenter study on isavuconazole treatment of invasive aspergillosis or invasive mucormycosis in pediatric subjects is underway and planned to be completed in 2 years (ClinicalTrials.gov; NCT03816176).

Nebulized Amphotericin B (NAB)

NAB is an option for maintaining remission in recurrent exacerbations. In one RCT (21 adults with asthma), non-liposomal NAB without concomitant azole therapy was found to be efficacious in maintaining remission in those with recurrent exacerbations. On the other hand, NAB had poor efficacy in inducing a response in patients with acute-stage ABPA or during an exacerbation of ABPA (113).

In a case report of a pediatric patient with end-stage CF lung disease with progressing symptoms, very poor lung function and severe bronchiectasis, treatment with NAB resulted in improvement of cough, dyspnea, hypoxia, 6-min walk test, reduction in oral corticosteroid dosages, and pulmonary function with no adverse events (114).

NAB has the potential to precipitate or worsen bronchospasm, especially the deoxycholate preparation; therefore, the first dose should be administered with caution and short-acting bronchodilator may be administered 15–30 min prior to NAB (113).

Monoclonal Antibodies

Corticosteroids can control the symptoms of most patients and their combination with antifungal drugs increases treatment success. However, some patients become steroid-dependent or experience adverse effects. In an effort to treat refractory patients or to reduce adverse effects, many physicians have tried monoclonal antibody treatment. As a monoclonal antibody, although omalizumab is the most preferred, mepolizumab and benralizumab are the most investigated.

Omalizumab

Omalizumab is a humanized monoclonal antibody against IgE recommended for the treatment of uncontrolled allergic asthma and chronic spontaneous urticaria. It is considered for use in the treatment of other allergic disorders such as ABPA because its mechanism of action is via IgE antagonism. Although omalizumab seems to facilitate ABPA control in asthma, evidence for use in patients with CF is currently limited to data from case reports. In addition, an RCT evaluating the safety and efficacy of omalizumab for the treatment of ABPA in patients with CF aged 12 years and older was designed, but this study was terminated prematurely due to adverse events (115). Another RCT was reported in 2015 which evaluated the clinical and immunologic effects of omalizumab in asthmatic ABPA patients. Thirteen patients with chronic ABPA were randomized to a 4-months treatment with omalizumab or a placebo followed by a 3-months washout period. The ABPA exacerbations were significantly less frequent during the active treatment phase compared with the placebo period (116). Van der Ent et al. reported the first case of a single dose of omalizumab treatment in a 12- year-old girl with CF and ABPA who showed a rapid and good improvement of clinical signs and lung functions (117). In a case series of Emiralioglu et al. six patients with CF and ABPA who had received omalizumab were reported. Omalizumab (300 mg dosage) was administered subcutaneously every 4 weeks to the patients who were treated previously with oral prednisolone and itraconazole. Decreased IgE levels, improvement in respiratory symptoms, and a steroid-sparing effect was shown with omalizumab treatment (118). A retrospective multicenter observational French study retrieved 32 patients with ABPA and CF (11 children and 21 adults) who had received omalizumab for more than 3 months. Among them, 14 patients were able to discontinue steroid treatment or reduce their daily dose during follow-up (119). Very recently, a retrospective study of 27 adult CF patients receiving omalizumab for asthma or ABPA was conducted by Koutsokera et al. to evaluate the efficacy and safety of treatment. Omalizumab was found to be effective in the improvement of respiratory functions in adult CF patients with difficult-to-control asthma or ABPA with no significant adverse effects during the study period (120).

Furthermore, a standard dose has not been established for omalizumab in patients with CF and ABPA. Different studies used various doses (ranging from 225 mg to 750 mg) and frequency of treatment (ranging from once per week to once

monthly) according to the weight and serum IgE level. However, the most commonly used regimen was 375 mg every 2 weeks (121). The duration of treatment is also controversial. As an example, Wong et al. have reported 2 patients with CF and steroid-dependent ABPA who were successfully able to be weaned off steroid therapy with the treatment of omalizumab monthly for 2 years (122).

In contrast to the above case reports, two studies on patients with CF with ABPA found no benefit with omalizumab treatment. In one of them, Brinkman et al., reported a 15-year-old patient who could not be weaned from steroid therapy for over 12 months with omalizumab treatment (123). Ashkenazi et al. also presented nine patients with CF and ABPA who were treated with 300–375 mg omalizumab every month but did not respond to treatment (124). However, in both studies, they administered the drug every month with a lower dose compared with other cases.

Omalizumab may be a promising treatment option also for CF patients with chronic bacterial infections since use of corticosteroids is of concern due to compromised immunity for these patients. In a recent retrospective cross-sectional study, no significant adverse events or worsening of infection due to omalizumab treatment were observed in patients with ABPA and chronic bacterial airway infection. Consequently, treatment with omalizumab was found to be effective and safe in patients with ABPA, regardless of concurrent chronic respiratory tract infections since it does not exhibit immonusuppressive effects (125).

Mepolizumab

Mepolizumab is an anti-IL-5 monoclonal antibody used for severe refractory eosinophilic asthma. Altman et al. were first to demonstrate mepolizumab as an additional and effective treatment option for severe ABPA resistant to corticosteroids, antifungal therapy, and omalizumab in a 58-year-old asthmatic woman (126). In this case, clinical improvement was achieved with the addition of 100 mg mepolizumab every 4 weeks to high-dose omalizumab treatment.

In another case, mepolizumab was shown to be effective as a monotherapy in a 64-year-old woman treated for severe bronchial asthma with ABPA exacerbation (127). In this case, after a successful 3-years treatment period with systemic corticosteroids and itraconazole, deterioration occurred. With the addition of 100 mg mepolizumab every 4 weeks, dramatic improvements were observed in symptoms, lung function, peripheral eosinophil counts, and chest imaging.

Benralizumab

Benralizumab is a monoclonal antibody directed against the alpha chain of the IL-5 receptor. Recently, two adult cases of ABPA with asthma were reported to switch to benralizumab after treatment with mepolizumab (128). Benralizumab is thought to clear bronchial mucus plugs, prevent irreversible airway damage, and improve the prognosis of patients with ABPA.

Dupilumab

Dupilumab is a humanized monoclonal antibody. It is a dual inhibitor of IL-4 and IL-13 pathways. In a case series, the clinical course of three adult subjects with asthma and ABPA treated with dupilumab over 6 months was presented (129). In this study, dupilumab was found to facilitate disease control in ABPA, with reduced symptoms and oral corticosteroid use.

Treatment Practices

In acute ABPA, systemic corticosteroids are the first choice of treatment. Although any of corticosteroid regimens as detailed above can be used, high-dose steroids should not be preferred as the first choice in the management of ABPA due to the more frequent adverse effects. The steroid dose should be adjusted according to the clinical and immunologic characteristics of disease. Antifungal agents should be added to therapy if there is a slow or poor response to corticosteroids, for relapse of ABPA, in corticosteroid toxicity, and corticosteroid-dependent ABPA. Azole therapy is usually begun with itraconazole; newer azoles are reserved for those who fail therapy or experience adverse reactions with itraconazole or fail to achieve optimal serum levels of itraconazole, despite receiving the maximum dose (104). Nonetheless, a small number of patients may require chronic corticosteroid therapy (3).

After starting treatment for acute ABPA, monitoring is performed with clinical evaluation, serum total IgE levels, spirometry, and chest radiography. It is not helpful to measure Aspergillus-specific IgE and IgG during treatment because their levels are not correlated with the reduction in the serum total IgE or clinical or radiologic improvement. Serum total IgE concentrations should be measured every 6–8 weeks, especially in the first year (130). The clinical effectiveness of therapy is evaluated through serum total IgE levels. The goal of therapy is not to achieve normal IgE levels but to decrease its levels by 35–50% at 8 weeks, which leads to clinical and radiographic improvement. During the treatment, serum total IgE levels are measured to determine the new baseline IgE concentrations (5). The lowest value achieved after treatment is taken as the new baseline. An increasing level (>100% of the new baseline) of total IgE along with worsening respiratory symptoms and the appearance of consistent radiologic findings suggest an exacerbation of ABPA (131). Twenty to 35% of relapses are asymptomatic and are detected radiographically and serologically. The treatment of the first exacerbation is similar to the treatment of acute disease. Greater than or equal to two exacerbations within 6 months of stopping therapy or worsening of clinical and/or radiologic condition, along with immunologic worsening (rise in IgE levels) on tapering oral steroids/azoles is steroid-dependent ABPA. Pulse steroids may be considered in such patients. If they are currently taking itraconazole, newer azoles may be considered. Omalizumab has shown promise in such cases but its use in patients with ABPA and CF requires more definitive clinical trials. Furthermore, it is also very important to identify and exclude any potential environmental exposure source of *A. fumigatus* because it can initiate exacerbations.

Remission may be considered if the patient has remained asymptomatic with stable IgE levels (persisting at/below baseline or increase by <50%) for at least 6 months without the requirement of corticosteroid or antifungal therapy. In the

remission period, monitoring may be performed every 3 months for a year and every 6 months thereafter with a clinical examination and serum total IgE levels. Chest radiography may be obtained if clinically indicated. Spirometry is performed in routine follow-ups and in response to changes in symptoms. Antifungal therapy is not used to prevent exacerbations given the potential toxicity and lack of proven benefit.

Another considerable point of management is that chronic respiratory tract infections are almost inevitable in ABPA patients with CF. These patients are especially vulnerable to *Pseudomonas aeruginosa* or *nontuberculous mycobacteria* because of the combined effects of structural deformities in the airways and compromised immunity caused by systemic and local administration of corticosteroids (132). In that case, monoclonal antibodies such as omalizumab may be a good choice since it can prevent the use or reduce the doses of systemic corticosteroids (125).

In summary, clinical improvement is generally achieved with proper diagnosis, follow-up, and treatment. At the same time, there is a considerable variation in treatment practices of patients with CF and ABPA, hence there is a pressing need for new guidance in both treatment and its duration.

AUTHOR CONTRIBUTIONS

BS, DA, BO, NE, EY, and UÖ have made contributions to the design, editing, and writing of this manuscript. All authors contributed to the article and approved the submitted version.

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Early Diagnosis and Intervention in Cystic Fibrosis: Imagining the Unimaginable

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Cystic fibrosis is the most common life-shortening genetic disease affecting Caucasians, clinically manifested by fat malabsorption, poor growth and nutrition, and recurrent sinopulmonary infections. Newborn screening programs for cystic fibrosis are now implemented throughout the United States and in many nations worldwide. Early diagnosis and interventions have led to improved clinical outcomes for people with cystic fibrosis. Newer cystic fibrosis transmembrane conductance regulator potentiators and correctors with mutation-specific effects have increasingly been used in children, and these agents are revolutionizing care. Indeed, it is possible that highly effective modulator therapy used early in life could profoundly affect the trajectory of cystic fibrosis lung disease, and primary prevention may be achievable.

Keywords: cystic fibrosis, cystic fibrosis transmembrane conductance regulator, corrector, potentiator, immunoreactive trypsin(ogen), sweat chloride test, newborn screening

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INTRODUCTION

Newborn screening (NBS) programs were first established almost 60 years ago in the United States after the seminal discovery that phenylalanine could be detected from a dried blood spot, ultimately leading to early diagnosis of phenylketonuria and avoidance of the severe neurocognitive complications characteristic of this inherited metabolic disorder (1). Other screening programs emerged, generally adhering to basic principles outlined in a report commissioned by the World Health Organization (2), and the number of diseases tested has grown in the last several decades. While there is variability between programs, some states and countries screen for as many as 50 treatable metabolic conditions, endocrinopathies, hemoglobinopathies, and genetic diseases, like cystic fibrosis (CF).

Occurring in roughly 1 in 3,000 live births in the United States, based on epidemiological and neonatal screening data, CF is the most common, life-shortening inherited disease of Caucasians (3). CF is caused by defective CF transmembrane conductance regulator (CFTR), a cAMP-regulated anion transporter expressed on the surface of various epithelia. Functionally linked to the epithelial sodium channel and other apical channels, CFTR abnormalities lead to reduced epithelial chloride conductance and sodium absorption, resulting in dehydration of the periciliary fluid layer and mucus on the airway surface that impairs mucociliary clearance (4, 5). Innate defenses are also compromised by altered bicarbonate secretion in the CF airway (6, 7). Together, these changes lead to progressive airway obstruction, allowing bacterial infection to become established and provoking a persistent neutrophilic inflammatory response that results in the gradual destruction of the airways and ultimately respiratory failure.

Before implementation of NBS for CF, children were typically diagnosed after developing symptoms consistent with fat malabsorption, often leading to nutritional failure. Some were identified shortly after birth when they presented with meconium ileus, which occurs in roughly 15% of children with CF (8, 9). Recurrent respiratory infections, often misdiagnosed as asthma or bronchiolitis, would occur during infancy, but are more common in older children. Indeed, children with milder, pancreatic sufficient phenotypes are often recognized later as their respiratory symptoms become more prevalent.

Adoption of NBS in many nations has led to earlier diagnosis and treatment, and the life expectancy of a child born with CF in many parts of the world has steadily improved (10, 11). In addition, newer, small molecule therapeutics have begun to dramatically change the disease (12, 13). In this article, we will review NBS for CF and describe existing and emerging therapies that have impacted the progressive respiratory decline of people with CF and how they may avert lung disease and other complications even before they begin.

NBS FOR CF

Early attempts to screen neonates for CF over 40 years ago relied on measuring albumin content in dried meconium (14), which had a high false-positive rate. However, it was discovered that young infants with CF had elevated, circulating levels of pancreatic enzymes and proenzymes, even children with pancreatic sufficient forms of the disease. In particular, trypsinogen, a pancreatic enzyme precursor released from the inflamed exocrine pancreas caused by inspissated secretions and destruction of acinar cells, can be detected in the blood of neonates with CF. Indeed, over 40 years ago, Crossley and colleagues showed that the serum immunoreactive trypsinogen (IRT) could be measured in blood spots dried on the Guthrie cards (15), and the ability to measure this analyte was paramount for development of a broad, population-based newborn program.

In the United States, methods for NBS differ between states and countries, but all invariably use some form of the IRT measurements as part of the screening process. It is important to note that IRT concentrations can be elevated in the absence of CF, particularly in neonates who are premature, have low Apgar scores, or experience perinatal stress. It was recognized that a single-tier approach had a lower sensitivity, so in the United States, most states have adopted two-tier protocols that involve serial IRT measurements repeated 1 to 2 weeks apart if the initial value is elevated, or IRT followed by genetic testing for specific *CFTR* mutations if abnormal. Some states have adopted a third-tier, using a protocol that involves repeated measurements of IRT levels, with DNA analysis performed if both concentrations are above the designated threshold (16, 17). Uniquely, California, a state that has a racially diverse population, has incorporated *CFTR* sequencing in their screen to identify CF in neonates with high IRT concentrations and only one mutation in their genetic panel (18).

The threshold defining an elevated IRT level varies between states. While some states will apply an absolute concentration to prompt further testing, others use percentile cutoffs that improve specificity. Serial IRT approaches without genetic analysis has benefits, allowing for identification of individuals with less common *CFTR* mutations in certain populations (19), but delaying time to a positive screen because the second specimen is obtained later. Genetic panels used in NBS are variable, and the number of *CFTR* mutations analyzed may differ from state-to-state. Genetic testing has the advantage of identifying people who are heterozygous for *CFTR* mutations, but also more likely to identify patients with mutations but normal or equivocal sweat chloride levels, referred to as CFTR-related metabolic syndrome or, in Europe, CF screen-positive, inconclusive diagnosis.

ADOPTION AND EVOLUTION OF CF NBS

In the United States, NBS for CF was slowly accepted, given the relative absence of data showing benefits of early diagnosis. Indeed, even the Cystic Fibrosis Foundation was hesitant to make a recommendation regarding NBS, stating the benefits of presymptomatic and early treatment were controversial (20). Nevertheless, screening programs were established in North America. In 1982, Colorado became the first state to implement NBS for CF, followed shortly thereafter by Wisconsin. Initially developed as part of a decade-long randomized controlled trial (21), NBS was added to the Wisconsin state-wide program in 1994 (22). A workshop held by the Centers for Disease Control and Prevention and Cystic Fibrosis Foundation in 2003 evaluated diagnostic testing and decision-making and provided recommendations for best practices for screening for CF (23). At the time of its publication, only eight states had implemented an NBS program, but within 7 years, all 50 states and Washington, DC, had screening programs for CF, with Texas being the last state to implement screening.

Similar to the US experience, there is considerable variability in screening programs among nations. Certainly, disease prevalence plays an influential role in the need for screening and early diagnosis (24, 25). Worldwide, Australia and New Zealand were pioneers, establishing NBS programs in 1981 (26). During the past two decades, the number of programs has rapidly increased in Europe (25, 27), with more than 20 European countries performing NBS at some level. Indeed, alternative screening approaches have been adopted in some countries. For instance, a four-tier screening algorithm was created in the Netherlands that involves measurement of IRT and pancreatitis-associated protein levels from the same dried blood spot (28), *CFTR* mutation panel, and, if indicated, extended genomic analysis. The Dutch experience highlights the complexity of such programs, and a reminder NBS is exactly that, a screening tool and not a diagnostic test.

Often overlooked, successful NBS depends on the accuracy of diagnostic testing. The diagnosis of CF is based on elevated sweat chloride concentrations (29). Any clinical concerns

Abbreviations: cAMP, cyclic adenosine monophosphate; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; FEV_1, forced expiratory volume in one second; IRT, immunoreactive trypsinogen; NBS, newborn screening.

for CF, regardless of the screening result, are an indication for sweat chloride measurement. While other approaches are available, the only reliable, validated diagnostic test for measuring sweat chloride concentration is the quantitative pilocarpine iontophoresis test, performed according to Clinical and Laboratory Standards Institute guidelines (30).

BENEFITS OF NBS FOR CF

NBS, regardless of disease, is successful only if early identification is feasible using simple, cost-effective means and can lead to improved clinical outcomes. For decades, the diagnosis of CF required clinical suspicion. Before screening, the median age of diagnosis was 6 months in the United States, and nearly 70% of affected children were identified by their first birthday (31). Malnutrition occurs early in life (32), and pulmonary involvement can begin early in infancy, despite the child appearing asymptomatic (33, 34). With widespread adoption of NBS in the United States, the age at diagnosis has shifted to <1 month, often before the child is symptomatic.

There was early evidence from the Netherlands almost 5 decades ago that screening in the neonatal period was associated with a survival benefit (35). While CF was added relatively late to US programs, there was growing evidence that delayed diagnosis would have serious implications for affected people. Because of low mortality rates in children, it is difficult to establish survival differences between screened and unscreened children with CF. Using data from the US Cystic Fibrosis Foundation Patient Registry of more than 27,000 patients, children identified by screening within a month of age and treated early had better survival compared to counterparts diagnosed symptomatically (36), supported by several subsequent studies (37–39).

The best evidence clearly showing benefits of NBS is its effect on early nutrition and growth. Before screening, children with CF were often diagnosed after becoming severely malnourished with vitamin deficiencies. These children frequently failed to achieve their growth potential and had evidence of impaired development of cognitive function, likely related to malnutrition. Investigators in Wisconsin unsurprisingly found that the diagnosis of CF was confirmed by a positive sweat test at a much younger age in a screened cohort as compared to controls. Moreover, children with CF identified by screening had significantly better height, weight, and head circumference percentiles, and these differences persisted throughout infancy and early childhood, especially the children who had pancreatic insufficiency and homozygous for the *Phe508del* mutation (40). Recently, a multicenter, longitudinal, observational cohort study examined the nutritional health of 231 American children with CF identified by NBS over a 3-year period and found significant improvement in nutritional status, with normalization of weight in the first year of life (41).

Malnourished children with CF have increased risk of chronic lung disease. A large study of 931 children with CF examining the effect of early nutrition on the development of lung disease highlighted the importance of earlier intervention. Children with better nutritional indices at 3 years of age had higher lung function measures at the age of 6 years (42). Thus, early diagnosis in order to optimize the nutritional status by starting enzymes at diagnosis and adding nutritional supplements as indicated can lead to improved pulmonary health.

There have been several studies that show children diagnosed with CF following NBS have fewer complications than those who were symptomatic at the time of diagnosis. Australian investigators compared the outcomes of children with CF identified early from NBS or diagnosed late. Unscreened patients had reduced height, lower pulmonary function measures, and increased rates of infection and colonization with *Pseudomonas aeruginosa* (43). Respiratory benefits persisted into adolescence (44, 45). Others have similarly shown benefits in the US and UK populations, with reduced *P. aeruginosa* infections, reduced treatment burden, and fewer hospitalizations (46–48). These findings emphasize not only the importance of early diagnosis and detection, but also the need for continued improvement of screening protocols with genetic advances.

In addition to the clinical benefits from early identification, screening programs for CF have economic benefits, with several studies revealing its cost-effectiveness (49, 50). Additionally, investigators have reported that the incidence or CFTR allele distribution decreased following implementation of NBS, although these observations need to be confirmed in larger studies (51, 52).

IMPLICATIONS OF NBS IN THE ERA OF MODULATOR THERAPIES

Management of CF has been directed at the downstream consequences of CFTR dysfunction, incorporating antibiotics, anti-inflammatory agents, inhaled mucolytics, and airway clearance techniques. Pancreatic enzyme replacement therapy and vitamin supplements treat pancreatic insufficiency and prevent nutritional deficits. These treatments have led to longer lives, even before widespread implementation of NBS. However, the emergence of novel small molecule therapeutics that target the basic defects has raised hope that CF lung disease can be prevented before it starts. These newer CFTR potentiators and correctors have mutation-specific effects that can restore CFTR function. These agents are revolutionizing care and have reduced respiratory symptoms, exacerbation frequency, and slowed progression of lung disease in people with CF (12, 13).

When ivacaftor was approved by the US Food and Drug Administration (FDA) 8 years ago, preceding clinical trials showed dramatic improvements in sweat chloride values along with improvements in weight gain, pulmonary exacerbation rates, and lung function measures in patients with *G551D* mutations, a class 3 *CFTR* gating defect (12, 53). Subsequent studies revealed improved lung mucociliary clearance that was sustained 3 months following treatment, which correlated with forced expiratory volume in 1 s (FEV_1) (54). More recently, several studies then evaluated the effectiveness of ivacaftor in younger children (55–57). A phase 3, multicenter trial examined the ivacaftor pharmacokinetics in young children and its effect on sweat chloride concentrations, growth parameters, and markers

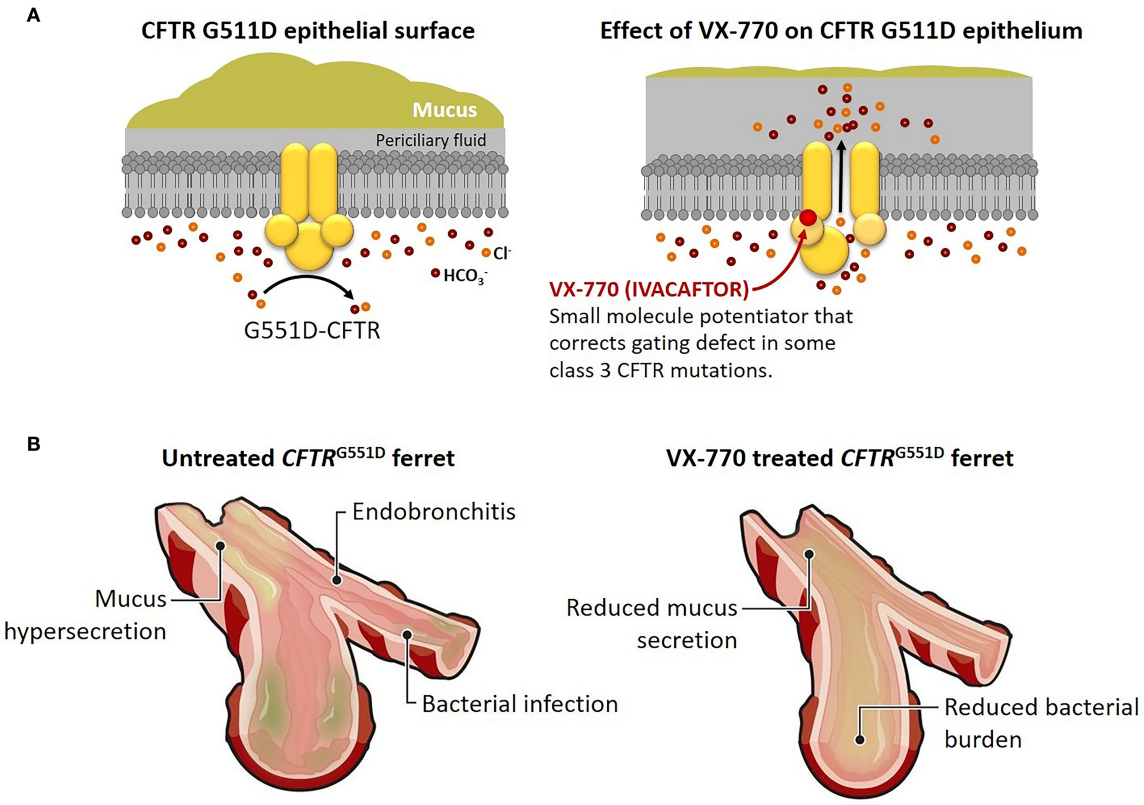

FIGURE 1 | Early treatment with VX-770 prevents pathological changes in a cystic fibrosis animal model. **(A)** Effect of the CFTR potentiator VX-770 (ivacaftor) on ion channel gating of the CFTR G551D mutation. The *G551D* mutation abolishes ATP-dependent gating, which results in reduced channel open probability, but treatment with VX-770 alters activity of the mutant CFTR, leading to greater chloride ion and bicarbonate ion secretion, reduced sodium ion absorption, and hydration of epithelial surfaces. **(B)** Effect of prenatal and postnatal treatment with VX-770 (ivacaftor) on the airways of young ferrets with G551D mutation. Mucus accumulation, bacterial infection, and endobronchitis develop early in untreated airways of young kits with *G551D* mutations, but treated animals avoided bronchial infection and inflammation until the drug was discontinued. Similar effects were seen in other affected organs, including the pancreas, intestines, and genitourinary tract. Modified from Ferkol (65).

of exocrine pancreatic function. Growth measures for age were normal throughout the study, and pancreatic function biomarkers also improved, suggesting that ivacaftor preserved exocrine pancreatic function (56). Other studies have shown beneficial nutritional effects in preschool children (55, 58). These findings were surprising. In CF, the exocrine pancreas is involved before birth, with obstruction of small ducts and acini seen as early as the second trimester (59). Disease progresses after birth, with pancreatic inflammation, fibrosis, and fatty infiltration, once thought to occur in early infancy (60).

In secondary analyses of GOAL (*G551D* Observational Study), lung clearance indices were significantly improved in treated children within 1 month of starting treatment and were maintained 6 months after beginning therapy (61). In fact, ivacaftor is now approved for use in children with certain mutations as young as 4 months of age.

Since the development of ivacaftor (Kalydeco (R)), three other therapies have been approved for use in the United States (62). Lumacaftor–ivacaftor (Orkambi®) was initially approved for use in 2015 for those with homozygous *Phe508del* mutations and is now available for use down to 2 years of age. Tezacaftor–ivacaftor (Symdeko®) was approved in 2018 and may now be used for those 6 years or older who have homozygous *Phe508del* mutations or *Phe508del* and a second specific mutation. Most recently, highly effective triple-drug therapy, elexacaftor–tezacaftor–ivacaftor (Trikafta® or Kaftrio®), was approved by the FDA and more recently the European Commission for use in CF patients 12 years or older who have one or two *Phe508del* mutations, which accounts for 90% of all affected individuals (13, 63). This treatment has shown the most promise in altering the clinical trajectory of those with CF. Trials showed marked reductions of sweat chloride and improved percent predicted FEV_1 values (13).

Modulator therapy is increasingly used in younger children and even infants, raising the prospect that CF could be prevented before it begins (55, 56). However, to be successful, primary prevention requires early diagnosis and treatment. NBS is an essential part of the success of early diagnosis and with the advent of modulator therapy use in younger and younger children, critically important.

Given these improvements, although purely speculative, primary prevention may be achievable. Using genetically modified ferrets that harbor *CFTR G551D* mutations, investigators showed the potential benefits of *CFTR* modulators (64). Like other animal models for CF, the newborn ferret is prone to meconium ileus, with 80% experiencing severe intestinal obstruction that leads to early death. However, when pregnant jills were treated with ivacaftor (VX-770) late in pregnancy, kits homozygous for the *G551D* mutation had markedly reduced incidence of neonatal bowel obstruction.

Postnatally, ivacaftor was administered to the kits, and they maintained pancreatic sufficiency and grew as well as wild-type littermates. In compound heterozygous (*G551D/KO*) ferrets, most remained pancreatic insufficient, but many maintained normal growth. Similarly, ivacaftor treatment protected the airways from bacterial infection and inflammation (**Figure 1**). Once treatment was discontinued; however, the benefits disappeared, and CF kits developed characteristic pancreatic and pulmonary pathology. These findings suggest the importance of early and sustained modulator treatment in maintaining *CFTR* function (65), and these agents are not a cure.

While fertility was not assessed, the vas deferens and epididymis appeared pathologically normal in male kits homozygous for the *G551D* mutation, in contrast to compound heterozygous (*G551D/KO*) ferrets. Thus, one could speculate obstructive azoospermia or congenital bilateral absence of the vas deferens could be prevented in certain patients. The pathogenesis of the male genitourinary defects begins *in utero*, likely related to accumulation of obstructing, thickened secretions that leads to degeneration of the vas deferens. Indeed, male fetuses with CF, between 12- and 18-week gestation, have a normal vas deferens, demonstrating that the defect occurs later in embryonic development (66).

It would be premature to consider clinical trials testing the efficacy of ivacaftor in preventing CF in neonates who have *G551D*. First, there would be few eligible subjects. Few people with CF are homozygous for class 3 *CFTR* defects (53). Moreover, treating a fetus by treating an unaffected pregnant mother would pose ethical issues; pregnant women and their unborn children are often excluded from pharmaceutical trials. These therapies are not without risk, including liver dysfunction and cataract development, and would likely prohibit use in an unaffected woman.

That said, we may soon have evidence of whether primary prevention of CF is feasible. In contrast to their male counterparts who have obstructive azoospermia, women with CF are generally fertile, and with improvements in care, a growing proportion are having children. Many women with CF are being treated with the newer, highly effective triple combination therapy, elexacaftor–tezacaftor–ivacaftor (13, 63). To maintain the mother's pulmonary and nutritional health, they often continue treatment throughout pregnancy at many centers.

While partners of pregnant women with CF typically undergo prenatal testing for *CFTR* mutations, occasionally they are missed, and children are born with CF. If their unborn child has CF and *Phe508del* mutation(s), he/she would indirectly be treated with elexacaftor–tezacaftor–ivacaftor *in utero*, as these small molecules can cross the placental barrier, thus leading to several interesting questions. Would combination therapy in this child prevent progressive airway disease, maintain pancreatic sufficiency, or preserve male fertility, paralleling what was described in the ferret model (65)? How would one assess the latter in young infants who typically do not have respiratory symptoms (67), and what would we use to demonstrate a treatment effect in the lung (67)? For primary prevention strategies to succeed, sensitive outcome measures are needed to detect the earliest changes in lung disease in infants and young children.

Furthermore, would it be unethical to withdraw a drug that prevented disease once the infant is born, despite lack of regulatory approval for young infants? If so, in the absence of clinical trials, how would we determine optimal dosing in this population?

Finally, would CFTR correction interfere with NBS of children born to women with CF, resulting in a false-negative screen? Could CFTR correction attenuate pancreatic injury and result in a negative IRT level? We may need to rethink our screening and diagnostic approach for such children.

While there are many significant gaps in available diagnostics and treatments between countries (68), we have entered a new era in CF, full of promise and possibilities. To achieve this potential, effective screening and diagnostic testing must be in place. Prenatal and neonatal screening programs mean that infants can be diagnosed and interventions begun before they are symptomatic. In some countries, CFTR genotyping is frequently performed early in life, and mutation- or class-specific *CFTR* modulators have already changed the lives of older infants and children. What was once unimaginable could become reality—primary prevention of CF might be achievable.

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REVIEW
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Toward the Establishment of New Clinical Endpoints for Cystic Fibrosis: The Role of Lung Clearance Index and Cardiopulmonary Exercise Testing

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As Cystic Fibrosis (CF) treatment advances, research evidence has highlighted the value and applicability of Lung Clearance Index and Cardiopulmonary Exercise Testing as endpoints for clinical trials. In the context of these new endpoints for CF trials, we have explored the use of these two test outcomes for routine CF care. In this review we have presented the use of these methods in assessing disease severity, disease progression, and the efficacy of new interventions with considerations for future research.

Keywords: lung clearance index (LCI), cardiopulmonary exercise testing (CPET), endpoints, cystic fibrosis, disease severity, disease progression

INTRODUCTION

Cystic Fibrosis (CF) treatment and patient management has improved dramatically over the last 20 years. The rise of new therapies and effective newborn screening has led to an extraordinary increase in overall survival and quality of life. Despite these improvements in clinical practice, the need for methods that can detect early pathological alterations and direct management remains important.

Spirometry is considered the gold standard endpoint for evaluating CF lung disease. Despite its catholic implication for routine CF assessment, it is not feasible from an early age. In children who are able to perform effort dependent lung function tests, spirometry has low sensitivity in detecting early structural and functional alterations (1). This inability to detect early-stage disease results in a "diagnostic gap" (2, 3) that can be crucial to detect if subsequent patient deterioration is to be avoided.

Cystic fibrosis clinicians and researchers have tried to fill this gap with methods that provide early and reliable deterioration assessment. The Multiple Breath Washout (MBW) test with Lung Clearance Index (LCI) and Cardio-Pulmonary Exercise Testing (CPET) have shown promise in detecting early changes in respiratory physiology.

LUNG CLEARANCE INDEX, LCI

Lung Clearance Index (LCI) is a measure of ventilation distribution inhomogeneity. It can be calculated with the Multiple Breath Washout Method (MBW). The above method measures the clearance of an inert gas (N2 or SF6) from the lungs during tidal breathing. The greater

inhomogeneity of ventilation, the higher LCI values are calculated (4). LCI sensitively detects pathology within the peripheral airways (5). This means it can provide information about the "silent lung zone," the lung compartments whose pathology is not detectable by conventional lung function tests such as spirometry (5, 6). LCI testing is of great value for lung diseases such as CF, for which early detection of structural damages and immediate interventions are crucial to prevent disease progression (7).

MBW is feasible from a young age to adulthood. It requires only passive cooperation with tidal breathing (8, 9). The success rate of the test is 72–99% except for infants and young children below 3.5 years of age, who might need sedation to achieve acceptable measurements. In experienced centers the test can still be used successfully in the majority of these younger children. In experienced centers, over 90% of children aged over 6 year can successfully complete this test and the success rate is even higher amongst adults (10–14).

Kent et al. have reviewed the reliability of LCI and found that the mean coefficient of variation (CV) and the intra-class correlation coefficient (ICC) for LCI measurements within one session were acceptable in both healthy controls and CF cases (15). Few studies have assessed the validity of LCI. Those that have been published demonstrated that LCI was able to distinguish CF subjects from healthy control subjects (16–21). Additionally, LCI could differentiate the disease severity among CF patients with mild to moderate lung disease as revealed by structural changes on high-resolution computed tomography (HRCT) and the patient's microbiological status (3, 7, 22–25). LCI is not well-tolerated by individuals with very severe lung damage, because of the time it takes to complete a measurement. For such individuals, LCI values do not accurately reflect the disease severity because of either totally obstructed or poorly ventilated lung units that do not participate in ventilation distribution. For such patients, spirometry may be a preferable option although this can also plateau at low levels in those with more severe disease (6).

When considering reference values, while LCI was considered an age-independent measure, the current literature suggests a different upper limit of normal (ULN) that is age dependent. The ULN in recent studies has been variably suggested as being; (i) 7.8–8.2 for infants (21, 26–28), (ii) 7.4–7.8 for preschoolers (26, 29), and (iii) 7.2–7.9 for school-aged children (23, 30, 31). Such values might well-depend on the particular equipment used.

Assessment Tool for Disease Severity

Spirometry has been a routine method for assessing lung function but is insensitive in detecting early airway damage (3, 32). In contrast, HRCT detects structural lung damage even in asymptomatic infants and children with normal spirometry (3, 33). Its use however is limited because of the necessary radiation exposure that occurs with scanning.

The superiority of MBW compared to spirometry regarding the sensitivity in detecting early demonstration of lung disease has been demonstrated in a large number of studies (3, 21, 23, 29, 31, 34, 35). In early stages of CF lung disease, many cases with normal FEV1% had abnormal LCI (3).

TABLE 1 | The role of LCI and CPET parameters in disease progression, prognosis, and intervention efficacy.

	LCI	CPET parameters
Disease progression	• Early detection of CF lung disease even though normal spirometry • Sensitivity to demonstrate structural damages shown on CT or MRI • Correlation with pathogens isolated from sputum cultures	• VE/VO2, VE/VCO2 reflect structural damage • VO2peak correlates with ventilation inhomogeneity
Prognosis	• Assessment tool in infancy and preschool age. Predictive for later lung function • Detection of the first isolation of *P. aeruginosa* even without symptoms • A predictor of PEx • Prediction of exercise intolerance and nocturnal hypoxemia	• Overall survival predicted by VO2peak, VE/VCO2 • PEx: • VE/VO2, VO2peak, PETCO2 predictive of future exacerbations
Intervention efficacy	• Assess the effectiveness of IV antibiotics, dornase alpha and hypertonic saline • Primary outcome measure of CFTR modulators	• Reliable outcome measure of interventions (intravenous antibiotics, CFTR modulator therapy, exercise prescription, transplantation assessment) • Richer data than spirometry in assessing therapeutic interventions • Usefulness as a meaningful endpoint for clinical trials

LCI also showed high sensitivity (92.3%) and positive predictive value (97.3%) in detecting structural damage as revealed by HRCT. A normal LCI can, in most cases, exclude the presence of HRCT abnormalities (3, 22). LCI has been found to relate in particular to the extent of bronchiectasis, and the presence of mucus plugging and emphysema (36). Such findings suggest that routine use of LCI is a reliable alternative to CT as a first line investigation to exclude lung damage whilst minimizing radiation exposure. Some studies recently investigated the performance of Magnetic Resonance Imaging (MRI) using hyperpolarized gas among patients with CF and also found a strong association between abnormalities detected and elevations in LCI (37–41).

LCI values have been investigated in relation to cough swab and sputum culture results among CF patients (16, 29, 42, 43). Patients with chronic lung infection with *Aspergillus fumigatus*, *Pseudomonas aeruginosa,* and *Stenotrophomonas maltophilia* had higher LCI levels than those with normal oral flora (44, 45). LCI values were associated with chronic infection with known CF pathogens (16) and pathogen load (42). Spirometry, LCI, and CT measures were all worse in patients colonized with *P. aeruginosa* compared to *Staphylococcus aureus*. It would appear that infection with the latter did not affect the subject's airways as significantly (44, 46, 47).

Ventilation inhomogeneity has been used to assess impacts on the daily life of CF patients. LCI has been shown to be predictive of exercise intolerance as assessed by cardiopulmonary exercise testing (48, 49). Chelabi et al. supported the utility of LCI to predict exercise limitation in children with normal FEV1 (50). Recently, Papale et al. evaluated the ability of LCI to predict nocturnal hypoxemia among CF patients and found that LCI was predictive of such abnormalities (AUC: 0.96, Youden index: 0.79) in stable patients with mild to moderate disease, FEV1 was predictive only in patient with more severe airways disease (AUC: 0.71) (51).

Assessment Tool for Disease Progression

LCI can be used to assess airway disease in infancy. Higher LCI, measured shortly after birth, correlated with a greater respiratory rate during the first year of life (52). Additionally, LCI values at the age of 3 months were predictive of LCI measurements during the first year of life (53). Normal LCI tended to remain stable (53) whereas those with raised levels tended to track at a higher level (54) despite there being minimal structural changes in this age group (55). Newly diagnosed patients discovered because of clinical symptoms had higher LCI values than those diagnosed through newborn screening (53). Abnormal LCI in preschool children is associated with many clinical parameters including F508del homozygous genotype, *P. aeruginosa* colonization and *nebulised dornase* alpha use. It is also predictive of spirometry in later childhood (56), as well as LCI during school age (57) and adolescence (58).

