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Special Issue Reprint

Cystic Fibrosis

A Disease with a New Face

Edited by
Janice Wang and Patricia A. Walker

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Cystic Fibrosis: A Disease with a New Face

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Guest Editors

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About the Editors

Janice Wang

Janice Wang is Professor of Medicine at the Zucker School of Medicine at Hofstra Northwell Health in New York. She serves as the Director of the Adult Cystic Fibrosis (CF) Clinical Program in the Division of Pulmonary, Critical Care and Sleep Medicine at Long Island Jewish Medical Center of Northwell Health and the Center's Adult Therapeutic Development Network Center Director. Her interests include CF, bronchiectasis, nontuberculous mycobacteria disease, sleep medicine, pulmonary medicine and critical care medicine. She has experience in the design and performance of investigator-initiated studies and serves as site-investigator for many industry-sponsored clinical trials to facilitate the performance of cutting-edge Phase 2 and 3 drug trials from the CF pipeline as well as innovative observational trials analyzing understudied CF-related complications. Dr. Wang demonstrates mentoring, teaching and educational leadership to physicians-in-training. She educates medical students, physicians and her patients with CF and their caregivers through local seminars, town halls and national conferences. She has received multiple grants as Principal Investigator from the Cystic Fibrosis Foundation.

Patricia A. Walker

Patricia A. Walker is a pulmonologist and long-standing investigator in Cystic Fibrosis (CF). She is the Co-Director and Adult Program Director of the Lenox Hill Hospital Cystic Fibrosis Center Northwell Health. She is also Director of the CF Therapeutic Development Center at Lenox Hill Hospital. She has served as site principal investigator or co-investigator for numerous studies including landmark trials evaluating treatment strategies for pulmonary exacerbations and therapeutic simplification in the era of highly effective CFTR modulator therapy. Patricia A. Walker is currently engaged in next-generation investigational studies, including mutation-agnostic genetic therapies and bacteriophage-based treatment approaches designed to address antibiotic-resistant airway infections. Participation in these studies reflects a commitment to expanding therapeutic options for individuals with rare CFTR variants or treatment-refractory disease and to ensuring that patients have access to emerging therapies as the field moves toward precision and gene-based medicine. In addition to clinical research, she has maintained a longstanding interest in reproductive health and aging in individuals with cystic fibrosis, particularly women with CF. She has participated in collaborative research initiatives addressing contraception, pregnancy planning, and the evolving health needs of women living longer with CF in the era of highly effective CFTR modulators. Education and mentorship have also been central to her career. Dr. Walker has trained and mentored residents, fellows, and junior faculty in pulmonary and cystic fibrosis medicine, emphasizing early recognition of CF and CFTR-related disorders in adult pulmonary practice. Many trainees have gone on to become leaders in CF care and research throughout the United States.

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Editorial

Cystic Fibrosis: A Disease with a New Face

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Medical advancements in the field of Cystic Fibrosis (CF), a rare, genetic disorder, have been profound over the last decade, allowing care to evolve in ways previously thought unimaginable by those living with CF and those caring for people with CF.

Once uniformly fatal in childhood, CF has become a chronic, increasingly adult disease characterized by extended survival, evolving clinical phenotypes, and unprecedented therapeutic opportunity.

Over the past several decades, the establishment of specialized CF centers providing multidisciplinary care, aggressive management of airway infection and inflammation, and improved nutritional support have steadily improved outcomes. However, no development has altered the trajectory of CF as fundamentally as the advent of highly effective cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapies.

The identification of the CFTR gene more than three decades ago provided the first molecular explanation for CF and laid the foundation for targeted therapy. Clinical care, however, remained focused on mitigating downstream consequences of CFTR dysfunction—airway infection, bronchiectasis, malnutrition, and progressive organ failure.

The introduction of CFTR modulators, particularly the combination of elexacaftor/tezacaftor/ivacaftor (ETI), marked a turning point. For most individuals with CF, these therapies substantially improve lung function, nutritional status, quality of life, and survival. As a result, CF is now increasingly recognized as a lifelong condition with a growing adult population and a shifting spectrum of disease manifestations.

Although most of the population in the U.S. with CF benefit from CFTR modulators, 6% of people remain with CF without a treatment targeting their specific genetic mutations [1]. Furthermore, worldwide, not all individuals with CF have the option for or access to highly effective modulator therapies.

The impact of this therapeutic revolution is reflected in contemporary survival estimates. Today, the median predicted survival for individuals born with CF in the United States approaches 65 years. These gains represent one of the most striking success stories in genetic medicine. At the same time, they introduce new challenges: the emergence of aging-related comorbidities, evolving reproductive and family planning needs, long-term medication effects, and the need to redefine what optimal CF care means in the era of disease modification.

Despite the remarkable success of CFTR modulators, CF has not been "cured". For those who do not have a modulator, or who do not respond, many challenges exist. Structural lung disease, once established, does not fully reverse, and chronic complications such as CF-related diabetes (CFRD), liver disease, bone disease, and malignancies continue to affect long-term outcomes. Moreover, as CF phenotypes become more heterogeneous

and milder forms of CFTR dysfunction are increasingly recognized, diagnosis itself has become more complex. CF is no longer solely a pediatric disease with a typical presentation. It is now frequently diagnosed in adulthood, and often in individuals with atypical or organ specific manifestations.

In this Special Issue of 16 manuscripts, "Cystic Fibrosis: A Disease with a New Face", we visit the past, review the present, and anticipate the future of CF as the medical and scientific fields of this orphan disease make remarkable advancements one would hope to see with every disease. We explore aspects of CF care that have transformed over time, with eight review articles evaluating newborn screening and diagnostic challenges in all ages, lung transplant, future therapeutics, comorbidities of an aging population, symptom burden, and palliative care; quality of life and well-being, reproductive medical care, nutritional care guidelines, and less studied in people with CF, but equally important, disordered eating behaviors [2–9].

In eight additional articles, original investigations demonstrate and reaffirm insights into the context of HEMT. Real-world data were used to evaluate the prevalence of CF-related diabetes (CFRD), adverse events, and drug-drug interactions with elexacaftor/tezacaftor/ivacaftor (ETI), providing valuable insight into the clinical impact of ETI since its commercial availability [10,11]. Three articles highlight how ETI-associated improvement is exhibited in areas of CF-associated liver disease, glycemic control in CFRD, and nocturnal oxygenation and lung function [12–14]. Appreciation for the complexities of CF as a highly variable phenotypic disease with the inheritance of complex alleles in Mexican families is shown in a featured article by Mendiola-Vidal et al. [15]. However, knowledge gaps remain, given the complex pathogenicity of CF, and are explored in studies evaluating serum levels of calcitonin gene-related peptide (CGRP) levels in people with CF and HEMT-restorative effects on pancreatic ductal epithelial cells in CF [16,17].

Together, these manuscripts reinforce a central theme: CF has become a biologically and clinically diverse disease in which traditional categories are increasingly inadequate. Modulator therapy has narrowed but not eliminated the gap between genotype and phenotype. As individuals with CF live longer and healthier lives, the full spectrum of CFTR dysfunction, from classic CF to CFTR-related disorders, becomes more visible. This complexity demands a more nuanced approach to diagnosis, long-term management, and research prioritization.

Looking forward, the future of CF therapy is both promising and demanding. Gene-based and mutation-agnostic therapies, including mRNA delivery, gene editing, and novel CFTR correction strategies, hold the potential to address the unmet needs of individuals who do not benefit from current modulators. At the same time, there is an increasing need for long-term observational data to define the durability, safety, and broader health effects of CFTR modulators across decades of use.

The growing adult CF population requires expanded expertise in managing metabolic, hepatic, oncologic, and reproductive conditions, as well as innovative models of care that support transitions across the lifespan.

The transformation of CF also carries implications beyond the disease itself. More than ever before, CF has become a paradigm for precision medicine, demonstrating how molecular diagnosis, targeted therapy, and comprehensive clinical infrastructure can converge to change the natural history of a genetic disease. However, this success also highlights the importance of equitable access, global implementation, and sustained investment in research to ensure that all individuals with CF benefit from ongoing scientific advances.

This Special Issue captures CF at a moment of extraordinary transition. The disease that once defined pediatric pulmonary medicine is now a complex, lifelong condition

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shaped by powerful therapies, diverse genetic and phenotypic expression, and expanding clinical horizons.

The articles presented here not only document how far the field has come but also illuminate the challenges and opportunities that lie ahead. As CF continues to take on a new face, continued innovation, careful study, and collaborative care will be essential to ensure that the promise of this new era is fully realized for all who live with this disease.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Review

Future Comorbidities in an Aging Cystic Fibrosis Population

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Abstract: Cystic fibrosis (CF) is an autosomal recessive disease due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. With the advent of highly effective modulator therapy targeting the abnormal CFTR protein, people with CF (PwCF) are living more than 40 years longer than the pre-modulator therapy era. As a result, PwCF are facing new challenges of managing similar comorbidities affecting the average aging population. While CF is notoriously identified as a chronic respiratory disease, the multisystem presence of the CFTR gene can contribute to other organ-related complications acutely, but also heighten the likelihood of chronic conditions not routinely encountered in this cohort. In this overview, we will focus on risk factors and epidemiology for PwCF as they relate to cardiovascular disease, dyslipidemia, CF-related diabetes, pulmonary hypertension, obstructive sleep apnea, CF-liver disease, bone health and malignancy. With increased awareness of diseases affecting a newly aging CF population, a focus on primary and secondary prevention will be imperative to implementing a comprehensive care plan to improve long-term morbidity and mortality.