A significant benefit of LCI has been predicting the risk of first isolation of *P. aeruginosa* in previously non-colonized patients. An increase of LCI by 1.18 could predict first colonization (sensitivity 52%, specificity 70%), despite 81% of such patients being free of respiratory symptoms and an almost stable FEV1% (59). LCI values have also been shown to be predictive of the risk of pulmonary exacerbations with a cut-off of LCI change of 1.37 predicting the likelihood of such clinical events (sensitivity: 47.8%, specificity: 79.6%) (44). Studies suggest that a larger proportion of exacerbation events had a deterioration of LCI compared to a decline in FEV1% (41.7 vs. 30.0%, *p*:0.012) (60). Vermeulen et al. showed that the baseline LCI value of a patient could also predict the risk of developing a pulmonary exacerbation during the following year. Higher baseline LCI values were associated with earlier pulmonary exacerbation (61).

Assessment Tool of Interventions Efficacy

A remarkable role of LCI is its utility in estimating the effectiveness of therapeutic interventions. LCI improved significantly in CF patients after receiving IV antibiotic treatment for a pulmonary exacerbation. The improvement of LCI was greater than the change in spirometry (25 vs. 15%), but neither LCI nor FEV_1 recovered to baseline values (60, 62, 63). In contrast there were no significant changes in LCI in studies assessing the effects of nebulized tobramycin (28-day on/off regime) (64, 65). The positive impact of inhalation of dornase alpha (66) and hypertonic Saline (67–69) has been demonstrated using LCI measurements. More recently LCI has been used as a primary outcome measure to assess the efficacy of CFTR potentiators (ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor) (70–72).

Although the value of LCI has been established in an increasing number of CF services, the main limitations on its more widespread use is that is a time-consuming test requiring specialist equipment and appropriately trained personnel.

Cardio-Pulmonary Exercise Testing

Cardio-pulmonary exercise testing (CPET) provides a comprehensive assessment of pulmonary, cardiovascular and muscular function. Its principle is quite simple; implementing maximal exercise in a patient while monitoring their cardiac function, pulmonary gas exchange, and progressive muscle oxidation. The test can provide useful data about early pathological alterations in each of these compartments and information about the extent to which these abnormalities impact on exercise performance.

Exercise Testing on a cycle ergometer or treadmill is feasible from the age of around 6 years (73). The test procedure varies in terms of duration depending on the protocol used and the information that need to be obtained (74). The suggested protocol for routine clinical practice does not usually last more than 10–15 min (73, 75) and results are immediately available for interpretation. The test protocol for CF patients has been standardized and reference value equations are available even for young children (76).

CPET's use in CF was mainly research-oriented until, in early 1992, when Nixon et al. demonstrated that peak oxygen uptake during a maximal cardiopulmonary exercise test (the amount of oxygen a CF patient's lung can absorb during maximal exertion) is a prognostic factor for survival. Patients with VO_2peak >80% predicted showed excellent 8-year survival, whereas patients with low maximal oxygen uptake (VO_2 peak <60%) had worse outcomes (77).

CPET has since been used for clinical and research purposes in CF with studies highlighting its role in assessing overall disease progression. CPET parameters have also been shown to reflect structural and functional abnormalities and in particular, disease prognosis.

Assessment Tool of Structural, Functional Abnormalities, and Disease Severity

Cystic fibrosis is characterized by progressive inflammation and impaired mucus excretion (78). This vicious cycle leads to airway remodeling and impaired gas exchange (79). Exercise testing is more sensitive than spirometry in detecting structural abnormalities, as shown by High Resolution Computed Tomography (HRCT) both in adults (80) and CF adolescents (81). CPET is also indicative of functional alterations taking place in CF lungs. CPET parameters have been shown to be associated with ventilation inhomogeneity (48) and reflect increased dead space ventilation in CF patients as the disease progresses (82). Van de Weert et al. found that low exercise capacity was associated with chronic *P. aeruginosa* colonization and changes in immunoglobulin levels in CF adolescents (83).

Assessment Tool for Disease Progression and Survival

As Cystic Fibrosis lung disease progresses and pseudomonas colonization is established, exercise testing parameters also deteriorate (84). However, the most clinically relevant contribution of CPET to clinical practice is the implications of test results for long term survival. Aerobic fitness as indicated by VO_2 Max has a key role in determining prognosis (85, 86). In addition other indices including VE/VO_2 and VE/VCO_2 have also proved useful as predictors of death or lung transplantation at 10-year follow-up (87, 88).

Assessment Tool of Intervention Efficacy

Exercise testing has shown additional benefits over spirometry in characterizing for example the efficacy of therapeutic interventions to treat pulmonary exacerbations (89, 90). It has also been used as an outcome measure for assessing the effectiveness of CFTR modulators (87, 91, 92) and as an incentive for as well as measure of the effectiveness of pulmonary rehabilitation (93–96). It has also been used for exercise prescription (97–100), pre- and post- transplantation assessment (101–103) and as a primary and secondary endpoint measure in clinical trials (95, 104, 105).

CONCLUSION

LCI and CPET parameters are sensitive measures for detecting early lung disease, and predicting outcomes including colonization with infecting pathogens, pulmonary exacerbations and long term survival, **Table 1**. These findings have led to the use of these methods for routine patient assessment in many CF centers. Whilst data confirming the sensitivity of LCI and CPET in the early detection of CF lung disease are increasing, there is still a need for more information about validity, age specific reference values and their best use as tools to measure the benefits of clinical interventions. Future research might usefully afford greater insight into the best use of these measures in assessing the needs of increasingly patients with CF.

AUTHOR CONTRIBUTIONS

EH, AK, VA, IT, E-AC, MG, FK, and JT have made contributions to the design, editing, and writing of this manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Pediatrics

BRIEF RESEARCH REPORT
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Early Interleukin-22 and Neutrophil Proteins Are Correlated to Future Lung Damage in Children With Cystic Fibrosis

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Cystic Fibrosis (CF) lung damage begins early in life. Lung function decline is associated with pulmonary infections, neutrophil infiltration and inflammation. In CF, neutrophils have an altered phenotype. In this pilot study, we aimed to determine if signals of dysfunctional neutrophil responses were evident early in life and whether these signals may be associated with lung damage in later childhood. We examined the pulmonary protein profiles of 14 clinical stable infants and pre-school children with CF employing the aptamer-based affinity platform, SOMAscan®. High resolution computed tomography (HRCT) was performed on all children after age 6 years and Brody score calculated. A Spearman's rank order correlation analysis and Benjamini-Hochberg adjustment was used to correlate protein concentrations in early life to Brody scores in later childhood. Early life concentrations of azurocidin and myeloperoxidase, were positively correlated with Brody score after age 6 ($p = 0.0041$ and $p = 0.0182$, respectively). Four other neutrophil associated proteins; Complement C3 ($p = 0.0026$), X-ray repair CCP 6 ($p = 0.0059$), C3a anaphylatoxin des Arginine ($p = 0.0129$) and cytokine receptor common subunit gamma ($p = 0.0214$) were all negatively correlated with Brody scores. Interestingly, patients with more severe lung damage after age 6 had significantly lower levels of IL-22 in early years of life ($p = 0.0243$). IL-22 has scarcely been reported to have implications in CF. Identification of early biomarkers that may predict more severe disease progression is particularly important for the future development of early therapeutic interventions in CF disease. We recommend further corroboration of these findings in prospective validation studies.

Keywords: cystic fibrosis, proteomics, bronchoalveolar lavage, neutrophils, biomarkers

INTRODUCTION

Cystic fibrosis (CF) is characterized by frequent pulmonary exacerbations resulting in bronchiectasis, irreversible lung damage and eventually lung failure. The path of disease progression is established early in life with lung damage already evident in patients with CF as early as 6 years of age (1). Bacterial infections and the associated inflammation are the most common cause of morbidity and lung function decline (2). One of the clinical hallmarks of CF is the increased

burden of neutrophils in the airways. Neutrophils are not only important effectors of bacterial phagocytosis, but they also dominate the inflammatory response. In the CF airways, neutrophils show a dysfunctional phenotype that deviates from their wild type counterparts (3).

High resolution computed tomography (HRCT) is accepted as a sensitive means of detecting early structural lung disease in CF, demonstrating abnormalities before the onset of clinical, spirometric, or plain radiographic abnormalities (4, 5). HRCT scanning is widely available and validated scoring systems have been developed (5, 6). Brody et al. (7) developed a sensitive, reproducible HRCT scoring system to evaluate CT features of CF lung disease suitable for use in children. Images are assessed for the lobar location and severity of morphological and parenchymal changes. These include bronchiectasis, peri-bronchial thickening, mucous plugging, atelectasis, consolidation, air/fluid levels, hyperinflation, and overall impression (6, 8).

We hypothesize that evidence of heightened neutrophil degranulation within the lungs of CF patients early in life will correlate with enhanced pulmonary injury later in life. Here we examined pulmonary proteins from bronchoalveolar lavage (BAL) in the first years of life that may be predictive of CF disease severity later in life. In CF, signals of early neutrophil dysfunction could be used to identify patients at risk of following a more severe disease pathway. We therefore were specifically interested in defining the presence or absence of specific proteins that have been implicated in neutrophil biological activity. This study aimed to identify BAL fluid proteins present in early life that correlate with severity of CF lung disease after age 6.

MATERIALS AND METHODS

BAL was obtained from 14 clinically stable infants and pre-school children (median age 2 years, range 1–5 years) with CF undergoing bronchoscopy as part of an annual surveillance program (**Table 1**). Clinical stability was defined as being asymptomatic with no change from baseline for 4 weeks prior to BAL. Bronchoscopy was performed through a laryngeal mask airway instilling 1 ml/kg sterile 0.9% NaCl per aliquot twice in the right middle lobe and twice in the lingula. Pooled aliquots were centrifuged at 10,000 × *g* for 10 min. The protein content of supernatants was determined by Quant-iT^{TM} protein assay kit (Thermo Fisher). A minimum concentration of 20/200 μl of each sample was shipped to SOMAscan for proteomic analysis. SOMAscan® is an aptamer-based affinity platform capable of measuring 1,305 human protein analytes in lung fluid with high sensitivity and specificity (9). High resolution computed tomography (HRCT), free breathing on inspiration was performed on all children during clinical stability at their most recent clinic (median age 6.5 years, range 5–10 years) and Brody score calculated by blinded radiologist (7). The Brody lobular score system has been shown to be a sensitive method for evaluating the progression of CF lung disease (6). There was a median of 4.5 years between initial BAL and follow-up HRCT (range 1–7 years). All statistical analyzes were performed using GraphPad PRISM version 8. $P < 0.05$ were considered statistically significant. To correlate protein concentration to Brody scores, a Spearman's rank order correlation analysis and Benjamini-Hochberg adjustment with a false discovery rate of $q = 0.10$ was performed.

TABLE 1 | Patient demographics and clinical details at time of BAL collection.

	Male	Female
Patient number (*n*, %)	7 (50)	7 (50)
Age (years) (mean, +/– SD)	2.1 (0.9)	2.5 (1.5)
BMI z score (median, +/– SD)	0 (0.89)	0 (0.82)
F508del/F508del (*n*, %)	3 (42.9)	3 (42.9)
F508del/G551D (*n*, %)	1 (14.2)	1 (14.2)
F508del/other (*n*, %)	3 (42.9)	3 (42.9)
Ivacaftor (*n*, %)	1 (14.3)	0 (0)
Long-term prophylactic antibiotics (*n*, %)	4 (57.1)	2 (28.6)
P. aeruginosa positive patients (*n*, %)	2 (28.6)	1 (14.3)
S. aureus positive patients (*n*, %)	5 (71.4)	4 (57.1)

BMI, body mass index; n, number; SD, standard deviation.
No patients were on Lumacaftor, prednisolone, antifungals, or inhaled antibiotics at the time of BAL collection.

RESULTS

SomaScan detected a total of 234 proteins and of these 18 were significantly correlated with Brody Scores. All proteins were detected at or above the limit of detection (LOD) of 38rfu (SomaScan white sheet). A total of 7 proteins implicated in inflammation and neutrophil function were observed to correlate with Brody scores (**Figure 1**). Early life concentrations of azurocidin and myeloperoxidase, (two neutrophil granule proteins) were significantly higher in patients with greater lung damage after age 6 ($p = 0.0041$ and $p = 0.0182$, respectively). Four immune proteins; Complement C3 ($p = 0.0026$), X-ray repair CCP 6 ($p = 0.0059$), C3a anaphylatoxin des Arginine ($p = 0.0129$) and cytokine receptor common subunit gamma ($p = 0.0214$) were all negatively correlated with Brody scores. Patients with more severe lung damage after age 6 had significantly lower levels of IL-22 in early years of life ($p = 0.0243$).

DISCUSSION

Sputum biomarkers of infection and inflammation have previously been suggested as correlates of present disease severity (10). In particular elastase, interleukin-8 (IL-8), matrix metalloproteinase (MMP)-9 and neutrophil counts were negatively correlated with current lung function (10, 11). Neutrophil elastase activity in BAL fluid in early life was associated with early bronchiectasis in children with CF (12).

Azurocidin is an antibacterial glycoprotein released from neutrophils upon degranulation in response to tissue injury or infection. Azurocidin recruits and activates monocytes resulting

Abbreviations: CF, cystic fibrosis; BAL, bronchoalveolar lavage; IL, interleukin; MMP-9, matrix metalloproteinase 9.

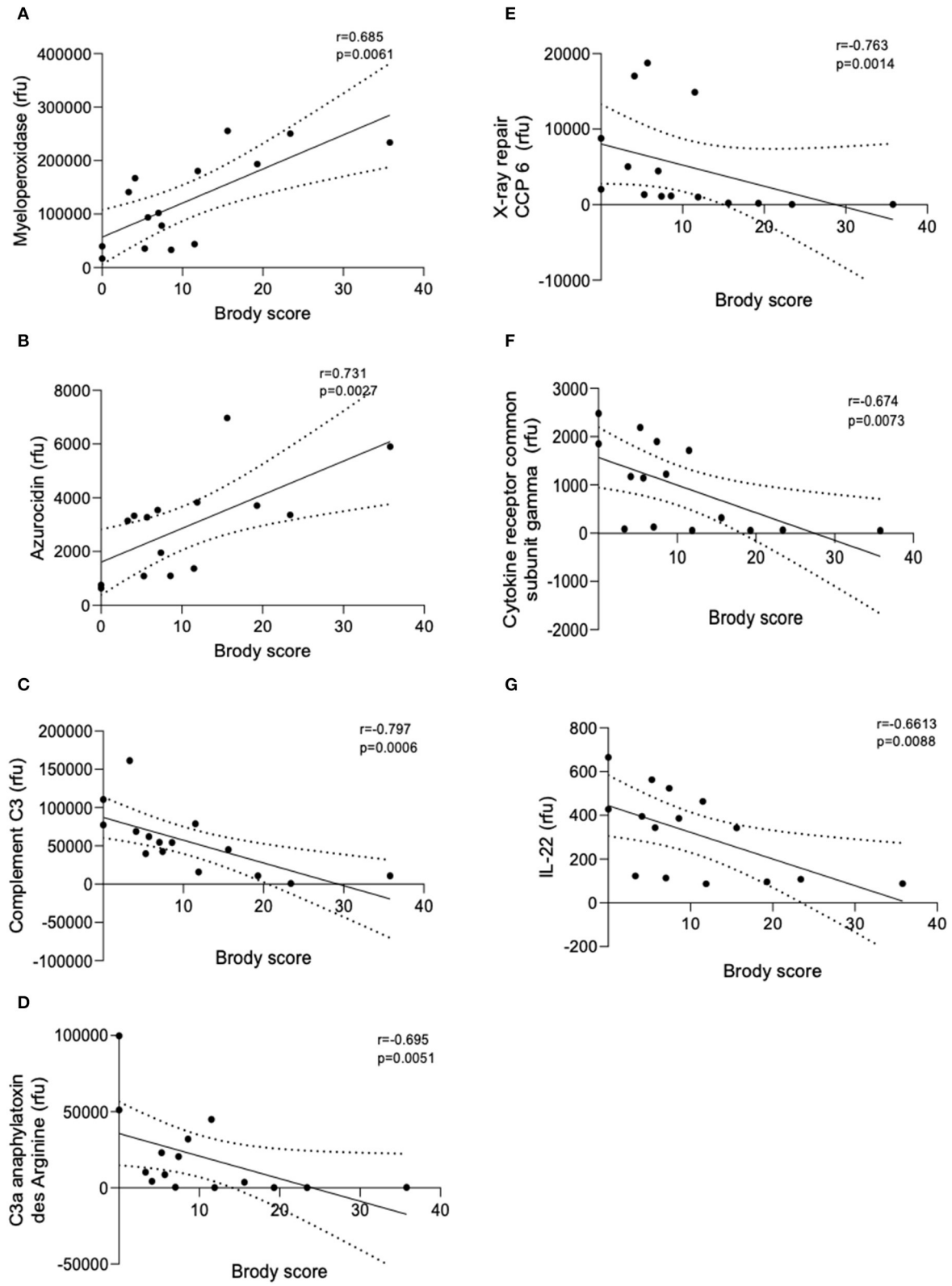

FIGURE 1 | Linear regression models of BAL protein concentrations correlated with Brody score. Linear regression analysis of the relative fluorescent units (rfu) of the seven proteins that correlated with Brody scores. **(A)** Myeloperoxidase, **(B)** Azurocidin, **(C)** Complement C3, **(D)** C3a anaphylatoxin des Arginine, **(E)** X-ray repair CCP-6, **(F)** Cytokine receptor common subunit gamma, and **(G)** IL-22. Spearman's Rho = r. Coefficient of determination = R^2. Dashed lines represent 95% confidence intervals.

in cytokine release and increased phagocytosis. Myeloperoxidase is a peroxidase enzyme that catalyzes the formation of reactive oxygen intermediates which play an important role in microbial killing. Myeloperoxidase, present in the lung fluid of CF patients, has been suggested to contribute to lung tissue destruction and the early pathogenesis of CF (13). Both of these neutrophil granule proteins were correlated to more severe lung damage after age 6 suggesting early disruptions in neutrophil function and excessive neutrophil degranulation as determinants of later disease severity. Of note, IL-8 and neutrophil elastase were not correlated to Brody score in this study however this may be due to the young age of patients and the relative stability at time of bronchoscopy.

Interleukin-22 (IL-22) is involved in mucosal host defenses including tissue repair and protection, increasing innate defenses and maintaining epithelial barrier functions. IL-22 has been shown to provide critical immunity against a number of pathogens including *Klebsiella pneumoniae*, *Streptococcus pneumoniae, Pseudomonas aeruginosa*, and *Aspergillus fumigatus* (14). It has been suggested that the presence of neutrophils in the lungs of CF patients results in an acquired deficiency of the IL-22 pathway due to IL-22 degradation by neutrophil proteases (15). IL-22 has also been negatively correlated with neutrophil recruitment in the lungs (16). Here we observed that low levels of IL-22 in early life were correlated with more severe lung damage after age 6. Low levels of IL-22 early in life may pre-dispose certain sub-populations of CF patients to poorer immunological responses to infection, increased neutrophil recruitment and so contribute to greater lung damage later in life. Complement C3, C3a, X ray repair CCP6, and common receptor cytokine subunit gamma were all negatively correlated with Brody scores. The complement component C3 plays a central role in the activation of the complement system. Derived from C3, C3a anaphylatoxin is a potent local inflammatory mediator. Xray repair CCP6 is a DNA repair protein and the common receptor cytokine submit gamma is integral to regulating immune system functions. Low levels of these proteins may lead to dysfunctional immune responses. Nonetheless, this study has a number of limitations. The main limitation of this study is its small sample size. The widely used aptamer-based assay, SomaScan, provides relative protein quantification and further studies should include absolute quantification methods in combination. Considering these limitations results should be interpreted with caution and larger validation studies should be conducted to corroborate these exciting findings.

CONCLUSION

Identification of early biomarkers is particularly important in order to intervene in the progression of CF disease. We have shown that several neutrophil proteins in early CF BAL correlate with more severe lung disease later in childhood and therefore may be useful in identifying particularly vulnerable patient populations. Additionally, IL-22 may be a novel target to investigate in the inflammatory process in CF. We recommend further corroboration of these findings in prospective validation studies. This is the first description of early life IL-22 levels correlating with worse CF lung injury in later life.

DATA AVAILABILITY STATEMENT

All Data relevant to this study is displayed in **Table 1**.

ETHICS STATEMENT

The ethics for this study was reviewed and approved by Tallaght University Hospital & St James's Hospital joint research ethics committee (2019-09 List 35(01)). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JR, SD, and PG: conception of study. PG: performed bronchoscopies and collected samples. JR and ER: carried out laboratory work. CO'L, JR, ER, JW, and RW: collated clinical data and analyzed data. TP: performed brody scoring. JR, ER, SD, and PG: wrote paper. All authors read and approved the final manuscript.

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REVIEW
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Sweat Testing and Recent Advances

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Cystic fibrosis (CF) is the most common fatal genetic disease of the Caucasian population. Sweat testing is the principal diagnostic test for CF, and it is used for the evaluation of infants with positive CF newborn screening (NBS) and in patients with clinical findings suggesting CF. This article describes the classical sweat test method in detail and also provides an overwiew of recent advances.

Keywords: cystic fibrosis, diagnosis, sweat test, Gibson-Cooke, chloride

INTRODUCTION

High concentrations of chloride (Cl^-) detected in sweat from patients in the early 1940s resulted in the development of the sweat Cl^- test (ST), and by 1959, the test was being used by Gibson and Cooke (1). Since the discovery of the cystic fibrosis (CF) gene, encoding the CF transmembrane regulator (CFTR) protein in 1989, more than 2000 mutations have been reported. The CFTR is located on the apical membrane of the epithelial cells in the exocrine secretory system that includes the sweat glands. Defective CFTR function mainly results in abnormal Cl^- transport across the Cl^- channels as well as diminished sodium transport across the cell membrane. Reduced Cl^- secretion and enhanced sodium reabsorption across the epithelial cells increases the viscosity of secretions, and in the sweat, Cl^- concentration is elevated (1–3).

Detection of elevated values of sweat Cl^- by the quantitative pilocarpine iontophoresis test (QPIT) performed via chloridometer is accepted as the gold standard in CF diagnosis. This technique is performed in three stages: cholinergic stimulation of sweating with iontophoresis, collection of the sweat sample, and measurement of sweat Cl^- concentration (4–9).

Well-organized and accurate ST procedures are especially important in countries with limited access to genetic testing. After the introduction of new CFTR modulators in the treatment of CF, ST has become even more important. Besides its role in the diagnosis of CF, normalization of sweat Cl^- concentrations after administration of modulator therapies is used as proof of their efficacy (10, 11).

Because QPIT is relatively complicated to carry out; the technique and the multiple steps of the process need to be well-understood (6). Several evidence-based guidelines on how to perform the test properly have been published; each including a detailed description about sweat induction, collection, analysis, and interpretation (4–6, 8, 9). The English language guidelines were developed by the Clinical and Laboratory Standards Institute (CLSI, USA), Multi-Disciplinary Working Group (UK), and The Australasian Association of Clinical Biochemists (AACB, Australasia) (4–6). National guidelines are also available in French and Turkish and are actively used in France and Turkey (8, 9).

The CLSI guideline for ST was revised in 2019 and is officially recommended by the American Cystic Fibrosis Foundation (CFF) (4). According to this guideline, there is a single agreed-upon methodology for pilocarpine iontophoresis and sweat collection but several acceptable methods for sweat analysis (7). Recent reports, however, state that there are still differences in laboratory techniques employed in testing in many countries, particularly lower income countries. Poor adherence to published guidelines suggests an inability to meet quality standards in laboratory diagnostics and, consequently, casts doubt on the accuracy of the results (10–12).

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Lack of access to pilocarpine, equipment, and trained laboratory staff, coupled with the relative difficulty of the recommended technique for QIPT have caused a search for an ST method that is easier to carry out. Additionally, collecting a sufficient amount of sweat can be challenging in infants (13, 14). For these reasons, some devices that use different, simpler methods have been developed for measuring sweat Cl^-, and the safety and efficacy of these methods are reported by controlled studies (15–20). In this study, we review the QIPT and the newer alternatives now being employed in the diagnosis of CF.

INDICATIONS

The ST is indicated for individuals suspected to have CF, either from positive NBS or the presentation of clinical features suggestive of CF. CF genotyping is recommended in all patients with positive or borderline ST results and also in patients in whom ST is not technically possible. It is also necessary to identify which CF patients are eligible for CF-mutation-specific therapy. The CFF recommends CF genotyping in all patients diagnosed with CF (7, 11, 12, 21).

Patients with CF may have a variety of clinical manifestations. Some neonates may have meconium ileus but have IRT levels in the normal range; in these cases, ST should be carried out. Young children may have pulmonary complications, such as recurrent pneumonia, chronic sinusitis, nasal polyps, or persistent and recurrent wheezing and coughing. The most common gastrointestinal findings are failure to thrive with malabsorptive stools and recurrent abdominal pain. Patients with these findings should be referred to the ST center (7, 21–23).

Because sweat Cl^- concentration can be temporarily elevated in the first day of life, ST should be done later than 48 h after birth and optimally at the 10th day (4). ST should be postponed in premature infants until they reach 2 kg of weight and more than 36 weeks corrected gestational age. Ideally, the child should be well-hydrated and should not have acute illness. ST can be carried out for subjects requiring oxygen via mask or nasal canula. Newborns and infants that are receiving open system oxygen in the incubator should not be tested because sparks can be produced during the iontophoresis phase of the sweat test when a low electric current is applied (4, 22, 23).

SWEAT COLLECTION

It is recommended to perform ST in an accredited care center by a trained technician (4–7). The ST is typically performed on the patient's arm or leg. The test starts with iontophoresis of pilocarpine, a parasympathomimetic alkaloid, which acts on the cholinergic receptors by mimicking acetylcholine, to stimulate sweat production by sweat glands. Collection of two simultaneous samples is recommended because of the variability of the test and insufficient sample risk (4).

In the original Gibson and Cooke method, iontophoresis is done by placing two electrodes on the patient's arm or leg and covering one of them with pilocarpine-soaked gauze and the other with deionized water–soaked gauze. An electric current of maximum 1.5 mA is then applied for 5 min to stimulate sweat production. The electrical stimulation is painless and causes no discomfort. Sweat is collected for a period of up to 30 min. For the gauze or filter paper method, the stimulated area must be 2×2 inches. The filter paper is then placed in a laboratory dish of known weight so that the quantity of the collected sweat can be calculated. The minimum quantity required for sweat collected from the gauze method is 75 mg (4). However, conventional procedures, such as those using gauze and filter paper, carry a significant risk of evaporation unless performed by trained and experienced staff. Errors made during sweat stimulation and collection and analysis can cause skin burns and also volumetric, gravimetric, condensate, and evaporation inaccuracies. This is especially significant in young, particularly preterm, infants (4, 13).

In 1983, the Wescor (Logan, Utah) Macroduct system of sweat collection was developed. This technique was easier to perform than the conventional QIPT and requires only 15 ml of sweat. Gel discs containing pilocarpine are utilized, and the iontophoretic current passing through these discs stimulates sweat production. For safety, the iontophoretic current source needs to be battery powered. Capillary tubing is used for collecting the sweat produced by induction of sweat glands.

The sample is analyzed using a variety of techniques. In young infants, however, there is still a higher risk of insufficient quantity (QNS) ST. McColley et al. reports that 27% of CF Centers in the United States state a mean QNS rate of 10.5% in infants 14 days old or younger (13, 24, 25). Collecting sweat from two sites is, therefore, recommended in infants. Bilateral testing increases the likelihood of collecting a sufficient specimen from at least one site. If the quantity of sweat collected from one site is insufficient for analysis, the sweat samples from various sites should not be combined; in this case, the ST should be repeated (26, 27).

Currently, both the Gibson and Cooke QPIT and the Macroduct® systems are recommended for sweat collection in CF diagnosis (**Figures 1**, **2**). The Chloridometer, the conventional device for sweat Cl^- analysis, also utilizes the Macroduct® coil for sweat collection (4).

In studies comparing these two systems, equivalent results are reported. The only significant difference between the Gibson and Cooke and Macroduct® QPIT sweat collection systems was sample matrix. The sample for the Gibson and Cooke QPIT is diluted because of the need for elution from the collection medium. The Macroduct® QPIT sample is collected into tubing and can be analyzed directly.

BIOCHEMICAL ANALYSIS

Coulometry is the unique method of sweat Cl^- analysis approved and described in detail in the CLSI guidelines; it involves coulometric titration with a chloridometer. A chloridometer measures the free Cl^- concentration in an acidic solution by allowing current to flow through a circuit. Free Cl^- ions bind with silver cations generated from silver electrodes to form silver Cl^- molecules; these no longer conduct the electrical current. The chloridometer measures how much current flows and how

FIGURE 1 | Iontopheresis and sweat collection by Gibson-Cooke method.

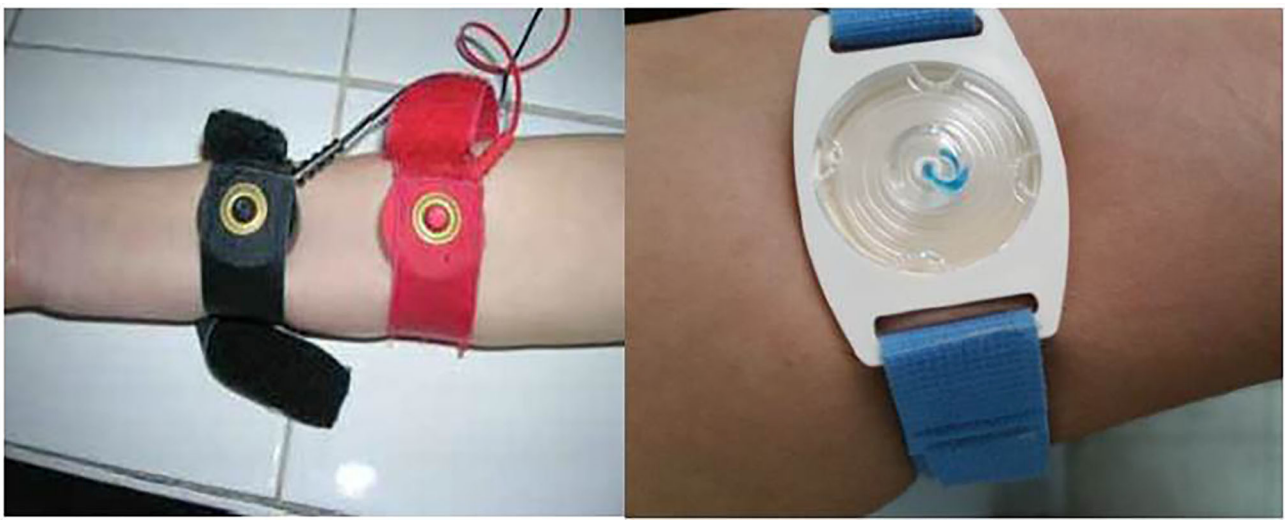

FIGURE 2 | Iontopheresis and sweat collection by Macroduct System.

long it flows for in order to determine how many free Cl^- ions are in the solution at the beginning of the process. Using a specimen volume of ≥15 μL, a chloridometer can measure Cl^- concentrations from 10 to 160 mmol/L (4).

Sweat collection and analysis should be performed on the same day, and the results and their interpretation should be reported to clinicians as soon as possible. Analysis of the sweat shortly after collection or within a few hours should be routine procedure for the ST center (21). Collected sweat should not be stored or transported via the coiled tubing system because of the evaporation risk. If a significant delay is expected between collection and analysis, the laboratory may store specimens in 0.2-mL microcentrifuge tubes for 72 h without significant evaporation (4). For the purpose of shipment, in the case of collection of sweat during clinical trials, the specimens can be stored frozen (−70°C) and accurately analyzed later.

The ion-selective electrode (ISE) method may also be used to measure Cl^-, but there are limited data available on its use. This method converts the activity of a specific ion dissolved in a solution into an electrical potential, which is measured by a voltmeter (3). The ISE technique is included in the UK and Australian ST guidelines as an acceptable method; however, it has not been validated systematically for accuracy of sweat Cl^- measurements. The CLSI suggests that, if it is used, the laboratory must validate the method against the traditional ST. The major concern about ISE measurement is that decreased

sensitivity at lower concentrations could lessen the precision of the result (4–6).

SWEAT CL⁻ INTERPRETATION

The cutoff value for sweat Cl^- testing is the same regardless of a patient's age (7, 28). A level of Cl^- of higher than 60 mmol/L in the sweat is indicative of CF; concentrations lower than 30 mmol/L are considered normal, and CF is unlikely. A level between 30 and 59 mmol/L is defined as intermediate (borderline), and repeated ST or additional diagnostic tests are required (28). After a positive ST result, either ST is repeated or genetic testing is performed to confirm the definite CF diagnosis (12). A diagnostic algorithm for CF for interpreting the results of the ST is presented in **Figure 3**.

INTERFERING FACTORS

Variability in sweat Cl^- levels is shown in previous studies, but sweat Cl^- biological variability, in both healthy people and CF patients is not well-known (29, 30). Results from a total of 5,960 tests from two CF centers were reported by Vermeulen et al. According to this study, in 90% of subjects, −3.2 and +3.6 mmol/L changes were obtained from samples taken on both sides collected at the same test occasion. However, two separate tests showed much higher variability with changes between −18 and +14 mmol/L in 90% of the subjects. Biological variability mostly affected the intermediate test results, and some of them returned to within normal range with the repeated tests in that study. On the other hand, sweat Cl^- values higher than 60 mmol/L showed small biological variability (30).

Another concern regarding ST is false positive cases. The most common reason for a false positive ST is technical error during the procedure, such as evaporation of the sweat sample. The incidence of this problem is reduced by correct implementation and adherence to recommended testing procedures and by ensuring that the test is performed in adequately equipped laboratories and by properly trained personnel (4).

Sweat Cl^- levels may also be elevated falsely in other pathologic conditions, including atopic dermatitis, ectodermal dysplasia, pseudohypoaldosteronism, untreated hypothyroidism, glycogen storage disease type I, carbonic anhydrase XII mutations, malnutrition, and anorexia nervosa. Elevated sweat Cl^- concentrations in non-CF patients may also be related to iatrogenic causes, such as mineralocorticoid, $NaCl^-$ perfusion, and topiramate treatment (21). The underlying mechanism for false positive results in many conditions is unknown. The possible sweat gland function impairment associated with the skin manifestations may be the reason for high levels of sweat Cl^- in patients with atopic dermatitis and ectodermal dysplasia (31). Hyperchlorhidrosis caused by otosomal recessive inherited Carbonic Anhydrase XII deficiency should be considered in the differential diagnosis of a positive ST, especially with the clinical findings of hyponatremic dehydration during infancy. High sweat Cl^- levels during treatment with topiramate may be the result of the inhibition of carbonic anhydrase isotypes in the sweat gland ducts (32).

REPORTING RESULTS

Name, surname, and the date of birth of the patient and the date and hour of the test should be recorded. The type of ST employed; the level of Cl^- measured; the unit of measurement; if the value is normal, borderline, or high; and the interpretation of the test result should all be specified in the test report. Cl^- concentrations in whole numbers should be reported using mmol/L units. In quantitative Cl^- measurements, mmol/L and milliequivalent per liter (mEq/L) are equivalent. It is not necessary to report the total sweat volume collected if an adequate volume was ensured (4).

OTHER ST METHODS

Conductivity

Although quantitative sweat Cl^- measurement is the unique approved method for CF diagnosis, sweat conductivity measurement is easier and also commonly used in many settings throughout the world. Conductivity depends on the concentration and mobility of the ions within a solution and reflects a non-selective measurement of ions. As the bicarbonate and lactate ions in the sweat affect the conductivity, results do not show the sweat Cl^- concentration (26, 27, 33). Mean sweat conductivity test results are ∼15–20 mmol/L higher than sweat Cl^- measurement. Several sweat conductivity measuring instruments are available.

A conductivity instrument was approved as a screening method (8). A sweat conductivity test result is defined as abnormal if the value is ≥50 mmol/L, and these patients should be referred for a quantitative ST to confirm the diagnosis of CF (4).

According to the CLSI guideline, conductivity should not be used as a diagnostic test for infants with a positive NBS result. These babies should be tested with quantitative ST (4). There are many studies, however, that have compared the conductivity ST method with conventional coulometric ST and that show adequate efficacy and safety (15, 33, 34).

Nanoduct® is one of the ST devices used with the conductivity method. It was developed especially for use with newborns, and only 3 μL of sweat is required. As soon as sweat enters the microconductivity cell, the ST result is shown on the display (35).

In the national NBS program for CF in Switzerland, Macroduct® collection (with Cl^- concentration measurement) and Nanoduct® test (measuring conductivity) methods were compared. Although only 60% of Macroduct® tests were successful at the first attempt, the Nanoduct® had a higher rate of successful outcome (79%), and it was as sensitive as the Macroduct® in identifying newborns with CF (sensitivity 98 vs. 99%, respectively) but less specific (specificity 79 vs. 93%) (35). Another study from the Netherlands also showed that Nanoduct® fails less often in newborns than the Gibson and Cooke/Macroduct® because it can operate with a small quantity of sweat, and it is, therefore, advocated that it can be used to

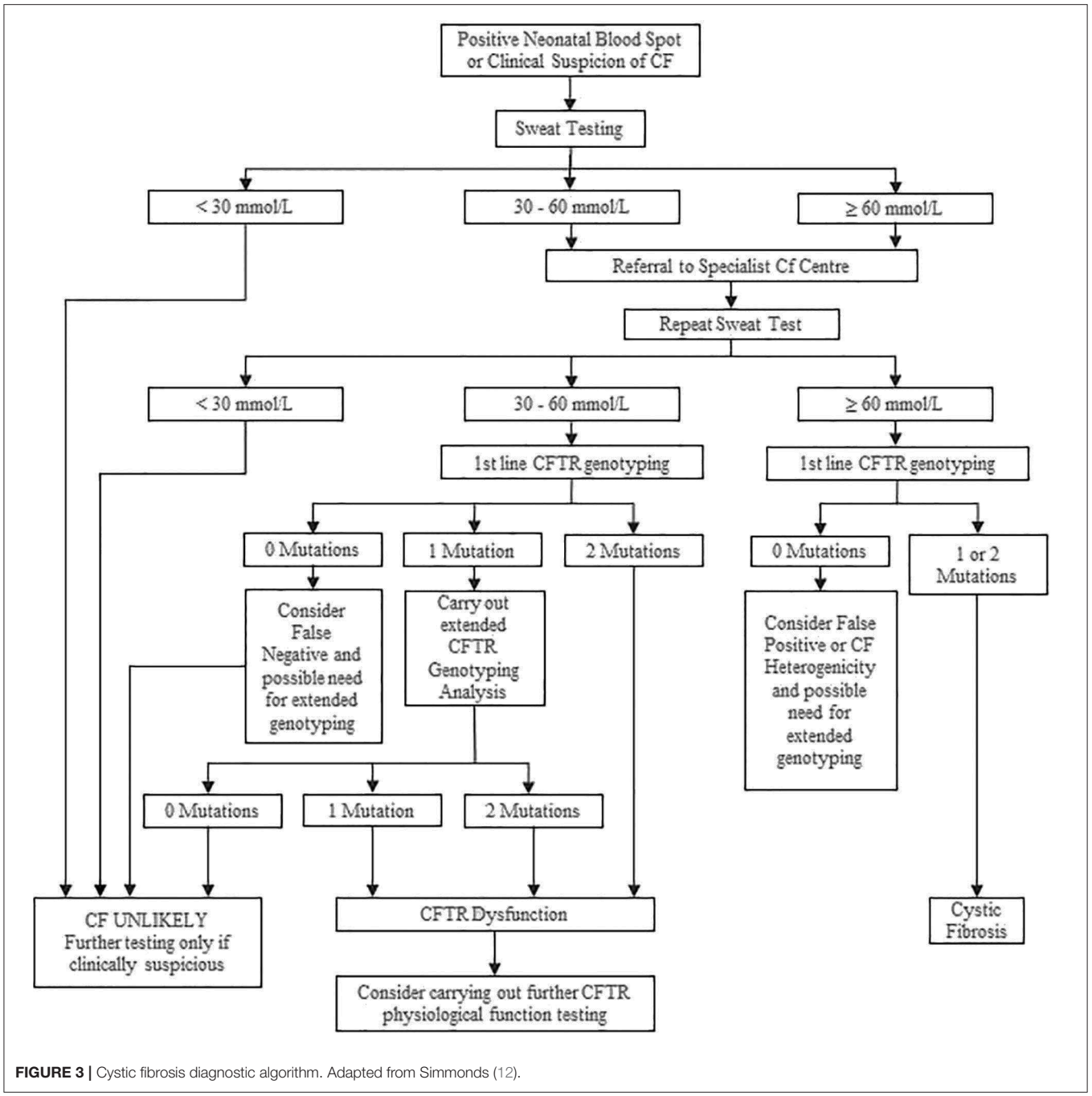

FIGURE 3 | Cystic fibrosis diagnostic algorithm. Adapted from Simmonds (12).

confirm the diagnosis CF in infants with a positive NBS test for CF (36).