Keywords: highly effective modulator therapy (HEMT); screening; dyslipidemia; cardiovascular disease; cystic-fibrosis-related diabetes (CFRD); pulmonary hypertension; obstructive sleep apnea; cystic-fibrosis liver disease (CFLD); osteoporosis; cancer

1. Background

Cystic fibrosis (CF) is an autosomal recessive genetic disease that was first described in 1938 by Dr. Dorothy H. Anderson at a time when most patients died in childhood [1]. In the 1950s, life expectancy for CF was less than a year, and by the 1970s, a mere 8 years [2]. Worldwide incidence of CF is estimated to be between 1/2000 and 1/6000 with higher rates among people of northern European descent, compared to other races. There are more than 70,000 people with CF (PwCF) worldwide. Approximately 32,000 PwCF live in the United States alone, with more than 1000 cases diagnosed annually [3]. Based on the 2021 U.S. Cystic Fibrosis Foundation (CFF) Patient Registry, 91.4% of registered PwCF are White, 9.8% are Hispanic, 3.5% are African American, and 5.1% are "other" [3,4]. The adult CF population is increasing and exceeding the pediatric population. In the United States, approximately 58% of the CF population are 18 years and older (Figure 1) [4]. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), which is responsible for encoding the CFTR protein, a chloride ion channel located on the epithelial cell surface of multiple organs, including the lungs, sinuses, pancreas, liver, gastrointestinal system, and reproductive system [3,5]. The major mutation responsible for the disease, Delta F508, was first discovered in 1989; however, more than 2000 additional mutations have now been identified [1,5]. Abnormal CFTR protein can lead to an increase in sodium absorption and a relative increase of chloride ion transport into the interstitium. The result is a net increase in sodium chloride absorption and water absorption via an osmotic gradient, ultimately causing impaired mucus hydration and

clearance. Although the majority of clinical manifestations in CF are respiratory in nature, the widespread expression of the CFTR channel can lead to complications in other organ systems [5]. Thirty-two percent of adults with CF aged 20–64 can have up to five or more comorbidities [6].

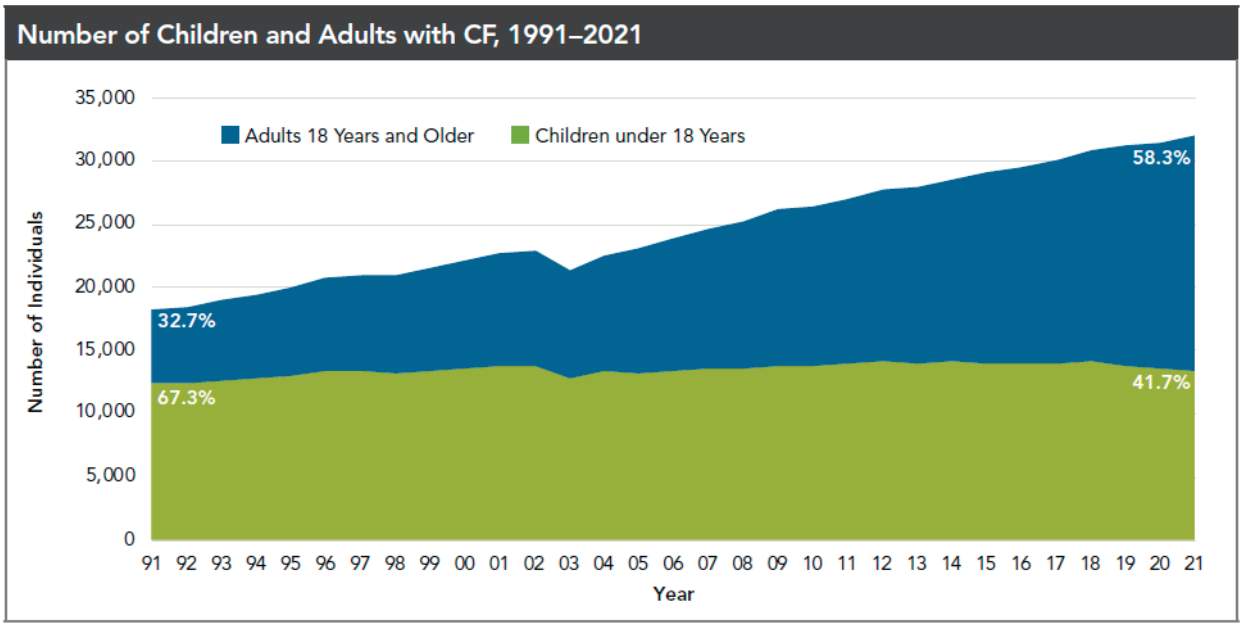


Figure 1. This graph represents cystic fibrosis (CF) patients under care at CF Foundation-accredited care centers in the United States, who consented to have their data entered. The adult cystic fibrosis population is exceeding the pediatric population, indicating the improving life expectancy of people with CF. Reproduced with permission from the Cystic Fibrosis Foundation Patient Registry 2021 Annual Data Report [4].

Recent advancements in CF therapies, primarily the CFTR modulators (or highly effective modulator therapy (HEMT)) underscore the impact that personalized medicine has had on redefining treatment paradigms and improving the course of disease. The first CFTR channel modulator approved was ivacaftor in 2012. Lumacaftor then followed in 2015, but was later replaced by tezacaftor, due to side effects, in combination with ivacaftor. The most recent modulator therapy was approved by the United States Food and Drug Administration in 2019. The drug, elexacaftor/tezacaftor/ivacaftor (ETI), is a combination of two protein correctors (elexacaftor and tezacaftor) and one potentiator (ivacaftor) which work to increase CFTR protein activity and mitigate disease progression in patients with at least one F508del mutation. Following the implementation of HEMT, survival for PwCF has improved significantly with the new median predicted age of survival being 53 years for those born with CF today [7]. A person-level microsimulation model based on known clinical outcomes and real-world data on the effectiveness of CFTR modulator therapy was developed to estimate survival and clinical benefits associated with ETI in patients with CF [7]. The median projected survival for patients with CF initiating ETI between ages 12 and 17 years was 82.5 years, a staggering estimate of at least 45 years more than for patients managed with best supportive care. It is imperative that clinicians of all specialties recognize the growing CF patient population and how this aging population will face new medical challenges and morbidities not routinely faced due to their short life expectancies. This chapter will review CF-related diabetes and osteoporosis, which are common comorbidities in CF, as well as those that may benefit from more attention in the future as the patient population grows in age and number: cardiovascular disease, dyslipidemia, pulmonary hypertension, obstructive sleep apnea, CF liver disease, and

malignancies. The prevalence of these conditions will likely increase and as such we should be prepared to address them.

2. Cardiovascular Disease

The prevalence of cardiovascular disease (CVD) is considered to be low, but may be under-recognized in PwCF given that symptomatic CVD typically presents in older age. The aging adult CF population will begin to face comorbidities commonly associated with increased CVD risk, as seen in the general population, such as obesity, hypertension, cystic-fibrosis-related diabetes (CFRD), and chronic kidney disease [8]. One multicenter cohort of 422 adults with CF and SARS-CoV-19 infection reported a history of ischemic heart disease in about 22.5% of the cohort [9,10]. CFRD likely has the highest prevalence among this population, occurring in about 40% to 50% of PwCF. It is speculated that the traditional diet long recommended for those with CF and failure to gain weight can expose patients to suboptimal food choices that include higher saturated fats and excessive simple carbohydrates intake [11]. Such diets are well known to be associated with development of dyslipidemia and diabetes. Atherosclerosis is inherently a chronic inflammatory process leading to vascular changes such as endothelial damage, deposition of fats, and atherosclerotic plaque formation. CF has been described as a chronic inflammatory disorder, noting that PwCF tend to have higher levels of C-reactive protein (CRP) compared to healthy counterparts [12]. The inflammatory state in CF is multifactorial; not only due to the dysfunctional CFTR, but due to the ensuing chronic respiratory tract infections, as well as local and systemic inflammatory-immune responses. This pro-inflammatory state exhibited in CF may have a cumulative effect on the vasculature, leading to increased oxidative stress, endothelial dysfunction and large arterial stiffness, contributing to early development of hypertension [12,13]. Consequently, heart rate can also be increased in parallel to myocardial work due to increased arterial stiffness. The augmentation index, a composite vascular parameter of arterial stiffness, was found to be elevated in PwCF compared to control groups and increased with aging and/or the presence of CFRD [13,14]. As previously mentioned, the dysfunctional CFTR protein can contribute to chronic inflammation as it stimulates production of interleukin-8 (IL-8), a strong chemotactic agent activating leukocyte infiltration and furthering inflammation [13,14]. CFTR is also found in smooth muscle cells and vascular endothelium; abnormal CFTR activity can disrupt calcium concentration and signaling in vascular smooth muscle cells, resulting in decreased activity of endothelial nitric oxide (NO) synthase and NO bioavailability. Without the vasodilatory, anti-inflammatory and antioxidant properties of NO, there would be an increased risk for progression toward CVD [15]. Interestingly, in mouse models, the restoration of CFTR function reduced total atherosclerosis, improved ischemia-reperfusion injury after cardiac ischemia, and can turn a vulnerable plaque into more stable phenotypes [15]. More awareness is needed for the prevention and treatment of CVD in the aging CF population in order to reduce the future comorbidities that may be related to untreated cardiovascular disease.