Coulometric Endpoint Method

The coulometric endpoint method utilizes an electrolysis reaction measuring the changes in resistance to the current flowing between electrodes. The concentration of the solution is equivalent to the current generated. Sweat is collected by a tube similar to Macroduct® coil that reduces the risk of sample evaporation. This method is approved by the CLSI, UK, and Australian ST guidelines (4–6).

The CFΔ Collection System® is a new-generation, ST analyzer manufactured by UTSAT, which is based on this coulometric end point method. Studies comparing this device with quantitative sweat Cl^- analysis demonstrate that the coulometric end point method is safe and can be as reliable as gold standard methods. This device is approved by CE, commercially available, and routinely used in Turkey, in some of the Middle East and African countries, and in Azerbaijan (18, 37).

This method was compared with both the titrimetric Cl^- measurement (Sherwood® Chloridometer 926S, Sherwood Scientific Ltd., Cambridge, UK) and the classical Gibson and

Cooke and manual titration methods. Bland–Altman plots were used to analyze the agreement between methods in the healthy controls and the CF subjects. Good agreement was obtained between the coulometric end point technique and the gold standart ST methods (18).

Ion Exchange Technology: Wearable Sweat Sensor

This technique is based on ion exchange technology accepted by the CLSI Guidelines and measures quantitative Cl– levels with the accuracy of the traditional method (19).

Recently, several reports have been published on wearable sweat Cl analyzer for CF diagnosis (16, 19). CF Quantum® Sweat Test System (CFQT) is one example that is awaiting approval by the FDA and the CE; it is manufactured by Medtronic Inc., Minneapolis, Minnesota. Sweat production is stimulated with pilocarpine via a portable, wearable electrode and collected by a Cl^- test patch. Finally, the sweat Cl^- value is calculated by an analyzer after scanning the patch with a camera. The time needed to achieve a reliable result is short, and the result is reported in 30 min with a small sample volume (9 μL). This technique was compared with the conventional coulometric method in both CF patients and healthy controls. The sweat Cl^- concentrations obtained from the wearable sensor showed excellent agreement with the conventional tests with a Pearson correlation coefficient of $p = 0.97$. Sweat Cl^- measurements for all healthy subjects were within the accepted threshold for normal (≤29 mEq/L; 16–27), and all individuals with CF were above the accepted threshold (≥60 mEq/L; 65–130), confirming CF diagnosis. The correlation coefficient between the CFQT and conventional ST was 0.98 [95% confidence interval (CI): 0.97–0.99]. The sensitivity and specificity of the CFQT in diagnosing CF was 100% (95% CI: 94–100%) and 96% (95% CI: 89–99%), respectively (16).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is used in the clinical laboratory on a routine basis to an increasing extent, mainly to determine the presence of oligoelements. It can also be employed for sweat Cl^- assay and provides accurate measurements, especially at low Cl^- concentrations (38). Pullan et al. used ICP-MS to analyze sweat Cl^- and sodium for the measurement of ST, collected via a Macroduct® sweat collector tube (39). Collie et al. demonstrated that both online (instrument based) and off-line (sample based) internal standard methods measuring Cl– were successful in providing accurate, reproducible results (17). Marvelli et al. conducted a study comparing this method with gold standard coulometric titration in 50 healthy volunteers and two CF patients. The method was then cross-validated by assaying 50 standard samples with Cl^- concentration values in the range 10–131 mM by both ICP-MS and coulometric titration. Bland–Altman plots confirmed the analogous concentration levels for coulometric titration and ICP-MS; bias had a value of −0.9 (95% CI = −1.96 ÷ 0.20) with lower and upper limits of agreement of −8.3 (95% CI = −10.18 ÷ −6.47) and 6.6 (95% CI = 4.71 ÷ 8.42), respectively. Consequently, the authors report good correlation between the two Cl^- analysis techniques (38). Although not involved in any of the current English language ST guidelines, ICP-MS is utilized by a number of ST laboratories, especially in Australia, the UK, and Italy. Authors confirm this method is as safe and accurate as the conventional coulometric method and suggest it be recognized as a candidate reference method for the monitoring and diagnosis of CF (17, 39, 40).

Capillary Electrophoresis: Skin Wipe Test (SWT)

In the SWT, unstimulated, spontaneously formed sweat is collected using a cotton swab moistened with deionized water, then extracted. The collection procedure is non-invasive and faster than the conventional ST method. Evaluation by SWT with contactless conductivity detection, typically performed in the biochemical laboratory, analyzes the whole "sweat ionome" (41).

Durc et al. compared SWT with the conventional coulometric method in 114 CF patients, 76 healthy carriers, and 58 controls. The SWT method with capillary electrophoretic analysis for CF diagnosis performed comparably with the conventional Macroduct® ST. The SWT method evaluated Cl^-/K^+ and $(Cl^-+Na^+)/K^+$ ion ratios for CF diagnosis. Two ion ratios, Cl^-/K^+ and $(Cl^-+Na^+)/K^+$, from the SWT samples and Cl^- values from the ST samples were evaluated to diagnose CF. Sensitivity of the SWT method using the Cl^-/K^+ ratio (cutoff value 3.9) was 93.9% compared with 99.1% when using the $(Cl^-+Na^+)/K^+$ ratio (cutoff value 5.0) and 98.3% in using Macroduct Cl^- (cutoff value higher or equal to 60 mmol/L). The method specificities were 97.8, 94.0, and 100.0%, respectively. The authors propose the SWT as a new diagnostic technique for CF (20).

ST for Outcomes in Clinical Trials

The increased use of CFTR modulators in the treatment of CF has highlighted the need for precise and accurate biomarkers to evaluate their efficacy. These therapies may not result in equivalent clinical improvement for all CFTR mutations, and the Cl^- concentration in sweat can serve as a useful biomarker of CFTR function, *in vivo*, in assessing the response to modulator treatments (18). In terms of the treatment response, studies show correlations between functional classes of CFTR variants and sweat Cl^- concentration (19, 20). In clinical use, baseline and serial sweat Cl^- measurements are usually used to monitor the effects of therapies targeting CFTR function in previously diagnosed CF patients (4, 16, 17, 21).

CONCLUSION

Sweat Cl^- concentration is the first-choice test to confirm a CF diagnosis. In addition to this, it is also essential in monitoring the efficacy of modulator treatments. All steps of ST are subject to a risk of error, resulting from inexperienced laboratory personnel or lack of appropriate quality assurance. Inaccurate methodology of the sweat collection, technical error, and misinterpretation of the results are all possible. Additionally, with the increasing frequency of NBS all over the world, the need for ST in the neonatal period and also in very low-weight babies is increasing.

These have all led to efforts to create easier to carry out but still reliable, ST methods and procedures. Although, as described in the paper, a number of newer methods have been developed and are being used, these methods still need careful interpretation in decision making for CF.

AUTHOR CONTRIBUTIONS

YG and BK have made contributions to the design, editing, and writing of this manuscript. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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Continuous Glucose Monitoring as a Valuable Tool in the Early Detection of Diabetes Related to Cystic Fibrosis

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Aims: We evaluated the impact of cystic fibrosis-related diabetes (CFRD) on lung disease and nutritional status.

Study Design: The retrospective cohort study evaluated the subjects' medical records from 2004 to 2019. All participants older than 10 years diagnosed by a 30-minutely sampled OGTT formed OGTT-CFRD subgroup. The participants diagnosed with continuous glucose monitoring (CGM) (at least two peaks above 11.1 mmol/l and more than 10% of recorded time above 7.8 mmol/l) formed a CFRD-CGM subgroup. The participants without CFRD formed a non-CFRD group. The longitudinal follow-up was made 2 years before and 3 years after insulin therapy initiation.

Results: Of 144 participants included, aged 10–55 years (44% males), 28 (19.4%) had CFRD. The HbA1c was significantly lower in the CGM-CFRD in comparison to the OGTT-CFRD subgroup (5.9 ± 0.62 and 7.3 ± 1.7% respectfully; $p = 0.04$). Subjects with CFRD were malnourished in comparison to non-CFRD, with significant improvements with insulin replacement therapy in regard to BMI Z-score (−1.4 ± 1.3 vs. −0.5 ± 1.2%, $p = 0.04$) and pulmonary exacerbation score ($p = 0.02$). In OGTT-CFRD subgroup there is an increase in FEV1 (62.7 ± 26.3 to 65.1 ± 21.7%, $p = 0.7$) and decrease in FVC (from 76.4 ± 24.2 to 71.2 ± 20%, $p = 0.003$) from diagnosis to second year of follow-up. In CGM-CFRD subgroup there was a decrease in FEV1 (from 58.2 ± 28.2 to 52.8 ± 25.9%, $p = 0.2$) and FVC-values (from 72.4 ± 26.5 to 67.4 ± 29.1%, $p = 0.08$).Chronic *Pseudomonas aeruginosa* infection was more prevalent in the CFRD group ($p = 0.003$).

Conclusion: Continuous glucose monitoring is a useful tool for insight of glucose impairment and diagnosis of CFRD. Early recognition of CFRD and therapeutic intervention has favorable effects on clinical course of the disease.

Keywords: cystic fibrosis related diabetes, continuous glucose monitoring, lung function decline, oral glucose tolerance test, hemoglobin A1c

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INTRODUCTION

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder in non-Hispanic White with a prevalence of 1: 2,500–5,000 live births (1). Many factors, especially early diagnosis, hypercaloric diet, and aggressive treatment of exacerbations, have led to a shift in patients' median life expectancy (2). Prolongation of life led to increased prevalence of cystic fibrosis-related diabetes (CFRD) (1, 3). Disorders in glucose metabolism can also occur in younger age (in 2% of children), but the majority of patients are diagnosed with CFRD during adolescence or in adults (in up to 40% of all cases) (4). The pathophysiological mechanism of CFRD is complex. It is primarily caused by insulin deficiency, but unlike diabetes mellitus type 1, β-cell damage in CF is not of autoimmune origin. Abnormal chloride channel function results in thick viscous secretions that lead to obstructive damage to the exocrine pancreas. Destruction of islet architecture is due to chronic inflammation, progressive fibrosis and fatty infiltration (5). Besides insulin insufficiency, insulin resistance also plays a role (6). There is peripheral insulin resistance, which arises from decreased glucose uptake by muscle, and hepatic insulin resistance, which is caused by impaired suppression of hepatic glucose production (7). Insulin resistance can get worse with acute pulmonary exacerbation, severe chronic lung disease and systemic glucocorticoid therapy (5).

What has been described as asymptomatic insulopenia in early childhood, if unrecognized, over the years lead to progressive malnutrition, loss of lung function, and increased number of exacerbations with a significant negative impact on survival and quality of life. Although its importance is validated and presented in official recommendations, the true prevalence of CFRD in CF centers varies, probably due to disagreement on age when screening is performed and different methodologies and diagnostic criteria in use[1]. In the last decade, the novel method for the early detection of CFRD has been suggested—continuous glucose monitoring (CGM) (8). Although it is not currently approved as diagnostic tool for CFRD by official position statements, its complementary use to OGTT leads to better prediction of symptomatic insulin deficiency and urges earlier treatment initiation. It was showed that the early stages of insulin deficiency may be contributing to catabolism and malnutrition, promoting lung bacterial growth and deteriorating lung function in CF. As the primary mechanism of CFRD is insulin deficiency, insulin replacement therapy is needed (9).

The aim of our research was to evaluate the impact of CFRD on lung disease, nutritional status and frequency of exacerbation. We hypothesized that early recognition of CFRD with CGM and prompt initiation of insulin therapy, was related to the less gradual decline of lung function, fewer pulmonary exacerbations and improvements in patients' nutritional status.

[1]ECFS Patient Registry (https://www.ecfs.eu/ecfspr).

METHODS

In this retrospective cohort study, data extracted from patients' history files from January 2004 until December 2019, were evaluated. All participants were treated in the national CF center—Mother and Child Health Institute of Serbia. Data included demographics, underlying diseases, nutritional status, CFRD evaluation, sputum microbiology, frequency of exacerbation, and lung function testing. Subjects were in clinically stable condition, at least 1 month apart from pulmonary exacerbation, without concomitant use of systemic glucocorticoid therapy. The 5 years follow-up period, covered time span of 2 years before and 3 years after the diagnosis. For the purpose of this research, an exacerbation was defined as a worsening of respiratory symptoms that required oral or intravenous antibiotic therapy.

CFRD Evaluation

The CFRD group consisted of subjects with diagnosis confirmed either by 30-minutely sampled oral glucose tolerance test (OGTT) or CGM (when become available), in asymptomatic patients on regular annual check-ups from 10 years of age (9). Participants with fasting hyperglycemia (above 7.0 mmol/l) were scheduled for further evaluation, no matter when screened previously.

The OGTT screening was performed using 1.75 g/kg of body weight of glucose up to maximum of 75 g. The blood glucose and insulin levels were measured over a period of 2 h at 30-min intervals. The participants were considered to have CFRD if the glycemia at 120 min was $\geq$11.1 mmol/l (OGTT-CFRD subgroup) (10).

A subgroup of subjects in whom CGM was used in diagnostic algorithm besides OGTT was presented separately. The CGM (available in our center from 2015), was performed within 3 months after OGTT in each subject, using the device applied outpatiently (Medtronic iPro^{TM}2 Professional CGM system). The device stayed *in situ* for 7 days in the home environment for all subjects. They were on their usual diet and being advised to measure a minimum four self-monitored blood glucose levels daily. They were advised keeping a diary for glucose measurements and dietary intake. The participants were considered to have CFRD based on interstitial blood glucose levels on CGM, if there were at least two peaks above 11.1 mmol/l and more than 10% of recorded time above 7.8 mmol/l (CGM-CFRD subgroup). The non-CFRD group consisted of all other CF patients older than 10 years, without CFRD. Glycated hemoglobin (HbA1c)-values were measured for every subject on each occasion, when OGTT was performed. Value of HbA1c at diagnosis in CFRD group and last obtained value in non-CFRD group, were considered to be important for the analysis.

Nutritional status for each year of follow-up was expressed as a Z-score of body-mass index (BMI). Pancreatic sufficiency was proved with the absence of symptoms of maldigestion, combined with normal values of fecal elastase. For the purpose of this study, CF liver disease was defined as palpation of an enlarged liver and/or spleen or changes different than fatty infiltration on routine annual assessment ultrasound.

Chronic use of inhaled corticosteroids (ICS) in low/moderate doses (<400 mcg/day of budesonide or equivalent) in year preceding the CFRD assessment, was registered for each patient. None of the patients were treated with chronic systemic corticosteroid therapy 3 months preceding baseline.

Lung Function

All study participants performed forced spirometry, according to official ERS guidelines (11) using a volume-constant method (MasterLab, Jaeger, Würzburg, Germany). The reference equations used for pulmonary function testing were those of Zapletal et al. (12). In the CFRD group the central event was the year when the diagnosis of CFRD was established. The best annual values of forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) in each year of follow-up were taken to calculate the 5-year trend value of these parameters. For the non-CFRD group, the best annual values of FEV_1 and FVC in the last 5 years of follow-up were considered for the analysis.

Statistics

Statistical data were evaluated by IBM SPSS Statistics 25 software program and were expressed as median and range. The choice of statistical test depended on the data type and distribution—parametric (ANOVA of repeated measurements), non-parametric (Chi-square test, Fisher's exact test). Correlations were evaluated by Pearson's and Spearman's rank correlation tests. To describe the relationship of two or more variables, linear regression was used.

The observation of lung function values trend, frequency of exacerbations and nutritional status was performed as well. In subjects with CFRD, central event was the insulin replacement therapy initiation. The trends of abovementioned parameters were calculated having in mind 2-year period preceding the CFRD diagnosis and 3-years of follow-up. In subjects from the non-CFRD group, last 5 years of follow-up was considered to be of interest, when calculating such a trend.

A statistically significant difference was considered to be $p < 0.05$. The study protocol was approved by the local Ethics committee (decision number 8/2). The research was conducted according to Declaration of Helsinki.

RESULTS

The study included 144 subjects, 28 (19.4%) subjects in the CFRD group and 116 (80.6%) in the non-CFRD group. The mean age at diagnosis of CFRD was 20.7 ± 9.6 years. The mean age in the OGTT-CFRD subgroup was 21.0 ± 8.8 years, and in CGM-CFRD subgroup 25.9 ± 8.7 years. In all patients in whom CGM measurement confirmed the diagnosis of CFRD, OGTT results were inconclusive for the diagnosis of CFRD. The average value of HbA1c in the CFRD group was 6.8 ± 1.6%, which was comparable to values from the non-CFRD group- −6.4 ± 1.2%. In subjects from the OGTT-CFRD subgroup, the value of HbA1c was significantly higher than in the CGM-CFRD subgroup (7.3 ± 1.7 and 5.9 ± 0.62% respectfully, $p = 0.04$). A careful reassessment of the patient's medical data did not show an association of possible comorbidities or therapeutic interventions and HbA1c levels. For the participants in the CGM-CFRD group, maximal measured glucose levels were from 13.5 to 15.2 mmol/l. In 12–33% of recorded time on CGM, glycemia was above 7.8 mmol/l, with three to seven peaks above 11.1 mmol/l.

The comparable number of subjects from CFRD and non-CFRD groups was homozygote for F508del mutation. Four pancreatic sufficient subjects from non-CFRD group, proved to be compound heterozygote with residual function mutation. Demographic characteristics are shown in **Table 1**.

Nutritional Status and Liver Disease

In the year when diagnosis was confirmed, subjects from the CFRD group were significantly more malnourished in compare to the non-CFRD group (−1.4 ± 1.3 vs. −0.5 ± 1.2, $p = 0.04$). In addition, weight gain on insulin replacement therapy resulted in significant improvement in BMI Z-score in CGM-CFRD subgroup (from −1.7 ± 1.4 to −1.4 ± 1.1, $p = 0.02$), but not in the OGTT-CFRD subgroup (from −1.2 ± 1.4 to −1 ± 1.5, $p = 0.07$)—**Figure 1**, **Table 1**.

Data analysis showed that liver disease was present more frequently in CFRD group ($p = 0.007$). It had been shown that participants with liver disease had lower FEV_1-values ($p = 0.03$).

Lung Function and Pulmonary Exacerbations

The inhaled ICS were used more frequently in the CFRD group ($p = 0.001$). In the CFRD group, the number of exacerbations decreased significantly after initiating insulin therapy ($p = 0.02$). The number of exacerbations didn't change significantly in the non-CFRD group ($p = 0.5$)—**Figure 1**.

Participants from CFRD group have significantly lower FEV_1 compared to non-CFRD group (61.6 ± 26 and 82.4 ± 24.4% respectfully, $p < 0.001$). Using correlation analysis it was showed that FEV_1 positively correlates with BMI ($r = 0.610$, $p < 0.001$) and negatively correlates with age ($r = -0.559$, $p < 0.001$) The 5-year trend values of lung function was examined and there was a statistically significant negative trend of FEV_1- and FVC-values over time in both groups ($p < 0.001$)—**Figure 2**.

As for the CFRD group, in OGTT-CFRD subgroup there is an insignificant increase in FEV_1 (from 62.7 ± 26.3 to 65.1 ± 21.7%, $p = 0.7$) and decrease in FVC (from 76.4 ± 24.2 to 71.2 ± 20%, $p = 0.003$) from diagnosis to second year of follow-up. In CGM-CFRD subgroup there was a decrease in FEV_1-values (from 58.2 ± 28.2 to 52.8 ± 25.9%, $p = 0.2$) and FVC-values (from 72.4 ± 26.5 to 67.4 ± 29.1%, $p = 0.08$) at the year of diagnosis and in the second year of follow-up—**Figure 2**. But there is no difference between CGM-CFRD and OGTT-CFRD subgroups in the second year of follow up in regard to FEV_1 and FVC (52.8 ± 25.9 vs. 65.1 ± 21.7%, $p = 0.06$ and 67.4 ± 29.1 vs. 71.2 ± 20%, $p = 0.9$, respectively)—**Table 1**.

Chronic colonization of the lower respiratory tract with *Pseudomonas aeruginosa* was more frequent in the CFRD group compared to the non-CFRD group ($p = 0.003$). However, the incidence of chronic colonization with *Burkholderia cepacia* and *Staphylococcus aureus* was not significantly different between groups.

TABLE 1 | Demographic data.

	CFRD	Non-CFRD	Total	*p*	CGM-CFRD	OGTT-CFRD	Total	*p*
Number	28	116	144		7	21	28	
Male, *n* (%)	13 (46.4)	51 (43.9)	64 (44.4)	0.84	4 (57.1)	9 (42.9)	13 (46.4)	0.67
Female, *n* (%)	15 (53.6)	65 (56.1)	80 (55.6)	0.84	3 (42.9)	12 (57.1)	15 (53.6)	0.67
Age	20.7 ± 9.6	18.6 ± 9.2	19.7 ± 9.4	0.62	25.9 ± 8.7	21.0 ± 8.8	23.5±8.7	0.32
Age at CF diagnosis	3.2 ± 3	2.7 ± 3.63	2.8 ± 3.3	0.91	3.6 ± 2.9	2.8 ± 3.1	3.2 ± 3	0.74
Age at CFRD diagnosis	20.7±9.6	/	20.7±9.6	/	25.9 ± 8.7	21.0 ± 8.8	23.5±8.7	0.32
Pancreatic insufficiency, *n* (%)	28 (100)	112 (96.6)	140 (97.2)	1	7 (100)	21 (100)	28 (100)	/
Liver disease, *n* (%)	19 (67.8)	44 (37.9)	63 (43.7)	**0.01**	5 (71.4)	14 (66.7)	19 (67.8)	1
FEV_1 2 years prior to baseline, (%)	66.1 ± 22.9	84.7 ±23.7	75.4 ± 23.3	**0.001**	57.5 ± 23.6	69.7 ± 23	66.1 ± 22.9	0.06
FEV_1 at baseline, (%)	61.6 ± 26	82.4 ± 24.4	72.8 ± 25.3	**0.001**	58.2 ± 28.2	62.7 ± 26.3	61.6 ± 26	0.81
FEV_1 at second year of follow up, (%)	57.7 ± 25.5	77.8 ± 27.1	67.8 ± 26.3	**0.001**	52.8 ± 25.9	65.1 ± 21.7	57.7 ± 25.5	0.06
FVC 2 years prior to baseline, (%)	77.4 ± 19.6	86.7 ± 17.8	82.1 ± 18.7	0.06	69.2 ± 22.5	81.2 ± 18	77.4 ± 19.6	0.05
FVC at baseline, (%)	76.3 ± 23.9	85.3 ± 17.9	80.8 ± 20.9	0.06	72.4 ± 26.5	76.4 ± 24.2	76.3 ± 23.9	1
FVC at second year of follow up, (%)	70.9 ± 21.7	83.1 ± 19.6	77 ± 20.6	**0.04**	67.4 ± 29.1	71.2 ± 20	70.9 ± 21.7	0.9
Homozygote for F508del mutation, *n* (%)	14 (50)	64 (55.2)	78 (54.1)	0.82	4 (57.1)	10 (47.6)	14 (50)	0.84
BMI Z-score 2 years prior to baseline (mean)	−1.2 ± 1.1	−0.4 ± 1.2	0.8 ± 1.1	0.05	−1.3 ± 1.1	−0.9 ± 1.3	−1.2 ± 1.1	0.8
BMI Z-score at baseline (mean)	−1.4 ± 1.3	−0.5 ± 1.2	0.9 ± 1.3	**0.04**	−1.7 ± 1.4	−1.2 ± 1.4	−1.4 ± 1.4	0.4
BMI Z-score at second year of follow-up (mean)	−1.1 ± 1.4	−0.6 ± 1.2	0.8 ± 1.3	0.07	−1.4 ± 1.1	−1 ± 1.5	1.1 ± 1.4	0.4
PA infection, *n* (%)	21 (75)	51 (44)	72 (50)	**0.003**	6 (85.7)	15 (71.4)	21 (75)	0.64
BC infection, *n* (%)	4 (14.3)	14 (12.1)	18 (12.5)	0.75	0 (0)	4 (19)	/	0.54
SA, *n* (%)	4 (14.3)	24 (20.7)	28 (19.4)	0.58	0(0)	4 (19)	/	0.54
Chronic ICS use	16 (57.1)	34 (29.3)	50 (34.7)	**0.01**	6 (85.7)	10 (47.6)	16 (57.1)	**0.02**
HbA1C (%)	6.8 ± 1.4	6.4 ± 1.2	6.6 ± 1.4	1	5.9 ± 0.62	7.6 ± 1.7	6.8 ± 1.4	**0.04**
Glycemia in 60 min during OGTT (mmol/l)	11.1 ± 2.3	8.5 ± 2.0	9.8 ± 2.5	0.27	9.1 ± 2.3	12.9 ± 2.3	11.1 ± 2.3	0.31
Glycemia in 120 min during OGTT (mmol/l)	9.1 ± 0.9	7.8 ± 2.5	8.3 ± 3.2	0.82	5.6 ± 0.6	12.5 ± 1.3	9.1 ± 0.9	**0.02**

CFRD, patients diagnosed with CFRD; CGM-CFRD, subgroup diagnosed with continuous glucose monitoring; OGTT-CFRD, subgroup diagnosed with oral glucose tolerance test; CF, cystic fibrosis; DM, diabetes mellitus; PA, Pseudomonas aeruginosa; BC, Burkholderia cepacia; SA, Staphylococcus aureus, FEV_1, forced expiratory volume in 1 s. Bold values represent statistically significant P < 0.05

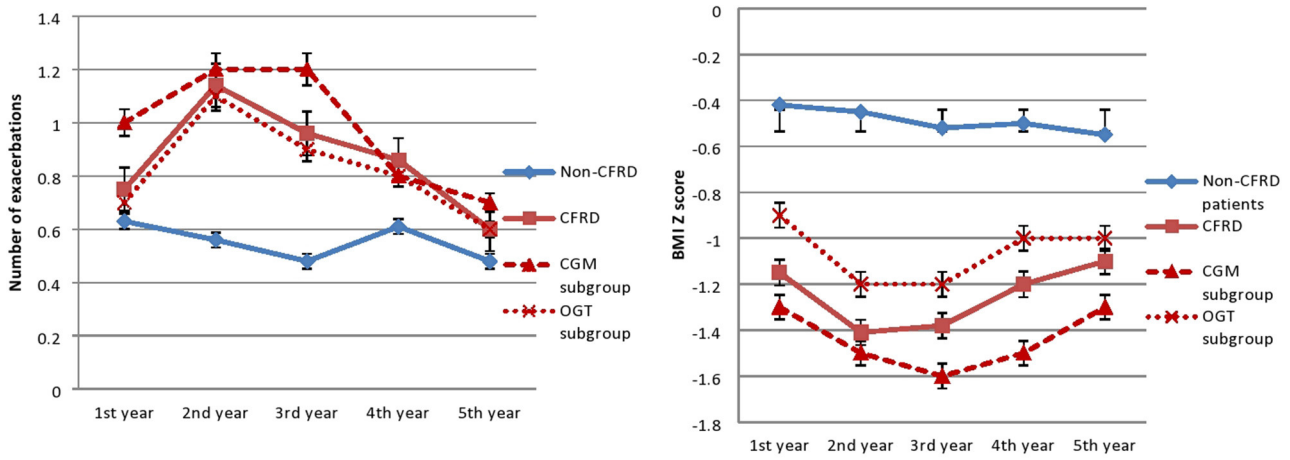

FIGURE 1 | Number of exacerbations and BMI Z-score. In the CFRID group the number of exacerbations significantly decreased ($p = 0.02$), and BMI Z-score significantly improved ($p = 0.04$) after initiating insulin therapy in the third year of follow-up.

DISCUSSION

We showed that the occurrence of CFRD has an important, unfavorable effect on the course of CF. This is reflected in more severe malnutrition, a further lung function impairment and frequent exacerbations of CF lung disease. In addition, we showed that CGM monitoring is useful in diagnosis of CFRD, even though OGTT results were not confirmative.

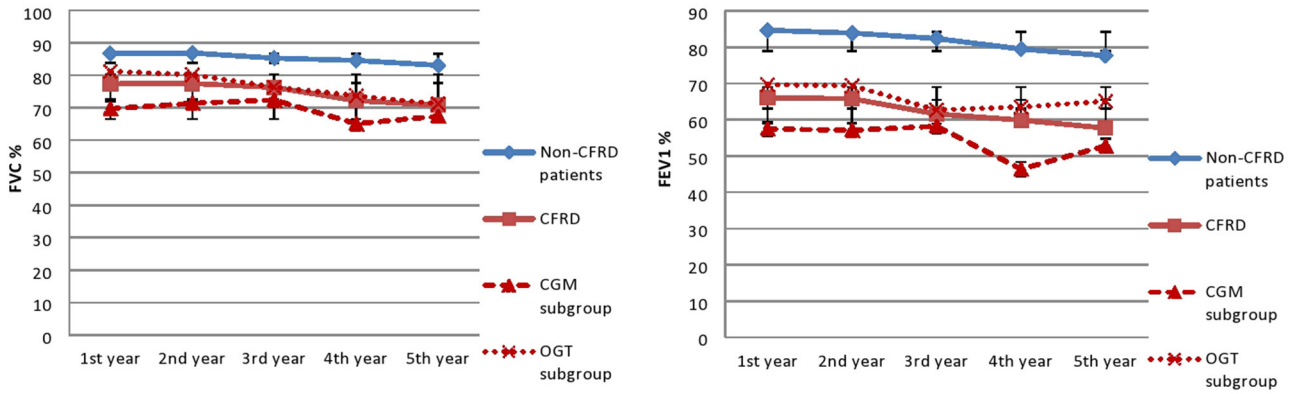

FIGURE 2 | Lung function trends. FVC, forced vital capacity; FEv_1, Forced expiratory volume in 1 s. In third year insulin therapy was started. There is a difference within each group during the observed period ($p < 0.001$), no significant difference was observed between groups.

Regular blood glucose control is an essential part of evaluating response to therapy and adjusting treatment regimens, but is not suitable because in requires frequent measurements. Although the study was not designed to evaluate sensitivity of CGM in the diagnosis of CFRD, the earliest glucose abnormalities are seen with it. This was also confirmed by the results of other research, where more than half of CF patients with normal glucose tolerance by OGTT demonstrate intermittent postprandial glucose levels above 11.1 mmol/l (6, 13). Hameed et al. found that having glucose level above 7.8 mmol/L for ≥4.5% of the time during CGM predict a greater decline in BMI Z-score and FEV_1 (5). Respiratory dysfunction correlates with the degree of insulopenia and the severity of glucose metabolism disorders (7). Interestingly, a beneficial effect was present in recent studies with the improvement of lung function parameters during insulin therapy (1). We confirmed previously published data showing that presence of CFRD negatively correlates with lung function and nutritional status (4, 14, 15). Chan et al. showed a negative correlation between CGM results (peak glucose, excursion above 7.8 mmol/l and percentage of time above 7.8 mmol/l), lung function, and BMI Z-score (16). Moreover, the results of our research showed that early diagnosis of CFRD no matter which diagnostic method was used, followed by the initiation of insulin therapy, had beneficial effects on lung function decline, frequency of exacerbations and BMI Z-score, regardless of patients' age. Although it is believed that HbA1c levels ≥6.5% is consistent with diabetes (5, 6), its levels were not higher in the CFRD group in compare to non-CFRD. The levels of HbA1c were lower in the CGM-CFRD subgroup, meaning that the normal HbA1c-value does not exclude CFRD. In addition, it was showed that some subjects from non-CFRD group had HbA1c levels ≥6.5%, but normal CGM and OGTT data. HbA1c is generally thought to underestimate hyperglycemia in CF, but not necessarily to overestimate average glycemia in this population. These results confirmed the limited significance of HbA1c in the diagnosis of CFRD, but do not preclude its significance in disease follow-up.

Since CFTR chloride channel defect plays a role in the β-cell and in insulin secretion, CFTR modulator therapy currently in use and new drugs in the pipeline, might impact CFRD prevalence in years to follow. In recent study of Gaines et al. (17) 36% of CFRD patients treated with CFTR modulators had markedly improved disease status. Several patients developed persistent hypoglycemia after CFTR modulator therapy initiation, probably due to relatively delayed reduction of insulin therapy. The beneficial effects of such therapy are probably related to its anti-inflammatory potential. Some other chronic anti-inflammatory therapy (e.g., oral azithromycin) has beneficial effects on the CF lung disease, but such effects of ICS have not been established, except for patients with associated asthma (18). Although the results of our research showed that ICS administration was significantly more common in patients with CFRD, it had been shown before that this therapy at low to median daily doses had no effect on glucose metabolism (5). So, it seems reasonable to believe that ICS therapy in CF should be used cautiously.

Our research has shown that CFRD is more common in patients with liver disease, which may be related to the complex mechanisms of glycoregulation that take place in the liver. Although pancreatic insufficiency (PI) is associated with a more severe clinical features, its representation in both groups is similar, which is different from the large studies that suggest positive correlations between CFRD and PI (19). However, the prevalence of PI should not be neglected because it reached 100% in the CFRD group, suggesting that the number of subjects was insufficient to achieve a significant statistical difference. Disease severity is unpredictable when mutations from two different classes are present, but mild CF phenotype is mostly present in subjects with at least one residual function mutation (mutation classes IV–VI). Four pancreatic sufficient patients from non-CFRD group and none from CFRD group proved to have at least one residual function mutation. In addition, susceptibility for development of CFRD in PI patients is in part influenced by genetic variants in alternative chloride channels and single-nucleotide polymorphisms for type

2 diabetes (20) which were not analyzed for the purpose of this research.

Dysregulation of glucose metabolism promote bacterial growth in the lungs and result in more frequent pulmonary exacerbations, with further impairment of respiratory function and life expectancy, as confirmed by the results of our study (5). We showed that chronic *P. aeruginosa* infection is more prevalent in the CFRD group. These results were comparable to the research of Leclerq et al. (13), although their definition of CFRD was less strict than we used in our study. In CF patients, airway mucous glucose levels become elevated at systemic glucose levels >8 mmol/l and these glucose-containing secretions are associated with growth of respiratory pathogens (6). Undiagnosed CFRD in patients chronically colonized with *P. aeruginosa*, may accelerate lung function decline despite adequate chronic suppressive antimicrobial therapy.

This study has several limitations. The sample is relatively small, which may interfere with the results and evaluation of the examined associations. Additional work is needed to demonstrate the CGM-thresholds at which insulin intervention may be beneficial. In addition, the study is limited to a single hospital location. Its strengths are in a timely evaluation of CFRD using CGM, underling its usefulness as a possible more accurate and earlier indicator of dysglycemia than the 2-h blood glucose on OGTT. It also included longitudinal follow-up, after the initiation of insulin therapy.

We believe early diagnosis of CFRD should be encouraged in regular clinical practice. It has beneficial effects due to timely initiation of insulin therapy that result in better glycoregulation, improved nutritional status and lung function, fewer pulmonary exacerbations and prolonged life expectancy. Regular CGM should be used complementary with OGTT and HbA1 in order to have better insight in patient's CFRD status. Finally, the authors would suggest that CGM criteria should be included in CFRD Clinical Care Guidelines in nearest future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee Mother and Child Health Institute. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BG and AS contributed for literature search, data collection and organization of database, study design, statistical analysis, manuscript preparation, and manuscript revision. IS contributed for data collection and organization of database, statistical analysis, and manuscript revision. ST contributed for literature search, manuscript preparation, and manuscript revision. PM contributed for manuscript preparation and manuscript revision. All authors contributed to the article and approved the submitted version.

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Long-Term Outcomes in Real Life of Lumacaftor–Ivacaftor Treatment in Adolescents With Cystic Fibrosis

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Background: The combination of the CFTR corrector lumacaftor (LUM) and potentiator ivacaftor (IVA) has been labeled in France since 2015 for F508del homozygote cystic fibrosis (CF) patients over 12 years. In this real-life study, we aimed (i) to compare the changes in lung function, clinical (e.g., body mass index and pulmonary exacerbations) and radiological parameters, and in sweat chloride concentration before and after initiation of LUM/IVA treatment; (ii) to identify factors associated with response to treatment; and (iii) to assess the tolerance to treatment.

Materials and Methods: In this tri-center, non-interventional, and observational cohort study, children (12–18 years old) were assessed prospectively during the 2 years of therapy, and retrospectively during the 2 years preceding treatment. Data collected and analyzed for the study were exclusively extracted from the medical electronic system records of the patients.

Results: Forty adolescents aged 12.0–17.4 years at LUM/IVA initiation were included. The lung function decreased significantly during and prior to treatment and increased after LUM/IVA initiation, becoming significant after 2 years of treatment. LUM/IVA significantly improved the BMI *Z*-score and sweat chloride concentration. By contrast, there was no significant change in exacerbation rates, antibiotic use, or CT scan scores. Age at LUM/IVA initiation was lower in good responders and associated with greater ppFEV1 change during the 2 years of treatment. LUM/IVA was well-tolerated.

Conclusion: In F508del homozygote adolescents, real-life long-term LUM/IVA improved the ppFEV1 trajectory, particularly in the youngest patients, nutritional status, and sweat chloride concentration but not exacerbation rates or radiological scores. LUM/IVA was generally well-tolerated and safe.

Keywords: cystic fibrosis, child, CFTR potentiator, CFTR corrector, lung function testing, nutritional status, sweat chloride

INTRODUCTION

Cystic fibrosis (CF) is an inherited genetic disease leading to *cystic fibrosis transmembrane conductance regulator* (CFTR) dysfunction. CFTR is involved in active excretion of chloride at the apical membrane of the epithelial cells (1–4). Among more than 2,000 mutations of the CFTR gene, the homozygous F508del represents the most frequent in France with a prevalence of up to 41% of patients (5). This mutation leads to a processing and trafficking defect of the CFTR protein resulting in an early degradation or a dysfunctional protein expressed into the apical membrane of the cells (1, 4, 6). As a consequence, epithelial mucus secretion is dehydrated, promoting pulmonary infection (7) and organ injury (e.g., pancreatic insufficiency), leading to impaired lung function, growth, and nutritional outcomes (8–10). Therapeutic approaches over the last 20 years based on symptomatic treatments (physiotherapy, fluidifiers, and pancreatic enzymes, etc.) (11, 12) slowed down the progression of CF and enhanced life expectancy (4, 10). Nevertheless, CF remains a severe disease, in which pulmonary exacerbations and poor nutritional status contribute to progressive lung function decline and death in young adults (4, 8, 10). The highest decline in lung function occurs during adolescence which is thus a pivotal period in the management of the disease (13).

In this context, therapeutic approaches have been developed to improved cell chloride secretion by targeting the defect in F508del-CFTR folding using in association: the CFTR corrector lumacaftor (LUM) (enhances trafficking and processing), and the CFTR potentiator ivacaftor (IVA) (increases the channel opening probability) (1, 4). Phase III studies have demonstrated good tolerance of the lumacaftor/ivacaftor association (LUM/IVA) and significant potential benefits in young children aged 2–5 years old (14) and 6–11 years (15, 16) [e.g., improved body mass index (BMI)], and in adolescents over 12 years of age and adults [i.e., improvement in BMI and lung function, assessed by the percentage of predicted forced expiratory volume in 1 s (ppFEV1), and decrease in the number of exacerbations usually defined by clinical deterioration treated with IV antibiotics] (17). The positive effect of LUM/IVA on lung function decline and BMI has been confirmed in patients over 12 years by the 96 weeks follow-up PROGRESS study (8) and more recently, in France, in the real-life study performed by Burgel et al. (18). However, the PROGRESS study mostly included CF adults (8), and the study performed by Burgel et al. followed CF adolescents only for a year after LUM/IVA initiation.

Thus, real-life studies are needed to confirm the ability of LUM/IVA to change the course of the disease and to evaluate its long-term effects, particularly in adolescents, who are at a critical stage of their development.

Thus, we conducted a pediatric prospective real-life cohort study including F508del homozygote adolescents followed-up within the French South-Western network (MUCOSUD) to assess the evolution of the ppFEV1 before and after the LUM/IVA initiation. The secondary objectives were to assess (i) the evolution of clinical [i.e., anthropometric parameters (weight, height, and BMI expressed as *Z*-scores) and number of pulmonary exacerbation, antibiotic use], biological (i.e., sweat chloride concentration), and radiological (i.e., the Bhalla CT-scan score with the percentage high attenuation volume (%HAV) mucus secretions sub-score) parameters; (ii) to identify parameters associated with response to treatment; and (iii) to assess the tolerance to treatment.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator; HAV, high attenuation volume; ppFEV1, percent predicted forced expiratory volume in 1 s; LUM/IVA, lumacaftor/ivacaftor.

METHODS

The study was conducted prospectively between February 2016 and December 2018 within the French South-West network of pediatric CF centers (tertiary-care university hospitals in Bordeaux, Toulouse, and Limoges) and the included CF adolescents (12–18 years old) eligible for LUM/IVA treatment (F508del homozygotes aged 12 years and older) not included in other CFTR modulator protocols study. Patients having stopped LUM/IVA within 3 months after initiation of treatment were excluded from the analyses. It was a non-interventional study. Indeed, LUM/IVA has been approved by the Food and Drug Administration and the European Medicines Agency, and labeled in France since 2015 for CF patients with F508del homozygous CFTR mutation over 12 years of age. Thus, at the time of the patient follow-up period, LUM/IVA was prescribed as part of the routine care of a patient. In addition, the use of the data collected and analyzed for the study were exclusively extracted from the medical records of the patients (MUCODOMEOS, https://www.vaincrelamuco.org/2019/05/09/mucodomeos-un-logiciel-adapte-aux-besoins-des-crcm-2684) after having obtained their informed consent. In this context and according to the French law in force, the approval of an ethics committee was not required.

Data Collected

All children were followed prospectively at least every 3 months before and after the LUM/IVA initiation as part of their disease follow-up (**Figure 1**). Briefly, weight, height, BMI, and ppFEV1 were assessed the day of the LUM/IVA initiation (M0) and at 3, 6, 12, and 24 months (M3, M6, M12, and M24) after the treatment initiation during routine clinical visits of the patients. To assess the effect of the LUM/IVA treatment on the disease course, theses parameters were retrospectively collected 12 and 24 months (M-12 and M-24) prior to the initiation of LUM/IVA. Anthropometric data (weight, height, and BMI) were automatically transformed as *Z*-scores by the MUCODOMEOS software based on the World Health Organization standardized values (19). The ppFEV1 was determined using the Global Lungs Initiative 2012 reference values. Before treatment, we chose retrospectively the best ppFEV1 value for each study date (±1 month). At initiation and during the 2 years of follow-up, the FEV1 was collected prospectively at each study visit. Good responders were defined as a ppFEV1 gain >5% at M24 compared to M0. The 5% threshold was chosen in accordance with previous studies (17, 20, 21). Adolescence is the period

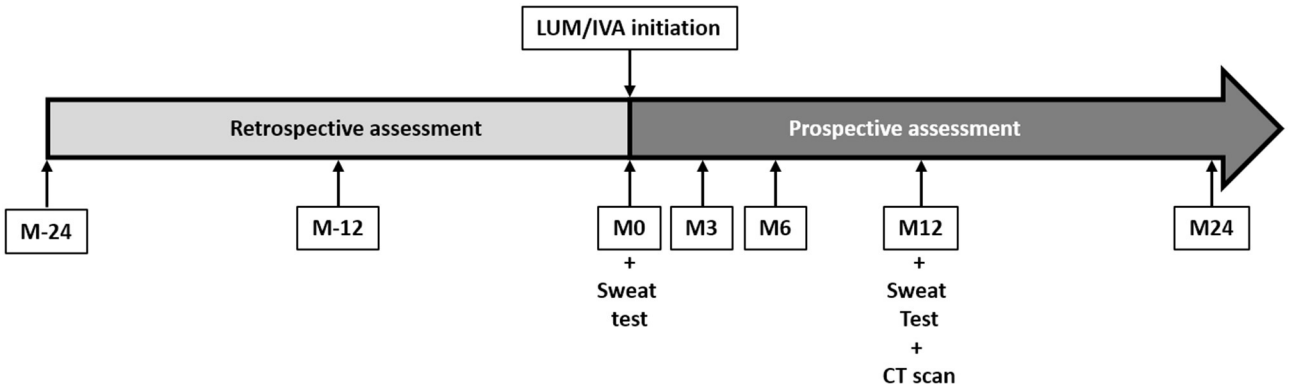

FIGURE 1 | Study design and chronology. Patients were included at the initiation of lumacaftor/ivacaftor (LUM/IVA) (M0). At 3 (M3), 6 (M6), 12 (M12), and 24 (M24) months of treatment, height, weight, body mass index, and lung function testing results were assessed prospectively. These parameters were also assessed retrospectively at 24 (M-24) and 12 (M-12) months before the LUM/IVA initiation. A sweat test was performed at M0 and M12. Computed tomography scans were performed at M12. The side effects of LUM/IVA treatment were assessed at each visit after initiation.

with the highest decline in lung function (14), and improvement in ppFEV1 is an "optimal" outcome. However, having a stable lung function may be considered a success. We therefore defined among the patients who were not good responders to 2 other groups: the no-decline patients characterized by a ppFEV1 gain between 0 and 5%, and the low/non-responders characterized by a decrease ppFEV1 at M24 compared to M0. To evaluate the evolution of the response to treatment of the patients, the presence of a good response, no decline response, or low/no response was evaluated by the ppFEV1 change at M3, M6, and M12 (vs. M0) using the same thresholds. The number of oral and/or intravenous antibiotics courses and the number of exacerbations [i.e., acute pulmonary exacerbation requiring oral or intravenous antibiotic therapy as defined previously for clinical research (22)] that occurred in the 2 years following treatment initiation and in the 2 years prior to treatment were collected. A sweat test was performed at M0 and M12 according to European recommendations (23).

CT scans performed in the radiology department of the inclusion centers before and after 1 year of treatment were centralized for the study evaluations. Two evaluations were performed: a visual CT Bhalla score (24) and an automated measurement of percentage high attenuation volume (%HAV) (25). Visual scorings were performed independently by two radiologists from Bordeaux University Hospital, with previous publication experience in CT scoring in CF (26–28). The means of these evaluations were kept for further analyses. The %HAV was automatically measured by using the Pulmo3D Syngo software (Siemens, Erlangen, Germany).

To assess treatment tolerance, plasma liver enzymes were monitored prior to the LUM/IVA treatment initiation and at each visit during the first year and annually thereafter. Eye examinations were performed prior to the start of treatment and monitored at 1 year. At each visit, we also collected clinical data regarding side effects and recorded adherence to treatment in order to avoid a lack of response due to poor adherence treatment.

Statistics

Analyses were performed only on available data using R software® (version 4.0). Figures were done using Prism software® (version 5.1). Data from the entire population were analyzed and comparison between subgroups according to the presence or absence of impaired lung function (ppFEV1 $< 80\%$) or according to the Z-scores BMI (Z-scores < 0 vs. Z-scores ≥ 0) at the LUM/IVA initiation) were performed. Normality was assessed using Q-Q plots. Parametric variables were compared using Mann–Whitney test or ANOVA, and results were expressed as mean ± standard deviation (SD). Non-parametric variables were compared with Mann–Whitney test for unpaired values or Wilcoxon test for paired values and with t-test for parametric variables. Results were expressed as the mean ± standard deviation or as the median and interquartile range [median (IQR_{25}; IQR_{75})]. Categorical variables were expressed as absolute values and as percentages. Categorial variables were performed using the Fisher's exact test or Chi square test. Correlations were performed using the Spearman test. A p-value < 0.05 was considered significant.

RESULTS

Population Characteristics

Among the 350 adolescents with CF followed within the French South-West network of pediatric CF centers (MUCOSUD), 50 were eligible for the LUM/IVA treatment of whom one had a contraindication for LUM/IVA (severe cirrhosis) and seven were included in new therapies clinical trials (**Figure 2**). LUM/IVA was initiated in the remaining 42 included patients but two discontinued the LUM/IVA treatment early (<3 months) and were excluded from the analyses (**Figure 2**).

The main characteristics of the 40 adolescents at initiation of LUM/IVA are summarized in **Table 1**. At M0, there was a negative correlation between sweat chloride and both ppFEV1 ($r = -0.46$; $p = 0.009$) and BMI Z-scores ($r = -0.42$; $p = 0.022$),

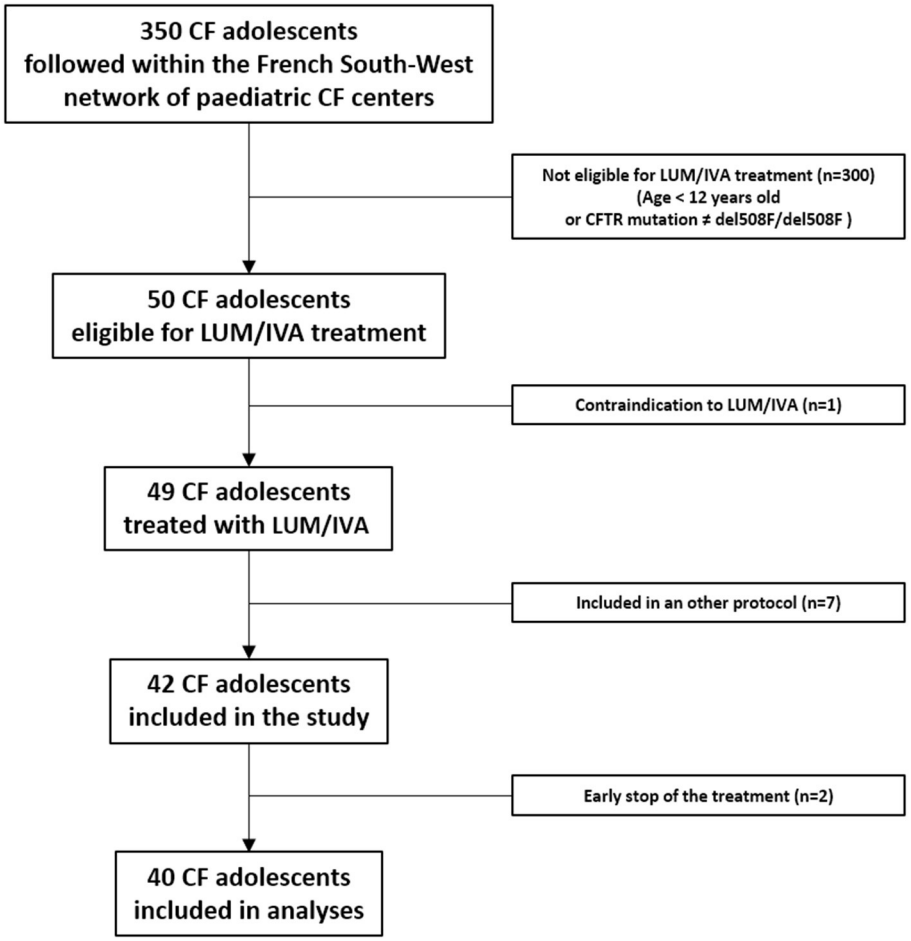

FIGURE 2 | Flow chart. CF, cystic fibrosis; LUM/IVA, lumacaftor/ivacaftor association; del508F/del508F, homozygous deletion of the codon for phenylalanine at position 508 in the cystic fibrosis transmembrane regulator (CFTR) gene.

and a positive correlation between ppFEV1 and BMI Z-score ($r = 0.46$; $p = 0.004$). Except for lower ppFEV1 and BMI *Z*-scores in patients with impaired lung function (ppFEV1 < 80%) at M0, there was no significant difference between patients according to the presence or the absence of impaired lung function at M0 (**Table 1**). Except for anthropometric parameters (i.e., weight, height, and BMI *Z*-scores) and sweat chloride, there was no difference between patients with or without BMI *Z*-scores < 0 at M0 (**Table 1**).

Lumacaftor/Ivacaftor Changes the Trajectory of Both ppFEV1 and BMI *Z*-Score and Improves Sweat Chloride in Adolescents With Cystic Fibrosis

From 2 years before (M-24) to LUM/IVA initiation (M0), the ppFEV1 decreased significantly from 88.3% ± 17.0 to 83.4% ± 16.5 ($p = 0.008$) (**Figure 3A**). By contrast, 2 years after LUM/IVA initiation (M24), the ppFEV1 had increased significantly to 89.2% ± 20.9 corresponding to an absolute change of +5.8% ± 12.1 compared with M0 ($p = 0.027$) (**Figure 3A**). To note, the ppFEV1 measured at M3, M6, and M12, as well as at M-12, were not significantly different compared with that measured at M0 (**Figure 3A**). In addition, the BMI *Z*-score was stable from M-24 to M0 ($p = 0.28$), increased after the LUM/IVA initiation with a significant improvement to 0.02 ± 0.92 at M24 corresponding to an increase of 0.31 ± 0.66 compared with M0 ($p = 0.006$) (**Figure 3B**). Interestingly, the weight *Z*-score (**Supplementary Figure 1A**) but not the height *Z*-score (**Supplementary Figure 1B**) increased significantly between M0 and M24. Moreover, sweat chloride improved significantly from the LUM/IVA initiation to M12 ($p = 0.002$) but remained higher than the upper limit of the normal (**Figure 3C**). Surprisingly, we did not find any significant difference in either the number of exacerbations, or oral or intravenous antibiotic use in the 2 years before and after the LUM/IVA initiation (**Supplementary Figures 2A–C**). We also did not find difference in the Bhalla or HAV scores in the 24 patients who underwent a CT scan before the start and after 1 year of the LUM/IVA initiation (**Supplementary Figures 3A,B**).

TABLE 1 | Characteristics of patients at lumacaftor/ivacaftor initiation.

	Total	ppFEV1 <80% at M0	ppFEV1 ≥80% at M0	*p*1	BMI *Z*-score <0 at M0	BMI *Z*-score ≥0 at M0	*p*2
N	40	18	22		24	16	
Age (years)	13.9 ± 1.7	13.5 ± 1.8	14.2 ± 1.5	0.111	13.8 ± 1.7	14.0 ± 1.8	0.814
Male	22/40 (55.0)	8/18 (44.4)	14/22 (63.6)	0.339	14/24 (58.3)	8/16 (50.0)	0.748
Weight *Z*-score	−0.40 ± 1.01	−0.71 ± 1.02	−0.15 ± 0.94	0.134	−1.00 ± 0.70	0.51 ± 0.63	**<0.001**
Height *Z*-score	−0.37 ± 1.06	−0.41 ± 1.17	−0.34 ± 0.99	0.860	−0.66 ± 1.07	0.06 ± 0.91	**0.030**
BMI *Z*-score	−0.28 ± 1.00	−0.68 ± 0.97	0.04 ± 0.94	**0.036**	−0.92 ± 0.61	0.75 ± 0.52	**<0.001**
Past history							
Meconial ileus	2/40 (5.0)	1/18 (5.6)	1/22 (4.5)	1.000	1/24 (4.2)	1/16 (6.3)	1.000
DIOS	1/40 (2.5)	0/18 (0.0)	1/22 (4.5)	1.000	0/24 (0.0)	1/16 (6.3)	0.400
CFRD	2/40 (5.0)	0/18 (0.0)	2/22 (9.1)	0.492	1/24 (4.2)	1/16 (6.3)	1.000
CFLD	9/40 (22.5)	6/18 (33.3)	3/22 (13.6)	0.253	7/24 (29.2)	2/16 (12.5)	0.272
P. Aeruginosa colonization							
None	18/40 (45.0)	7/18 (38.9)	11/22 (50.0)		10/24 (41.7)	8/16 (50.0)	
Intermittent	11/40 (27.5)	5/18 (27.8)	6/22 (27.3)	0.713	8/24 (33.3)	3/16 (18.8)	0.598
Chronic	11/40 (27.5)	6/18 (33.3)	5/22 (22.7)		6/24 (25.0)	5/16 (31.3)	
S.Aureus colonization							
None	8/40 (20.0)	4/18 (22.2)	4/22 (18.2)		5/24 (20.8)	3/16 (18.8)	
Intermittent	3/40 (7.5)	0/18 (0.0)	3/22 (13.6)	0.264	1/24 (4.2)	2/16 (12.5)	0.618
Chronic	29/40 (72.5)	14/18 (77.8)	15/22 (68.2)		18/24 (75.0)	11/16 (68.8)	
ppFEV1	83.3 ± 18.3	68.2 ± 12.7	95.6 ± 11.7	**<0.001**	80.1 ± 18.2	88.1 ± 18.0	0.109
Sweat chloride (mmol/L)	104.7 ± 16.6	99.2 ± 17.8	110.3 ± 14.8	0.189	110.1 ± 14.2	97.3 ± 17.4	**0.039**
Treatment							
PERT U/Kg/j	7302 ± 1727	7554 ± 1887	7107 ± 1609	0.506	7741 ± 1738	6600 ± 1507	0.116
Proton pump inhibitor	21/40 (52.5)	9/18 (50.0)	12/22 (54.5)	1.000	13/24 (54.2)	8/16 (50.0)	1.000
Ursodeoxycolic acid	16/40 (40.0)	7/18 (38.9)	9/22 (40.9)	1.000	10/24 (41.7)	6/16 (37.5)	1.000
Laxative treatment	5/40 (12.5)	0/18 (0.0)	5/22 (22.7)	0.053	1/24 (4.2)	4/16 (25.0)	0.138
Inhaled antibiotic	17/40 (42.5)	10/18 (55.6)	7/22 (31.8)	0.200	10/24 (41.7)	7/16 (43.8)	1.000

M0, day of lumacaftor/ivacaftor initiation; ppFEV1, percent of predicted forced expiratory volume in 1 s; BMI, body mass index; DIOS, distal intestinal obstruction syndrome; CFRD, cystic fibrosis related diabetes; CFLD, cystic fibrosis liver disease; P. Aeruginosa, Pseudomonas Aeruginosa; S. Aureus, Staphylococcus Aureus; PERT, pancreatic enzymes replacement therapy.

Results are expressed as n/N (%) for categorical variables or as mean ± standard deviation for quantitative variables. Comparisons between patients with ppFEV1 < 80% compared with those with ppFEV1 ≥ 80% at M0 (p1) or between (patients with BMI Z-score < 0 compared with those with BMI Z-score ≥ 0 at lumacaftor/ivacaftor initiation (p2) were performed using the Fisher's exact test for categorical variables or the Mann–Whitney test for quantitative variables. A p-value ≤ 0.05 was considered significant.

Subgroup Analyses of the Effect of Lumacaftor/Ivacaftor on Changes in ppFEV1, BMI *Z*-Score, and Sweat Chloride

In patients with impaired lung function (ppFEV1 < 80%) at the LUM/IVA initiation, the ppFEV1 increased significantly from M0 to M24 (ppFEV1 absolute change of +7.3 ± 10.8%) after a significant decrease prior to the LUM/IVA initiation (ppFEV1 absolute change of −9.4 ± 13.3%) (**Figure 4A**). The same trend was observed in patients without impaired lung function at M0, but the difference was not significant (**Figure 4A**). Of note, the ppFEV1 was already lower at M-24 prior to the start of LUM/IVA and remained lower during the study period in patients with ppFEV1 < 80% at M0 compared with those with ppFEV1 ≥ 80% (**Figure 4A**). In addition, the decrease in ppFEV1 in the 2 years prior to the LUM/IVA initiation was greater in patients with impaired lung function than those without at M0 (**Figure 4B**); however, the ppFEV1 change from M0 to M24 was not different between the two groups (**Figure 4B**) as well as between M-24 and M24 (ppFEV1 change: −2.8 ± 17.9 vs. 3.4 ± 15.8, $p = 0.086$). Prior to treatment, the BMI *Z*-score remained stable in the two groups (**Figure 4C**). After the LUM/IVA initiation, in both groups of patients (with or without impaired lung function), the BMI *Z*-score increased, with a significant improvement at M24, but remained significantly lower in patients with BMI *Z*-score < 0 at M0 vs. the others, from M-24 to M24 (**Figure 4C**). In the two groups, the sweat chloride decreased significantly after a year of treatment (**Figure 4D**).

In patients with a BMI *Z*-score < 0 at the LUM/IVA initiation, the ppFEV1 decreased significantly in the 2 years prior to the LUM/IVA initiation but remained stable in the following 2 years (**Figures 3C,D**), whereas in patients with BMI *Z*-score > 0 at M0, the ppFEV1 did not change before the LUM/IVA initiation and increased in the following 2 years with a significant improvement at M24 (**Figure 5A**). Indeed, the decrease in ppFEV1 in the 2 years prior to the LUM/IVA initiation was greater in patients with BMI *Z*-score < 0 than at M0 for those without (**Figure 5B**), whereas the increase in ppFEV1 from M0 to M24 was not

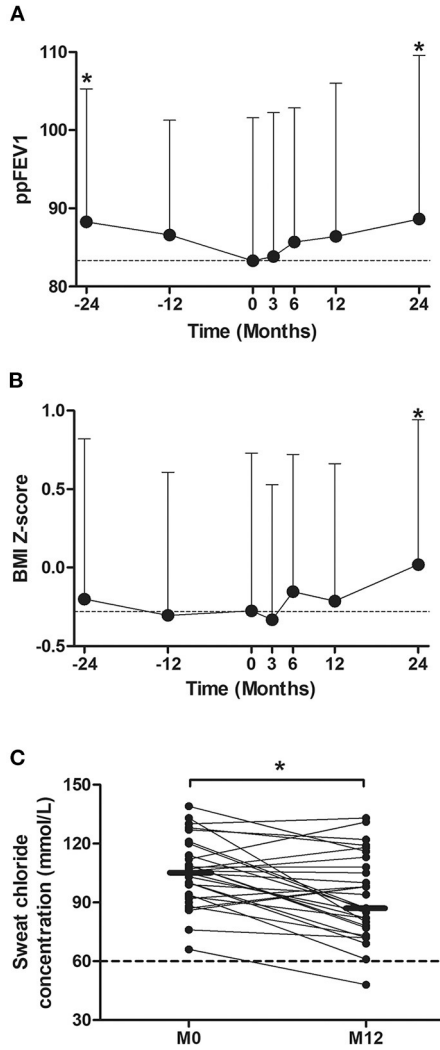

FIGURE 3 | Evolution of the percent predicted forced expiratory volume in 1 s (ppFEV1), body mass index (BMI) *Z*-score, and sweat chloride before and after the initiation of lumacaftor/ivacaftor (LUM/IVA). Evolution of ppFEV1 **(A)** and BMI *Z*-score **(B)** from 2 years before to 2 years after lumacaftor/ivacaftor (LUM/IVA) initiation (M0). Evolution of sweat chloride from M0 to 12 months after lumacaftor/ivacaftor (LUM/IVA) initiation **(C)**. Data are plotted at each timepoint using all available and were represented at the mean ± standard deviation **(A,B)**. Comparisons with data obtained at M0 were performed using the Wilcoxon paired test (*$p < 0.05$).

different between the two groups (**Figure 5B**). The BMI *Z*-score decreased significantly between M-24 and M0 in patients with a BMI *Z*-score < 0 at the LUM/IVA initiation and increased significantly after as early as 6 months of treatment (**Figure 5C**). In contrast, in patients with a BMI *Z*-score ≥ 0 at the LUM/IVA initiation, the BMI *Z*-score did not change significantly from M-24 to M24 (**Figure 5C**). A decrease in sweat chloride was observed in both patients with and those without a BMI *Z*-score < 0, but the difference was only significant for the first group (**Figure 5D**).

The Number of Good Responders Increased in Time and Was Associated With Age at the Lumacaftor/Ivacaftor Initiation

During the LUM/IVA treatment, the number of low/non-responders decreased, and the number of patients in both the no decline group and the good responders group increased significantly with time (**Figure 6A**). After 2 years of treatment, 11/40 (27.5%) were in the no decline group and 22/40 (55.0%) were good responders. Among these 22 patients, 14 (35% of the entire population) had an improvement in ppFEV1 of over 10% (**Figure 6A**). However, the percentage of good responders was also not significantly different according to the presence or the absence of impaired lung function or BMI *Z*-score $<$ 0 at the LUM/IVA initiation (**Figure 6B**). Due to the small size of the no decline group and the low/non-responder at M24, we then compared the results of the good responders, defined by their ppFEV1 change >5% at M24, with those of the other patients. In the two groups, there was no change in the BMI *Z*-score in the 2 years prior to the LUM/IVA treatment, but this parameter increased in the 2 years after with a significant improvement at M24 only in the group of good responders (**Supplementary Figure 5A**). Indeed, the improvement in the BMI *Z*-score was greater in the good responders than in other patients (**Supplementary Figure 5B**). Surprisingly, there was no significant difference between the two groups of responders in the improvement of sweat chloride ($p = 0.983$), HAV scores ($p = 0.482$), and Bhalla scores ($p = 0.576$). When characteristics assessed at M0 of good responders and the other patients were compared, only the age at the LUM/IVA initiation was significantly different and lower in good responders (**Figure 6C** and **Table 2**). Moreover, there was a significant reverse correlation between age at M0 and change in ppFEV1 after 2 years of treatment (rho $= -0.33$; $p = 0.04$) (**Figure 6D**), whereas no association was found between the change in ppFEV1 between M0 and M24 and ppFEV1 (rho $= -0.09$; $p = 0.57$), BMI *Z*-scores (rho $= 0.00$; $p = 1.00$), and the sweat chloride concentrations (rho $= -0.03$; $p = 0.87$) at M0, sweat chloride improvement between M0 and M12 (rho $= -0.11$; $p = 0.56$), or BMI *Z*-score change between M0 and M24 (rho $= -0.11$; $p = 0.50$).

Lumacaftor/Ivacaftor Is Well-Tolerated and Adherence Is Satisfactory

Side effects observed in the 40 patients included were transient chest tightness ($n = 1$), increased anxiety ($n = 1$), transient diarrhea ($n = 3$), and increased aspartate and alanine aminotransferase >5 times the upper limit of normal ($n = 1$). Except for this last patient who temporarily interrupted the treatment, all the others maintained their treatment for at least 2 years independently of the observed response and without any difference between good responders and other patients. No cataract or lens opacities were detected.

Early cessation of treatment (<3 months) occurred in only two patients, due to psychological depression in one case and social difficulties in the other.

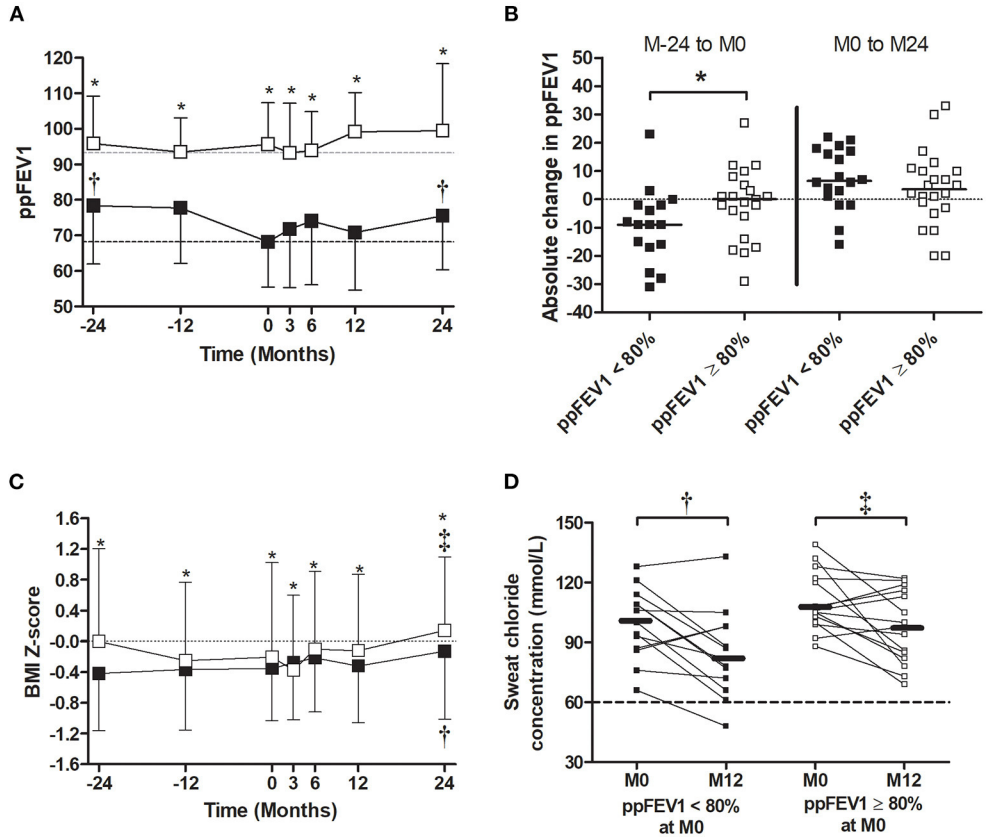

FIGURE 4 | Evolution of the percent predicted forced expiratory volume in 1 s (ppFEV1), body mass index (BMI) *Z*-score, and sweat chloride before and after the initiation of lumacaftor/ivacaftor (LUM/IVA) in patient with or without ppFEV1 <80%. Evolution of ppFEV1 **(A)** and BMI *Z*-score **(C)** 2 years prior (M-24) to 2 years after (M24) LUM/IVA initiation (M0) in patient with (black square) or without (empty square) ppFEV1 < 80%. Absolute changes of ppFEV1 between M-24 to M0 [**(B)** left] and M0 to M24 [**(B)** right]. Evolution of sweat chloride from M0 to M12 in patients with or without lung function **(D)**. Comparisons with data obtained at M0 were performed using the Wilcoxon paired test **(A,B,D)** and between the two groups using the Mann–Whitney test. *, †, and ‡Indicate significant difference ($p < 0.05$) compared with M0 in patients with ppFEV1 < 80% (†) and in patients with ppFEV1 ≥ 80% (‡) or between groups (*).

DISCUSSION

This real-life study highlights the long-term effectiveness and safety of LUM/IVA on lung function and nutritional outcomes in CF adolescents in real life. We found a significant improvement in the ppFEV1, from worsening in the 2 years preceding treatment to a significant increase during the 2 years of the LUM/IVA therapy, particularly in patients with ppFEV1 < 80% or BMI *Z*-score < 0 at the LUM/IVA initiation and a concomitant improvement in BMI *Z*-score and sweat chloride concentration after the LUM/IVA treatment.

The impact of LUM/IVA on ppFEV1 appears to be greater in the adolescents in the present study compared with that reported in the pivotal phase III randomized controlled trials, TRANSPORT (+2% to 4% after 24 weeks of LUM/IVA) (17) and PROGRESS (+0.5% at 96 weeks) (8). In the long term, the PROGRESS study demonstrated a slowing down of lung function decline in treated compared with paired non-treated patients from the US registry, but with the persistence of a declining trend in ppFEV1 (8). In comparison, in our study, we showed an increase in ppFEV1 in treated patients. Both the TRANSPORT and PROGRESS studies mainly involved CF adults (8, 17), whereas we focused on CF adolescents only. Our results are consistent with the slowing down of lung function decline after a year of LUM/IVA treatment in adolescents with CF included in the French real-life cohort (18), and with another study performed by Loukou et al. (29). In our study, we confirmed the short term effect at 6 months of LUM/IVA treatment with a proportion of good responders close to a previous report (20). Moreover, we demonstrated both a sustained long-term effect up to 2 years after the LUM/IVA initiation and a significant number of late responders. We also demonstrated that in some patients who were not good responders, ppFEV1 stability was observed, and this represents a satisfactory response since adolescence is the period of greatest decline in lung function. In addition, we demonstrated some benefits in adolescents with impaired lung function or BMI *Z*-score < 0, and at a lesser extent in those with normal ppFEV1 or BMI *Z*-score > 0 at the start of the treatment (i.e., ≈50% of good responders). Patients with impaired lung function or BMI *Z*-score < 0 had also the worst sweat chloride

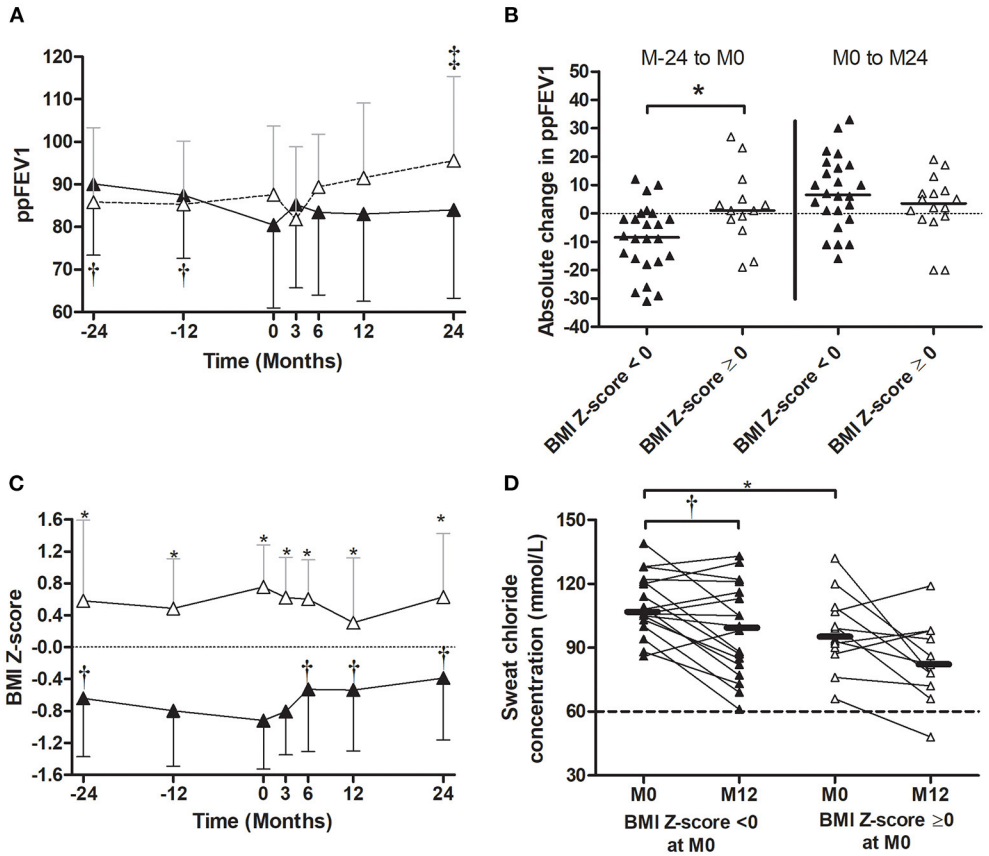

FIGURE 5 | Evolution of the percent predicted forced expiratory volume in 1 s (ppFEV1), body mass index (BMI) *Z*-score, and sweat chloride before and after the initiation of lumacaftor/ivacaftor (LUM/IVA) in patient with or without BMI *Z*-score < 0. Evolution of ppFEV1 **(A)** and BMI *Z*-score **(C)** 2 years prior (M-24) to 2 years after (M24) LUM/IVA initiation (M0) in patient with (black triangle) or without (empty triangle) BMI *Z*-score < 0. Absolute changes of ppFEV1 between M-24 to M0 [**(B)** left] and M0 to M24 [**(B)** right]. Evolution of sweat chloride from M0 to M12 in patients with or without BMI *Z*-score < 0 (D). Comparisons with data obtained at M0 were performed using the Wilcoxon paired test **(A,B,D)** and between the two groups using the Mann–Whitney test. *, †, and ‡ Indicate significant difference ($p < 0.05$) compared with M0 in patients with ppFEV1 < 80% (†) and in patients with ppFEV1 ≥ 80% (‡) or between groups (*).

which may be related to a poorer nutritional status or greater CFTR dysfunction. Thus, it was not surprising that restoring CFTR function and/or nutritional status with LUM/IVA has a better effect in these patients. Moreover, we highlighted an inverse correlation between the age at the LUM/IVA initiation and improvement in ppFEV1 after 2 years of treatment. This suggests that early initiation of the treatment at a younger age regardless of pulmonary function or growth parameters may be more beneficial. This was in accordance with the improvement in lung clearance index at 24 weeks of treatment in younger patients (9.1 ± 1.5 years old) with normal lung function as previously described in a 6–11 year phase III study (15).

Interestingly, we observed significant improvement in growth parameters (i.e., weight and BMI *Z*-score), particularly in adolescents with poor BMI *Z*-scores at the start of LUM/IVA as demonstrated in younger patients included in the LUM/IVA phase III (14) or in CF adults (8).

As previously described in an industry-sponsored and larger real-life studies (8, 14, 15, 17–20), we confirmed the improvement induced by LUM/IVA regarding CFTR function, with a significant decrease in sweat chloride concentration, particularly in the most severe patients (low ppFEV1 or BMI). However, we did not find any difference in sweat chloride concentration improvement between responders and non-responders, nor any association between this parameter and ppFEV1 or BMI *Z*-score improvement as previously demonstrated (15, 17, 19, 21).

In contrast, we could not confirm the decrease in pulmonary exacerbation rates and antibiotics consumption in real life in CF adolescents treated with LUM/IVA as found in placebo-controlled and long-term industry sponsored studies (i.e., decrease in exacerbation rates by 30% to 39%) (8, 17) or in a previous real-life study (i.e., decrease in exacerbation rates by 54%) (30). However, in this last study, patients were older (31 ± 11.6 years old) (8, 14, 26) and/or had worse lung function (ppFEV1: 37.4 ± 11.3 %) (30) and/or had a higher rate of exacerbations treated by intravenous antibiotics (3.4 ± 2.4/year) at the time of initiation of LUM/IVA (30) compared with our patients. Moreover, adolescents in our cohort had <2

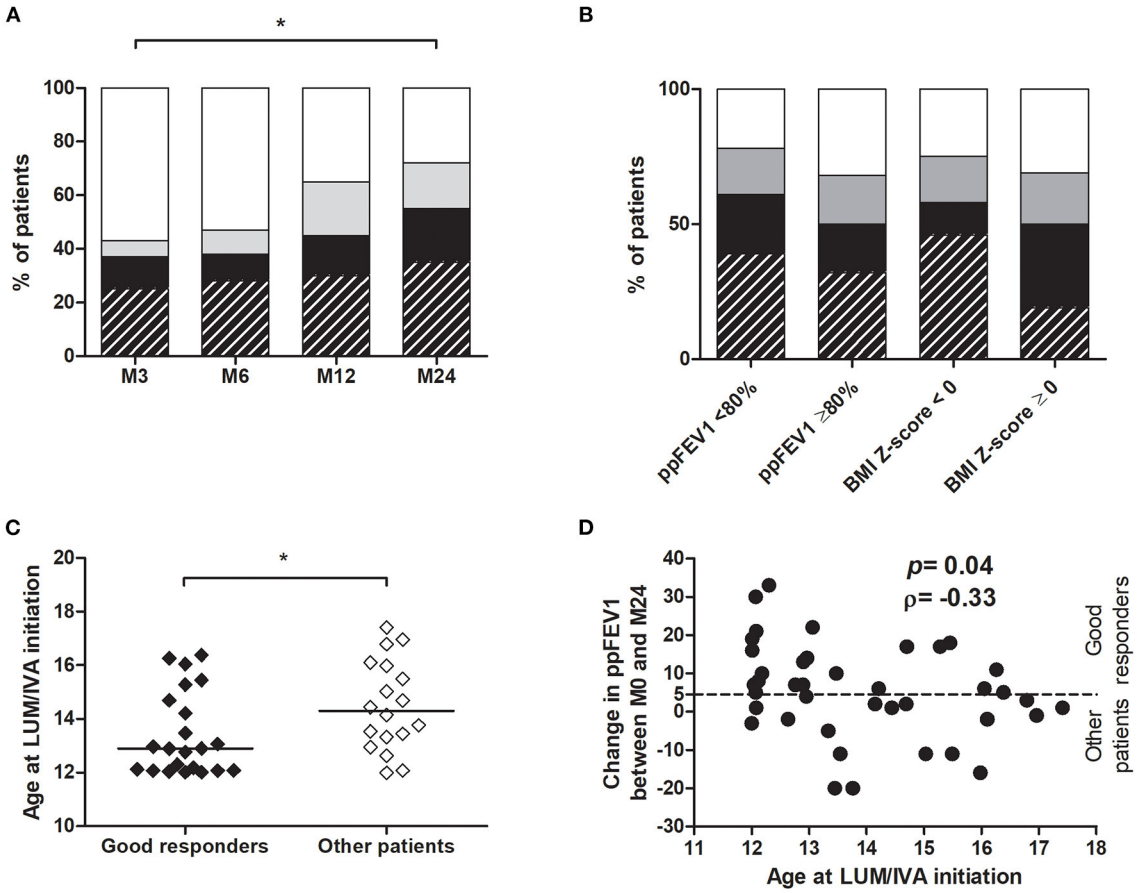

FIGURE 6 | Evaluation of LUM/IVA treatment response over time and factors associated with a good response. For each time point, responses of patients were evaluated by the ppFEV1 change at the time point compared with M0 **(A,B)**. Patients with a good response to treatment (ppFEV1 change ≥5%) are represented in hatched black (ppFEV1 change >10%) or black (ppFEV1 change >5%), those in the no decline group (ppFEV1 change between 0 and 5%) in gray and the low/non responders (ppFEV1 decline) in white **(A,B)**. Comparisons between good responders (hatched black and black) and other patients at 3 (M3), 6 (M6), 12 (M12), and 24 months (M24) after LUM/IVA initiation was performed using the Chi-square test **(A,B)**. Age at initiation of LUM/IVA was compared between good responders, defined by their ppFEV1 change >5% at M24, and other patients (no decline group + low/non responders) **(C)**. Comparisons between groups were performed using the Mann–Whitney test. *Indicates $p < 0.05$. Correlation between age at LUM/IVA initiation and ppFEV1 change between LUM/IVA initiation (M0) and 24 months after initiation (M24) from all patients **(D)** was performed using the Spearman test and the coefficient of correlation ρ is shown.

exacerbations per year, and <1 intravenous antibiotic course per year, thus, the effect of LUM/IVA is more difficult to demonstrate. In agreement with our results, in young adults with F508 homozygous mutations, Tessel et al. found no difference in the annualized rate of exacerbations (31).