3. Dyslipidemia

Prevalence of dyslipidemia in PwCF is unclear but likely has been low due to low survival rates and lifetime nutritional challenges. As the life expectancy and longevity of patients with CF increases, dyslipidemia will likely be an additional comorbidity to address [16]. PwCF carry several risk factors for dyslipidemia, including their CF-specific diet, exocrine pancreatic function, CF liver disease, pro-inflammatory state, and corticosteroid exposure. A high-fat, high-calorie diet is a long-established and integral part of CF management, particularly for pancreatic insufficient (PI) patients, alongside the use of pancreatic enzyme replacement therapy (PERT). This diet was first introduced in a Toronto CF clinic in the early 1970s, and was associated with improved nutritional outcomes, thus becoming standard of care through integration into the CF nutritional guidelines [16]. A retrospective cross-sectional review of PwCF performed between 2005 and 2007 showed that pancreatic sufficient (PS) CF patients were more likely to develop dyslipidemia compared to PI-CF

patients. These findings translated to triglycerides, low-density lipoprotein (LDL), total cholesterol, and high-density lipoprotein (HDL) levels [16,17]. The significant differences in lipid concentrations seen in this study between PI and PS patients suggest that impaired lipid absorption in PI patients may be a contributing factor. Another study at the University of Minnesota measured the fasting lipid profiles among 192 CF patients and found them to have higher triglyceride and lower cholesterol concentrations when compared to their age-related cohort in the general population. It is hypothesized that hypertriglyceridemia in patients with CF may be related to pro-inflammatory cytokines which promote hyperlipidemia during severe stress by inhibiting lipoprotein lipase activity, thus decreasing triacylglycerol clearance and stimulating hepatic lipogenesis [16–18]. There are currently no dyslipidemia screening guidelines in PwCF, but given the findings mentioned, this be serve as a prudent area for development. In the general population, the United States Preventive Services Task Force (USPTSF) strongly recommends that men over the age of 35 and women over the age of 45 get screened at least once for dyslipidemia since there is evidence that people at increased risk for coronary heart disease can be asymptomatic. The question of using lipid-lowering strategies in PwCF needs to be further explored. While dyslipidemia is associated with atherosclerotic CVD in the general population, there has been a paucity of literature on the impact of CVD in PwCF. It is worth noting that atherosclerotic disease has been incidentally found in autopsies of CF patients. General lipid-lowering therapeutics may not be appropriate for all patients with CF given their side effect profiles that include inhibition of fat absorption, body fat reduction, and weight loss [18,19]. Dyslipidemia in CF is heterogenous in presentation, and the underlying molecular mechanisms are not fully understood [20]. For instance, Nowak et al. showed that low HDL was common in both PI- and PS-CF patients, supporting the concept that fat malabsorption alone would not fully explain low HDL levels [20]. Again, chronic low-grade inflammation plays a major role; cytokine TNF-alpha mediated inhibition of hepatic lipoprotein lipase in patients with CF was associated with higher triglyceride levels. The impact of HEMT and associated longer life expectancy warrants further longitudinal studies following lipid profiles and disease development in PwCF to fully understand the risks associated with dyslipidemia in CF [20].

4. Cystic Fibrosis Related Diabetes

Cystic fibrosis related diabetes (CFRD) is a common comorbidity among PwCF with an estimated prevalence of 20% in adolescents and up to 40–50% in adults with CF. Survival is significantly impacted by CFRD status, 60% of PwCF with normal glucose tolerance live to age 30, compared to only 25% of those with the disease [21]. The pathophysiology of CFRD is complex, and not fully understood; however, CFRD includes reduced and delayed insulin secretion, insulin resistance, and dysfunction of the entero-insular axis. It is associated with accelerated decline in pulmonary function and worsened nutritional status [21]. Research suggests the CFTR protein plays an important role in insulin and glucagon secretion, as well as protection of β cells from oxidative stress. At present, there is still a lack of consensus on appropriate means to diagnose as well as manage CFRD. As onset of CFRD can occur in early childhood, current recommendations are for screening to begin annually at age 10 with the oral glucose tolerance test (OGTT). HbA1c is not routinely recommended for CFRD diagnosis as it may be falsely normal. More recently, there is an increasing move towards continuous glucose monitoring in select circumstances given its utility in identifying daily glucose fluctuations, finding that its use is more practical and effective [21]. At this time, CFRD is associated more with microvascular (i.e., diabetic retinopathy, nephropathy, neuropathy) than macrovascular complications. Diabetic retinopathy is seen in 5–27% of CF patients with CFRD [21]. It has been found that most CF adults with diabetic retinopathy were diagnosed with CFRD, on average, 12 years prior. It has also been shown that patients with CFRD have increased arterial stiffness. As previously mentioned, arterial stiffness along with hyperlipidemia, hypertension, and external risk factors can contribute considerably to CVD. Case reports

have documented CFRD patients suffering from single and multi-vessel cardiac disease [21]. According to the current clinical guidelines, the only recommended treatment for CFRD is insulin [21]. It is hypothesized that older PwCF, particularly with mild CF severity, may develop diabetic manifestations due to a type 2 diabetes-like pathophysiologic pathway (i.e., insulin resistance) rather than from insulin secretory endocrine pancreatic dysfunction as seen more commonly in patients with CFRD. Therefore, with the advent of HEMT, it remains to be seen how CFRD prevalence and management will be affected. CFRD adds another complex condition to CF, already ripe with its distinct complications.

5. Pulmonary Hypertension

CF patients develop progressive respiratory disease and/or failure, placing them at increased risk of developing pulmonary hypertension (PH) and subsequent right ventricular dysfunction. The pathophysiology of developing PH in CF is complex and likely multifactorial, including a state of chronic airway and systemic inflammation which can lead to pulmonary vascular remodeling and endothelial dysfunction. Chronic hypoxia is a contributing insult through its vasoconstrictive effects and ensuing effects on pulmonary vascular cell proliferation and pulmonary arteriolar hypertrophy [22]. While PH in CF may be classified as group III, the clinician should also evaluate patients for left ventricular systolic and diastolic dysfunction, particularly in older patients [22,23]. At present, epidemiologic studies using the gold-standard right heart catheterization (RHC) to evaluate for PH in the general CF population are lacking. The predominant research on PH in CF patients have used transthoracic echocardiography (TTE); however, this method can underestimate the true prevalence of PH. A recent transplant study found that 57% of CF patients had PH before lung transplant, but PH did not significantly alter the risk of death after transplant. A study by Hayes et al. found a significant risk of death associated with PH in CF patients, especially in older PwCF [24]. The hazard ratio for mortality risk in CF patients with mild and severe PH were 1.747 and 2.284, respectively ($p < 0.001$). There is a paucity of data investigating PH in the younger CF population, and currently no CF-specific recommendations for PH-directed therapy [24].

Determination for whether PH needs to be screened for at an earlier age is a concept that needs to be further explored in the age of increasing CF survival. It is known that patients with advanced CF lung disease (ACFLD) are at high risk for PH, which is associated with a worse mortality. ACFLD patients should undergo routine screening as these risks play a major role in their candidacy for lung transplantation. In fact, PH is a defining criterion for ACFLD. ACFLD is defined by (1) a forced expiratory volume in one second (FEV1) of <40% predicted when stable; (2) referred for lung transplantation evaluation; or (3) having at least one feature of an intensive care unit admission for respiratory failure, hypercarbia, daytime oxygen requirement at rest, PH, severe functional impairment, and/or a six-minute walk test distance of <400 m [25]. ACFLD care consensus guidelines recommend baseline echocardiography screening and evaluation for hypoxemia. Oxygen supplementation is a mainstay of treatment in order to reduce mPAP, pulmonary vascular resistance and right ventricular dysfunction [25]. While sildenafil, a pulmonary vasodilator, is commonly used in practice and appears to be safe, there remains insufficient evidence for clinical efficacy in patients with ACFLD [25]. Awareness of PH-related signs and symptoms clearly requires particular attention; however, screening is fundamental for early diagnosis and intervention.

6. Obstructive Sleep Apnea

Patients with CF commonly exhibit upper airway (UA) inflammation and obstruction. Chronic or acute inflammation is a common cause of UA collapse during sleep, posing a risk for obstructive sleep apnea (OSA) which is associated with intermittent hypoxic events and sleep disruption [26]. Hypoxia can contribute to the development of PH, leading to right ventricular dysfunction or cor pulmonale. Evaluation of nocturnal hypoxia is part of a thorough PH workup to screen and address for risk factors. Sleep-related hypox-

emic events have been studied using polysomnography in the younger CF population, including infants and children, with 70% found to have mild to moderate OSA. The most common site of airway obstruction is the oropharyngeal tract and can be due to tonsillar hypertrophy, increased size of uvula, or enlargement of lateral pharyngeal walls. Studies demonstrate a close relationship between OSA and the occurrence of oropharynx structural alteration and chronic rhinosinusitis [26,27]. OSA is strongly associated with CAD, heart failure, hypertension, and cardiac arrhythmias [27]. In adults with CF, OSA can also affect neurobehavioral function as seen with greater levels of fatigue and lower levels of activity and mood. Poor sleep can be a symptoms burden in CF and should be screened routinely with consideration for OSA as a contributing cause. Obesity also predisposes patients to developing OSA, making it another risk to be wary of when caring for PwCF.