We did not find difference in Bhalla (24) scores or HAV (25) scores after a year of treatment as demonstrated in CF adults with advanced disease only (25). However, such results have not been demonstrated in CF children who have a lower level of disease severity. Indeed, CT scans were not included in the industry-sponsored clinical trials of LUM/IVA involving CF children (8, 9, 14–19). In addition, our results are in agreement with the lack of modification in the visual Eichinger score previously demonstrated in CF before and after treatment despite lung clearance index improvement (32). However, a possible lack of sensitivity in the imaging scores may have impacted these results, as the visual scores may not detect a variation of <50% in lung injury due to their scoring systems (32). Nevertheless, it does not reduce the usefulness of treating young patients before irreversible lesions are established. To note, this lack of improvement in imaging scores could also be the consequence of scans performed too early, since the results on ppFEV1 or BMI were only significant after 2 years of treatment in our population.

In our study, the side effects of the treatment were consistent with those observed in the industry-sponsored studies (8, 15, 17) or real-life cohorts (18, 29), and in favor of good tolerability and patient adherence. Interestingly, we observed psychological side effects such as increased anxiety and depression in one patient leading to an early cessation of treatment as previously described in real life (33) but not in the industry-sponsored studies (8, 15, 17).

Our study had several limitations. First, the number of patients included is low compared with other real-life studies and there is no control-group. However, these patients accounted

TABLE 2 | Patient characteristics at initiation of lumacaftor/ivacaftor.

	Other patients	Good responders	*p*1
N	18	22	
Age (years)	14.5 ± 1.67	13.4 ± 1.6	**0.044**
Male sex	10/18 (55.6)	12/22 (54.6)	1.000
Weight *Z*-score	−0.50 ± 1.17	−0.32 ± 0.87	0.514
Height *Z*-score	−0.56 ± 1.10	−0.22 ± 1.03	0.178
BMI *Z*-score	−0.35 ± 1.14	−0.22 ± 0.90	0.888
Past history			
Meconial ileus	1/18 (5.6)	1/22 (4.5)	1.000
DIOS	1/18 (5.6)	0/22 (0.0)	0.450
At inclusion			
CFRD	1/18 (5.6)	1/22 (4.5)	1.000
CFLD	4/18 (22.2)	5/22 (22.7)	1.000
P. Aeruginosa colonization			
None	9/18 (50.0)	9/22 (40.9)	
Intermittent	2/18 (11.1)	9/22 (40.9)	0.085
Chronic	7/18 (38.9)	4/22 (18.2)	
S.Aureus colonization			
None	5/18 (27.8)	3/22 (13.6)	
Intermittent	1/18 (5.6)	2/22 (9.1)	0.587
Chronic	12/18 (66.7)	17/22 (77.3)	
ppFEV1	83.4 ± 13.5	83.2 ± 21.8	0.828
Sweat chloride concentration	103.1 ± 15.4	106.1 ± 18.2	0.592
Ballah score	12.9 ± 3.4	13.7 ± 5.6	0.405
HAV score	3.27 ± 0.46	3.31 ± 0.56	0.965
Treatment			
PERT U/Kg/j			
Proton pump inhibitor	11/18 (61.1)	10/22 (45.5)	0.360
Ursodeoxycolic acid	8/18 (44.4)	8/22 (36.4)	0.748
Laxative treatement	2/18 (11.1)	3/22 (13.6)	1.000
Inhaled antibiotics	10/18 (55.6)	7/22 (31.8)	0.200
Within the 2 years prior treatment			
No. of exacerbations	4.0 [2.0; 5.3]	3.0 [1.0; 6.3]	0.913
No. of IV ATB courses	1.0 [0.0; 3.0]	1.0 [0.0; 4.3]	0.865
No. of oral ATB courses	1.5 [0.8; 3.3]	1.5 [0.0; 3.0]	0.677
First 2 years of LUM/IVA			
Change in *Z*-score BMI	0.10 ± 0.80	0.44 ± 0.48	0.154
Change in ppFEV1	−4.9 ± 7.9	13.7 ± 7.9	<0.001
No. of exacerbations	4.0 [2.8; 6.3]	3.5 [1.0; 6.0]	0.330
No. of IV ATB courses	2.0 [0.0; 3.3]	0.5 [0.0; 3.5]	0.819
No. of oral ATB courses	2.5 [0.8; 4.0]	1.0 [1.0; 2.3]	0.179

M0, day of lumacaftor/ivacaftor initiation; ppFEV1, percent predicted forced expiratory volume in 1 s; BMI, body mass index; DIOS, distal intestinal obstruction syndrome; CFRD, cystic fibrosis-related diabetes; CFLD, cystic fibrosis liver disease; P. Aeruginosa, Pseudomonas Aeruginosa; S. Aureus, Staphylococcus Aureus; PERT, pancreatic enzyme replacement therapy.

Results are expressed as n/N (%) for categorical variables or as mean ± standard or at median with IQR [median (IQR_{25}; IQR_{75}) deviation] for quantitative variables. Comparisons between good responders (change in ppFEV1 ≥ 5% after 2 years of treatment by LUM/IVA) and other patients were performed using the Fisher's exact test for categorical variables or the Mann–Whitney test for quantitative variables. A p-value ≤ 0.05 was considered significant.

for 84% of our patients eligible for the LUM/IVA treatment in our centers. Second, spirometric tests performed 2 years and 1 year before and after T0 were selected to match the best ppFEV1 obtained at ±1 month from the chosen time point. We use this method to avoid the transient effect of exacerbation on ppFEV1. Third, pre-treatment data were assessed retrospectively. However, such data included standard items, which were uniformly and prospectively recorded during routine follow-up using the same software in the entire South-West network. This data is also channeled to the national registry, decreasing the risk of bias due to missing data. Fourth, only exacerbations requiring antibiotics have been considered, in contrast to less severe pediatric exacerbations due in most instances to viral infections. However, compared with previous clinical trials we assessed exacerbation requiring IV and/or oral antibiotics and not only those requiring IV courses which represent only a subset of antibiotic-treated exacerbations especially in adolescents. Fifth, the adherence to treatment was assessed at each visit but was based on the reports of children or their parents, and this represents an important limit of real-life studies.

CONCLUSION

We confirm that in real life, adolescents given long-term LUM/IVA improve their ppFEV1 and BMI *Z*-score, which have been associated with survival in cystic fibrosis. Adolescents with impaired lung function had a greater treatment benefit on lung function, but early introduction appeared to promote better response to the treatment, with good tolerance. In the era of CFTR modulators, real-life studies help the clinicians understand what type of results are really to be expected according to specific age groups.

TAKE-HOME MESSAGE

This real-life study showed short- and long-term benefits of lumacaftor/ivacafor association in adolescents with cystic fibrosis, particularly in the youngest ones, by changing trajectories and improving lung function and nutritional parameters.

AUTHOR'S NOTE

This study focuses on the long-term safety and efficacy of lumacaftor/ivacaftor in children with cystic fibrosis in real-life.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SB designed and supervised the study, with contributions from AM and MM. SB, AM, LR, FG, CC, RE, JL, MM, GD, MF, and EM included and followed the patients. The statistical analysis was performed by SB, FB, and RE. SB, MM, and AM were major contributors in writing the manuscript and contributed equally to the manuscript. All authors have approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.744705/full#supplementary-material

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Conflict of Interest: SB, FG, CC, RE, FB, and MF conducted clinical trials with Vertex pharmacological agents, on behalf of the European Cystic Fibrosis Society-Clinical Trials Network (ECFS-CTN) and within the scope of ECFS-CTN activities.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Patient and Provider Experience With Cystic Fibrosis Telemedicine Clinic

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In response to the novel coronavirus (COVID-19) pandemic, all in-person cystic fibrosis (CF) appointments were converted to telemedicine visits at UCSF Benioff Children's Hospital. The purpose of our study was to learn about the experiences that patients, families, and providers had with telemedicine visits and to assess their interest in using telemedicine in the future. Our hypothesis was that most patients, families, and providers want to continue telemedicine visits in the future. An anonymous 11-question survey was distributed to patients, families, and providers in November and December 2020. The survey was completed by 46 of 72 families (64% response rate) and 24 of 25 providers (96% response rate). Thirty-seven families (80%) and 21 providers (88%) were satisfied with their telemedicine experience. Thirty-three families (72%) want to have telemedicine visits in the future. Thirty-five families (76%) and 22 providers (92%) were satisfied with their experience using Zoom. Forty families (87%) and 19 providers (90%) want 2 or more visits each year to be via telemedicine. Our study showed that most families and providers were satisfied with telemedicine, would like to continue using telemedicine, and prefer to have at least 2 of the 4 recommended annual CF visits via telemedicine. Our survey identified the following benefits to telemedicine: decreased travel time, decreased cost, and avoiding exposure to COVID. However, we need to ensure that we do not exacerbate existing health disparities for families that do not speak English and/or do not have the internet capabilities to support telemedicine technology.

Keywords: cystic fibrosis, quality improvement, telehealth, telemedicine, patient experience, provider experience

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INTRODUCTION

On March 11, 2020 the novel coronavirus disease (COVID-19) outbreak was declared a pandemic by the World Health Organization. Two days later, the United States declared COVID-19 a national emergency. In response, UCSF Benioff Children's Hospital Oakland and San Francisco converted all in-person Cystic Fibrosis (CF) appointments to telemedicine visits via Zoom, a secure web-based platform that provides video and telephone services. The first telemedicine CF visit was conducted on March 27, 2020.

Prior to the COVID-19 pandemic, telemedicine was generally thought of as a way to increase access to subspecialty care in rural and/or underserved areas. A prior study by Kane and Gillis found that only 15.4% of physicians worked in a practice that utilized telemedicine to interact with patients (1). In pediatrics, the percentage was even lower: only 11.8% of pediatric physicians

worked in a practice that utilized telemedicine to provide patient care. The survey utilized in this study defined telemedicine as "the use of technology as a substitute for an in-person encounter with a health care professional." Although the Kane and Gillis study found that video conferencing was the most commonly used telemedicine modality, there are many types of telemedicine including: telephone calls, facilitated virtual visits with examination equipment, remote monitoring of chronic disease via various measurements (e.g. glucose, blood pressure, weight, etc.), storage and forwarding of health data (e.g. imaging, photos of rashes), and patient portals (e.g. MyChart) (2).

In a systematic review to assess the utility of telemedicine to monitor symptoms, monitor adherence to prescribed therapies, and provide therapeutic intervention to adults and children with CF, the investigators found that monitoring symptoms and collecting objective data (e.g. spirometry data) via telemedicine is feasible in patients with CF. However, there was a high rate of non-compliance with data reporting; therefore, the benefits of telemedicine in CF patients could not be determined (3). Since that 2012 systematic review, the use of telemedicine in the care of patients with CF has changed dramatically, in large part due to the COVID-19 pandemic.

The COVID-19 pandemic forced us to utilize telemedicine to provide multidisciplinary medical care for many patients, including CF patients. Our study was designed to help us understand the telemedicine experience of patients with CF as well as CF care team members, and to assess their interest in continuing to use telemedicine in the future. Our hypothesis was that most patients, families, and providers want to continue telemedicine visits in the future.

MATERIALS AND METHODS

This cross-sectional study was performed at UCSF Pediatric Cystic Fibrosis Center. The study population consisted of CF patients (ages 0–22 years) and their families as well as CF care providers (including pulmonologists, gastroenterologists, pharmacists, social workers, respiratory therapists, physical therapists, dieticians, nurses, and pediatric residents).

Patients were seen in the multidisciplinary Cystic Fibrosis clinic virtually, using the Zoom platform with breakout rooms. Patients were scheduled with all providers on the same day. Interpreters were provided to all families when indicated. In-person visits with the CF care team were completed in limited cases as clinically indicated (e.g. sick patients). Patients were scheduled for separate in-person visits in the pulmonary function laboratory for pulmonary function tests (PFTs) and throat/sputum cultures when indicated.

Study participants were asked to complete either a patient or provider survey about their experience with telemedicine visits, experience using Zoom, interest in using telemedicine in the future, benefits of telemedicine, benefits of in-person visits, and the frequency in which they would like various measurements/diagnostic studies (e.g. height and weight, PFTs, throat cultures, vital signs, physical exams, labs, chest x-rays) to be obtained. The families of CF patients either received a link to the Qualtrics survey in English via email or had the survey administered verbally, either via Zoom or over the phone. Non-English-speaking families had the survey administered verbally with the assistance of a medical interpreter ($n = 12$). Families were sent reminders to fill out the survey via email and via text message. The 72 families who receive regular CF care at UCSF received the patient survey. A total of 25 CF providers received a link to the Qualtrics provider survey via email. All survey data were de-identified and collected from November to December 2020. Telemedicine visits were ongoing at the time the survey was conducted. At the time of the survey, patients had had more than one telemedicine visit and at least one visit within 3 months of the survey. The patient and provider surveys can be found in **Supplemental Materials Appendices 1 and 2**.

Per UCSF IRB, this study was exempt from further review.

RESULTS

Clinic Metrics

Three hundred and sixty-two (362) encounters were entered into the Cystic Fibrosis Registry in 2019 and 375 encounters were entered in 2020. In 2019, the average length of in-person visits was 134 minutes. In 2020, telemedicine visits lasted between 30 and 90 minutes. Of our 72 CF patients followed in our center, only 4 patients (6%) were seen in-person and only 4 patients (6%) had audio-only telemedicine visits (due to difficulties with internet connection or trouble setting up Zoom) between March 2020 and January 2021. The percent of patients with at least 4 visits per year increased from 88% in 2019 to 95% in 2020. The only patients who did not have at least 4 visits were patients who moved in/out of our center during 2020 or were diagnosed with CF mid-year. All patients were seen by a respiratory therapist, dietitian, and social worker at least once in 2020. Ninety-seven percent of patients 7 years and older had at least one PFT and one culture. Ninety-six percent of our patients completed annual labs. The clinic no-show rate improved by 40% during the transition to telemedicine.

Patient Survey

Of the 72 families who received the patient survey, 46 families responded (64% response rate). Of the 46 respondents, there were families with children age 0–24 months ($n = 4$), 2–5 years ($n = 13$), 6–12 years ($n = 12$), 13–17 years ($n = 10$), and older than 18 years ($n = 7$).

Of the 46 respondents, 37 (80%) were overall satisfied with their telemedicine visits. Forty-one respondents (91%) felt that all their questions and concerns were addressed during their telemedicine visits. Thirty-five respondents (76%) were satisfied with their experience using Zoom. However, seven reported having internet connection difficulties and two reported having difficulty using or locating the Zoom ID.

Thirty-three respondents (72%) indicated that they would like to have future visits via telemedicine. When specifically asked about the number of telemedicine visits they preferred per year, 47% of families preferred 2 out of 4 recommended annual CF visits be provided virtually. Forty families (87%) wanted 2 or more visits each year to be telemedicine visits (**Figure 1**).

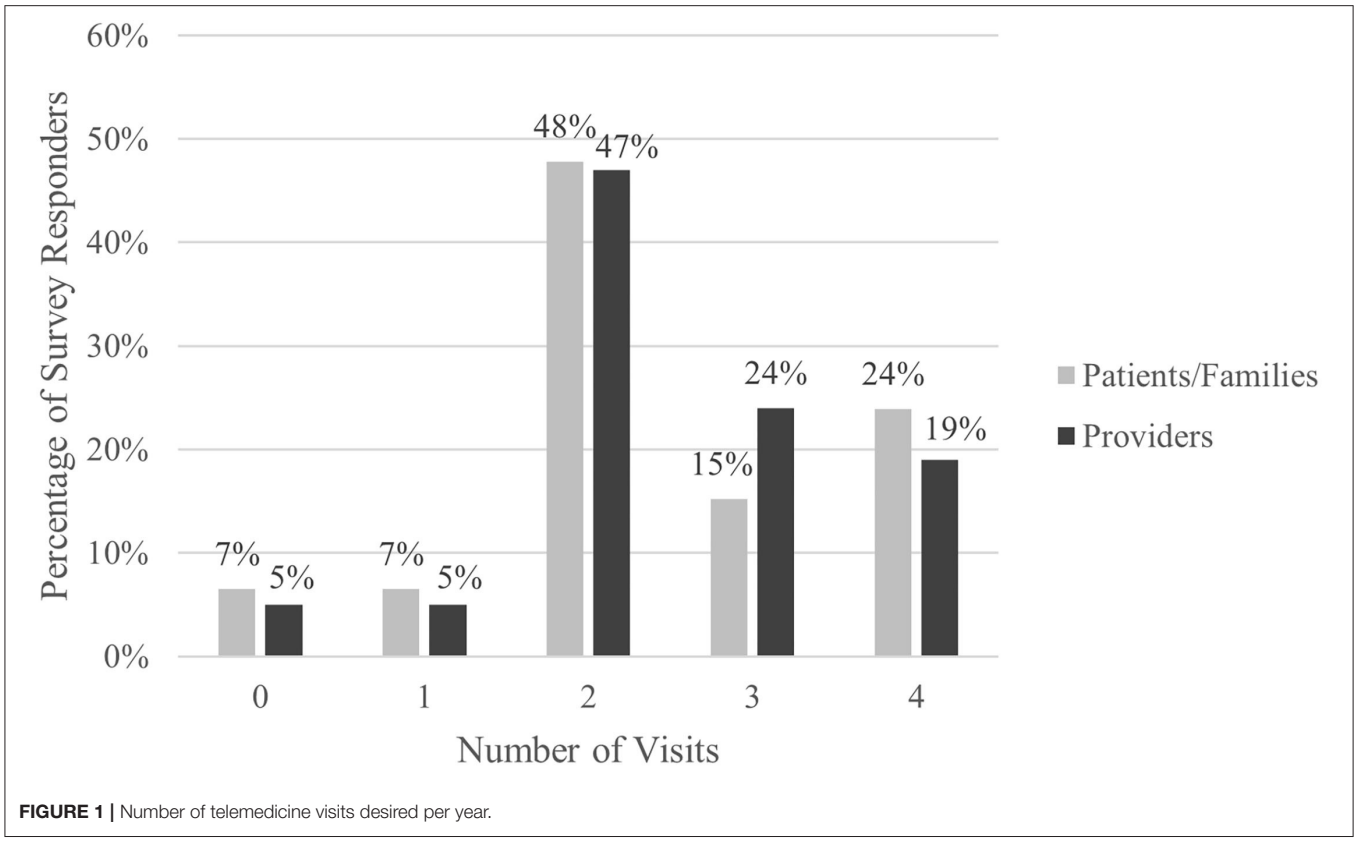

FIGURE 1 | Number of telemedicine visits desired per year.

However, there were 3 families (7%) who reported that they wanted all 4 visits to be in-person.

Respondents identified the following benefits of telemedicine visits: decreased cost, decreased travel time, decrease in the amount of time missing work/school, do not have to arrange for childcare for other children, concerns about COVID, and concerns about poor air quality from fire smoke. Decreased travel time and concerns about COVID were the two most common reasons that families preferred telemedicine visits. One respondent commented that they "felt the doctors were more focused," "there was less waiting," and the "kids did not get bored."

The most common reasons for wanting an in-person visit were: to have a physical exam done and to complete PFTs, labs, throat culture, etc. on the same day. Some felt an in-person visit was more personal ($n = 12$). Two respondents reported that they preferred in-person visits because they experienced technical difficulties. Families also preferred in-person visits if their child was sick or if there was an urgent matter. Additional comments from the patient surveys can be seen in **Table 1**.

When families were asked about the frequency in which they would like various clinical data obtained (e.g. height and weight, PFTs, throat cultures, vital signs, physical exams, labs, and chest x-rays), the responses varied based on the clinical data/test type. In general, most families wanted each of these types of clinical data obtained 1–2 times per year (**Figure 2**).

Provider Survey

Of the 25 CF providers who received the provider survey, 24 providers responded (96% response rate). The respondents included: pulmonologists ($n = 6$), gastroenterologists ($n = 2$), pharmacists ($n = 2$), social workers ($n = 3$), respiratory therapists ($n = 5$), physical therapists ($n = 1$), dieticians ($n = 3$), nurses ($n = 1$), and pediatric residents ($n = 1$).

Of the 24 respondents, 21 (88%) were overall satisfied with telemedicine visits and satisfied with the care that they provided. Twenty-two respondents (92%) were satisfied with their experience using Zoom, 18 (75%) were satisfied with their experience using Zoom breakout rooms, and 17 (71%) were satisfied with interpreter experience via Zoom. Despite most providers feeling satisfied with their experience, providers identified various difficulties with telemedicine (**Table 2**).

When providers were specifically asked about the number of telemedicine visits they preferred per year, 47% of providers preferred 2 out of 4 recommended annual CF visits be provided virtually. Ninety percent of providers wanted 2 or more visits each year to be telemedicine visits (**Figure 1**). One respondent (5%) reported wanting all 4 visits to be in-person.

Providers identified the following benefits of visits via telemedicine: decreased cost, decreased travel time, concerns about COVID, concerns about poor air quality from fire smoke, convenience for patients and families (especially those who live far away), and a reduced no show rate. Decreased travel time

TABLE 1 | Comments and suggestions from the patient survey.

Reasons families would like to have telemedicine visits
If I don't need to bring the kids in because they are not sick, I can have the appointment via telemedicine.
I have concerns about exposure. I also have concerns that through televisions the care is not acceptable.
Feel the doctors are more focused – seems to be less waiting around and kiddos don't get bored!
Driving to San Francisco makes me nervous.
Reasons families would like to have in-person visits
I feel that the staff get to know our son better and build a stronger relationship over time.
If my child is feeling sick.
Additional comments
I prefer when the doctors are together in the appointment so it is shorter. Otherwise I do like my child to see everyone.
Having everything possible sent to PCP for PCP to do like throat swabs.
I think weight and height are important but it is easy to do at home. Overall I've had a great experience with telemedicine visits.
A lot of the information can be given ahead of time – would maybe be helpful to know when the nurse is going to call pre-meeting for info or send a secure form for us to fill in with height, weight, any issues, etc.
I'm happy with these visits. They are thorough and convenient.
I would like to thank the doctors for taking care of my kids.
I would like all visits to be in person.
I would like to alternate video visits with in-person visits. I feel more secure with a physical exam that my daughter is OK.
If there is an urgent matter I would like to have an in-person appointment. If it is not urgent, I am ok with a telephone visit.
We would like to return to in-person visits whenever possible.
Given CF patients are at high risk and you cannot run clinics at full capacity, it would be great to have at least 2 of the 4 annual visits in person. It makes me nervous as a parent to have my child not seen in person because this disease can creep up on you and I really want to make sure we are staying ahead of it, especially given the coronavirus, and knowing it's not going away anytime soon.
Everything goes well.
Parking is too expensive.

and concerns about COVID were the two most common reasons that providers wanted to have telemedicine visits. One responder noted that having a separate, additional telemedicine visit to discuss nutrition is sometimes effective because they can spend more time with patients and their families.

The most common reasons providers cited for wanting an in-person visit were: an in-person visit feels more personal, they prefer to do in-person demonstrations/teaching, and it is easier to get labs and tests done on the same day as a visit. Providers also commented that they would like to have in-person visits to perform a physical exam and because it can be hard to tell when patients and families are engaged when having a conversation through a screen.

When providers were asked about the frequency in which they would like various clinical data obtained (e.g. height and weight, PFTs, throat cultures, vital signs, physical exams, labs, and chest x-rays), the responses varied based on the clinical data/test type. Overall, most providers wanted each of these types of clinical data to be obtained 1–2 times per year (**Figure 3**).

DISCUSSION

Our results demonstrate that 80% of our patients and 88% of our providers were satisfied with their telemedicine visits. Seventy-two percent of families reported wanting to have telemedicine visits in the future, and both patients and providers preferred to have at least 2 of the 4 recommended annual CF visits via telemedicine. These findings are similar to prior studies reporting that the majority of patients and providers want to have future visits via telemedicine (4–6). Interestingly, our data demonstrated a trend of differences between different provider types: respiratory therapists, physical therapists, and social workers tended to prefer more in-person visits; dieticians preferred fewer in-person visits; and physician and nurse providers had varied opinions. Future studies should explore these differences in a larger cohort.

The CF Foundation recommends that all pediatric CF patients have 4 visits per year with a multidisciplinary team of specialists (including pulmonologists, gastroenterologists, pharmacists, social workers, respiratory therapists, physical therapists, dieticians, and nurses) in order to properly care for patients with this chronic and multisystemic disease (7, 8). Because of the number of providers involved in each visit, our visits prior to the pandemic typically lasted over 2 h and sometimes had a significant amount of down-time. With telemedicine, there was less down-time between providers and less time spent rooming patients. During telemedicine visits, families are often more comfortable because they are at home and they do not need to obtain childcare for their other children. Additionally, patients can drift in and out of the visit easily, which allows providers to visually examine patients and ask them questions directly while also minimizing the child's boredom during lengthy visits. This added benefit of telemedicine visits also allows providers to have more focused conversations with the patient's primary caregivers.

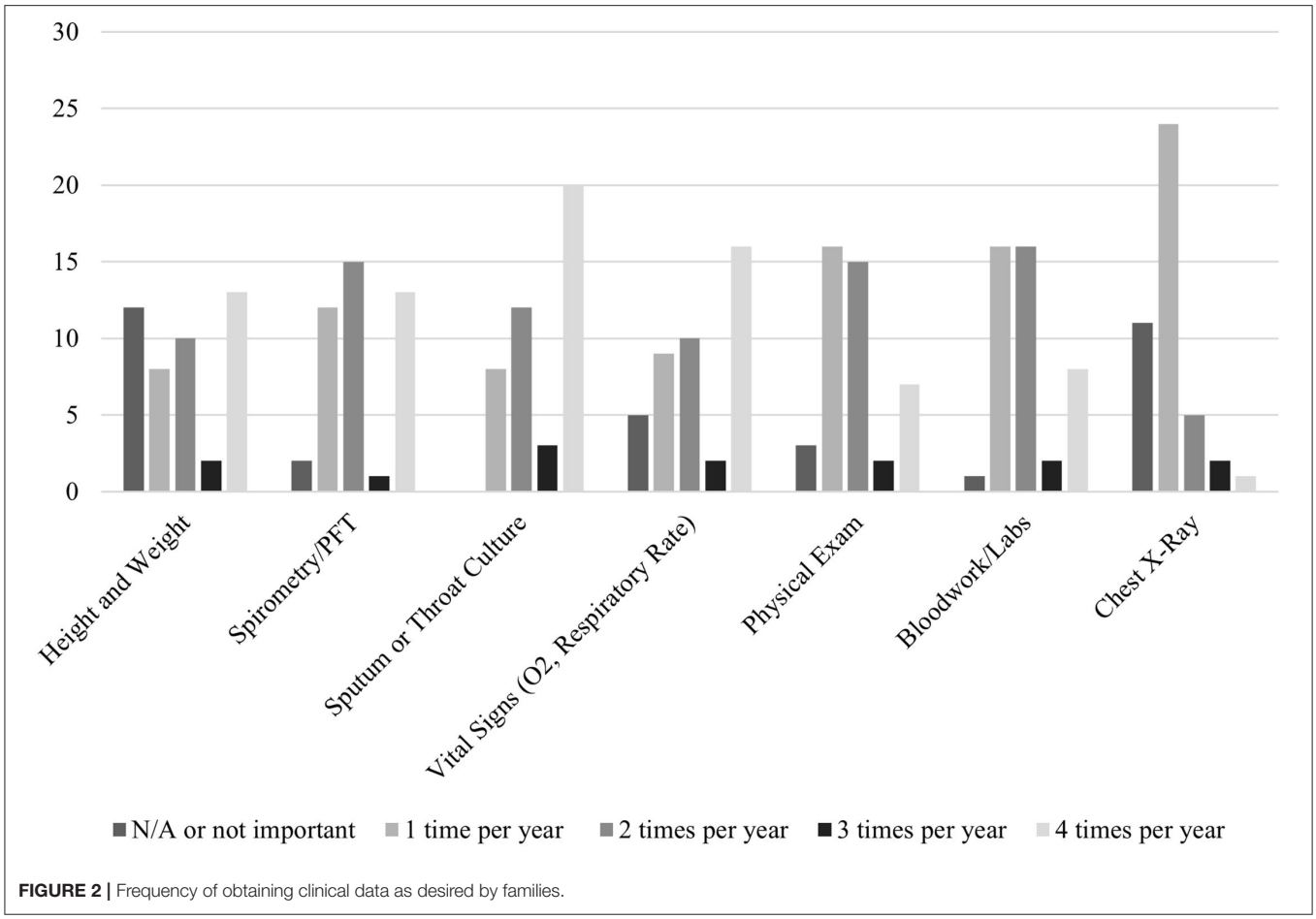

FIGURE 2 | Frequency of obtaining clinical data as desired by families.

TABLE 2 | Provider-identified difficulties with telemedicine.

Issues with internet ($n = 2$)
Issues with Zoom ($n = 2$)
Found it difficult to communicate with other providers ($n = 2$)
Difficult to move in and out of different breakout rooms ($n = 2$)
Medical interpreters cannot be called from breakout rooms ($n = 2$)
It is difficult to obtain accurate anthropometric measurements ($n = 1$)
Concerns about the privacy of patients and their families ($n = 1$)

The UCSF Pediatric CF Program serves a wide catchment area that spans from the Oregon border to Central California. Our patient population is also diverse: 28% of our patients are non-white and/or Hispanic and approximately 8% of our families speak only Spanish. We pride ourselves on caring for this underserved population and want to ensure that families that live farther away and/or only speak Spanish receive the best quality of care. Some families have previously noted that it is difficult to attend in-person clinic visits due to travel costs and concerns about missing work or school. In our study, we found that most families are satisfied with telemedicine visits and would like to continue using telemedicine after the pandemic. With telemedicine, we can increase access to care for families who live far away or have difficulty coming to in-person visits for various reasons.

In the era of new born screening (NBS) and cystic fibrosis transmembrane conductance regulator (CFTR) modulators, we have seen that our patients are healthier. Even prior to the pandemic, some families were starting to push back on the need for 4 in-person visits each year when they felt their child was healthy. While more research is needed to determine the optimal clinic regimen for the future, and whether family and provider satisfaction changes over time, as the pandemic improves, our data suggests that adding telemedicine as an additional care delivery modality, can improve clinic attendance, and still allow patients and care centers to meet the Cystic Fibrosis Foundation care guidelines.

As we move forward, we must ensure that there is proper support in place to ensure that telemedicine visits go smoothly and that our CF care team members continue to provide high-quality patient care. Some of the logistical components include: providing families with scales and tape measures so they can report accurate height and weight measurements, providing home pulse oximeter devices to measure heart rate and oxygen saturation, home spirometers to remotely monitor FEV1, timely communication with families to ensure they have the appropriate Zoom ID and are able to connect to the internet, and

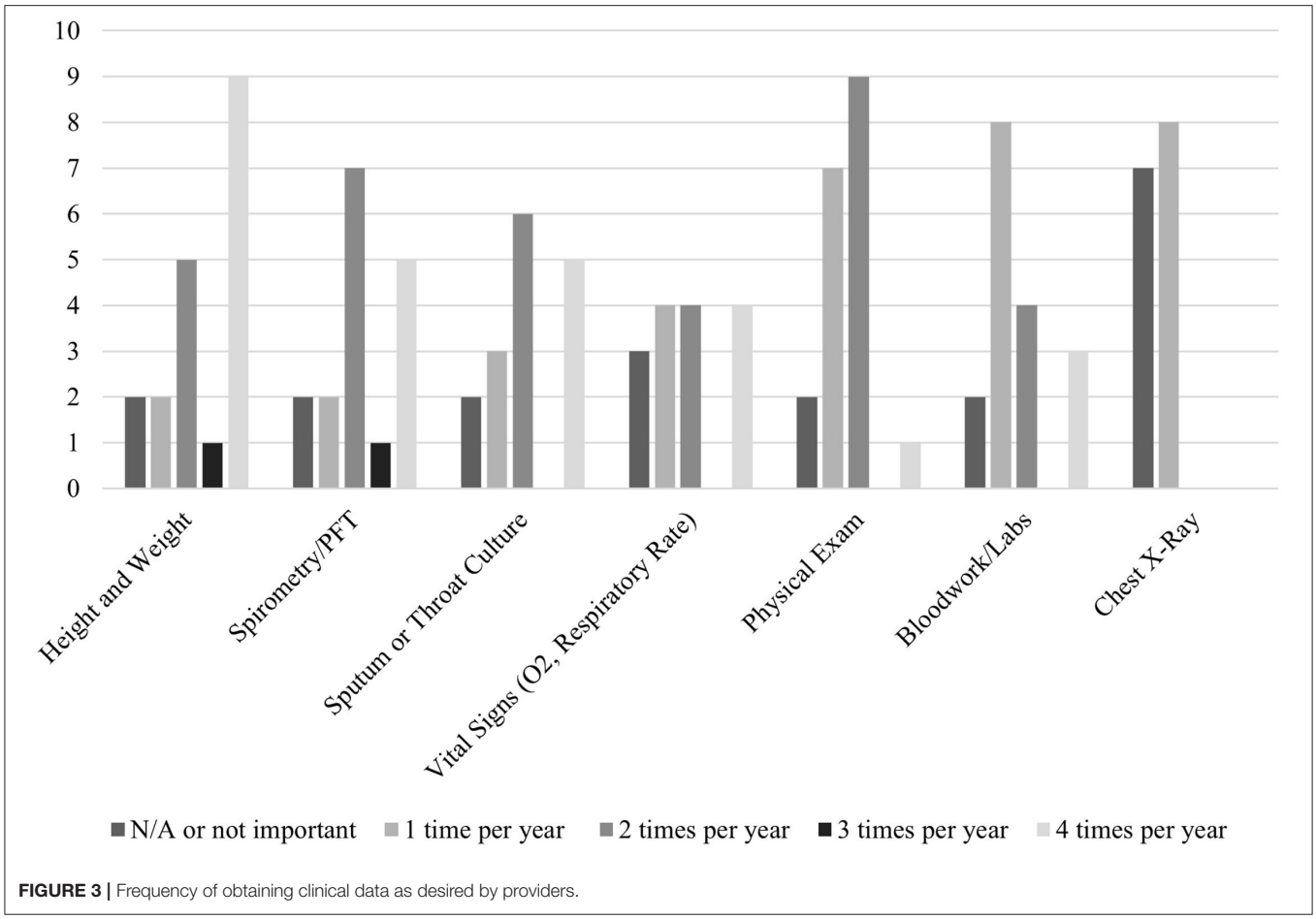

FIGURE 3 | Frequency of obtaining clinical data as desired by providers.

adequate support from medical interpreters. Anecdotally, our low socioeconomic and non-English-speaking families had more difficulty connecting to the Zoom platform, which accounted for the 4 patients who had audio-only telemedicine visits. This lack of video-capability prevented the providers from visually examining the patient during the telemedicine visit. To prevent inadvertently increasing health disparities, we will need to assess each family's access to internet and comfort using Zoom (and other telemedicine technology) before replacing future in-person visits with telemedicine visits.

Some other challenges associated with telemedicine visits include difficulty obtaining lung function testing, throat cultures, and laboratory testing (especially with frequent liver function lab monitoring in the era of CFTR modulator therapy) and providing mental health screening. With access to more reliable portable spirometry devices at home, we may be able to further decrease the number of in-person appointments and decrease travel costs/time for our families. Mental health screening is also difficult to perform via Zoom; it can feel impersonal and there is no guarantee of privacy, as other family members may be able to overhear the conversation. However, we were able to successfully complete mental health screening via video visits and families were able and willing to schedule a separate in-person visits for lung function testing, labs, and respiratory cultures.

One limitation of this study is our small sample size (only 46 families and 24 providers completed our surveys), as this study only involved one CF center. Unfortunately, this made it challenging to draw conclusions from subgroup analyses, including differences in opinions between patients and different provider types. Our study was also limited by our low response rate (only 64% of families responded to the survey), which could have potentially biased our results. Since our survey data was de-identified, we were not able to determine specific characteristics associated with responders vs. non-responders. Communication with families was another limitation; not all families speak English and/or respond reliably to emails. Because of this, our non-English-speaking families completed the survey verbally; the surveys were administered by two research assistants with the help of interpreters. Another limitation was that our patient survey did not include a question regarding patient/family demographics (e.g. ethnicity/race, preferred language, etc.) so we were not able to examine the difference in experience between white and

non-white patients/families. Our patient population also includes patients from lower socioeconomic status (many of our patients are on Medicaid), so this data may not be generalizable to other populations. Also, since Zoom was used in this study, our results may not be generalizable to other centers that use other telemedicine platforms.

In conclusion, our study has shown that most families and providers were satisfied with telemedicine and would like to continue using telemedicine in the future. There are several benefits to telemedicine including convenience, decreased travel time and cost, and avoiding exposure to COVID. However, we will need to ensure that we do not inadvertently exacerbate existing health disparities for families that do not speak English and/or do not have the internet capabilities to support telemedicine technology. Future studies are needed to evaluate access and satisfaction of telemedicine technology in patients with racial and ethnic differences, as well as assess satisfaction over time, especially as the pandemic recedes. Further studies are also needed to correlate quality of care with care delivery modalities to develop optimal care delivery guidelines.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KH was involved in the project's conceptualization, methodology, data collection and analysis, and manuscript writing (original draft and editing). FN was involved in the project's conceptualization, methodology, manuscript writing (editing), and supervision. MC was involved in the project's conceptualization, manuscript writing (editing), and supervision. NL was involved in the project's manuscript writing (editing) and supervision. EG was involved in the project's conceptualization, data collection, manuscript writing (editing), and supervision. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.784692/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BRIEF RESEARCH REPORT
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Assessing the Utility of an Outpatient Exercise Program for Children With Cystic Fibrosis: A Quality Improvement Project

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Children with cystic fibrosis (CF) (cwCF) suffer from inadequate weight gain, failure to thrive, and muscle weakness. The latter may be secondary to disuse atrophy (muscle wasting or reduction in muscle size associated with reduced physical activity and inflammation). Handgrip strength (HGS) is a reliable surrogate for muscle strength and lean body mass. Data from our CF center have shown an association between low HGS and forced expiratory volume in 1 s (FEV_1) in cwCF. High-intensity interval training (HIIT) improves physical strength. Therefore, we devised a project to assess implementing a HIIT exercise program in the home setting, in order to improve physical strength in cwCF with HGS $\leq$ 50th percentile. Patients were instructed to complete 3–5 sessions of HIIT exercises per week. Wilcoxon matched-pairs signed-rank tests were used to compare HGS, FEV_1, and body mass index (BMI) percentile at baseline and at a follow-up clinic visit. Follow-up was limited due to the COVID pandemic. Adherence to the HIIT regimen was poor. A total of twenty-nine cwCF participated in the program. However, a total of 13 individuals reported some form of moderate activity at follow-up and therefore constituted our final study population. There was a statistically significant increase in absolute grip strength (AGS) and FEV_1 for these individuals. Even though the home HIIT protocol was not followed, the project demonstrated that moderate physical activity in cwCF can lead to significant improvement in HGS and overall physical strength.

Keywords: grip strength, CF, pediatrics-children, exercise, FEV_1

INTRODUCTION

Cystic fibrosis (CF) is a multisystemic disorder affecting more than 30,000 individuals across the United States. Due to pulmonary and gastrointestinal manifestations, people with CF (cwCF) often suffer from inadequate weight gain, failure to thrive, and muscle weakness. The latter may be secondary to disuse atrophy associated with reduced physical activity and inflammation (1). CwCF are generally weak and are less likely to participate in a vigorous activity when compared with their peers (2). Though cwCF and their families are generally aware of the benefits of physical activity, barriers such as pulmonary exacerbations and low lung function can impact their ability to regularly participate in physical activity (3).

Regular exercise improves muscle weakness in individuals with and without chronic conditions. For cwCF, physical activity augments airway clearance, an integral part of maintaining pulmonary health, and slows the rate of decline in lung function (4). Additionally, physical activity increases aerobic capacity and improves muscle strength and lean body mass (LBM) (5). LBM is positively correlated with nutritional status and also lung function in cwCF (6, 7), when compared with body mass index (BMI). Despite its ease of measurement in the clinical setting, BMI is an imperfect measurement because of its inability to differentiate between fat mass and LBM.

Handgrip strength (HGS) has been shown to be a reliable surrogate for LBM and is a practical method of assessing physical strength in the clinic setting. Data from our CF center indicate that there is a positive association between HGS and forced expiratory volume in 1 s (FEV_1) in cwCF (8). High-intensity interval training (HIIT) improves physical fitness in children and improves the quality of life in patients with cardiometabolic disorders (5, 9, 10). HIIT exercises are offered to the individuals in our CF center during their inpatient stay for pulmonary exacerbations, in an attempt to promote muscle strengthening and augment airway clearance. We devised this project to address muscle weakness in our pediatric CF population. The goal of the project was to assess the utility of implementing a HIIT exercise program in the home setting, in order to improve physical strength.

METHOD

Study Population

CwCF ages 12–18 years, attending our Pediatric CF center, were included in this quality improvement (QI) project. This age group was identified as most likely to participate in HIIT exercises. Our physical therapist (PT) evaluated individuals at baseline to ensure that they were able to perform HIIT exercises. Individuals with HGS of ≤50th percentile for age who were able to perform HIIT exercises were offered the intervention of an individualized HIIT home training program. Individuals included for final analysis met criteria of HGS ≤ 50th percentile and had returned for serial grip strength measurements (see **Supplementary Figure 1**). This manuscript originated from data from a QI project in our center.

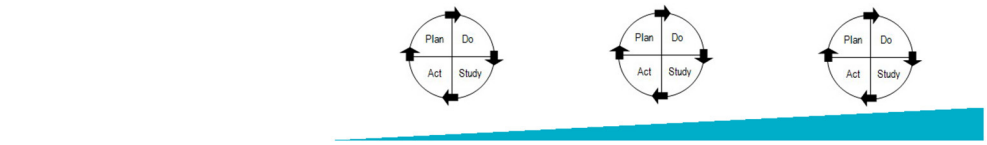
Project Design and Measurement

The QI project was reviewed by our Medical School Institutional Review Board (IRBMED), and it was determined that it does not require institutional review board (IRB) approval because it does not satisfy the definition of research under 45 CFR 46.102(l) and 21 CFR 56.102(c). The project was undertaken in a series of Plan-Do-Study-Act (PDSA) cycles (**Figure 1**). The first PDSA cycle involved identifying cwCF aged 12–18 years with HGS ≤ 50th percentile and offering them a HIIT exercise program to be done at home. Trained personnel measured each participant's grip strength with a calibrated Jamar Plus digital hand dynamometer (Patterson Medical, Warrenville, IL, USA) using the American Society of Hand Therapists' measurement protocol. For the measurement, individuals were seated with their shoulders adducted, elbow flexed at 90°, and forearms in a neutral position. The handle was positioned such that the individuals were able to wrap their thumb around one side of the handle and their fingers around the other side, with their intermediate phalanges covering the face of the handle and without the tips of their fingers coming into contact with the palm of their hand. Three measurements were taken on each hand, alternating between hands with a 10- and 15-s break between each measurement. Individuals were encouraged to squeeze harder until the number on the digital read-out stopped rising (11). Age- and gender-specific percentiles for absolute grip strength (AGS) were determined from the pre-published percentile charts, originating from our center, that were derived from data collected by two survey cycles of the National Health and Nutrition Examination Survey (8).

HIIT is a training technique involving periods of high-intensity exercises alternating with low-intensity exercises or rest (12). The HIIT home training program offered to the project participants was individualized and included 5 min of a warm-up and 16–24 min of HIIT exercises followed by stretching, to be done 3–5 times per week. Individuals were allowed to choose 8 exercises from a list of exercises that are considered high intensity, including jumping jacks, burpees, and squats. Each exercise was followed by a rest period of 1–2 min until the patient returns to baseline work of breathing as assessed by the 15 count breathlessness scale. Verbal and written instructions were provided during the initial clinic visit by our PT, who was then available for consultation by phone between visits.

The second PDSA cycle involved follow-up of the individuals who were offered the home exercise program. This was done by our PT. The type and frequency of activity that individuals were involved in were recorded. If individuals did not report completing the HIIT training, they were questioned for any additional activity or exercise they were completing. The exercise program was then adapted for patients participating in non-HIIT activity, to allow them to complete exercises of their choice. This was done in order to encourage patients to remain active. All reported activities were classified according to metabolic equivalents (MET) values listed in the Youth Compendium of Physical Activities (13) and defined as very light/light (<3.0 METs), moderate (3.0–5.9 METs), or vigorous (≥6.0 METs) activities. If an individual reported no activity, barriers were addressed, and additional education was provided.

In the third and final PDSA cycle, we evaluated adherence to the exercise program and repeated HGS measurements in those individuals able to return for an in-person visit. In our center, individuals are followed up every 3 months. Initially, we planned to obtain HGS measurements at these quarterly visits; however, with the onset of the coronavirus pandemic, many in-person clinic visits were converted to virtual visits. Therefore, we obtained grip strengths when individuals and/or staff were available during an in-person encounter. Follow-up HGS measurements were then compared with baseline measurements. The medical record was also reviewed to document interval change in spirometry, BMI, and BMI percentile.



	Test Cycle 1	Test Cycle 2	Test Cycle 3
Test Description:	Evaluating physical strength through measurement of Hand Grip Strength (HGS)	Evaluating Home Exercise Program during the COVID Pandemic through Virtual Visits	Evaluating HGS and adherence to Home Exercise Program at in-person visits
Test Population:	cwCF age 12-18 years	cwCF age 12-18 with HGS ≤ 50th percentile	cwCF with HGS ≤ 50th percentile
Location of test:	CS Mott Pediatric CF center		CS Mott Pediatric CF center
Duration (# hours/days/months):	4 months	6 months	4 months
Executed by:	Physical Therapist and Registered Dieticians	Physical Therapist	Physical Therapist, MDs
Test Results:	37.8% of patients were found to be weak as evidenced by HGS ≤ 50th percentile.	Poor adherence to the HIIT program, but individuals participated in other forms of moderate exercise	Patients who participated in moderate physical activity recorded statistically significant increases in HGS
Action (Adapt, Adopt or Abandon):	Provide High Intensity Interval Training program to patients to be done at home 2-3 times a week	Adapt: Encourage continued regular exercise despite the Stay at Home Order	Adopt

FIGURE 1 | Plan-Do-Study-Act (PDSA) ramp describing test cycles 1 to 3 of the home exercise program.

TABLE 1 | Baseline characteristics of children with CF with HGS ≤ 50th percentile ($N = 29$).

Baseline characteristic	Median (range)
Age (years)	15.99 (12.04–18.87)
Time to follow-up (months)	8 (2–10)
BMI (kg/m^2)	21.37 (17.56–31.29)
BMI percentile	68.16 (19.73–98.79)
Maximum AGS (kg)	24 (11.8–45.8)
AGS percentile	10 (4–50)
FEV_1 (percent predicted)	85 (47–104)
	***N* (percent)**
Individuals on highly effective modulators	21 (72)
Individuals on at least moderate activity	9 (31)

AGS, absolute grip strength; BMI, body mass index; FEV_1, forced expiratory volume in 1 s.

Statistical Analysis

Wilcoxon matched-pairs signed-rank test was used to compare HGS, lung function, BMI, and BMI percentile at baseline and follow-up assessments of the entire cohort. A multiple linear regression model was used to determine independent predictors of increased HGS. For all comparisons tests, a p-value of < 0.05 was considered statistically significant. R version 4.0.5 (14) was the statistical software used for analysis.

RESULTS

One hundred three cwCF between the ages of 12 and 18 had HGS assessed over a 4-month period. Of those tested, 39 (37.8%) were found to have HGS ≤ 50th percentile. Twenty-nine of the 40 children returned for follow-up measurement. The median time to follow-up was 8 (2–10) months (**Table 1**), due to delayed in-person follow-up because of the pandemic. The median AGS was 24 kg [11.8–45.8] with the median AGS percentile at the 10th percentile. In contrast to AGS, the median BMI and BMI percentile were within the normal range at 21.3 kg/m^2 and 68th percentile, respectively.

The initiation of the project coincided with the approval of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA), a highly effective modulator for individuals with CF at 12 years of age and older. Sixty-three percent of our cwCF had been on ELX/TEZ/IVA at the time of the baseline measurement. Four children taking tezacaftor–ivacaftor at enrollment changed to ELX/TEZ/IVA after the baseline assessment. These 4 individuals were excluded from the analysis to eliminate possible confounding factors.

Of the 29 children with serial grip strength measurements, only 2 reported participating in the HIIT program, with one discontinuing after a month due to the perceived difficulty of the exercises. Thirteen (44.8%) of the 29 individuals with HGS data had information on participation in an activity at baseline and follow-up and had been on ELX/TEZ/IVA at baseline. These

TABLE 2 | Comparison between Visit 1 (baseline) and visit 2 (follow-up) in children with CF with HGS ≤ 50th percentile ($N = 13$).

	Visit 1	Visit 2	Percent change	*p*-value
AGS (kg)	24.1 (11.8–45.8)	26.7 (19–49.8)	11.25	<0.001
AGS percentile	10 (4–50)	22.2 (4–70)	100	<0.001
Absolute BMI (kg/m^2)	21.37 (17.56–31.29)	22.25 (17.26–31.56)	3.88	0.04
BMI percentile	68.16 (19.73–98.79)	70.84 (21.71–98.77)	3.93	0.98
FEV_1 (% predicted)	85 (47–107)	95 (52–123)	11.76	0.006

Parameters for Visits 1 and 2 are represented as median (range). AGS, absolute grip strength; BMI, body mass index; FEV_1, forced expiratory volume in 1 s; CF, cystic fibrosis; HGS, hand grip strength.

13 individuals constituted our final study group. Within this group, there was an 11.2% increase in median AGS and a 100% increase in median AGS percentile at visit 2 (**Table 2**). There was a small increase in median BMI and BMI percentile. We also looked at lung function, which was assessed at every follow-up visit. There was an increase in FEV_1 of 11.7%, which was statistically significant.

We then divided this cohort of 13 patients into two groups: one with stable activity (n of 7) and one with increased activity (n of 6). The group with stable activity was already participating in at least moderate activity at baseline and continued to participate in a similar activity at follow-up. The group with increased activity included children who were inactive at baseline but reported at least moderate activity at follow-up. Though both groups had increases in AGS [median percent change 6.8% for the stable group vs. 10.9% for the increased group], there was no significant difference between the two groups in the baseline-to-follow-up percent change in grip strength or grip strength percentile. In addition, there was no significant difference in the percent change in BMI or lung function between the groups. To determine factors associated with percent change in HGS, we analyzed the data using a multiple linear regression model with the percentage change in HGS and percentage change in HGS percentile as the dependent variables and change in BMI, time to follow-up, female gender, and increased activity as independent variables. From this model, there was a significant association between female gender and lower average percent change in AGS percentile ($p < 0.01$). Though the average percent change in AGS and AGS percentile in the increased activity group was positive in the model, this was not statistically significant.

DISCUSSION

Physical strength is decreased in cwCF for a variety of reasons including poor nutritional status and decreased physical activity. HGS is an indicator of physical fitness and general muscle strength in children and adults (15). It is also a more practical method of assessing LBM. When compared with BMI, LBM does not incorporate fat mass; therefore, it better correlates with nutritional status and lung function in cwCF (16). Data from our CF center indicate a positive association between HGS and lung function in children with CF (8).

We first set out to identify children in our CF center who were weaker, as evidenced by low AGS. Almost 40% of children and adolescents had HGS ≤ 50th percentile for age. Interestingly, their average BMI was within normal limits. This is in keeping with prior studies that demonstrated the inadequacy of BMI in assessing body composition and nutritional status. In a pilot study done in children with CF who were hospitalized, Gibson et al. found that mean HGS z-scores were very low compared with the standard, whereas mean BMI z-scores were much closer to the standard (16). Similarly, among 201 medically stable CF individuals aged 6–21 years in our pediatric center, 40.7% of individuals with a BMI ≥ 50th percentile were weak for their size as evidenced by an HGS measurement < 25th percentile (8). Therefore, HGS may be more sensitive in detecting early changes in muscle mass than BMI.

In an attempt to improve physical strength in those children with low HGS, we devised a HIIT workout plan. HIIT increases LBM in healthy adults. It has similar effects on increasing fitness as a moderate-intensity aerobic training and in a shorter time. Therefore, time-efficient HIIT workouts may be more appealing to cwCF. Brown et al. used a 12-week program of 3 sessions and found that their intervention of HIIT exercises resulted in increased LBM and reduction in total body fat percentage in healthy young adult females (5). Smaller studies and case reports in cwCF have shown HIIT exercises to be safe and effective in improving exercise capacity and in a shorter time than standard exercise programs (17, 18). Verbal and/or written guidelines on exercise have been shown to be effective in increasing self-reported exercise activity in children with CF. A randomized trial is presently underway in CF adolescents and adults, which may give us insight into the efficacy of the web-based intervention in promoting physical activity (19).

We employed a combination regimen including verbal instruction by our PT, who also provided handouts with exercises that could be incorporated into a HIIT regimen. Though follow-up was severely affected by the pandemic, which led to decreased patient retention and longer time to follow-up, 6/29 (20%) of eligible children were motivated to start a moderate exercise program on their own (increased activity group). Only 2 children participated in the HIIT program. The remaining individuals chose different aerobic activities, which included swimming, cheerleading, weightlifting, walking, and running. Competing priorities, degree of difficulty, and lack of interest in the HIIT program were identified as barriers to participation in the program. Twenty-four percent (7/29) who were already participating in at least moderate activity at baseline (stable activity group) continued to participate in aerobic activities

of their choice at their follow-up visit. Following the HGS measurement, the explanation of the benefits of exercise, and the encouragement of cwCF to exercise by the PT, both groups demonstrated significant improvement in AGS, AGS percentile, and FEV_1 percent predicted. This would suggest that even in patients who are active at baseline, there is a potential of continuing to increase physical strength and lung function. With the knowledge that the increased activity group had a higher percentage change in AGS, we created a multiple linear regression model to ascertain predictors for percentage change in grip strength. After change in BMI, time to follow-up, and gender were controlled, the average difference in AGS was positive but not statistically significant. This may have been due to our small cohort. Given that females are generally weaker than their age-matched male counterparts secondary to reduced muscle mass, it was not surprising that the female gender had a much lower average percent change in the AGS percentile in the model.

Though HGS increases with age, the absolute increases in values in our cohort exceeded that which would have been expected throughout the project. More frequent follow-up may have further improved/encouraged other children to continue or start an activity. In addition, it was clear that individuals either continued to participate in an exercise of their choosing or commenced aerobic exercises other than the HIIT regimen that was presented to them. This suggests that the message to cwCF is that implementing home exercise is important and that any activity (at least moderate in nature) would be adequate in improving their muscle strength and potentially their lung function. Therefore, emphasis should be placed on encouraging moderately intense physical activity at regular intervals regardless of its nature.

The Cystic Fibrosis Foundation recommends that cwCF maintain a BMI of at least the 50th centile. This has been positively associated with better nutritional status and lung function. Similarly, better LBM is associated with better lung function and may correlate even more than BMI (6). Ionescu et al. found that cwCF with low fat-free mass (FFM) (which is approximately equal to LBM) had a lower mean FEV_1% predicted compared with the normal FFM individuals (20). A recent publication from our CF center revealed a statistically significant association between HGS and percent predicted FEV_1 across all BMI percentiles (8). Hence, by increasing HGS and therefore increasing LBM through physical activity, an individual may see positive effects on lung function. It is hypothesized that this improved lung function is due to increased respiratory muscle strength associated with optimizing overall LBM (21). In our cohort, there was a statistically significant change in percent predicted FEV_1 at follow-up.

Despite the significant improvement seen with the HGS, we recognize that our QI project had some limitations. Our cohort was limited by the small number of children who qualified for this program. Additionally, the majority of the children included in this QI initiative were started on the highly effective modulator ELX/TEZ/IVA within 3 months of beginning the project. This presents a possible confounder; however, we eliminated the children who started ELX/TEZ/IVA after baseline grip strength assessment. Also, since we did not analyze patients who participated in very light/light activities (<3.0 MET), we could not comment on whether lower-intensity exercises could also lead to changes in AGS improvement. Furthermore, our cohort excluded patients with HGS > 50th centile; therefore, we cannot comment on whether stronger patients can also increase their HGS or whether their participation in exercise could have accounted for higher HGS measurements. A longer follow-up period would have allowed us to look at the sustainability of moderate exercise on improving HGS and lung function in children with CF. However, there have been studies in children with CF which demonstrate the feasibility of long-term aerobic training programs of up to 3 years (22).

CONCLUSION

A significant number of cwCF are weaker than their age-matched peers. Regular physical activity involving at least moderate aerobic exercise can increase HGS and LBM which may, in turn, have positive effects on physical fitness, lung function, and overall quality of life in this population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

DA, CT, CI, and SB contributed to the acquisition of the data. AH contributed to the analysis and interpretation of the data. DA, AF, and SN contributed to the interpretation of the data. DA wrote the first draft of the manuscript. All authors contributed to the conception and design of the project, manuscript revision, and read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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What Is Most Suitable for Children With Cystic Fibrosis—The Relationship Between Spirometry, Oscillometry, and Multiple Breath Nitrogen Washout

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Introduction: In cystic fibrosis (CF), pathological lung changes begin early in life. The technological progress currently gives many diagnostic possibilities. However, pulmonary function testing in children remains problematic.

Objectives: Our study aimed to correlate the results of impulse oscillometry (IOS) with those of multiple breath nitrogen washout (MBNW) in our pediatric CF population. We also compared those parameters between the groups with and without spirometric features of obturation.

Methods: We collected 150 pulmonary function test sets, including spirometry, IOS, and MBNW in patients with CF aged 12.08 ± 3.85 years [6–18]. The study group was divided into two subgroups: IA (without obturation) and IB (with obturation). We also compared Sacin, Scond, and oscillometry parameters of 20 patients aged 14–18 years who reached the appropriate tidal volume (VT) during MBNW.

Results: Statistical analysis showed a negative correlation between lung clearance index (LCI) and spimoetric parameters. Comparison of subgroups IA ($n = 102$) and IB ($n = 48$) indicated a statistically significant difference in LCI ($p < 0.001$) and FEV1z-score ($p < 0.001$), FEV1% pred ($p < 0.001$), MEF25z-score ($p < 0.001$), MEF50 z-score ($p < 0.001$), MEF75 z-score ($p < 0.001$), R5% pred ($p < 0.05$), and R20% pred ($p < 0.01$). LCI higher than 7.91 was found in 75.33% of the study group, in subgroup IB—91.67%, and IA—67.6%.

Conclusions: LCI derived from MBNW may be a better tool than IOS for assessing pulmonary function in patients with CF, particularly those who cannot perform spirometry.

Keywords: cystic fibrosis, pulmonary function tests, lung clearance index, impulse oscillometry, spirometry

INTRODUCTION

Pathological changes in the lungs that contribute to a reduction in the quality of life and an increase in mortality appear reasonably early in patients with cystic fibrosis (CF). Despite technological advances and available devices, pulmonary function testing in children remains problematic. The challenges are proper assessment and timely intervention, which are essential to delaying and minimizing disease progression. A gradual decrease in lung function is associated with some physiological factors such as airway obstruction and ventilation heterogeneity (1, 2). These changes should be able to be detected in lung function tests. Not all available techniques are useful due to the difficulties associated with the lack of cooperation in children. Spirometry is considered to be the gold standard of lung function measurements in children older than 6 years and adults (3). This test is routinely used to assess lung function in children with CF. However, spirometry may not be feasible during acute exacerbations and in children with deteriorating lung function. They may not be able to perform spirometry as per standard guidelines; it requires forced expiratory maneuvers (4). It has also been shown that spirometry may not be very sensitive in detecting mild to moderate lung damage in children with CF (5). What is more, during spirometry, a quite large amount of aerosol is generated, which is essential in the coronavirus disease 2019 era (6). Hence, there is a need for a lung function test that is sensitive enough to pick up early abnormalities and that can also be performed easily in preschool children and children with advanced bronchopulmonary disease, who are unable to perform spirometry (4). In our article, we tried to correlate the parameters obtained during the performance of pulmonary function tests using various techniques. We also tried to find a more suitable technique that would better complement the spirometry test for patients who are unable to perform it properly. This is a very important issue in routine clinical practice in CF centers.

The impulse oscillometry (IOS), which is a variant of forced oscillation technique, described by Dubois over 50 years ago, allows evaluation of the mechanical properties of the respiratory system with a minimal need for patient cooperation (7, 8). The IOS technique uses pressure oscillations at a standard square pressure wave, at a frequency of 5 Hz from which all other applied frequencies are derived using spectral analysis. IOS measures the airway impedance (Zrs), which is comprised of two components: resistance (Rrs), which is the real part, and the imaginary part, which is the reactance (Xrs). The resistance reflects the relationship between the applied pressure and the resultant flow. It represents the total respiratory system resistive properties (extrathoracic and intrathoracic airways, lung parenchyma, and chest wall). The resistance at low frequencies (i.e., 5 Hz) reflects the total airway resistance, whereas the resistance at high frequencies (i.e., 20 Hz) reflects large airway resistance. The difference between resistance at 5 Hz and resistance at 20 Hz reflects the small airway resistance. The reactance is a component of impedance that encompasses the capacitive and inertive properties of the lung. It reflects the elasticity of the lung and is negative in sign. Other important parameters in IOS are frequency at which the reactance crosses zero is called resonant frequency (Fres) and AX, which represents the sum of all reactance components at all frequencies before the resonant frequency (6, 9). IOS can differentiate between small and large airway obstruction and is more sensitive than spirometry for peripheral airway disease. It has been used to study various respiratory disorders, especially asthma, and is suitable for measuring bronchodilatory response and bronchoprovocation testing (8). IOS has also been studied in other respiratory diseases such as chronic obstructive pulmonary disease, interstitial lung disease, and supraglottic stenosis (10).

The second technique, which could be helpful, is multiple breath nitrogen washout (MBNW). The lung clearance index (LCI), which is derived from MBNW, is another evolving sensitive tool to assess lung function in children (4). MBNW was introduced in the mid-1950s, but years later, it was put into clinical use. Unfortunately, the performance of MBNW is also much harder for people who cough a lot (pulmonary exacerbations, worse lung disease) and have inflamed airways, which is due to the dry air that they have to inhale during the measurement. Recent years have brought several studies on the IOS technique. In this method, sound waves are superimposed on normal tidal breathing, and the disturbances in flow and pressure caused by the external waves are used to calculate parameters describing the resistance to airflow and reactive parameters that mostly relate to efficient storage and return of energy by the lung (8).

Spirometry, in contrast to IOS or MBNW, is associated with a forced expiration, during which flow restrictions are measured. In IOS, resistance (Rrs) and airway reactance (Xrs) can be measured during tidal breathing. It seems that MBNW and probably IOS are more interesting in those patients with normal spirometry. Therefore, it is worth considering how these techniques are correlated with each other. Data on the comparison of IOS and MBNW parameters with spirometry results in children with CF are limited (4, 10–16).

In our study, we tried to correlate the parameters detected during spirometry, IOS, and MBNW to assess their usefulness in evaluating lung function in children with CF. We also compared those parameters between the groups with and without spirometric features of obturation.

This study was presented in poster form at the European Cystic Fibrosis Society Conference in Liverpool on June 6, 2019.

MATERIALS AND METHODS

We conducted a retrospective observational study in Cystic Fibrosis Center in Dziekanow Lesny from March 2017 to June 2019. CF was diagnosed in patients based on clinical features, sweat chloride measurements, and genetic tests according to the current diagnostic criteria (17, 18). Data regarding genotype and medical history were obtained from the clinical records. Patients who were able to perform spirometry, IOS, and MBNW with single-use mouthpieces met the eligibility criteria. The exclusion criteria were lack of cooperation, pulmonary exacerbation, severe clinical condition precluding a patient from performing MBNW, e.g., dyspnea, hemoptysis, and other severe complications of CF.

The protocol was approved on January 10, 2019, by the local ethics committee at the Institute of Mother and Child in Warsaw (opinion number 4/2019). After obtaining approval from the local ethics committee, the participants, and caregivers gave their informed consent for the use of their test results in the study.

All patients were divided into two subgroups depending on the results of the index FEV1/FVC z-score (ratio of forced expiratory volume in 1 s and vital capacity). Group IA ($n = 102$) did not present any evidence of obstruction, and group IB ($n = 48$) showed the features of obstruction in spirometry (FEV1/FVC z-score < -1.65). Criteria for obstruction were established in accordance with the recommendations of the Polish Pulmonary Society in 2005 (19) and based on the guidelines of ERS 1993 and Quarnjer 1989 (20).

To assess inhomogeneity in the acinar (Sacin) and conducting (Scond) airway regions, in the second part of the research, 20 patients with CF aged 15–18 years were extracted from the main group. Those patients were chosen due to their ability to perform MBNW and other tests correctly and to reach the appropriate tidal volume (VT) values required in the standardization documents. In this group, patients performed at least three acceptable and repeatable tests in MBNW, oscillometry, and spirometry. All tests were performed in the morning, starting from IOS, next MBNW, and spirometry. Before the tests, each patient performed the drainage.

We also created two subgroups based on the normal LCI values for healthy children: equal or below (group IC) and above (group ID), a cutoff point of 7.91 (21). This part of the study aimed to analyze the correlation between LCI and parameters such as MEF25, MEF50, MEF75 (maximal expiratory flow at 25, 50, and 75% of FVC), R20, R5 (resistance at 20 and 5 Hz), X5 (reactance at 5 Hz), Ax (area of reactance), and Fres (resonat frequency) in these subgroups.

Pulmonary Function Measurements

All tests were performed according to the American Thoracic Society and European Respiratory Society guidelines (22–24). All the tests were performed on the same day. Measurements of spirometry and IOS were conducted using Vyntus IOS, Jaeger system (CareFusion, Hochberg, Germany). MBNW tests were performed using Exhalyzer D (EcoMedics AG, Duernten, Switzerland, software version 3.2.0).

Spirometry maneuvers were performed according to the American Thoracic Society and European Respiratory Society guidelines until three repeatable and acceptable measurements were achieved. The spirometer was calibrated every day before measurements with a 3-L syringe. Patients performed spirometry in a sitting position. They would have to achieve at least three reproducible attempts. Test results were considered reproducible if the difference between the two largest values of forced vital capacity FVC was <0.150 L, and the difference between the two largest forced expiratory volume in 1 s (FEV1) values was <0.150 L. The test was considered to be carried out correctly if it met other criteria such as back extrapolated volume (BEV) < 5% of FVC or 0.100 L, no cough in the first second of expiration, no glottic closure after 1 s of expiration, no evidence of obstructed mouthpiece and/or spirometer, no evidence of a leak, reaching expiratory plateau < 0.025 L in the last 1 s of expiration, or expiratory time > 15 s. We use reference values from Quanjer et al. (25). Reference values from Zapletal et al. (26) were used for MEFs.

The environmental conditions in Exhalyzer D were updated daily. Flow and gas calibrations were performed before measurements. MBNW tests were carried out using single-use mouthpieces and nose clips. Patients performed at least three attempts. The calculations included results from a minimum of two correctly performed and repeatable trials (24). Acceptable maneuvers were defined by the software and also based on the operator's observation of the subject's behavior during testing. Quality control of the study concerned an adequate starting and end-tidal inert gas concentration, stability over 30 s, and regular breathing. We also made sure that there was no evidence of significant trapped gas release with larger breaths, apneas (may significantly decrease functional residual capacity), or sighs (may significantly elevate functional residual capacity) (24).

Oscillometry is potentially affected by upper airway artifacts in the form of swallows, vocal cord closures, coughs, incorrect positioning of the tongue, or mouth leaks; therefore, each test was couched by a technician. According to recommendations, the three replicates used to derive indices had a coefficient of variability of Rrs of ≤15% in children, at the lowest oscillation frequency (27, 28).

Statistical Analysis

Statistical analysis consisted of calculating means and standard deviations. We used the Spearman test to find a correlation between parameters from spirometry, oscillometry, and MBNW. Analyses were performed for the whole group and for subgroups divided according to spirometric criteria of obstruction, i.e., FEV1/FVC z-score < -1.65 and also for subgroups depending on the LCI value (IC where LCI $\leq$ 7.91 and ID > 7.91). All values expressed as z-scores were based on sex- and age-specific regression equations. The normality of the data distributions was determined by the Shapiro–Wilk test and graphical analysis. The homogeneity of variance was examined using the Brown–Forsythe test. In the analysis of relationships between unpaired quantitative variables for which no normal distribution was obtained, the non-parametric Mann–Whitney was used. In the analysis of relationships between unpaired quantitative variables, for which a normal distribution and homogeneity of variance were obtained, the Student's *T*-test was used for unpaired samples. The level of statistical significance was set at $p < 0.05$. Data were analyzed with STATISTICA version 13.3.

RESULTS

Characteristic of the Study Population

We examined 150 children with CF and adolescents aged 6–18 years who were enrolled (12.08 ± 3.85; 69 males; 81 females). The study group included 61 people with the "*F508del /F508del*" genotype, 72 people with the "*F508del*/other" genotype, and 17 people with mutations specified in the CFTR2 database as pathogenic other than *F508del*. **Table 1** shows the biometric characteristics of the whole study group and the

TABLE 1 | CF patients study group and subgroups characteristics: subgroup IA without spirometric features of obturation, IB with obturation features, IC [LCI (≤7.91)] and ID (LCI (>7.91)].

Parameter	Group	Subgroup IA	Subgroup IB	Subgroup IC	Subgroup ID
N	150	102	48	36	114
AGE	12.08 ± 3.85	11.74 ± 3.94	12.80 ± 3.57	10.2 ± 3.86	12.47 ± 3.77
FEV_1 %pred	89.51 ± 17.29	96.26 ± 14.26	75.14 ± 14.17	99.89 ± 14.88	86.23 ± 16.75
FEV_1 z-score	−0.90 ± 1.49	−0.31 ± 1.20	−2.14 ± 1.26	0.01 ± 1.26	−1.18 ± 1.45
FVC % pred	98.47 ± 14.47	99.32 ± 14.10	96.67 ± 15.22	103.31 ± 15.18	96.95 ± 13, 96
FVC z-score	−0.16 ± 1.25	−0.08 ± 1.19	−0.33 ± 1.35	0.25 ± 1.26	−0.29 ± 1.22
FEV_1%FVC %	90.38 ± 11.27	96.49 ± 6.06	77.42 ± 8.45	96.67 ± 9.42	88.40 ± 11.12
FEV_1%FVC z-score	−1.18 ± 1.47	−0.43 ± 0.92	−2.75 ± 1.15	−0.34 ± 1.15	−1.44 ± 1.47
MEF_{25} %pred	−1.39 ± 1.69	88.14 ± 36.70	32.96 ± 16.12	99.81 ± 46.12	61.58 ± 34.18
MEF_{25}%z-score	70.82 ± 40.70	−0.59 ± 1.24	−3.13 ± 1.12	−0.32 ± 1.60	−1.73 ± 1.57
MEF_{50} z–score	−0.77 ± 1.75	0.11 ± 1.15	−2.63 ± 1.31	0.28 ± 1.40	−1.10 ± 1.73
MEF_{50} % pred	87.44 ± 33.40	103.99 ± 24.82	52.27 ± 18.87	108.31 ± 30.30	80.85 ± 32.69
LCI	10.16 ± 3.22	9.04 ± 2.19	12.52 ± 3.75	7.03 ± 0.60	11.15 ± 3.07
S^*_{acin}VT	0.12 ± 0.09	0.10 ± 0.07	0.167 ± 0.11	0.03 ± 0.02	0.14 ± 0.09
S^*_{cond}VT	0.06 ± 0.03	0.05 ± 0.03	0.08 ± 0.03	0.07 ± 0.07	0.07 ± 0.03
R5%pred	114.89 ± 41.83	110.95 ± 43.14	123.25 ± 37.96	107.61 ± 50.64	117.18 ± 38.60
R20% pred	110.55 ± 28.80	107.10 ± 30.7	117.87 ± 22.90	108,67 ± 31,19	111.14 ± 28, 12
R5–R20 [kPa/(l/s)]	0.12 ± 0.11	0.12 ± 0.12	0.11 ± 0.07	0.12 ± 0.12	0.12 ± 0.10
X5 %pred	123.88 ± 85.02	114.65 ± 72.54	143.48 ± 105.03	107.11 ± 72.92	129.17 ± 88.13
X20%pred	27.51 ± 125.40	34.26 ± 133.41	13.17 ± 106.28	36.42 ± 133.54	24.70 ± 123.20
F_{res}%pred	122.34 ± 42.60	117.89 ± 40.13	131.79 ± 46.46	113.56 ± 40.89	125.11 ± 42.93
AX	1.18 ± 1.05	1.18 ± 1.10	1.18 ± 0.95	1.18 ± 1.11	1.18 ± 1.03
Hight [m]	1.49 ± 0.19	1.48 ± 0.20	1.52 ± 0.18	1.45 ± 0.21	1.51 ± 0.18
HIGHT z–score	0.23 ± 0.99	0.31 ± 1.02	0.05 ± 0.93	0.39 ± 1.28	0.17 ± 0.88
WEIGHT [kg]	42.30 ± 15.49	42.05 ± 16.35	42.82 ± 13.64	36.12 ± 15.38	43.30 ± 15.46
WEIGHT z–score	0.00 ± 1.03	0.12 ± 1.03	−0.28 ± 0.99	0.005 ± 1.13	−0.006 ± 1.00
BMI	18.29 ± 3.16	18.42 ± 3.37	18.02 ± 2.65	17.82 ± 2.91	18.44 ± 3.23
BMI z–score	−0.14 ± 1.04	−0.02 ± 1.03	−0.4 ± 1.03	−0.26 ± 1.16	−0.10 ± 1.00

main spirometric, MBNW, and IOS parameters. There is also a description of the subgroups.

Pulmonary Function Measurements

Correlation Between Lung Function Parameters of Subgroups: With and Without Spirometric Features of Obstruction

In our study, we compared subgroups of patients with and without spirometric features of obstruction. We found a statistically significant difference between the values of LCI ($p < 0.001$), FEV1z-score ($p < 0.001$), FEV1% pred ($p < 0.001$), MEF25z-score ($p < 0.001$), MEF50 z-score ($p < 0.001$), MEF75 z-score ($p < 0.001$), R5% pred ($p < 0.05$), and R20% pred ($p < 0.01$). There was no statistically significant difference in AX, Fres, Fres% pred, R5, and X5 and X20 in both subgroups.

What is more, a correlation between LCI and FEV1/FVC %pred and FEV1/FVC z-score was found only in the group without obturation (rSpearman = −0.34 and rSpearman = −0.34). In the case of the study of the correlation of parameters from IOS measurements and spirometry, correlations were observed in both groups. We compared parameters such a R5 z-score, R5 % pred, R20 %pred, R20 z-score, X5%pred, X5 z-score, R5-R20, Ax and Fres, Fres %pred with FEV1 %pred, FEV1 z-score, FEV1/FVC %pred, FEV1/FVC z-score, MEF25 %pred, MEF25 z-score, MEF50 %pred, MEF50 z-score, MEF75%pred, and MEF75 z-score. We found some correlations in both groups but not as much strength as in the case of LCI and IOS. We presented the results in **Table 2**. In addition, we observed a significant negative correlation between LCI and MEF25 %pred, MEF25 z-score, MEF50 %pred, MEF50 z-score, R20Hz, and R5 %pred. Studies on the correlation between LCI and reactance at 5 and 20 Hz showed mostly no relationship between these parameters in patients with CF. Only the X5% pred parameter, in this case, shows a weak correlation with LCI (rSpearman = 0.32). There were no significant correlations between LCI, X20Hz, and R5-R20 Hz parameters in both groups. All significant correlations between lung function test parameters are shown in **Table 3**.

Correlation Between LCI, Spirometry, and Impulse Oscillometry Parameters in Subgroups: With LCI ≤ 7.91 and LCI > 7.91

Based on normal values for healthy children (21), we distinguished that 75.33% of all enrolled patients had LCI

TABLE 2 | Correlations between IOS and spirometry parameters in subgroups: subgroup IA without spirometric features of obstruction and IB with obstruction features.