7. CF Liver Disease

Cystic fibrosis associated liver disease (CFLD) is the third leading cause of mortality in CF patients [28]. It is estimated that the prevalence of CFLD increased from 203.4 to 228.3 per 1000 adult patients during 2008–2013 [28]. Adult-onset CFLD is being increasingly reported, and while the true prevalence of CFLD in adults varies, it may be approaching as high as 40% [28–30]. Advanced CFLD with portal hypertension affects about 7% of PwCF [28–30]. CFLD is associated with male sex, F508del homozygosity, and previous history of meconium ileus [28]. CFTR dysfunction can give rise to abnormally viscous biliary secretions, ductal obstruction, and subsequent hepatic cirrhosis. It is estimated that up to 40% of patients can develop liver disease throughout their lifetime. CFLD incidence has been shown to increase between 1% and 2% every year from birth up to 25 years of age [28]. A small percentage of these patients will develop cirrhosis within the first decade of their lives. Patients can develop complications from portal hypertension and subsequent variceal bleeding. PwCF and CFLD have more than three times the risk of death compared to those without liver disease [28,30]. Treatment of CFLD with ursodeoxycholic acid has remained controversial, although it is well tolerated and commonly used in practice. There are no current medical therapies available to reverse liver disease in CF, and patients with uncomplicated CF cirrhosis likely do not benefit from liver transplantation. Mendizabal et al. compared the outcomes of 203 patients with CF vs. patients without CF who underwent a liver transplant and concluded that by five years post-transplantation, both children and adults with CF had lower survival rates than non-CF patients [28,31]. The severity of liver disease alone is not sufficient for determining optimal timing for liver transplant, and other factors such as the stage of lung disease must also be strongly considered. The prevalence of CFLD has been increasing in the past few years, and the burden of CFLD has not diminished especially in CF patients in late adolescence and early adulthood. Annual screening with abdominal examinations, radiographic and laboratory testing (e.g., liver function and coagulopathy testing) is recommended in CF patients with CFLD [28,31]. Some hepatic abnormalities include gallbladder changes, hepatic steatosis, and sclerosing cholangitis. Serum markers of liver disease are frequently monitored following the initiation of HEMT. A key area of interest is the long-term effect of HEMT in preventing the development of hepatic fibrosis and advanced CFLD, which will hopefully be elucidated with further observational studies [32]. A diagnosis of liver cirrhosis can be a barrier to lung transplantation; thus, it is important to understand CFLD in order to develop new strategies to prevent progressive liver damage.

8. Bone Health

CF bone disease (CFBD), primarily due to osteoporosis, is a well-recognized morbidity in CF, the prevalence for which can be guaranteed to increase among the aging CF population. Fracture risks have been estimated to be twice as high in PwCF compared to the general population. Poor bone health contributes to increased prevalence of kyphosis in PwCF and reduced lung function, and negatively affects tolerability to perform effective airway clearance techniques [33]. CFBD affects less than 5% of children with CF, 20% of

adolescents with CF, and up to 65% of patients older than 45 years [34]. Bone mineral density (BMD) in the pediatric CF population age is half that of the non-CF population [34]. In young adults with CF, average BMD of the spine and distal radius is also reduced [34]. These findings have not changed over a 15-year period since 1995, despite improvements in lung function and with diagnosis and treatment for vitamin D deficiencies [35] This observation demonstrates that the underlying predisposition for CFBD is multifactorial and includes not only vitamin D deficiency and a chronic inflammatory state from CF lung disease, but may also be attributed to other established causes such as pancreatic insufficiency, vitamin K deficiency, systemic corticosteroid use, CFRD, hypogonadism, malnutrition, and physical inactivity [35]. Intermittent episodes of pulmonary exacerbations (Pex) play a role by driving inflammatory cytokine levels (e.g., tumor necrosis factor- α) which potentially leads to increased levels of osteoclast precursors and increased osteoclast activity, bone resorption, and decreased bone formation [36,37]. With antibiotic treatment for Pex, osteoclast activity is reduced back to baseline; however, cumulative bone loss will have occurred over time with every Pex.

The CFF consensus statement recommends BMD screening with dual energy X-ray absorptiometry (DXA) as the gold standard for all adults 18 years and older with CF and for children at least 8 years old with any of the following clinical criteria: (1) ideal body weight is less than 90% of the goal; (2) forced expiratory volume in 1 s (FEV1) is less than 50% predicted; (3) glucocorticoid use of greater than or equal to 5 mg/day for at least 90 days per year; (4) delayed puberty; and/or (5) a history of fractures [38]. At 20 years of age and older, BMD is measured at the lumbar spine and proximal hip (see Table 1 for CFF guidelines for bone health screening). Preventative care through screening, early diagnosis, and treatment are an essential part of CF care. Nutritional management is a major part of bone health, and registered dieticians within CF care centers and clinicians should be attentive to routine monitoring. Treatment includes bisphosphonates such as alendronate, risedronate, and ibandronate, which act by inducing osteoclast apoptosis, thereby inhibiting bone resorption [38]. Another available treatment is denosumab, a monoclonal antibody against receptor activator of nuclear factor $\kappa\beta$ ligand (RANKL), that inhibits osteoclast formation and reduces bone resorption.

Table 1. Cystic Fibrosis Foundation guideline recommendations for bone health monitoring in people with CF [39]. CFRD = cystic fibrosis related diabetes; Pex = pulmonary exacerbation; BMD = bone mineral density.

BMD T/Z Score	Interpretation	Management	Follow-Up DXA Screening
$\geq$1.0	Normal	• Monitor • Maintain nutritional health	Repeat screening every 5 years
Between -1.0 and -2.0	Osteopenia	• Address calcium, vitamin D insufficiency • Minimize corticosteroid use • Improve glycemic control in CFRD • Optimize respiratory health to reduce Pex • Consider bisphosphonate therapy in patients with history of fragility fractures, BMD loss of > 3–5%, and/or are awaiting transplant	Repeat every 2 to 4 years
≤ -2.0	Osteoporosis	• Start treatment (e.g., bisphosphonate, denosumab)	Annually

9. Cancer

Prior to therapeutic advancements with HEMT, the prevalence of cancer in CF was rare as it was primarily a pediatric disease due to short life expectancy. Between 1971 and 1986, a study in the United Kingdom identified 7 children with CF out of 20,071 children registered with childhood cancer [40]. With improved life expectancy and more than half of the CF population now living to an adult age, cancer incidence will likely increase with age. The U.S. CFF Patient Registry data from 1990 to 2009 revealed an increased prevalence of digestive tract cancer, testicular cancer, and lymphoid leukemia [41]. There

are several potential risks for cancer in PwCF. Repeated exposure to radiation from X-rays and CT scanning over the lifetime of a person with CF is concerning for the development of acute lymphoblastic leukemia and thyroid cancer [41]. Another mechanism for increased cancer risk lies in the evidence that the CFTR gene serves as a tumor suppressor gene in the intestinal tract in humans and animal models [42,43]. Reduced CFTR expression and carrier status for CFTR mutation, even in people without a clinical diagnosis of CF, are recognized as risk factors for colorectal cancer (CRC), cancers of the gallbladder and biliary tract, thyroid cancer, and unspecified NHL [42–44].

Reported digestive tract cancers include those of the esophago-gastric junction, biliary tract, small bowel, and colon. The risk of CRC in adults with CF is 5–10 times greater than the general population and is associated with carrier status for the more severe CFTR mutations, (classes I, II, and III) compared to the milder classes (IV and V) which have residual CFTR function. CF comorbidities and the pathophysiology itself also contribute risk to digestive tract cancers. For example, dysfunctional CFTR activity at the intestinal epithelia level can lead to dehydration, promote bacterial overgrowth, which can disrupt the gut microbiome and epithelial turnover, contributing to cancer risk [41,45,46]. Common CF comorbidities of diabetes, gastroesophageal reflux, inflammatory bowel disease, vitamin D deficiency, and high-fat diets are recognized risk factors for digestive tract cancers.

Through a multidisciplinary task force, the CFF published CRC screening recommendations specific to PwCF, recommending colonoscopy as the screening examination in PwCF beginning at age 40, with re-screening every 5 years. Earlier re-screening by 5 years as opposed to 10 years in the general population is based on the high rate of adenomatous polyps and advanced adenomas within 5 years [46,47]. Bowel preparation for colonoscopy requires a more intensive regimen, consisting of multiple small-volume washes over one larger volume regimen to fully optimize colonoscopic visualization [45,47]. Procedures other than colonoscopy are not recommended at this time; flexible sigmoidoscopy is limited to the descending colon, and computed tomography (CT) colonography does not provide a means for biopsy. Currently, there are not enough data to recommend stool-based tests. Positive screening with adenomatous polyps requires surveillance colonoscopy in 3 years or sooner if indicated. CF recipients of solid organ transplants who are 30 years of age or older and have fully recovered from their transplant should have CRC screening within 2 years of transplant unless they had a negative colonoscopy within the past 5 years. Rescreening for transplant recipients is every 5 years.

PwCF who are post-lung transplant recipients are at a 10-fold higher overall cancer risk compared to the general population, with the most common cancer among CF patients being non-Hodgkin lymphoma (NHL), followed by CRC [48]. Post-transplant lymphoproliferative disease (PTLD) consists mainly of abnormal B-cell proliferation occurring within one year of transplant [49]. Based on the International Society for Health and Lung Transplantation Registry, 17% of a cohort of 30,598 adult recipients of lung transplants between 1999 and 2011 had CF; PTLD developed in 2% of CF recipients compared to 1% for non-CF recipients in this cohort [50]. Recipient seronegativity for Epstein–Barr virus (EBV) and cytomegalovirus (CMV), and EBV and CMV mismatch were found to be of higher prevalence among CF compared to non-CF patients in the cohort. Awareness of PTLD is crucial as clinical presentation can be extremely variable. Mainstay treatments include antiviral medications and immunoglobulin therapy, reduction of immunosuppression, surgical resection and irradiation therapy, and chemotherapy [50].

Since HEMT enhances CFTR activity, it is hypothesized that long-term use may reduce cancer risk; however, this remains to be seen as we follow registry data and future comorbidities. Certain malignancies are known to be of higher prevalence in the CF compared to the general population, and therefore need special consideration. Clinicians should be aware of important cancer screenings that are both unique to PwCF, but also the standard of care in the general population.

10. Conclusions

The advent of HEMT over the last decade has changed the course of treatment, improving quality of life through exacerbation prevention, but also improving life expectancy by decades for PwCF. Until a cure for CF is found, morbidity directly related to CF remains significant. The CF population has always needed to contend with unique risk factors affecting morbidity and mortality, but as a population that is beginning to experience increased longevity, with this will come the need for new screening guidelines focusing on preventative care. The general population not afflicted with CF has important guideline-based screening measures for diabetes, dyslipidemia, cardiovascular health, bone health and malignancy. All of these same considerations must now be applied to the aging CF population, but with consideration for the pathophysiology which raises their inherent likelihood for adverse outcomes. More research is necessary to follow PwCF longitudinally to devise proper population-specific screening guidelines. In the interim, CF care teams must remain vigilant in the care of PwCF according to accepted guidelines, which will likely evolve with future incorporation of age-related comorbidities.

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Review

Evolving Nutritional Needs in Cystic Fibrosis

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Abstract: The course of cystic fibrosis (CF) as a nutritional illness is diverging since the introduction of highly effective modulator therapy, leading to more heterogeneous phenotypes of the disease despite CF genetic mutations that portend worse prognosis. This may become more evident as we follow the pediatric CF population into adulthood as some highly effective modulator therapies (HEMT) are approved for those as young as 1 year old. This review will outline the current research and knowledge available in the evolving nutritional health of people with CF as it relates to the impact of HEMT on anthropometrics, body composition, and energy expenditure, exocrine and endocrine pancreatic insufficiencies (the latter resulting in CF-related diabetes), vitamin and mineral deficiencies, and nutritional health in CF as it relates to pregnancy and lung transplantation.

Keywords: cystic fibrosis; nutrition; highly effective modulator therapy; body mass index; pancreatic insufficiency; cystic fibrosis-related diabetes; cystic fibrosis pregnancy

1. Background

Nutritional manifestations of cystic fibrosis (CF) are a principal focus of interest in the evolving landscape of CF care. People with CF (PwCF) have a higher prevalence of malnourishment compared to the general population as a consequence of its underlying pathophysiology in multiple organ systems, particularly the pancreas. Historically, CF has been linked to nutritional malabsorption causing failure to thrive and death in children. In 1938, autopsy findings from pediatric patients with CF were reported by Dr. Dorothy Andersen to show extensive pancreatic ductal obstruction and fibrosis and mortality due to severe malnutrition [1]. About 90% of individuals suffer from exocrine pancreatic insufficiency (PI) which causes malabsorption of lipids and fat-soluble vitamins, weight loss, diarrhea, risk for zinc deficiency, and stunted growth in children. CF is, in fact, also strongly linked to high-energy and nutrient needs due to its respiratory manifestations, increased metabolic rate and chronic inflammatory state [2]. Additionally, CF-related diabetes, anorexia, dysphagia, bone disease, and chronic medication usually result in nutritional concerns as well. CF nutritional care encompasses nutrition education, counseling, specialized diets to maintain body mass index (BMI) goals, and respiratory and overall health. Nutritional and lung health are strongly correlated; PwCF who have optimized nutrition and BMI have reduced risk of infection, morbidity and mortality [2].

Earlier CF therapies targeted nutritional malabsorption and respiratory clearance and infections with the use of pancreatic enzyme replacement therapy (PERT), inhaled antibiotics, and mucolytic treatments. Fast forward to the era of cystic fibrosis transmembrane conductance regulator (CFTR) modulators, also referred to as highly effective modulator therapy (HEMT), and the majority of PwCF are now benefiting from these treatments that aim to restore CFTR activity at the cellular level. Modulators correct the defective

CFTR protein and improve chloride ion transport in epithelial cells of multiple organs, thereby improving organ function and delaying organ injury [3]. For PwCF who qualify for modulator therapy, it is an imperative standard of care. Commercially available CFTR modulators include ivacaftor (IVA), lumacaftor/ivacaftor (LUM/IVA), and tezacaftor/ivacaftor (TEZ/IVA). These drugs have demonstrated various effects on nutrition, weight, and body mass index (BMI) over the last decade. For example, IVA had positive effects on weight gain, yet TEZ/IVA and LUM/IVA produced either no significant changes or mixed effects in weight and BMI [4]. The latest approved modulator therapy is known as elexacaftor/tezacaftor/ivacaftor (ETI), tradename Trikafta®. Trikafta is a triple drug modulator therapy, and was approved in 2019 by the United States Food and Drug Administration (FDA) for PwCF ages 12 and older. In contrast to prior modulators' variable effects on anthropometric markers, ETI has shown significant improvements in weight and BMI. In 2021, ETI approval expanded to those 6 years of age and older with at least one F508del mutation and has made dramatic improvements in lung function as measured by forced expiratory volume in one second (FEV1), reduced pulmonary exacerbations and increase in weight and BMI [4,5]. About 90% of the CF population in the United States carries at least one F508del mutation and are therefore eligible for or receiving treatment with ETI [6]. In 2023, ETI became FDA approved for children with CF ages 2 to 5 years who have at least one copy of the F508del mutation or certain mutations that are responsive [7].

Following ETI use, long-term oxygen, non-invasive ventilation, and enteral nutrition needs have reduced by up to 50% in PwCF, as well as reductions in hospitalizations and lung transplantations [8]. The PROMISE study (A Prospective Study to Evaluate Biological and Clinical Effects of Significantly Corrected CFTR Function) is a real-world observational study evaluating the effectiveness of ETI across multiple clinical outcomes for up to 4.5 years (NCT04038047) [9]. One aspect to be explored is to determine whether fecal elastase values, which are diagnostic of PI (i.e., fecal elastase $\leq$ 200 μg/g), may increase in younger PwCF while on HEMT; this would support the hypothesis that ETI may safeguard against or normalize exocrine pancreatic function to some degree [10]. These positive changes were already observed in case studies of older children with PI who began IVA, and thus ETI may provide similar benefits [11–13].

The clinical changes seen thus far with ETI mean a promising future for how HEMT will affect nutrition in PwCF. Emphasis also remains on the continued nutritional care of patients who are ineligible for HEMT or remain underweight or malnourished despite HEMT use, such as those with advanced CF lung disease. This review covers the impact of HEMT on anthropometrics, body composition, energy expenditure, exocrine PI, CF-related diabetes, vitamin and mineral deficiencies, and nutritional health in CF as it relates to pregnancy and lung transplantation.

2. Anthropometric Parameters

Anthropometry, the study of body measurements, has been invaluable in our understanding of nutrition and overall growth, and medical and physical health in PwCF. Anthropometric outcomes commonly evaluated in clinical CF care and investigational studies are BMI percentiles and absolute value for pediatric and adult CF patients, respectively. Values that are associated with optimal lung function for pediatric patients include 50th percentile or above in weight-for-length for individuals up to age 2, greater than 50th percentile for BMI for ages 2–20, and for adults, a BMI of at least 22 for females and 23 for males. While weight gain is helpful in order to depict overarching anthropometric changes, significant detections in BMI are more pertinent due to the established correlation between BMI and lung function. As previously mentioned, ETI improves BMI status in PwCF and potentially PI, enabling BMI milestones to be achieved sooner and more easily with a probable decrease in caloric supplementation. These improvements were observed in people carrying at least one copy of the F508del mutation, across multiple published studies of varying duration; see Table 1.

While there are fewer studies assessing anthropometrics in the pediatric CF population, especially for ages 6–11, weight and BMI outcomes parallel that of adult trials [14]. ETI is associated with substantial weight gain as seen in underweight individuals with eventual normalization of BMI, although in PwCF with a normal BMI, continued weight gain may potentially lead to increased prevalence of obesity [15]. It is unclear whether ETI-associated weight gain is compensatory or excessive, especially considering this weight gain may be a response to years of malnutrition. Burgel et al. suggests that the degree of weight gain may be attributed to previously achieved weight gain from prior CFTR modulator use such as that observed in F508del homozygous individuals treated with LUM/IVA [8]. However, Graeber et al. showed weight gain after ETI to be equivalent or greater in patients treated with a modulator at baseline compared to no prior modulator [16]. Improved weight following HEMT may also be a downstream effect of a reduced hyperinflammatory state and reduced energy expenditure. In most PwCF, energy expenditure is higher than that of those without CF due to a systemically higher inflammatory state, particularly from the respiratory system where there is chronic bacterial colonization and airway inflammation. Evidence supports HEMT-associated reduction in such inflammation which can conserve energy and body weight [17].

Weight-related outcomes for PwCF with pancreatic sufficiency and/or CF-related diabetes (CFRD) are inconclusive. Pancreatic-sufficient patients did not have any significant weight change following ETI therapy across studies, likely because their baseline weight and BMI were higher compared to those with PI. In addition, individuals with CFRD had mixed results with weight gain. Scully et al. showed that in a CFRD cohort, there was an overall statistically significant increase in body weight by 5.6 kg ($p < 0.045$), but a non-statistically significant increase in BMI of 1.3 kg/m^2, likely because a larger weight increase is needed to detect a change in BMI [18]. Petersen et al. found a significant annualized BMI gain of 1.47 kg/m^2 per year based over a 20-month period in patients with CFRD [19].