Parameter	Group I A r Spearman*	Group I B r Spearman*	Parameter	Group I A r Spearman*	Group I B r Spearman*
R20 %pred vs. FEV1 %pred	−0.26	−0.35	R20 %pred vs. FEV1 z–score	−0.26	−0.34
R5 %pred vs. FEV1 %pred	−0.33	−0.36	R5 %pred vs. FEV1 z–score	−0.34	−0.33
X5 %pred vs. FEV1 %pred	−0.36	−0.29	X5 %pred vs. FEV1 z–score	−0.37	–
AX vs. FEV1 %pred	–	−0.33	AX vs. FEV1 z–score	–	−0.33
Fres vs. FEV1 %pred	–	−0.33	Fres vs. FEV1 z–score	–	−0.33
Fres %pred vs. FEV1 %pred	–	−0.40	Fres %pred vs. FEV1 z–score	–	−0.38
R5%pred vs. FVC% pred	−0.25	–	R5%pred vs. FVC% z–score	−0.25	–
X5 % pred vs. FVC %pred	−0.35	–	X5 % pred vs. FVC z-score	−0.35	–
R5 %pred vs. FEV1/FVC %pred	−0.22	−0.33	R5 %pred vs. FEV1/FVC z–score	−0.22	–
R5–R20 vs. FEV1/FVC %pred	–	−0.39	R5–R20 vs. FEV1/FVC z-score	–	−0.34
X20 %pred vs. FEV1/FVC %pred	–	0.31	X20 %pred vs. FEV1/FVC z-score	–	–
AX vs. FEV1/FVC %pred	–	−0.39	AX vs. FEV1/FVC z–score	–	−0.37
Fres vs. FEV1/FVC %pred	–	−0.41	Fres vs. FEV1 /FVC z–score	–	−0.39
Fres %pred vs. FEV1/FVC %pred	–	−0.37	Fres %pred vs. FEV1 /FVC z–score	–	−0.29
R5-R20 vs. MEF25 %pred	–	−0.36	R5–R20 vs. MEF25 z–score	–	–
AX vs. MEF25 %pred	–	−0.35	AX vs. MEF25 z–score	–	−0.29
R5%pred vsMEF25 % pred	−0.30	–	R5%pred vs. MEF25 z–score	−0.32	–
R20% pred vs. MEF25 %pred	−0.23	–	R20% pred vs. MEF25 z-score	−0.24	–
X5 %pred vs. MEF25 %pred	−0.38	–	X5 %pred vs. MEF25 %pred	−0.38	–
Fres vs. MEF25 %pred	–	−0.34	Fres vs. MEF25 z–score	–	–
Fres % pred vs. MEF25 %pred	−0.23	−0.36	Fres % pred vs. MEF25 z-score	−0.24	–
R20 %pred vs. MEF50 % pred	−0.21	−0.36	R20 %pred vs. MEF50 z-score	–	−0.35
R5 %pred vs. MEF50 % pred	−0.25	−0.31	R5 %pred vs. MEF50 z-score	−0.23	−0.29
R5-R20 vs. MEF50 % pred	–	−0.34	R5-R20 vs. MEF50 z–score	–	−0.29
X5%pred vs. MEF50 %pred	−0.33	–	X5%pred vs. MEF50 z-score	−0.33	–
X20 vs. MEF50 % pred	–	0.30	X20 vs. MEF50 % z-score	–	–
AX vs. MEF50 % pred	–	−0.40	AX vs. MEF50 z–score	–	−0.36
Fres vs. MEF50 % pred	–	−0.40	Fres vs. MEF50 z–score	–	−0.36
Fres %pred vs. MEF50 % pred	–	−0.34	Fres %pred vs. MEF50 z-score	–	−0.30
R5-R20 vs. MEF 75 %pred	−0.34	−0.44	R5-R20 vs. MEF75 z-score	−0.33	−0.45
X20 % pred vs. MEF 75 %pred	0.34	0.39	X20 % pred vs. MEF z-score	0.34	0.39
AX vs. MEF 75 %pred	−0.41	−0.52	AX vs. MEF 75 z–score	−0.41	−0.53
Fres vs. MEF 75 %pred	−0.42	−0.55	Fres vs. MEF z–score	−0.42	−0.55
Fres % pred vs. MEF 75 %pred	−0.29	−0.34	Fres % pred vs. MEF 75 z-score	−0.29	−0.34

*$p < 0.05$.
"–", no statistically important correlation was found.

above the upper reference limit for healthy children of 7.91 accordingly, 37 patients with LCI ≤ 7.91 (group IC), and 113 patients with LCI > 7.91 (group ID). Moreover, in the group with confirmed obstruction (subgroup IB), LCI values were above 7.91 in a greater percentage of children (91.67%) than in the group without obturation (subgroup IA; 67.6%). The analysis of LCI values showed that in the group of children with normal LCI (≤7.91), there was no significant correlation with the main spirometry and oscillometry parameters. When LCI is above 7.91, this correlation is statistically significant.

We observed statistically significant differences in parameters such as FEV1%pred ($p < 0.001$), FEV1 z-score ($p < 0.001$), FVC%pred ($p = 0.020$), FVC z-score ($p = 0.030$), FEV1/FVC%pred ($p < 0.001$), FEV1/FVC z-score ($p < 0.001$), MEF25%pred ($p < 0.001$), MEF25z-score ($p < 0.001$), MEF50%pred ($p < 0.001$), MEF50z-score ($p < 0.001$), MEF75%pred ($p = 0.041$), MEF75z-score ($p = 0.042$), and R5%pred ($p = 0.030$) in groups IC (LCI ≤ 7.91) and ID (LCI > 7.91), which we presented in **Table 4**. Correlation between LCI, spirometry, and IOS parameters are presented in **Table 5**. These correlations were observed only in group ID (LCI > 7.91).

Correlation Between Sacin, Scond, and IOS and Spirometry Parameters

Based on the analysis performed on 20 persons, no significant correlation was found between the Sacin and IOS parameters. We found correlation between Scond and R5Hz, R20Hz (rSpearman = 0.56 and rSpearman = 0.55), and spirometric parameters such

TABLE 3 | Correlation between lung function parameters of CF patient subgroups: subgroup IA without spirometric features of obstruction and IB with obstruction features.

	r Spearman	p Spearman
Subgroup IA		
LCI vs. FEV1/FVC %pred	−0.34	<0.05
LCI vs. FEV1/FVC z-score	−0.34	<0.05
LCI vs. MEF_{25} z-score	−0.46	<0.05
LCI vs. MEF_{25} %pred	−0.45	<0.05
LCI vs. MEF_{50}%pred	−0.32	<0.05
LCI vs. MEF_{50} z-score	−0.32	<0.05
LCI vs. R5 %pred	0.21	<0.05
LCI vs. R20	−0.24	<0.05
LCI vs. X5%pred	0.32	<0.05
Subgroup IB		
LCI vs. MEF_{25} z-score	−0.37	<0.05
LCI vs. MEF_{50}%	−0.35	<0.05
LCI vs. MEF_{50} z-score	−0.37	<0.05
LCI vs. R5	−0.29	<0.05
LCI vs. R5 %pred	0.34	<0.05
LCI vs. R20	−0.35	<0.05
LCI vs. X5%pred	0.44	<0.05

TABLE 4 | Statistically significant diferences in spirometry and IOS parameters in subgroups: subgroup IC (LCI ≤ 7.91) and subgroup ID (>7.91).

	Subgroup IC (LCI ≤ 7.91)	Subgroup ID (>7.91)	*P*-value
FEV1%pred	99.89 ± 14.88	86.23 ± 16.75	<0.001
FEV1 z-score	0.01 ± 1.26	−1.18 ± 1.45	<0.001
FVC %pred	103.31 ± 15.18	96.95 ± 13.96	0.020
FVC z-score	0.25 ± 1.26	−0.29 ± 1.22	0.030
FEV1/FVC%pred	96.67 ± 9.42	88.40 ± 11.12	<0.001
FEV1/FVC z-score	−0.34 ± 1.15	−1.44 ± 1.47	<0.001
MEF25%pred	99.81 ± 46.12	61.58 ± 34.18	<0.001
MEF25 z-score	−0.32 ± 1.60	−1.73 ± 1.57	<0.001
MEF50%pred	0.28 ± 1.40	−1.10 ± 1.73	<0.001
MEF50 z-score	108.31 ± 30.30	80.85 ± 32.69	<0.001
MEF75%pred	92.65 ± 21.50	83.78 ± 21.65	0.041
MEF75 z-score	−0.64 ± 1.38	−1.22 ± 1.47	0.042
R5%pred	107.61 ± 50.64	117.18 ± 38.60	0.030

as FEV1/FVCz-score (rSpearman = −0.62), MEF25 (rSpearman MEF25%pred = −0.69; rSpearman MEF25z-score = −0.75), MEF50 (rSpearman MEF50%pred = −0.60, rSpearman MEF50z-score = −0.58), and MEF75 (rSpearman MEF75%pred = −0.46, rSpearman MEF75z-score = −0.45).

DISCUSSION

During the last few decades, conventional spirometry was the method of choice for assessing respiratory status in patients with CF. However, this test is not applicable in small children, as adequate coordination and cooperation are required. Additionally, spirometry is not sensitive enough for evaluating early CF lung disease (29, 30). For these reasons, scientific interest in other non-invasive methods such as IOS and MBNW has been reintroduced over the last 15 years (31). These techniques seem to be alternative diagnostic tools to monitor lung function.

TABLE 5 | Correlation between LCI, spirometry, and IOS parameters in subgroup ID (LCI > 7.91).

Parameter	*r* Spearman	*p* Spearman
LCI vs. FEV_1%pred	−0.62	<0.05
LCI vs. FEV_1z-score	−0.61	<0.05
LCI vs. FVC%pred	−0.41	<0.05
LCI vs. FEV_1/FVC%pred	−0.52	<0.05
LCI vs. FEV_1/FVC% z-score	−0.51	<0.05
LCI vs. MEF_{25}%pred	−0.62	<0.05
LCI vs. MEF_{25}%z-score	−0.64	<0.05
LCI vs. MEF_{50}%pred	−0.55	<0.05
LCI vs. MEF_{50}%z-score	−0.55	<0.05
LCI vs. MEF_{75}%pred	−0.33	<0.05
LCI vs. MEF_{75}%z-score	−0.33	<0.05
LCI vs. R20	−0.26	<0.05
LCI vs. R20%pred	−0.26	<0.05
LCI vs. R5	−0.25	<0.05
LCI vs. R5%pred	0.36	<0.05
LCI vs. X5%pred	0.36	<0.05

The main parameter of MBNW-LCI was presented to be more sensitive than spirometry or plethysmography parameters in monitoring early lung disease in children (31, 32). LCI values increase with lung disease severity (33). On the other hand, it is a quite difficult test for patients with exacerbations because dry air may increase coughing. For this reason, oscillometry seems to be the simplest to be performed by all patients, but it is worth considering whether its results are sufficiently related and correspond to other techniques. In this paper, we tried to investigate the relationship between oscillometry, spirometry, and MBNW results in 150 children with CF aged 6–18 years. Based on our analysis of the observed results, we showed that LCI tends to increase in patients with lower values of spirometry parameters such as FEV1 z-score and FVC z-score. We also received statistically significant moderate negative correlations between LCI and MEF25z-score, MEF50z-score. However, we did not document a significant correlation between LCI and reactance at 5 and 20 Hz or R5-R20. Presumably, the presence of homogenic central obturation does not influence reactance significantly.

There is a limited number of studies with patients with CF of which the aim was to evaluate and monitor pulmonary functions with IOS (4, 10–16). The relationship between spirometry and IOS results in children with CF is contradictory. Toprak et al. (10), in the study comparing clinical severity scores and classic spirometry with IOS results and thoracic high-resolution computed tomography scores in children with CF, observed that

patients with FEV1 below 80% exhibited significantly higher (resistance) R5 and R10 values and significantly lower (reactance) X5 values on IOS. Moreau et al. (11) evaluated the relationship between IOS parameters and spirometry results in 30 children with CF. They found a significant negative correlation between the raw IOS values (R5, Z, and Fres) and spirometry parameters. In their research, correlations were insignificant when percent predicted values were analyzed. Raj et al. (4) found a significant moderate negative correlation between IOS parameters (R and percent predicted) and spirometric parameters. X5 and R20 were weakly correlated with spirometric parameters. The authors attempted to identify IOS breakpoints to identify patients with lung dysfunction at FEV1 $<$ 40, 60, and 80%. Discriminatory power for Z5% and R5% was high at all FEV1 cutoffs (4).

In the already cited study of Moreau et al. (11) determining the relationship between IOS data and spirometry results in children with CF aged 4–19 years, it was observed that IOS measurements presented an insufficient sensitivity to detect and follow bronchial obstruction in patients with CF, which we also observed. What is more, in the case of both IA and IB subgroups, spirometric parameters correlated with LCI to a greater extent than IOS parameters. Our research also showed that diagnosis of obstruction FEV1/FVC z-score ≤ -1.64 suggests that LCI no longer correlates as strongly with changes in the FEV1/FVC z-score parameter. However, in children without diagnosed obstruction (FEV1/FVC > -1.64), the mean LCI was lower than in children with obturation. What is more, in this case, LCI coefficient correlated with FEV1/FVC z-score parameter. Our research carried out in a pediatric group of patients with CF suggests that there is a moderate significant negative correlation between LCI and selected IOS parameters (R5%pred, X5%pred, R5, R5%pred, R20, and R20%pred).

It is known that patients with CF have reduced lung compliance due to parenchymal lung disease in addition to airflow limitation (34). However, large amounts of sputum accumulated in the airways of patients with CF may hinder measurements, especially IOS. For this reason, the IOS technique may be more useful in conditions such as asthma and chronic obstructive pulmonary disease (8, 35). The findings of a review prepared by Galant et al. (36) suggest that IOS can add value to traditional clinical and spirometric assessment and thus improve management of asthma in children and adults, as well as have the potential to detect early dysfunction of the peripheral airways, which may result in better outcomes. In another trial, it was suggested that IOS and tomography could be used safely in early detection of impairment of lung function, but authors concluded that further studies are needed to evaluate the utility of IOS in clinical monitoring of children with CF who are not compliant with spirometry maneuvers (10).

CONCLUSIONS

The described pulmonary function tests correlate similarly with spirometry, which is considered the gold standard. The presented research results suggest that the MBNW test detects disturbances in gas flow and distribution more than IOS. In the case of obstructive diseases such as asthma, the IOS technique seems to be a good diagnostic tool; nevertheless, in the case of CF, it seems justified to use it as a supplementary test. What is more, based on current evidence, we should remember that spirometry cannot be substituted by oscillometry and also by MBNW in monitoring the respiratory status of children and adolescents with CF. However, those techniques could be complementary to each other.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Persistent Pulmonary Interstitial Emphysema With Respiratory Infection: A Clinicopathological Analysis of Six Cases and Detection of Infectious Pathogens by Metagenomic Next-Generation Sequencing (mNGS)

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Background: Persistent pulmonary interstitial emphysema (PPIE) is known to be related to mechanical ventilation and preterm. However, PPIE is also reported rarely in non-ventilated and full-term infants. Its relationship with respiratory infection is rarely reported in the literature. PPIE is difficult to diagnose and always mimics other congenital thoracic malformations (CTMs), such as congenital cystic adenomatoid malformation (CCAM).

Objective: The objective of this study was to evaluate clinicopathological and radiographic features of PPIE with respiratory infection and to detect the possible infectious pathogens.

Methods: From January 2011 to December 2019, six cases were confirmed pathologically with PPIE from a large cohort of 477 resected CTMs in West China Hospital of Sichuan University. Clinical and radiographic features were obtained from patients' medical records and follow-up. The present study aimed to analyze clinicopathological and radiographic features and to detect the infectious pathogens by metagenomic next-generation sequencing (mNGS).

Results: The six PPIE cases included four girls and two boys, ranging from 2 months to 5 years; 100% (5/5) of the available cases were full-term and without mechanical ventilation. CCAM were suspected in 66.7% (4/6) patients; 66.7% (4/6) cases affected a single lobe, and 33.3% (2/6) cases affected both lung lobes. Clinically, all six PPIEs were presented with symptoms of respiratory infection and diagnosed with pneumonia. All six patients were treated by surgery after anti-infective treatment. The pathologic characteristics showed lung cysts with variable size along the bronchovascular bundles, the cysts had a discontinuous fibrotic wall with a smooth inner surface lined with uninucleated and/or multinucleated macrophages. *Streptococcus pneumoniae* was detected in patient No. 1. Human beta-herpesvirus 5 was detected in patient No. 2. *Neisseria mucosa*, *Neisseria sicca*, *Prevotella melaninogenica*, *Prevotella histicola*, and *Fusobacterium nucleatum* were detected in patient No. 5, and no infectious pathogen was detected in 50% (3/6, No. 3, No. 4, and No. 6) of cases.

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Conclusion: Six rare cases of PPIE with respiratory infection were treated by surgery after anti-infective treatment. All five available cases were full-term infants without mechanical ventilation. The histological characteristics of PPIE were the wall of cysts composed of a thin layer of discontinuous fibrous tissue and lined with uninucleated or/and multinucleated macrophages.

Keywords: congenital thoracic malformations, pulmonary interstitial emphysema, respiratory infection, infants, metagenomic next-generation sequencing

INTRODUCTION

With routine prenatal ultrasound scans performed, more and more congenital thoracic malformations (CTMs) are diagnosed in infants (1, 2), but the incidence is rare, which is present in 1 per 10,000–35,000 births (3). The differential diagnosis of CTMs contains congenital cystic adenomatoid malformation (CCAM), pulmonary sequestration (PS), bronchogenic cyst, congenital lobar emphysema (CLE), persistent pulmonary interstitial emphysema (PPIE), and so on.

Pulmonary interstitial emphysema (PIE) is a rare cystic disease of infants (4). PIE is an air leak syndrome, characterized by gas dissecting pulmonary interstitium along the bronchovascular bundles. There are three clinical types of PIE, including acute IPE, local persistent PIE (LPPIE), and diffuse persistent PIE (DPPIE) (5, 6). Acute IPE is <7 days in duration, diffuse persistent PIE is observed when small cysts are noted in all lobes of the lung, and local persistent PIE affects only one lobe (7). Chest computed tomography (CT) scan sometimes was limited to diagnose PIE. CT showed cystic lung lesions mimicking CCAM in the postnatal period (8). The definitive diagnosis is histological. A histological diagnosis of PIE was established through the wall of cysts composed of a thin layer of discontinuous fibrous tissue and lined with uninucleated or/and multinucleated macrophages (9, 10).

PIE is known to be related to mechanical ventilation and preterm (11, 12). However, it is also reported rarely in both non-ventilated and full-term infants (13, 14). Pursnani et al. (14) showed a 3-month-old infant with LPPIE who had no history of respiratory distress syndrome (RDS) and mechanical ventilation; the patient had a medical history of viral pneumonia 1 month prior to surgery, indicating respiratory infection may be related to PPIE.

However, there were just a few reports of PPIE with respiratory infection (13–22), and possible infectious pathogens were still unclear. With the development of molecular methods of identification, the metagenomic next-generation sequencing (mNGS) is a novel, rapid, simple, and convenient approach to the clinical identification of infectious diseases.

In the present study, we report six rare cases of PPIE with respiratory infection, followed by successfully surgical treatment. To our best knowledge, it is the first time to detect the possible infectious pathogens in PPIE by using mNGS.

Abbreviations: CTM, congenital thoracic malformations; PIE, Pulmonary interstitial emphysema; PPIE, Persistent pulmonary interstitial emphysema; CT, Computed tomography; mNGS, metagenomic next-generation sequencing; CCAM, congenital cystic adenomatoid malformation; PS, pulmonary sequestration; CLE, congenital lobar emphysema.

MATERIALS AND METHODS

Case Series and Clinicopathological Features

From January 2011 to December 2019, 477 resected CTMs in West China Hospital of Sichuan University were retrospectively rescreened independently by two pathologists (P Zhou and LL Jiang). According to the histological criteria of PIE, six PPIEs were enrolled in the present study.

Clinical and radiographic features were obtained from patients' medical records and follow-up. We retrospectively collected age, sex, term, mechanical ventilation, prenatal ultrasound, clinical features, radiographic features, affected sites, and the diameter of the cystic lesions.

Special Stain

Special stains (acid fast stain, Gomori's methenamine silver staining, and Giemsa) and TB-PCR (Qiagen) were carried out for all cases according to the manufacturer's protocol.

DNA Extraction, Library Construction, and Sequencing

DNA was extracted from available blocks with the TIANamp Micro DNA Kit (DP316, Tiangen Biotech) following the manufacturer's protocol. We constructed DNA libraries according to the standard protocol through end-repaired adapter added overnight and by applying polymerase chain reaction amplification to the extracted DNA. To measure the adapters before sequencing, quality control was carried out using a bioanalyzer (Agilent 2100, Agilent Technologies, Santa Clara, CA, USA) combined with quantitative PCR. DNA sequencing was then performed with the BGISEQ-100 platform.

Data Processing and Analysis

High-quality sequencing data were generated after removing low-quality, low-complexity, and shorter reads. The data mapped to the human reference genome (hg19) were excluded using a powerful alignment tool called Burrows–Wheeler Alignment to eliminate the effect of the human sequences. The database used for the present study includes 6,350 bacteria, 1,798 viruses, 1,064 fungi, and 234 parasites, which all relate to human disease. Finally, the mapped data were processed after filtering out duplicate reads for advanced analysis. The SoapCoverage from

TABLE 1 | Clinical features of the six cases with PPIE.

No.	Sex	Age	Other defects	Ultrasound finding	Term	Mechanical ventilation	CT diagnosis	Clinical magnification	Affected site	Diameter (cm)
1	M	3 y	None	None	Full term	None	CCAM	Pneumonia for 10 days	Right lower lobe	2.5
2	F	3 y	None	None	Full term	None	CCAM	Pneumonia for 1 month	Right upper lobe	1.8
3	F	1 y	None	None	Full term	None	CCAM	Pneumonia for 1 month	Both lower lobes	5.6
4	M	11 m	None	None	NA	NA	Cystic lesion	Recurrent pneumonia for 7 months	Right lower lobe	3.8
5	F	5 y	Ventricular septal defect	None	Full term	None	Cystic lesion	Recurrent pneumonia for 3 months	Both lung lobes	-
6	F	2 m	None	None	Full term	None	CCAM	Pneumonia for about 20 days	Right lower lobe	2.6

F, female; M, male; y, years; m, months; CCAM, congenital cystic adenomatoid malformation; NA, not available.

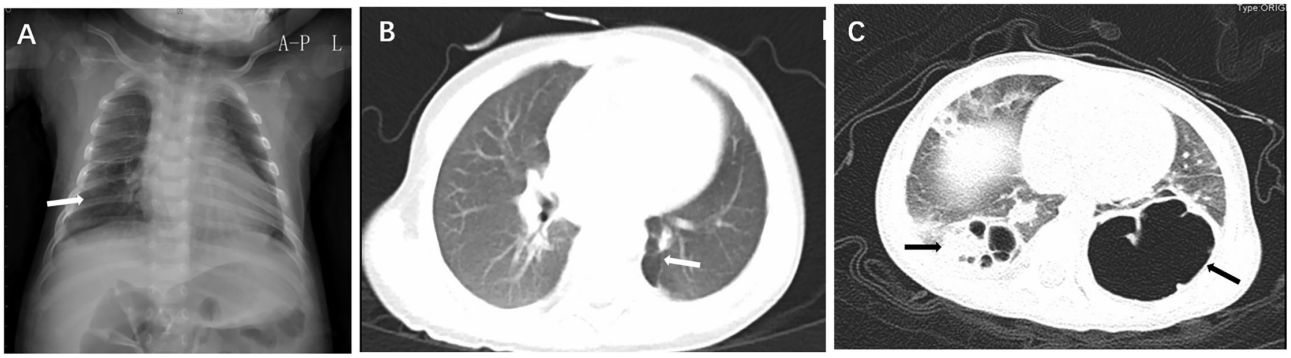

FIGURE 1 | Chest X-ray showed translucency of the right lower lobe (**A**, arrow); CT scan of the chest showed a single cyst at the left lower lobe (**B**, arrow) and bilateral cysts in left and right lower lobes (**C**, arrows).

the SOAP website was used to calculate the sequence depth and genomic coverage for each species.

RESULTS

Clinical Characteristics

From January 2011 to December 2019, 477 CTMs were retrospectively rescreened in West China Hospital of Sichuan University. CTMs were consisted of congenital cystic adenomatoid malformation (CCAM) (286, 60%), pulmonary sequestration (PS) (143, 30%), bronchogenic cyst (29, 6.1%), congenital lobar emphysema (CLE) (13, 2.7%), and persistent pulmonary interstitial emphysema (PPIE) (6, 1.3%).

All six PPIEs were treated by surgery after suggested anti-infective treatment therapy. The clinical characteristics of the six patients are shown in **Table 1**. There were four girls and two boys, ranging from 2 months to 5 years old. We collected the follow-up data of all patients except for No. 4. Patient No. 4 had the wrong phone number. The other available patients (5/5, 100%) were all full-term without mechanical ventilation. Clinically, all six cases of PPIE were presented with symptoms of respiratory infection and diagnosed with pneumonia. The common symptoms of the patients were cough, fever, and expectoration. Before surgical treatment, all six patients received suggested anti-infective treatment therapy. The cystic lesions were located at a single lobe among 66.7% (4/6) patients, who were identified with local PPIE, and both lung lobes among 33.3% (2/6) patients, who were identified with diffuse PPIE.

Besides this, patient No. 5 suffered the disease Langerhans cell histiocytosis (LCH) in the left submandibular lymph node for 1 year. She was asymptomatic when she received the suggested chemotherapy treatment of four courses; she suffered recurrent pneumonia for 3 months, and the routine examination of the chest CT found the diffuse cystic lesions in both lung lobes. In addition, she had the defect of ventricular septal defect.

Imaging Findings

Prenatal ultrasound did not find any lesion in all six patients. Chest X-ray showed translucency of the affected site (**Figure 1A**).

Chest CT scan of all the cases showed cystic lesions. According to the CT findings, CCAM was suspected in 66.7% (4/6 cases). The radiographic patterns of PIPE ranged from a cystic lesion or multicystic lesion of one or more lobes to a diffuse multicystic involvement of all lobes. CT scan of the chest showed a single cyst (**Figure 1B**) and bilateral cysts in both lungs (**Figure 1C**). The affected site was the right lower lobe in 50% (3/6 patients), the right upper lobe in 16.7% (1/6 patient). The local multicystic pattern in 66.7% (4/6) PPIE cases included a wide range of sizes for the cystic lesions (1.8–3.8 cm). There are 33.3% (2/6 patients) affected in both lung lobes.

Pathological Examination

All the patients were treated by surgery after suggested conventional anti-infective treatment. The resected affected site was the right lower lobe in 50% (3/6 patients), the right upper lobe in 16.7% (1/6 patient). There are 33.3% (2/6 patients) (No. 3 and No. 5) affected in both lung lobes; the resection of the right lower lobe was made in patient No. 3, and the resection of the left lobe was made in patient No. 5.

The gross specimens showed multiloculated cysts with variable size within the pulmonary parenchymal, and the cysts

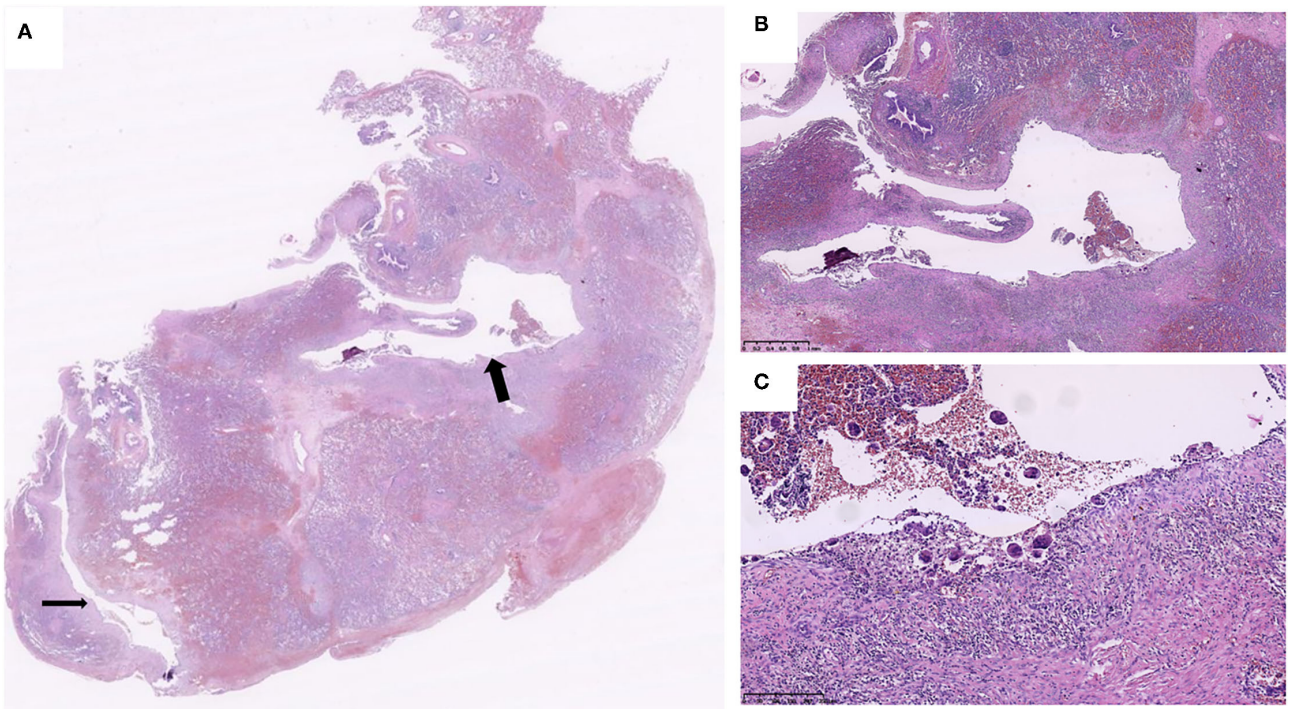

FIGURE 2 | Two air cysts (arrows) were around the bronchovascular bundles **(A)**, and the cystic walls primarily consisted of connective tissue. The cystic walls are consisted of discontinuous connective tissue and lined with uninucleated and multinucleated macrophages **(B,C)** (Case 2).

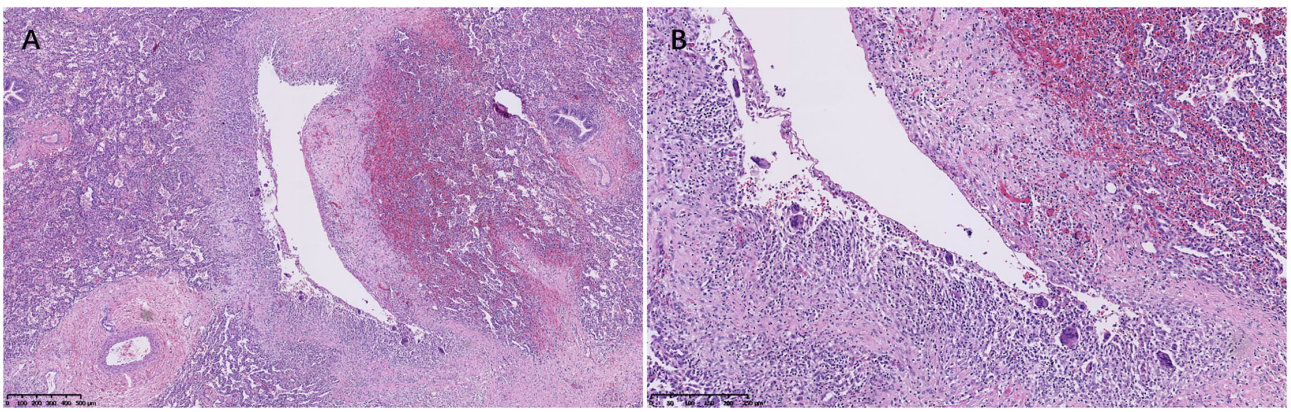

FIGURE 3 | An air cyst was around the bronchovascular bundles **(A)**, and the cystic walls consisted of connective tissue lined with uninucleated and multinucleated macrophages **(B)** (Case 3).

TABLE 2 | Infectious pathogens and metagenomic next-generation sequencing (mNGS) results.

Case.	Sex	Age	mNGS results	Specific reads (*n*)
No.1	M	3 y	*Streptococcus pneumoniae*	84
No.2	F	3 y	Human beta-herpesvirus 5	20
No.3	F	1 y	Not found	
No.4	M	11 m	Not found	
No.5	F	5 y	*Neisseria mucosa*	587
			Neisseria sicca	247
			Prevotella melaninogenica	258
			Prevotella histicola	174
			Fusobacterium nucleatum	239
No.6	F	2 m	Not found	

F, female; M, male; y, years; m, months.

had a smooth inner surface. Some cysts contained a small amount of clear fluid.

Microscopically, histological observation found that the walls of the cysts were adjacent to interlobular septa or bronchovascular bundles, the wall of cysts was composed of a thin layer of fibrous tissue, and the thin band of fibrous tissue was discontinuous. Small collections of uninucleated and multinucleated macrophages lined the surface of the main cysts (**Figures 2**, **3**). The giant cells contained from 2 to 40 centrally placed nuclears.

The adjacent parenchymal surrounding the cysts showed mild to marked atelectasis and inflammatory cells infiltrates in all cases (**Figures 2**, **3**), containing histocytes, lymphocytes, plasma cells, and neutrophils, indicating there was inflammation along the cysts. Mucosal edema, cellular debris of the bronchi**,** mucus, and inflammatory exudates of the bronchi can be seen.

Special Stain and TB-PCR

Special stains (acid fast stain, Gomori's methenamine silver staining, and Giemsa) and TB-PCR (Qiagen) were carried out for all six cases according to the manufacturer's protocol. None was seen in the case series, indicating it did not contain any identifiable organism or foreign material in all six cases.

Infectious Pathogens Detected by mNGS

The next-generation sequencing was performed from blocks of resected lung samples for each patient. In the current study, mNGS successfully identified the infectious pathogens in all patients, and the pathogens detected are shown in **Table 2**. Before surgical treatment, all the six patients received suggested anti-infective treatment therapy. Therefore, infectious pathogens were detected in three cases. *Streptococcus pneumonia* (specific reads: 84) was detected in patient No. 1. Human beta-herpesvirus 5 (specific reads: 20) was detected in patient No. 2. *Neisseria mucosa* (specific reads: 587), *Neisseria sicca* (specific reads: 247), *Prevotel lamelaninogenica* (specific reads: 258), and *Prevotella histicola* (specific reads: 174) and *Fusobacterium nucleatum* (specific reads: 239) were detected in patient No. 5. No infectious pathogen was detected in 50% (3/6) cases (patients No. 3, No. 4, and No. 6).

DISCUSSION

The pathophysiology of PIE is a result of air leakage into the interstitium from alveolus due to disruption of the alveolar wall basement membrane, which may dissect along the bronchovascular bundles and radiate outward to the periphery of the lung, mediastinum, and pericardium. PIE included local persistent PIE, acute PIE, and diffuse persistent PIE (5, 6). Diffuse persistent PIE is observed when small cysts are noted in all lobes of the lung (7), and acute IPE is <7 days in duration.

PIE is a rare condition that commonly affects newborn infants with a history of prematurity with positive pressure mechanical ventilation (11, 12). However, it is also reported rarely in both unventilated and full-term infants (13, 14). There are only a few cases reported for PIE developing in unventilated neonates (5, 13, 14, 21, 23–25). In our study, six rare PPIEs from a large cohort of 477 resected CTMs and five available cases were full-term infants without mechanical ventilation.

Chest CT sometimes may show air surrounding the bronchovascular bundles in patients with PIE (5). A multi-institutional study found that about 82% patients with PPIE had the characteristic CT findings with central lines and dots surrounded by radiolucency (26). Chest CT is not an effective diagnostic tool for PIE presenting as multiple cysts with various sizes in one or more lobes of the lung (15). In particular, CT showed cystic lung lesions mimicking CCAM (8). When a patient does not have classical CT features, PIE should be differentiated from other cystic lung lesions, including CCAM, CLE, bronchogenic cyst, cystic lymphangioma, and so on. According to the CT findings and clinical features, CCAM was suspected in 66.7% (4/6 cases) in the present study. Besides this, there was a rare case in which prenatal ultrasound found cystic lesions in the previous literature (27). Messineo et al. reports a male infant suffering from type I CCAM at 20 weeks of gestation with ultrasound scanning, which was diagnosed with PIE after surgery (27). Prenatal ultrasound did not find any lesions in the lungs of six patients with prenatal ultrasound examination in the present study, which indicates that the PPIE may be formed after birth.

Persistent PIE is pathologically characterized by irregularly shaped and multiloculated cysts of various sizes along the bronchovascular bundle. The cysts are air-filled spaces in the

TABLE 3 | Review of published literatures of PPIE with respiratory infection.

References	Cases	Sex	Age	Term	Mechanical ventilation	Pneumothorax	Mediastinal shift	Pneumo mediastinum	Infectious pathogen	Affected site	Surgical resection
Toledo Del Castillo et al. (20)	1	M	18 d	Full-term	Yes	No	Yes	No	RSV	Left lung	No
Aiyoshi et al. (19)	1	F	22 m	Full-term	Yes	Yes	No	Yes	RSV	Right upper lobe	No
Gala (22)	1	NA	At birth	Preterm	Yes	No	No	No	Not reported	Left lung	No
Sherren and Jovaisa (21)	1	F	87 y	NA	Yes	Yes	No	No	Not reported	Both lung lobes	No
Lee and Im (13)	1	F	6 w	Full-term	No	Yes	NA	Yes	Not reported	Right upper and middle lobe	No
Pursnani et al. (14)	1	M	3 m	Full-term	No	Yes	No	No	Not reported	Right upper and middle boles	Yes
Crosswell and Stewart (17)	1	M	At birth	Preterm	No	No	No	No	Not reported	Left lower lobe	No
Yao et al. (18)	1	F	9 w	Preterm	Yes	Yes	No	No	*Candida albicans*	Left upper lobe	Yes
O'Donovan et al. (16)	1	F	At birth	Preterm	Yes	No	Yes	No	*Staphylococcus aureus*	Right lung	No
Cohen et al. (15)	4	2F and 2M	45 d to 2 y	Full-term	No	No	1 with mild deviation	No	Not reported	2 right upper lobe, 1 right lower lobe and 1 left upper lobe	Yes

F, female; M, male; y, years; m, months; w, weeks; NA, not available; RSV, respiratory syncytial virus.

parenchymal and composed of a thin band of fibrous connective tissue. The degree of the fibrosis may vary the duration of PIE. The uninucleated and/or multinucleated macrophages lining the cyst walls are the typical pathological features (9, 10). The typical features of air cysts surrounding the bronchovascular bundles with fibrous tissue lining the uninucleated and/or multinucleated macrophages were both demonstrated among all six cases in our present study.

The differential pathological diagnosis was made with other CTMs, such as CCAM, PS, bronchogenic cyst, CLE, and so on. CCAM is characterized by a lack of communication between the lesion and the tracheobronchial tree and a proliferation of irregularly dilated terminal bronchiole-like structures (28). PS is characterized with non-functioning lung tissue that receives systemic arterial blood supply and does not communicate with the adjacent tracheobronchial tree (29). Bronchogenic cyst is lined with pseudostratified columnar respiratory epithelium, cartilage plate, smooth muscle, and bronchial glands (30). CLE showed lobar hyperinflation with overdistention of normally formed alveoli and without destruction of alveolar walls, aspiration pneumonia, infection, and proximal bronchial obstruction (31); 477 resected CTMs consisted of CCAM (60%), PS (30%), bronchogenic cyst (6.1%), CLE (2.7%), and PIE (1.3%) in the present study.

A standard treatment strategy for PIE has not yet been established. The critical treatment is to be able to maintain sufficient gas exchange (4). Surgical resection of PIE is controversial, and several studies advocate a conservative medical approach (26, 32). A conservative medical approach needs close clinical and radiological monitoring. When there is difficulty in making the diagnosis, the absence of classical CT features, and non-surgical options failed or progressive syndrome, or severe complications, surgery should be considered (33). Jassal et al. report a case that illustrates that extensive bilateral PPIE associated with a persistent pneumomediastinum can resolve spontaneously, thus demonstrating that conservative management without surgical intervention may be appropriate for some children (32). Infants with PPIE and weighing < 1,000 g are at significant risk of mortality and associated morbidity of PPIE (34).

The mechanism of production of PIE is the disruption of the alveolar wall basement membrane with subsequent dissection of air into the interstitial space. Given et al. (35) show that PIE in bronchiolitis is thought to occur secondary to inflammation, leading to mucosal edema, increased secretion, and cellular debris, resulting in expiratory obstruction of the small airways; the resulting check-valve effect leads to hyperinflation then alveolar rupture. Another study (13) demonstrates that pneumonia may contribute to the development of pulmonary air leaks by at least three mechanisms: first, air trapping from mechanical or check-valve obstruction within the bronchi by mucus and inflammatory exudates; second, reduced strength or direct disruption of the alveolar lining from parenchymal inflammation or necrosis as commonly seen in necrotizing pneumonia; and third, decreasing lung compliance.