While significant changes in weight and BMI have been seen with ETI, it is vital to continue to vigorously trend the anthropometric effects of HEMT in PwCF. The anticipated "Strength and Muscle Related Outcomes for Nutrition and Lung Function in CF" (STRONG) trial is a prospective observational trial that will be studying markers of anthropometrics, body composition, sarcopenia and frailty and compare them to dual-energy X-ray absorptiometry (DXA) output in patients of 18 years and older with CF (NCT05639556). The data gathered will also be analyzed for psychosocial and clinical outcomes in the context of varying lung function severity [15]. Long-term observations will help us to further understand the effect of HEMT on nutrition and elucidate questions such as whether weight gain plateaus, signaling a metabolic equilibrium, or maintains a rising trajectory, posing a risk for obesity.

Table 1. Effect of elexacaftor/tezacaftor/ivacaftor (ETI) on weight and body mass index outcomes in individuals who possess at least one F508del mutation. In the referenced observational studies, all participants were initiated on ETI, and changes in BMI/weight are noted. For interventional trials, ETI was compared to control (either placebo or tezacaftor/ivacaftor). * Anthropometrics based on Wang et al. (2012) Handbook [20]. BMI = body mass index; RCT = randomized controlled trial, CFTR = cystic fibrosis transmembrane conductance regulator; ETI = elexacaftor/tezacaftor/ivacaftor; LS = least squares mean; CFRD = cystic fibrosis related diabetes.

Study	Study Design and Duration	Age Range	F508del Mutation	The Effect of ETI on Weight/BMI
Burgel et al. (2021) [8]	Prospective observational study; 8 months	12+	Homozygous, Heterozygous	All participants were initiated on ETI and had an average weight gain of + 4.2 kg ($p < 0.00001$) No significance or mentions of BMI

Table 1. *Cont.*

Study	Study Design and Duration	Age Range	F508del Mutation	The Effect of ETI on Weight/BMI
Carnovale et al. (2022) [5]	Retrospective observational study; 48 weeks	12+	Homozygous, Heterozygous	Mean (95% CI) absolute increase in BMI after various treatment intervals: - 4 weeks: 0.66 (0.37, 0.95; $p < 0.0001$) - 12 weeks: 1.57 (1.19, 1.94; $p < 0.0001$) - 24 weeks: 2.02 (1.56, 2.48; $p < 0.0001$) - 48 weeks: 2.08 (1.63, 2.52; $p < 0.0001$)
Fajac et al. (2022) [21]	Randomized controlled trial; 24 weeks	12+	Homozygous, Heterozygous	For LS mean absolute change, ETI group had significant increases in BMI compared to controls: - F508del heterozygous: 13.2 (9.8, 16.6) - F508del homozygous: 7.5 (1.6, 13.3)
Graeber et al. (2021) [16]	Prospective observational study; 16 weeks	12+	Homozygous, Heterozygous	All participants started ETI and had the following changes in BMI: - Heterozygous: +1.1 (0.4–1.9), $p < 0.001$ - Homozygous with tezacaftor use at baseline; +1.2 (0.5–1.5), $p < 0.001$ - Homozygous without CFTR modulator at baseline; +0.3 (0.0–1.3), $p < 0.01$
Heijerman et al. (2019) [22]	RCT, double- blind, active controlled; 4 weeks	12+	Homozygous	Weight status/BMI in ETI compared to tezacaftor/ivacaftor (+0.60 kg/m^2, 95% CI 0.41–0.79; nominal * $p < 0.0001$)
Middleton et al. (2019) [23]	RCT, double-blind, placebo-controlled; 24 weeks	12+	Heterozygous	Compared to placebo, participants on ETI had the following changes: BMI > 20 y: +1.04 kg/m^2 ($p < 0.001$)—BMI z-score < 20 y: +0.3 (95% CI 0.17–0.43)
Nichols et al. (2022) [24]	Prospective, observational study; 6 months	11+	Homozygous, Heterozygous	After 6 months of ETI treatment for all participants: +1.24 kg/m^2 for BMI and +0.3 for BMI-z-score
O'Shea et al. (2020) [25]	Observational Study; 7 months	Adults, age not defined	Homozygous, Heterozygous	All participants had an average increase in BMI by 1.5 kg/m^2 ($p < 0.00001$)
Petersen et al. (2021) [15]	Retrospective study; 14 months	20+	Homozygous, Heterozygous	All patients were initiated on ETI and significant changes in BMI were detected in all genotypes with a mean difference of +1.65 kg/m^2: - Highly significant annualized difference in BMI trajectory = 1.47 kg/m^2/yr (95% CI: 1.08 to 1.87, $p < 0.0001$) - Highly significant changes ($p < 0.0001$) in annualized BMI trajectory for subgroups: CFRD, pancreatic insufficiency, prior modulator use, no prior modulator use, heterozygous, and homozygous; no change in BMI status for pancreatic sufficient

Table 1. *Cont.*

Study	Study Design and Duration	Age Range	F508del Mutation	The Effect of ETI on Weight/BMI
Scully et al. (2021) [18]	Prospective cohort; 10 months	18+	Homozygous, Heterozygous	No significance in change in BMI after ETI therapy for total cohort: - Change in BMI was greatest in CFRD subgroup +1.3 kg/m^2 (p = 0.082). - Significant increase in body weight by 5.6 kg in CFRD subgroup (p < 0.045)
Sutharsan et al. (2021) [26]	RCT, double-blind, active- controlled; 24 weeks	12+	Homozygous	BMI increase for ETI compared to tezacaftor/ivacaftor: - For ≥20 y: [95% CI: 1.44 kg/m^2 (1.07–1.82)] - For <20 y: BMI z-scores [95% CI: 0.51 (0.20–0.82)]
Zemanick et al. (2021) [14]	Non-randomized controlled trial; 24 weeks	6–11	Homozygous, Heterozygous	All pediatric patients were initiated on ETI and had the following significant z-score changes: - Weight-for-age z-score: +0.25 [0.16–0.33, p < 0.001] - BMI-for-age z-score: +0.37 [0.26–0.48, p < 0.001]

Energy Expenditure and Body Composition

More research is needed to understand the impact of HEMT on body composition, including fat mass and fat-free mass, energy expenditure, and dietary needs. Consumption of a fat-containing food with HEMT is imperative for absorption. Individuals with PI must also take PERT in conjunction with a fat-containing food and the medication. This factor may lead to an overall increase in caloric intake compared to pre-HEMT initiation, albeit LUM/IVA and TEZ/IVA did not result in significantly comparable weight gain compared to ETI [4]. Thus, the observed increased weight and fat mass may be attributed to the physiological effect of the drug as opposed to changes in caloric intake.

Body composition has become a key determinant of clinical outcomes in CF. Fat-free mass is positively correlated with lung health and overall outcomes while the opposite relationship can be said for fat mass as well [26]. With significant increases in weight and BMI across studies for those with CF and PI, understanding the way in which this weight is being deposited in the body has been a topic of investigation. Prior studies on IVA found an increase in fat mass and either an unchanged or increased effect on fat-free mass [27]. Gur et al. (2022) showed promising results for 18 individuals with CF taking ETI regarding the changes in body composition, bone mineral density (BMD), and exercise capacity. An increased weight of 2.5 kg (p = 0.05) and BMI of 0.9 kg/m^2 (p = 0.05) was observed. Hip and spine BMD increased significantly in the treatment group; (0.73 ± 0.098 to 0.81 ± 0.12 g/cm^2 for hip [p = 0.017]; and 0.76 ± 0.14 to 0.82 ± 0.14 g/cm^2 for spine [p = 0.025]) compared to no difference in hip or spine BMD for the control group [28]. These results provide promising information for how ETI may change the progression of CF bone diseases. Additionally, there was a significant increase in lean body mass (p = 0.017) and a non-statistically significant change in fat mass percentage (p = 0.069) [26]. Six-minute walk testing used to assess endurance and aerobic capacity also significantly improved by a distance of approximately 50 additional meters (p = 0.046) [26].

Gur et al. (2022) suggests some mechanisms that may be responsible for the changes above, including reduced resting energy expenditure, increased fat absorption and decreased gut inflammation. Increased lean body mass is associated with reduced inflammation and improved lung function and CF disease severity. This is early evidence of how ETI is truly changing body composition and physiology [29]. Extended observations may reveal the potential for increased physical activity and weight gain through muscle mass, underscoring the need for multidisciplinary coordination of care between a dietitian and physical therapist to optimize the interplay between nutritional and physical health. While smaller case series have yet to demonstrate improved physical activity with muscle mass following initiation of ETI, continued research of longer duration may reveal more promising data [30].

3. Comorbidities with Nutritional Implications

CF, although primarily a lung disease, also causes a variety of gastrointestinal (GI) manifestations which are important for diagnosis, prognosis, and quality of life [31]. Common comorbidities include exocrine and endocrine PI, acute pancreatitis, gastroesophageal reflux disease, small-intestinal bacterial overgrowth, distal intestinal obstructive syndrome, and CF liver disease. GI-related symptom burden is high and includes abdominal pain, bloating, abnormal stools, constipation, and heartburn, found in as many as 80% of PwCF [32]. The nutritional implications of GI manifestations in CF are very influential in growth and development in children and malnutrition in all PwCF. Here, we review PI, vitamin and mineral deficiencies, CF-related diabetes, CF-related liver disease, and kidney stone disease in CF.

3.1. Exocrine Pancreatic Insufficiency

Exocrine pancreatic insufficiency (PI) is the most common GI complication of CF. The CFTR mutation contributes to impaired bicarbonate secretion, as well as obstruction of the pancreatic ducts by mucus, leading to fibrotic damage of the pancreas.

This damage causes inadequate pancreatic enzyme production, specifically of lipase, protease and amylase, which are important for digestion, absorption, and nutrient utilization of fats, proteins and carbohydrates, respectively. A fecal elastase-1 test measures the amount of elastase, which when low in level (fecal elastase ≤ 200 $\mu g/g$) is diagnostic of PI. Malabsorption due to PI can cause significant nutritional consequences of weight loss, malnutrition, and deficiencies in fat-soluble vitamins A, D, E, and K. Symptoms of PI include steatorrhea, abdominal bloating and distention. Most people with CF and PI will require pancreatic enzyme replacement therapy (PERT) to aid with nutrient utilization and prevent adverse nutritional outcomes and manage PI symptoms. Acid-suppressing medications including histamine H2 antagonists and proton pump inhibitors are also often prescribed to enhance the efficacy of PERT, as they work more effectively in a basic pH. Potential side effects of PERT may include abdominal cramps, nausea, vomiting, constipation, diarrhea, bloating and, in people taking high doses of enzymes, fibrosing colonopathy. It has been suggested that fibrosing colonopathy could be due to the presence of methacrylic acid copolymer in the capsule and not due to the high doses of enzymes [33]. Since the discontinuation of the use of the polymer there have been no reports of fibrosing colonopathy [34]. Polypharmacy is an enormous lifelong burden in majority of PwCF. A call for efforts to simplify or reduce medical burden was conveyed as one of CF patients' top priority. PERT is no exception as it needs to be taken with food several times per day in numerous pill counts.

As mentioned previously, the PROMISE study aims to follow the real-world, long-term impact of ETI on GI comorbidities; one hypothesis is that it may delay the onset of PI or restore some exocrine function of the pancreas to the point of feasibly reducing PERT dosing [10,35]. Anecdotally, since the implementation of HEMT therapy, CF providers are recognizing the potential opportunity to reduce and/or withdraw PERT and acid-suppressing medications, but there is not enough evidence at this time to support this

practice [36]. It is essential to continue to monitor for PI-related signs and symptoms and reassess PERT dosing at every clinic encounter.

3.2. Vitamins and Mineral Supplementation

Deficiencies in vitamins and minerals are particularly common in PwCF and PI, although other contributing factors include uncontrolled fat malabsorption, poor adherence to PERT, and comorbidities such as short bowel syndrome and CF-related liver disease. Regular monitoring of fat-soluble vitamins and minerals annually and following supplement dosing changes are recommended. See Table 2.

3.2.1. Vitamin A

Vitamin A is important for embryonic development and healthy bones, vision, and immune system. Vitamin A also has anti-oxidant properties that are protective in respiratory epithelial cells and overall cardiovascular health [37]. In CF, normal levels of vitamin A are linked to overall better lung function. Vitamin A can be consumed in two forms: preformed vitamin A (retinol, retinyl esters) and provitamin A carotenoids, which primarily include beta-carotene, alpha-carotene, and beta-cryptoxanthin in the human diet. Preformed vitamin A is found in foods such as dairy, liver, eggs, and fortified foods such as breakfast cereals. Provitamin A is found in plant-based foods (e.g., fruits, vegetables). Beta-carotene conversion into retinol occurs during states of deficiency and high oxidative stress [38]. Current recommendations for Vitamin A level range from 450–3000 mcg retinol equivalents per day; however, recommendations are not based on clinical trials [38,39]. Vitamin A deficiency is becoming rare in PwCF, although annual monitoring is still suggested based on its risk factor associated with PI. Elevated retinol serum is another clinically relevant concern related to vitamin A level and has been associated with liver and bone disease and increased intracranial pressure in the general population [37].

3.2.2. Vitamin D

Vitamin D is important for normal bone mineralization and overall bone health by regulating calcium and phosphorus levels [40]. Compared to the general population, PwCF are at a greater risk of developing osteopenia and increased bone fractures. Causes for vitamin D deficiency include PI and decreased internal absorption, inadequate dietary intake or production via sunlight exposure, glucocorticoid use, and reduced fat stores. Vitamin D forms are ergocalciferol and cholecalciferol (Vitamin D3), the latter being the recommended form for supplementation. The primary source of ergocalciferol is plants and can be manufactured synthetically, whereas cholecalciferol is synthesized in the body from 7-dehydrocholesterol after exposure to the sun and is more effective in increasing vitamin D levels in the bloodstream longer than ergocalciferol [41]. The recommended daily goal for vitamin D ranges between 800–2000 IU daily and is the same recommended dose as the general population. Vitamin D should be monitored annually at the end of the winter season to assess the effects of limited sun exposure and to adjust supplemental doses as needed [41].

3.2.3. Vitamin E

Vitamin E plays a role in protecting the body against harmful oxidative damage. Normal vitamin E levels range from 23 μmol/L to 46 μmol/L, and plasma levels over 80 μmol/L are considered excessive. There are eight forms of vitamin E, but alpha-tocopherol is the only form that meets human requirements. Foods rich in vitamin E include nuts, seeds, and oils. Vitamin E is best measured using a vitamin E to total cholesterol ratio, as vitamin E circulates in the blood bound to lipoproteins. Thus, elevated total cholesterol would falsely lower vitamin E levels unless both values are interpreted using a ratio. Vitamin E/lipid ratio (mg/g) is calculated based on the following formula: vitamin E (mg/L) ÷ [Total Cholesterol (g/L) + Triglycerides (g/L)]. Normal range for this ratio is 1.4–5.71 mg/g, while deficiency is indicated by a ratio < 0.8 mg/g [42]. Vitamin E deficiency is rare, but can have

detrimental effects, such as peripheral neuropathy, myopathy, and pigmented retinopathy. The current recommended dosages of vitamin E range from 100 IU to 400 IU per day for PwCF [43]. The specific mechanism of action for most of its effect is still unknown. Elevated vitamin E is common in patients who are post-solid organ transplant and is associated with risk for major bleeding.

3.2.4. Vitamin K

Vitamin K is essential for blood clotting, bone formation, and cellular growth and is protective against free-radical induced cellular damage. Dietary sources are dark leafy green vegetables, such as kale, broccoli, and spinach. Causes of vitamin K deficiency are PI and frequent antibiotic use (causing changes in intestinal flora), commonly observed in PwCF. Vitamin K monitoring is assessed using prothrombin time and intake should be monitored carefully in patients on anticoagulants. Goal daily level recommended ranges; the Cystic Fibrosis Foundation (CFF) consensus guidelines recommend a vitamin K dose of 0.3–0.5 mg daily for children and 2.5–10 mg weekly for adults [44]. Additional vitamin K supplementation is commonly prescribed short- and long-term for patients with history of hemoptysis and concern for recurrence, but there are no clinical trials assessing the efficacy and dosing of vitamin K in the prevention of hemoptysis. In a retrospective observational study, vitamin K was prescribed to 38 adult CF patients in the setting of hemoptysis during an acute pulmonary exacerbation. Average vitamin K dose was 10 mg and two patients developed a thromboembolism. The benefit of vitamin K in CF patients with hemoptysis remains unclear [45].

With HEMT, vitamin deficiencies may be lessening, leading to overdosing in supplementation. Sommerburg et al. (2021) studied the effect of LUM/IVA on concentrations of lipid-soluble vitamins in CF patients. The study showed that vitamin D levels increased slightly after one year although this was not significant. Retinol levels increased following two years of LUM/IVA treatment; vitamin E levels decreased after one and two years on LUM/IVA [46]. Individuals might be prescribed modular formula vitamins or may need adjustments in their vitamin regimen. Modulator formula vitamins are lower in preformed vitamin A (retinol) and higher in pro-form vitamin A (beta carotene). Since most vitamin recommendations are not based on clinical trials, future research is needed to determine proper vitamin supplementation, especially in patients on HEMT.

Table 2. Vitamins and minerals of interest in cystic fibrosis. The above-listed vitamins and minerals are commonly found to be deficient as a result of poor absorption and pancreatic insufficiency. Daily goals of these vitamins and minerals are general recommendations from expert consensus rather than evidence-based practice and may not be CF-specific [47,48]. Adapted with permission from Sankararaman et al, 2022, John Wiley and Sons.