There was obvious inflammatory reaction in the present six rare cases. There are few reports of pulmonary air leakage

with respiratory infection. There were just 10 previous articles including 13 patients with PPIE with respiratory infection from a PubMed search (13–22). Review of published literature of PIE with respiratory infection is shown in **Table 3**. Gala (22) did not mention the sex of the case, so there were seven females and five males, ranged from birth to 87 years. Only 46.2% (6/13) patients with PPIE were treated with surgery. 66.7% (8/12) were full-term, 33.3% (4/12) were preterm. 46.2% (6/13) patients had mechanical ventilation. There was pulmonary air leakage, pneumothorax (5/13 cases), pneumomediastinum (2/13 cases), and mediastinal shift (3/12 cases). According to the previously reported PIE patients with respiratory infection, there were nine patients without certain infectious pathogens reported (13–17, 21, 22). Two patients were infected by respiratory syncytial virus (RSV), and the two were infected by *Candida albicans* (18) and *Staphylococcus aureus* (16). In our study, the common symptoms of the patients were cough, fever, and expectoration. Special stains (acid fast stain, Gomori's methenamine silver staining, and Giemsa) and TB-PCR did not find any identifiable organism or foreign material in our study. No infectious pathogen was detected in 50% (3/6) cases with pneumonia prior to surgery, which may be associated with clinical infectious symptoms being controlled with conventional anti-infective treatment before surgery. *Streptococcus pneumonia* was detected in patient No. 1, whose infectious symptoms were present during surgery. *Streptococcus pneumonia* is a significant human pathogen and a leading cause of bacterial pneumonia in children (36), and *Streptococcus pneumoniae* is a frequent cause of severe community-acquired pneumonia among children in Beijing of China (37). Human beta-herpesvirus 5 (HHV-5) (specific reads $n = 20$) was detected in patient No. 2 without immunodeficiency. *Neisseria mucosa*, *Neisseria sicca*, *Prevotel lamelaninogenica*, *Prevotella histicola*, and *Fusobacterium nucleatum* were detected in patient No. 5. Patient No. 5 has suffered the disease Langerhans cell histiocytosis (LCH) in the left submandibular lymph node for 1 year and has received the suggested chemotherapy treatment of four courses, and then she suffered recurrent pneumonia for 3 months, the detected infectious pathogens may be associated with respiratory infection after chemotherapy treatment for LCH. *Neisseria mucosa* and *Neisseria sicca* are known as common commensals of the upper respiratory tract (38), however, which sometimes are associated with respiratory diseases. Previous studies show that *Neisseria mucosa* and *Neisseria sicca* are consistent with respiratory microbiome from pediatric tracheostomy tubes without granulomas (39), and *Neisseria mucosa* caused pulmonary coin lesion in a child with chronic granulomatous disease (40). A case of spontaneous pulmonary abscess with cavitation caused by *Neisseria mucosa* in a chronically neutropenic child is reported (41).

CONCLUSION

Six rare cases of PPIE with respiratory infection were treated by surgery after anti-infective treatment. All five available cases were full-term infants without mechanical ventilation. The diagnoses of PPIE are based on characteristic radiographic imaging and histopathology. The histological characteristics of PPIE were the wall of cysts composed of a thin layer of discontinuous fibrous tissue and lined with uninucleated or/and multinucleated macrophages.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: DNA Data Bank of Japan (DDBJ), and BioSample Submission ID: SSUB020649.

ETHICS STATEMENT

Ethics approval was obtained from the respective Ethics Committees of West China Hospital, Sichuan University, China (No. 2020892). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

PZ collected data, analysis and drafted the initial manuscript, and reviewed and revised the manuscript. WW and YF performed data analysis, drafted, and revised the manuscript. YT and LJ reviewed and revised the manuscript. YZ and ZL performed data analysis. All authors read and approved the final manuscript.

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Short-Term Effects of Elexacaftor/Tezacaftor/Ivacaftor Combination on Glucose Tolerance in Young People With Cystic Fibrosis—An Observational Pilot Study

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Background: The effect of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) on glucose tolerance and/or cystic-fibrosis-related diabetes (CFRD) is not well understood. We performed an observational study on the short-term effects of ELX/TEZ/IVA on glucose tolerance.

Methods: Sixteen adolescents with CF performed oral glucose tolerance tests (OGTT) before and 4–6 weeks after initiating ELX/TEZ/IVA therapy. A continuous glucose monitoring (CGM) system was used 3 days before until 7 days after starting ELX/TEZ/IVA treatment.

Results: OGTT categories improved after initiating ELX/TEZ/IVA therapy ($p = 0.02$). Glucose levels of OGTT improved at 60, 90, and 120 min ($p < 0.05$), whereas fasting glucose and CGM measures did not change.

Conclusion: Shortly after initiating ELX/TEZ/IVA therapy, glucose tolerance measured by OGTT improved in people with CF. This pilot study indicates that ELX/TEZ/IVA treatment has beneficial effects on the endocrine pancreatic function and might prevent or at least postpone future CFRD.

Keywords: cystic fibrosis, elexacaftor/tezacaftor/ivacaftor therapy, oral glucose tolerance test (OGTT), continuous glucose monitoring, glucose tolerance

INTRODUCTION

Within the last years, CFTR modulators that target the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel have become available to many people with cystic fibrosis (CF) (1). They may either potentiate CFTR channel activity at the epithelial cell surface or correct the defect by helping CFTR reach the cell surface (2–4). The latest and most efficient CFTR drug

combines elexacaftor, tezacaftor, and ivacaftor (ELX/TEZ/IVA, one potentiator and two correctors, respectively). Several randomized controlled trials showed excellent results concerning the safety and efficacy of ELX/TEZ/IVA (5–7) and improved respiratory scores such as lung function (1, 8).

However, little is known about the effects of ELX/TEZ/IVA on other organs affected by CFTR dysfunction. Cystic fibrosis-related diabetes (CFRD) represents one of CF's most frequent extrapulmonary complications and is associated with lung function decline, poor nutritional status, and increased mortality (9–11). Reductions in islet size and beta-cell area seem to lead to glucose responsiveness abnormalities and insulin secretory defects (12, 13). However, the exact mechanisms leading to islet dysfunction are still unclear. Whilst several studies have shown CFTR channel activity in rodent and human islet cells (13, 14), others reported minimal or absent CFTR expression in human pancreatic endocrine cells, suggesting that insulin secretory defects are due to islet mass reduction, intra-islet inflammation, or inflammatory mediators (12, 15, 16).

Favoring the hypothesis of CFTR expression in islet cells, it is speculated that CFTR modulators may positively affect glucose tolerance and insulin secretion. Studies investigating mono- or dual-CFTR modulators present contradictory results. While several authors report improved glucose tolerance after starting therapy with ivacaftor (17–22) or lumacaftor/ivacaftor (23), others did not find an impact on glucose or insulin levels regarding CFTR modulators (24–26). A study in adults with CF—with and without a diagnosis of CFRD—reported an improvement of hyperglycemia and glycemic variability after initiation of ELX/TEZ/IVA (27).

We hypothesize a beneficial effect of ELX/TEZ/IVA treatment on glucose tolerance and insulin secretion: the aim of our study was thus to investigate the short-term effects of ELX/TEZ/IVA on glucose tolerance in insulin-naïve adolescents with CF by assessing oral glucose tolerance test (OGTT) and continuous glucose monitoring (CGM) before and after starting ELX/TEZ/IVA therapy.

MATERIALS AND METHODS

Study Population

In Switzerland, ELX/TEZ/IVA was approved by the Swissmedic in December 2020 for all CF individuals 12 years or older with at least one F508del CFTR mutation. All patients in the CF outpatient clinic of the University Children's Hospital of Bern, Switzerland, qualifying for therapy with ELX/TEZ/IVA, were notified about approval of ELX/TEZ/IVA during routine outpatient visits in the first quarter of 2021. We recommended ELX/TEZ/IVA to all eligible patients and asked patients, parents, or caregivers to participate in our study. We excluded patients with known CFRD to include insulin naïve participants only.

The study was performed in the University Children's Hospital of Bern, University of Bern, Switzerland, and approved by the Ethics committee of Bern, Switzerland (ID 2021-00982). Informed written consent was obtained from all parents or caregivers and teenagers $\geq$ 14 years.

Study Design

An OGTT was performed in median (IQR) 3 days (3–42) before (first study visit) and 26 (24–40) days after (second visit) initiating ELX/TEZ/IVA treatment. CGM measures were recorded 3 days before until 7 days after the start with ELX/TEZ/IVA therapy. Height and weight were obtained on both study visits, lung function measurements, HbA1c, and a sweat chloride test was performed (**Figure 1**).

Oral Glucose Tolerance Tests Measures

A standardized 3-h OGTT was performed before and after initiating ELX/TEZ/IVA therapy. After an overnight fast, an intravenous line was placed for blood draws. Participants consumed 1.75 g/kg (maximum 75 g) dextrose. Blood sampling was performed at baseline and 60, 90, 120, and 180 min (min) post-dextrose ingestion. We determined plasma glucose concentration, serum insulin levels, and c-peptide levels (28), as well as HbA1c. OGTT results were categorized into normal glucose tolerance (NGT) [normal fasting (<5.6 mmol/L) and 2-h (<7.8 mmol/L) plasma glucose], indeterminate glucose tolerance (INDET) (normal fasting and 2-h plasma glucose, 1-h plasma glucose value of $\geq$ 11.1 mmol/L), impaired fasting glucose (IFG) (fasting glucose 5.6–6.9 mmol/L, normal 2-h plasma glucose), impaired glucose tolerance (IGT) (fasting glucose 5.6–6.9 mmol/L, 2-h plasma glucose 7.8–11.0 mmol/L), and CFRD (fasting glucose > 6.9 mmol/L, 2-h plasma glucose $\geq$ 11.1 mmol/L). The classification was based on the American Diabetes Association (ADA) criteria (29) and international guidelines for screening and treating CFRD (30). Due to low numbers of IFG, IGT and IFG were both categorized into the IGT group.

Continuous Glucose Monitoring Measures

CGM systems track glucose levels in interstitial fluid via a sensor inserted into the subcutaneous fat tissue. A transmitter connected to the sensor wire sends real-time data wirelessly to a receiver. The receiver displays real-time glucose levels and historical trends and provides acoustic alarms when certain glucose thresholds (e.g., hypo- and hyperglycemia) are reached.

Participants wore a Dexcom G6 CGM-System (Dexcom, Inc., San Diego, CA, United States) at the upper arm inserted by specialized diabetes nurses using an automatic applicator. Families were instructed on how to use the CGM device and react to alarms personally. Additional written information was provided. As the exact time point of medication intake on Day 1 was not assessed, we excluded CGM measures of this day to include only measurements prior to or after the start of therapy. Furthermore, we included only days of measurement where all individuals wore a sensor to have an equal number of days for evaluation. Thus, for the standardized evaluation of glucose data by CGM, we included the following measures: (a) glucose measurements of Day 1 (start 4 pm) to Day 3 (stop 12 pm) before initiating ELX/TEZ/IVA therapy, approximately 2.5 days of measuring time; (b) glucose measurements of Day 2 post-ELX/TEZ/IVA (start 12 pm) and Day 6 (stop 12

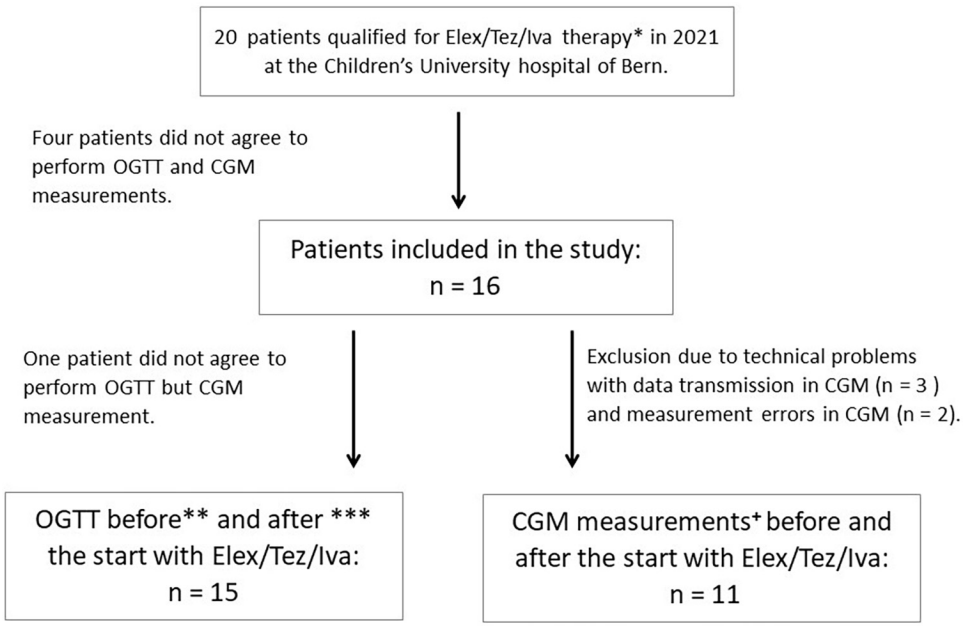

FIGURE 1 | Flowchart of study design. *Patients with known CFRD were excluded. **OGTT was performed 3 days to 6 weeks before the start with ELX/TEZ/IVA, except for three patients in whom OGTT was performed earlier (10 weeks, 4 months, and 6 months before the start) due to organizational reasons during routine clinical visits and disagreement of the patients to perform another OGTT. ***OGTT was performed 3–6 weeks after starting ELX/TEZ/IVA treatment, except for two patients in whom OGTT was performed after 8 and 10 weeks, respectively. All patients continued using ELX/TEZ/IVA until the second OGTT was performed. $^{+}$CGM measures were performed 3 days before and 7 days after starting with ELX/TEZ/IVA. In one patient, CGM was only installed 2 days before the initiation of ELX/TEZ/IVA therapy due to organizational reasons in routine clinical visits.

pm) for analysis after the initiation of ELX/TEZ/IVA therapy, resulting in a total of 5 days of measuring time. We used all available measuring points from CGM measurements generating mean, minimum, and maximum glucose values. Furthermore, we calculated the percentage of time of glucose levels within the following categories: very low ($\leq$2.7 mmol/L), low ($\geq$2.8 and $\leq$3.3 mmol/L), normal ($\geq$3.4 and $\leq$7.7 mmol/L), high ($\geq$7.8 and $\leq$11.1 mmol/L), and very high ($\geq$11.2 mmol/L), according to guidelines (31, 32) and as implemented in previous studies (24, 27).

Lung Function Testing

Ventilation inhomogeneity was derived from N2-multiple breath washout (MBW) measurements performed according to guidelines using the manufacturer's software (Spiroware V 3.2.1, Eco Medics AG, Duernten, Switzerland; reloaded with Spiroware V 3.3.1) (33, 34) with the primary outcome lung clearance index (LCI). N_2MBW trials were quality controlled, and mean values from tests with at least two trials of acceptable quality were included for analysis (35). Spirometry and body plethysmography (Jaeger MasterScreen Body plethysmography, CareFusion, Hochberg, Germany) was performed according to ERS/ATS guidelines (36). Outcomes were forced expiratory volume at 1 s (FEV_1), the ratio of FEV_1/FVC (forced vital capacity), and mid forced expiratory flow (MFEF). Z-scores for spirometry were derived from published reference equations; normal lung function's upper and lower limits were defined as $\pm$1.64 z-scores (37). Outcome parameters from body plethysmography were residual volume (RV)/total lung capacity (TLC) and specific resistance (sReff).

Statistical Analysis and Power Calculation

Descriptive statistics were expressed as median and interquartile ranges or numbers and percentages, as appropriate. We used the Wilcoxon's signed rank test to compare values before and after ELX/TEZ/IVA initiation. To investigate associations between the different parameters, we used Spearman rank correlation, Kruskal–Wallis test, and Fisher's exact test. The area under the curve (AUC) for glucose, insulin, and plasma C-peptide levels was calculated using the trapezoidal estimation. A p-value of $\leq$ 0.05 was considered statistically significant.

We used existing data of the effect of mono- or dual-CFTR modulators on glucose tolerance and insulin secretion for sample size calculation (17, 23). Assuming an alpha level of 0.05 (one-sided), a statistical power of 80%, an effect size (= difference of 2-h glucose values before/after starting ELX/TEZ/IVA treatment) of 2.0 mmol/L, and a standard deviation (SD) of 2.8 mmol/L, we estimated a minimal sample size of n = 14 (two-sample paired-means t-test). Statistical analyses were performed using Stata^{TM} (Stata Statistical Software: Release 13; StataCorp LP, College Station, TX, United States). Figures were generated using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, United States).

TABLE 1 | Demographic data of study population.

		Before ELX/TEZ/IVA therapy	Under ELX/TEZ/IVA therapy	*P*-value
Demographics *n* = 16	Sex (m)	6 (37.5)		
	Age (years)	13.8 (13.0; 15.4)		
	Weight (z-score)	−0.29 (−1.08; 0.23)	−0.25 (−0.94; 0.28)	0.5
	BMI (z-score)	−0.47 (−0.74; 0.33)	−0.25 (−0.83; 0.38)	0.09
	Pancreatic insufficiency	16 (100)		
CFTR variant	F508 homozygot	9 (56)		
	F508 heterozygot	7 (44)		
	Previous modulator use	5 (31)		
	– Ivacaftor/Tezacaftor	4 (25)		
	– Ivacaftor/Lumacaftor	1 (6)		

Results are displayed as median (IQR) or total numbers (%) as appropriate. CFTR, cystic fibrosis transmembrane conductance regulator.

RESULTS

Study Population

In total, 16 adolescents with CF with a median age (IQR) of 13.8 years (13.0; 15.4) were included in the study (**Figure 1**). Baseline characteristics were assessed at the first study visit. All participants performed lung function measurements before and 4–6 weeks after initiating ELX/TEZ/IVA treatment. Details on demographic data are given in **Table 1**.

Glucose Tolerance Before and After the Start With Elexacaftor/Tezacaftor/Ivacaftor

OGTT was performed in 15 people with CF in median (IQR) 3 (2–42) days (IQR) before and 26 (24–40) days after starting treatment with ELX/TEZ/IVA. OGTT improved after starting with ELX/TEZ/IVA ($p = 0.02$). Before ELX/TEZ/IVA treatment, two participants were diagnosed with (previously unknown) CFRD, and six could be categorized as IGT, two as INDET, and five as NGT. After treatment, no participant fell into the category CFRD, two into the category IGT, four into INDET, and nine into NGT (**Figure 2**). Thus, seven participants improved in OGTT, and seven participants remained stable. Of the latter, five participants had a normal result (NGT) in both tests. One participant changed from INDET to IGT (**Figure 2**).

We compared glucose, insulin, and plasma C-peptide levels at time points 0, 60, 120, and 180 min of OGTT, respectively (**Table 2** and **Figure 3**). After starting with ELX/TEZ/IVA, compared to pre-treatment measurements, plasma glucose levels were lower at 60, 90, and 120 min of OGTT, but no difference was found for fasting glucose and glucose levels at 180 min of OGTT (**Figure 3A**). Insulin and C-peptide levels were lower at 120 min and 180 min, whereas no differences were found for fasting and early secretion of insulin (at 60, 90 min) and C-peptide (at 60, 90 and 120 min) (**Figure 3B**). The AUC decreased for plasma glucose (**Figure 4**) and insulin after introducing ELX/TEZ/IVA (**Table 2**). Results did not change when excluding participants with NGT ($n = 5$) before initiating ELX/TEZ/IVA therapy (difference in OGTT categories: $p = 0.02$). HbA1c values did not change before and after starting with ELX/TEZ/IVA (**Table 2**).

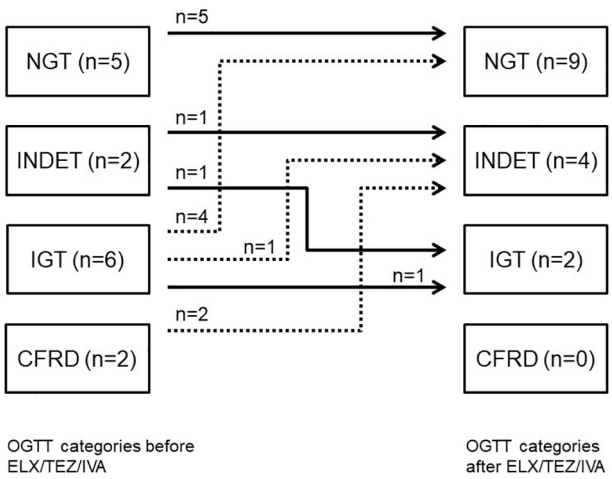

FIGURE 2 | OGTT categories before and after starting ELX/TEZ/IVA treatment in people with cystic fibrosis. Numbers of study participants and OGTT categories and individual changes after starting ELX/TEZ/IVA treatment. OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; INDET, indeterminate glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; CFRD, CF-related diabetes.

Continuous Glucose Monitoring

CGM was performed in 11 participants 3 days (median, min–max 2–4) before and 7 (6–7) days after the initiation of ELX/TEZ/IVA therapy (**Table 2**). We excluded the measurements on the first treatment day to standardize analyses, as the precise time of first medication use was not documented. To have genuinely only readings before and after initiation, we analyzed measurements from 2 days before and 5 days after therapy started (for details, see "Materials and Methods" section). We calculated mean, minimum, and maximum glucose levels before and after starting with ELX/TEZ/IVA and found no difference (**Figure 5**). Average sensor readings were within normal glucose ranges before and after the start with ELX/TEZ/IVA [median (IQR) 6.68 (6.22; 6.94) mmol/L and 6.60 (6.06; 7.46) mmol/L, $p = 0.60$, respectively]. As described in detail in the methods section, we classified glucose levels into different categories (very low, low, normal, high, and very high). We did not find a difference in the percentage of

TABLE 2 | Differences in glucose tolerance (OGTT and CGM) and lung function before and after initiation of ELX/TEZ/IVA therapy in people with cystic fibrosis.

		Before ELX/TEZ/IVA therapy	Under ELX/TEZ/IVA therapy	*P*-value
OGTT categories	NGT	5 (33.3)	9 (60)	**0.02**
and measures	INDET	2 (13.3)	4 (26.6)	
***n* = 15**	IGT/IFG	6 (40)	2 (13.3)	
	CFRD	2 (13.3)	0	
	Fasting plasma glucose (mmol/L)	5.21 (5.12; 5.35)	5.15 (4.85; 5.35)	0.2
	60 min plasma glucose (mmol/L)	10.86 (9.61; 12.48)	9.74 (8.28; 11.62)	**0.03**
	90 min plasma glucose (mmol/L)	9.62 (7.63; 11.38)	9.08 (6.5; 10.18)	**0.04**
	120 min plasma glucose (mmol/L)	7.67 (5.9; 9.35)	5.78 (4.9; 7.2)	**0.03**
	180 min OGTT plasma glucose (mmol/L)	4.27 (3.98; 5.25)	3.82 (3.37; 4.67)	0.6
	Average AUC blood glucose (mmol/L*min)	1383.75 (1212.45; 1542.45)	1262.10 (1079.85; 1423.5)	**0.008**
	Fasting insulin (mU/L)	6.4 (5.2; 10)	8 (4.5; 9.1)	0.7
	60 min insulin (mU/L)	53.4 (41.8; 71.9)	61.5 (35.3; 78)	0.6
	90 min insulin (mU/L)	60.4 (19.9; 80.9)	54.7 (34.3; 81.3)	0.7
	120 min insulin (mU/L)	58 (33.2; 79.5)	32.65 (14.1; 45.3)	**0.01**
	180 min insulin (mU/L)	13.4 (8.6; 24.1)	8.7 (4.9; 12.1)	**0.006**
	Average AUC blood insulin (mU/L*min)	8014.5 (5,160; 10,434)	6,924 (4,353; 9,330)	**0.02**
	Fasting plasma C-peptide (ng/ml)	1.16 (0.98; 1.53)	1.26 (0.93; 1.62)	0.6
	60 min plasma C-peptide (ng/ml)	8.46 (6.2; 9.7)	6.92 (4.68; 7.7)	0.8
	90 min plasma C-peptide (ng/ml)	8.62 (5.41; 9.81)	7.85 (5.16; 10.8)	0.5
	120 min plasma C-peptide (ng/ml)	8.12 (5.54; 9.8)	5.95 (3.57; 7.85)	0.08
	180 min plasma C-peptide (ng/ml)	3.52 (2.09; 4.97)	2.02 (1.58; 3.18)	**0.005**
	Average AUC plasma C-peptid e (ng/ml*min)	1067.4 (900.9; 1196.7)	930.45 (736.05; 1145.85)	0.05
CGM measures	Average sensor glucose (mmol/L)	6.68 (6.22; 6.94)	6.6 (6.06; 7.46)	0.6
***n* = 11**	Minimum sensor reading (mmol/L)	4 (3.1; 4.2)	4 (3.7; 4.5)	0.2
	Maximum sensor reading (mmol/L)	13.15 (12.2; 14.0)	13.1 (12.4; 14.9)	0.3
	% time glucose ≥ 11.2 (very high) (mmol/L)	3.0 (0.6; 4.46)	1.15 (0.84; 2.08)	0.3
	% time glucose ≥ 7.7 and ≤ 11.1 (high) (mmol/L)	17.56 (9.78; 21.73)	18.0 (12.28; 27.09)	0.2
	% time glucose ≥ 3.4 and ≤ 7.7 (normal) (mmol/L)	81.11 (77.38; 85.97)	81.13 (68.83; 86.6)	0.5
	% time glucose ≥ 2.8 and ≤ 3.3 (low) (mmol/L)	0 (0; 0.15)	0 (0; 0)	0.4
	% time glucose ≤ 2.7 (very low) (mmol/L)	0 (0; 0)	0 (0; 0)	–
Additional laboratory	HbA1c%	5.7 (5.4; 5.9)	5.6 (5.5; 5.9)	0.6
results *n* = 16	HbA1c ≥ 5.8% (*n*,%)	7 (47)	6 (40)	0.6
	Sweat chloride (mmol/l)	95 (93; 104)	51 (32; 59)	**0.002**
Lung function	LCI (TO)	8.05 (6.82; 10.22)	6.84 (6.39; 7.89)	**0.003**
data	FEV_1 (z-score)	−0.71 (−2.39; −0.32)	−0.39 (−1.08; 0.4)	**0.007**
***n* = 16**	MFEF (z-score)	−0.59 (−2.05; 0.15)	0.07 (−0.46; 0.9)	**0.001**
	FEV_1/FVC (z-score)	−0.42 (−1.23; −0.02)	0.31 (−0.33; 0.78)	**0.0009**
	RV/TLC (%predicted%)	27.76 (21.12; 37.04)	23.09 (19.5; 27.63)	**0.006**
	sReff (%predicted)	179 (125.5; 249.5)	144 (102; 175)	**0.01**

Differences between OGTT and CGM measures as well as additional laboratory results and lung function data in people with cystic fibrosis (CF) before and after the initiation of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) treatment. Results are displayed as median (IQR) or total numbers (%) as appropriate. CFTR, cystic fibrosis transmembrane conductance regulator; CGM, continuous glucose monitoring; OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; INDET, indeterminate glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; CFRD, CF-related diabetes; AUC, area under the curve; LCI, lung clearance index; FEV_1, forced expiratory volume at 1 s; FVC, forced vital capacity; MFEF, mid expiratory flow; RV/TLC, ratio of residual volume over total lung capacity; staf, specific resistance. Significant p-values (≤0.05) are marked in bold.

the respective glucose level time before and after ELX/TEZ/IVA initiation. No episodes of hypoglycemia (very low and low category) were observed after starting ELX/TEZ/IVA treatment. Episodes of hyperglycemia were similar before and after starting ELX/TEZ/IVA treatment (**Table 2**).

Lung Function and Additional Analyses

Lung function measurements and additional analyses were performed on the day of OGTT. All lung function parameters from N_2-MBW (LCI), spirometry (FEV_1, FEV_1/FVC, and MFEF), and body plethysmography (RV/TLC, sReff) improved under ELX/TEZ/IVA treatment (**Table 2**). Sweat chloride was pathologic (>60 mmol/L) in all people with CF before ELX/TEZ/IVA therapy and improved in all, normalizing (<30 mmol/L) in three and reaching borderline results (30–60 mmol/L) in ten. Weight and BMI did not change before and under ELX/TEZ/IVA (**Table 2**). Changes in OGTT [categorized into (i) improvement, (ii) no change and (iii) deterioration],

plasma glucose, insulin, and C-peptide levels did not correlate with lung function parameters (meaning differences between the first and second measurement) and were not associated with the reduction of sweat chloride (data not shown).

DISCUSSION

Summary

In this observational pilot study, glucose tolerance measured via OGTT improved in adolescents with CF shortly after initiating ELX/TEZ/IVA treatment, whereas glycemic profiles assessed by CGM did not show any changes. Lung function parameters and sweat chloride levels improved as well. Data of this observational pilot study indicate that ELX/TEZ/IVA improves respiratory outcomes—as shown previously (1, 38)—in people with CF and positively affects endocrine pancreatic function.

Comparison With Literature

In our study, OGTT results generally improved, as did plasma glucose levels at 60, 90, and 120 min, and late plasma insulin levels (90 and 120 min) under ELX/TEZ/IVA therapy. Our data is in line with a previous study investigating glucose tolerance before and after 1 year of lumacaftor/ivacaftor therapy in 40 adult people with CF with IGT or newly diagnosed and untreated CFRD (23). The authors reported an improvement of OGTT overall and in 2 h plasma glucose. Improvement of glycemic control or insulin secretion was observed under ivacaftor therapy (22, 39). In our study population, there was no new diagnosis of CFRD under ELX/TEZ/IVA treatment, but we could record a resolution of CFRD into IGT. These results align with data from patients with ivacaftor therapy, in whom one-third of patients with CFRD showed resolution (40). We found no difference in fasting glucose and early insulin secretion or improved HbA1c values after ELX/TEZ/IVA treatment initiation. Others found improvement under ivacaftor in all parameters mentioned above but not in OGTT values in teenage and adolescent patients with CF (18, 24). Further, a small study in five patients with NGT, IGT, or CFRD reported improved insulin response in OGTT 4 weeks after starting with ivacaftor, whereas glucose levels did not improve (17). However, several studies investigating glucose tolerance under lumacaftor/ivacaftor treatment (from 4 weeks up to 12 months) could not find differences for any assessed parameters: OGTT, plasma glucose, insulin, and C-peptide levels (25, 26, 41). We did not observe differences in CGM before and after the start with ELX/TEZ/IVA, our results are in line with a study investigating CGM before and after the start with ivacaftor in nine teenagers (24). A recent study in adults using ELX/TEZ/IVA reported improvement of CGM data after initiation of therapy. However, the main improvement could be found in patients with CFRD. Furthermore, CGM measures in this study were performed 3–12 months after the start with ELX/TEZ/IVA; thus, a further improvement in our cohort is possible (27).

Several studies investigating people with CF with impaired glucose tolerance reported low early-phase and high late-phase insulin secretion (42, 43). Our study showed that the late-phase insulin levels decreased after ELX/TEZ/IVA therapy initiation—an effect we interpret as insulin-resistance-improvement: less insulin is needed for lower glucose levels, and the initial insulin secretion is sufficient. The observed insulin secretion pattern after starting ELX/TEZ/IVA therapy approximates the pattern described in healthy people (43). The lower insulin levels under ELX/TEZ/IVA combined with lower glucose concentrations could be interpreted as higher insulin sensitivity.

The study population had no higher incidence of hypo- or hyperglycemic episodes under ELX/TEZ/IVA treatment.

The discrepancy between studies might be due to differences in study design, small participant numbers, and different CFTR modulators/correctors or combinations studied. Studies on lumacaftor/ivacaftor in patients with F508del mutation showed fewer CFTR activating effects than ivacaftor alone in patients with G551D mutation (44), which might explain the missing effect on glucose tolerance in studies investigating lumacaftor/ivacaftor.

All lung function parameters improved after initiation of ELX/TEZ/IVA. Our results are comparable with those of a larger previous study in children (45). Median improvement of lung function data (e.g., LCI and FEV1) in our study is in line with

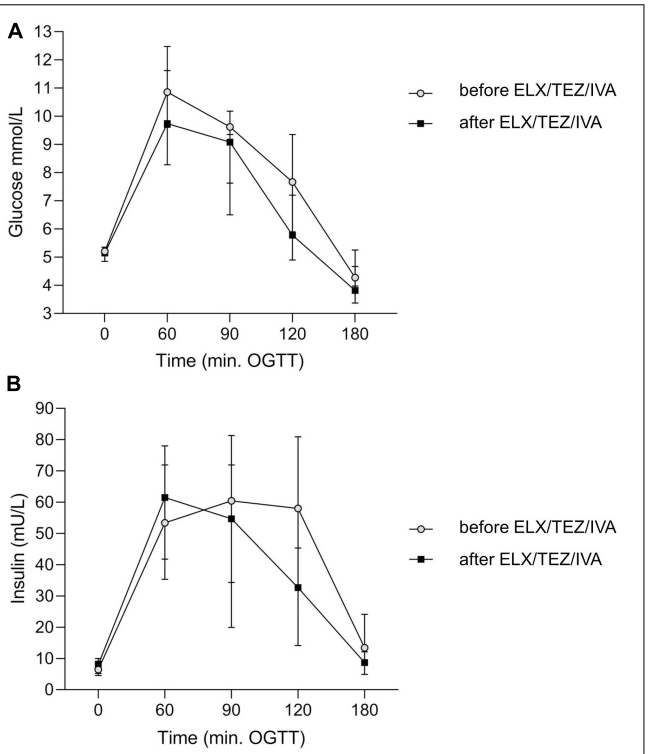

FIGURE 3 | Time course of plasma glucose **(A)** and insulin **(B)** levels before and after the start of ELX/TEZ/IVA treatment at different time points of the OGTT in people with cystic fibrosis. After starting ELX/TEZ/IVA treatment **(A)**, plasma glucose levels (in mmol/L) were lower at 60, 90, and 120 min of OGTT (all $p < 0.05$), but no difference was found for fasting glucose and glucose levels at 180 min of OGTT. **(B)** Plasma insulin levels (in mU/L) were lower at 120 min ($p = 0.04$) and 180 min ($p = 0.006$), whereas no differences were found for fasting and early insulin secretion. OGTT, oral glucose tolerance test.

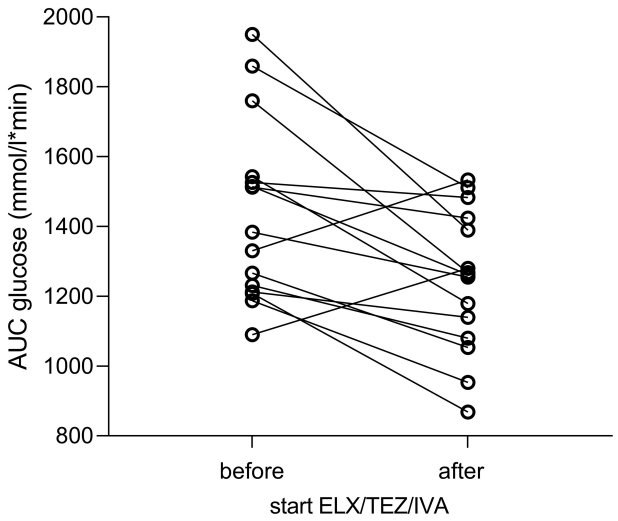

FIGURE 4 | Area under the curve of plasma glucose levels before and after initiating ELX/TEZ/IVA therapy in people with cystic fibrosis. The area under the curve (AUC) decreased for plasma glucose (in mmol/l*min) after introducing ELX/TEZ/IVA ($p = 0.008$).

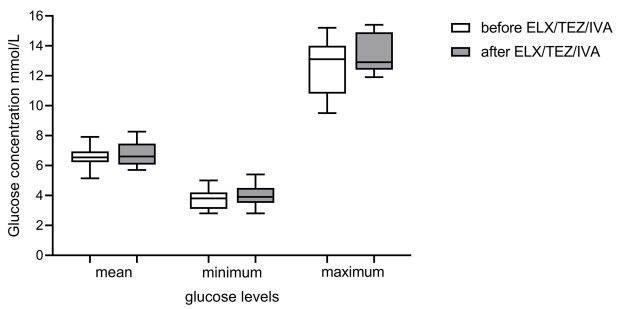

FIGURE 5 | Glucose levels of continuous glucose monitoring (CGM) prior and after initiation of ELX/TEZ/IVA therapy in people with cystic fibrosis. Mean, minimum and maximum glucose levels (mmol/l) of CGM measurements prior (measurements of 2 days) and after (measurements of 5 days) starting ELX/TEZ/IVA therapy. Displayed are boxplots with medium and interquartile range.

mean improvement in this larger cohort. This is also true for the improvement of sweat chloride (45).

Physiological Considerations and Clinical Implications

The mechanisms of how CFTR modulators may affect the pancreatic function and thus glucose homeostasis are not fully understood. Insulin deficiency is the primary defect in CFRD, but insulin resistance and impairment of the entero-insular axis play contributory roles (46). A dysbalance between pancreatic β-cell mass and insulin secretion (42, 47), and pancreatic islet loss are discussed as underlying pathophysiology (12). CFTR protein expression in pancreatic α- and β-cells and an early presence of abnormalities in insulin secretion imply a direct role of CFTR dysfunction (13, 14, 48). CFTR seems to play a role in glucose-induced electrical activities leading to CFTR-caused defect in β-cells and diminished insulin secretion, which was shown to be restored by lumacaftor *in vitro* (48). However, others did not detect CFTR activity in pancreatic cells (12), postulating that CFTR function indirectly impacts insulin secretion: an improved incretin secretion by gastrointestinal neuroendocrine cells and decreased inflammation could, e.g., lead to indirect β-cell effects (16). Another possible mechanism could be a relief of islet inflammation by CFTR restoration, as CF islets contain inflammatory interleukins (16, 49). CFTR restoration might improve the local environment of islets by enhancing pancreatic ductal function (16). Furthermore, one mechanism that leads to insulin resistance is an impaired insulin-induced glucose transporter 4 (GLUT), inhibiting glucose entering into dependent cells and impairing subsequent signaling pathways (50). Recently, Gu et al. demonstrated that GLUT4 translocation to the cell membrane was abnormal in CFTR knockout mice muscle fibers (51). Modulating CFTR by ELX/TEZ/IVA could positively influence GLUT4 membrane transportation and improve insulin resistance.

As we found short-term beneficial effects of ELX/TEZ/IVA on glucose tolerance, the "historical" pathophysiology of CFRD caused by β-cell loss cannot be the whole truth. Our data indicate that CFTR modulators might prevent CFRD in CF people—an early use assumed. In our study, participants with IGT and INDET improved; both categories are predictors for CFRD (32). Two subjects with unknown CFRD resolved after initiation of therapy.

In the clinical setting, it might thus be recommended to re-evaluate pathological glucose tolerance after beginning therapy with ELX/TEZ/IVA and before insulin treatment by repeating pathologic OGTTs and repetitive pre- and postprandial glucose measurements.

Strengths and Limitations

Our study assessed the short-term effect of ELX/TEZ/IVA combination therapy on glucose tolerance in CFTR naïve adolescents with CF. A vast strength is the comprehensive assessment of various outcomes of glucose homeostasis (OGTT, CGM) and other functional and laboratory outcomes. A limitation is the small number of participants included in the study. Therefore, more discrete effects in glucose homeostasis and lung function might not be detected. However, we met the beforehand defined sample size calculation based on our power analysis. It is known that improvement in respiratory function and consecutively in exercise capacity can affect glucose tolerance (52, 53). Our study did not include a detailed physical activity protocol, so we cannot exclude a certain influence of (more) physical activity under ELX/TEZ/IVA treatment on our results.

CGM measurements were performed early after initiation of ELX/TEZ/IVA therapy; thus, we do not know if changes occurred at the time of OGTT measurement. However, as CGM measurements before starting ELX/TEZ/IVA were also normal, we believe a further improvement is unlikely. As we only assessed the short-term effect, we cannot comment on whether the positive effect of ELX/TEZ/IVA on glucose tolerance is transient, sustainable, or if there is even a further improvement. In addition, due to the time range of OGTT (4–10 weeks) after therapy initiation, we cannot explicitly differentiate between very early

and later effects. A long-term follow-up of our participants will answer those additional questions.

CONCLUSION

This short-term observational study showed that glucose tolerance improves after initiating ELX/TEZ/IVA therapy in teenagers with CF, suggesting that ELX/TEZ/IVA also benefits pancreatic endocrine function. Early treatment with ELX/TEZ/IVA might postpone change future CFRD in adolescents with CF, highlighting that pathological OGTT results should be repeated after initiation or under ELX/TEZ/IVA treatment. While this has to be confirmed in larger longitudinal studies, it supports the early initiation of the CFTR modulator therapy before measurable organ dysfunction occurs, not considering respiratory outcomes alone.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was performed in the University Children's Hospital of Bern, University of Bern, Switzerland, and approved by the Ethics committee of Bern, Switzerland (ID 2021-00982). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

IK: conceptualization, methodology, visualization, writing—original draft, formal analysis, investigation, and data curation. EK: investigation, visualization, and writing—original draft. MB and LK: investigation, writing—review, and editing. CF: methodology, writing—review, and editing. PL: conceptualization, methodology, resources, project administration, writing—review, and editing. CC: conceptualization, writing—review, and editing. CB: conceptualization, methodology, resources, project administration, writing—original draft. All authors contributed to the article and approved the submitted version.

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