Vitamin/ Mineral	Dietary Sources	Important Roles	CF-Specific Relevance	Daily Goal Intake
Vitamin A	Dairy, liver, eggs, fortified foods (e.g., cereals)	Embryonic development, bone health, vision, Immune system	Deficiency may lead to poor eye, skin, and respiratory health	Infants: 1500 IU Toddlers: 5000 IU 4–8 years: 5000–10,000 IU Adults: 10,000 IU
Vitamin D	Salmon, tuna, milk, eggs, fortified cereal, cheese	Bone health	Higher prevalence of osteoporosis in CF versus general population	Infants: 400–500 IU 1–10 years: 800–1000 IU Adults: 800–2000 IU
Vitamin E	Nuts, seeds, oils	Antioxidant, protection of cells from oxidative stress	Protective of cell membranes, blood cells and nerves	Infants: 40–50 IU Toddler: 80–150 IU 4–8 years: 100–200 IU Adults: 200–400 IU

Table 2. *Cont.*

Vitamin/ Mineral	Dietary Sources	Important Roles	CF-Specific Relevance	Daily Goal Intake
Vitamin K	Leafy greens, liver, soybean, canola oil	Coagulable function, bone health	Essential for blood clotting, and strong bones	Infants/Toddlers: 0.3–0.5 mg Adults: 2.5–10 mg/weekly
Iron (Fe)	Red meat	Oxygen-carrying capacity		Infants: 0.27 mg Toddlers: 7 mg–11 mg 4–8 years: 10 mg Adults: 8 mg
Zinc	Red meat, chicken, fortified cereals, beans, nuts, whole grains	Immune system, wound healing		Infants: 1 mg/kg/day 4–8 years old: 15 mg/day Adults: 25 mg/day
Magnesium	Whole grains, nuts, legumes, avocado	Muscle and nerve function, bone health		Currently no formal guidelines for treatment of magnesium in CF
Calcium	Dairy, Milk	Bone health		Same recommendation as the general population Infants: 200 mg Toddlers: 700 mg 4–8 years: 1000 mg Adults 1000–1300 mg

3.3. Endocrine Pancreatic Insufficiency—Cystic Fibrosis-Related Diabetes

Cystic fibrosis-related diabetes (CFRD) is one of the most common extrapulmonary complications of CF. While CFRD only affects 2% of children, prevalence increases markedly with age, such that almost 20% of CF adolescents and 50% of CF adults will be diagnosed [49]. CFRD is associated with weight loss and reduced pulmonary status, and consequently an increase in morbidity and mortality [50]. Accordingly, there is great interest in the role of emerging CF therapies on the progression of CFRD. Despite the commonality and morbidity of CFRD, its pathogenesis has overlapping features shared by Type 1 diabetes mellitus (T1DM) and Type 2 DM. While the most well-understood cause of CFRD is decreased insulin secretion from the pancreas, it is likely a downstream consequence of defects in the CFTR protein encoded by mutations in the CF gene [51–53]. The CFTR protein is a chloride ion channel regulating the transport of chloride and bicarbonate in epithelial cells of many organs, the pancreas being one of them. In the presence of defective CFTR protein, thick secretions build up in the pancreatic ducts, leading to inflammatory and destructive injury to the pancreas and loss of insulin-producing beta cells. The lack of insulin is similar to that seen in T1DM, although the pathogenesis is not due to auto-antibodies as seen in T1DM [53,54]. Normal CFTR function is also responsible for beta-cell depolarization and release of insulin as well as alpha cell hyperpolarization and inhibition of glucagon release [51]. When CFTR is defective, there is dysregulation of beta and alpha cells, resulting in reduced insulin and increased glucagon release, respectively, and subsequently hyperglycemia. While HEMT has led to a significant improvement in the pulmonary and nutritional health of patients, there remains limited reports describing its role on glucose metabolism and its implications in the development and course of CFRD [52].

Large, randomized, controlled trials have yet to be seen reporting on the comprehensive impact of ETI on CFRD; such trials may not ever be feasible as HEMT is the current standard therapy for all eligible PwCF. However, a number of small studies have been published and add to our understanding of the role of ETI in CFRD. Korten et al. reported oral glucose tolerance test (OGTT) changes in 16 adolescents with CF 4–6 weeks after the initiation of ETI. Improvements at the 60-, 90- and 120-min intervals on OGTT were

noted, indicating improvement in glucose tolerance, but no significant changes in insulin and c-peptide levels were found [55]. Similarly, in a study of 20 pediatric and adult CF patients followed for 10.5 months after initiation of ETI, there was no change identified in insulin resistance or insulin secretion, as measured by the homeostasis model assessment (HOMA-IR) and c-peptide index, respectively [56]. Unlike Korten et al., however, they did not find any significant changes in the 60- or 120-min interval on the OGTT; nor were any changes found in HbA1c or in the mean value of total glucose increases during OGTT post initiation of ETI [56]. In a single-center retrospective subgroup analysis of 134 patients with CFRD, no improvement in HbA1c was found over a mean of 12 months after the initiation of ETI [22]. Lastly, in a prospective, single-center, observational study reporting outcomes of continuous glucose monitor (CGM) measures after the initiation of ETI in adult CF patients, CGM measures of glucose control improved at a median of 7.1 months after ETI initiation [12]. In the larger-scale prospective, multi-center PROMISE study, preliminary data reported from this study did not show consistent improvements in glycemic outcomes, although this was limited to LUM/IVA and did not report on ETI [35].

While our understanding of the effects of ETI on insulin secretion and glycemic outcomes continues to evolve, it is important to also consider the nutrition guidelines that impact current diabetes care. Historically, high-calorie, high-fat diets have been recommended for all CF patients with malnutrition, including those with CFRD. However, modern CFRD medical nutrition guidelines prioritize good nutritional status while maintaining normal blood glucose values [57]. Recently, a prospective study of 18 adults with CF demonstrated a correlation between lower glycemic index foods and more stabilized glucose values [58]. Additionally, physical activity has been proven to lower post-prandial glucose values in adults with CF [59]. Although the benefits of ETI on glucose-control outcomes have yet to be fully understood, ETI has led to improvements in caloric intake, gastrointestinal function, and intestinal absorption, all of which are thought to contribute to weight gain and improvements in malnutrition [51]. Paradoxically, this has also led to an increased prevalence of obesity [51] and hypercholesterolemia in PwCF [60]. How these downstream effects of ETI will specifically affect patients with CFRD is yet to be seen; there have been limited studies focusing on nutrition and lifestyle management for CFRD since the introduction of ETI. There is a strong need to better understand the role of HEMT on CFRD; it is clear though that as HEMT continues to improve nutritional status in PwCF and as the population grows, CFRD dietary interventions will have to evolve as well, including education on quantity and quality of macronutrients (e.g., carbohydrates) alongside physical exercise recommendations [60,61].

3.4. Macronutrient Needs for Patients with CF and Advanced Liver Disease

The liver is commonly affected in those living with CF and is the third leading cause of death in PwCF. CF-related liver disease (CFLD) with cirrhosis and without cirrhosis is prevalent in 3.1% and 3.3%, respectively, of CF patients in the USA [62]. The long-term effect of CFTR modulators remain unclear and is under investigation in the PROMISE study, although there is evidence of benefit in the liver [35,61]. In a small study of 20 patients treated with LUM/IVA, there was a reduction in hepatic steatosis [63]. Treatment with ETI at an early age may even reduce or prevent severe CFLD. CFLD encompasses multiple liver-involved manifestations, such as neonatal cholestasis, elevated liver enzymes, steatosis, gallbladder and biliary tract disorders, cirrhosis, and portal hypertension. CFLD is classified into three categories: preclinical disease, CFLD without cirrhosis and portal hypertension (PH), and CFLD with cirrhosis and PH [64]. Treatment for CFLD includes treating the underlying cause for liver disease and monitoring nutritional status through the assessment of adequate caloric intake, PERT dosing, vitamin supplementation, particularly of the fat-soluble vitamins A, D, E, and K, and insulin regimens for those with CFRD.

Patients with CFLD are at risk for malnutrition and micronutrient deficiencies due to decreased oral intake associated with anorexia, delayed gastric emptying, early satiety, increased energy expenditure, and malabsorption [65]. In CFLD, there is an estimated

higher daily caloric intake of about 130–150% above general recommendations. Dietitians and CF clinicians must pay particular attention to patients' intake of protein, fat, and carbohydrates. Patients with CFLD are more likely to need a higher intake of protein of 3 g/kg/day consumed in small, frequent daily portions to avoid stress on the liver [60]. Higher fat intake is also necessary to help meet increased energy demands in CFLD. In patients with cirrhosis, carbohydrates may not be properly utilized due to insulin resistance and glucose intolerance. They are at an increased risk of developing CFRD, and thus annual diabetes screening is important. Malnutrition is common in people with CFLD. Body mass index should not be the only indicator of nutritional status, rather the additional use of mid-arm circumference (MAC), triceps skinfold thickness, and hand grip should be used particularly in patients with ascites and/or edema. If patients cannot achieve adequate intake, enteral tube feeding such as nasogastric or gastrostomy tube can be used to maintain adequate nutritional status.

Nutritional supplementation may be necessary to provide additional protein intake, along with the use of PERT to optimize fat absorption, absorption of micronutrients and fat-soluble vitamins to prevent deficiency. PERT dosing should be monitored annually to ensure absorption of essential fatty acids and nutrients, but it is important to be aware that malabsorption can happen in advanced CFLD that is unrelated to PERT dosing. Fat-soluble vitamins A, D, E, K, should be monitored every 6 to 12 months [66]. All vitamins should be taken with meals and enzymes if patients have PI. Vitamins K and A should be closely monitored in those with CFLD. Vitamin K deficiency is very common in CF although it is more common in those with CFLD. Prothrombin time should be monitored to access vitamin K stores indirectly. Patients with CFLD should receive an additional 5–10 mg of vitamin K daily, depending on the prolongation of prothrombin time [67]. In cirrhosis, retinol-binding protein is low because of the absence of hypoalbuminemia, causing low retinol levels. Patients with low retinol levels of less than 20 mcg/dL should receive vitamin A supplementation. It is important to monitor vitamin A levels closely since high levels of vitamin A can lead to hepatic toxicity. Salt supplementation should be avoided for those with cirrhosis and portal hypertension due to the risk of developing or worsening ascites.

3.5. Nutritional Influences in Kidney Stone Disease in CF

People with CF are at higher risk for kidney stone disease (KSD) or nephrolithiasis, with reported prevalence rates of 4.6% to 5.7%, over twice that of the general population. Active KSD screening in CF has yielded as high as 21% prevalence [68–71]. KSD may present urgently or emergently with acute pain and urinary symptoms requiring acute care visits or hospitalization. Nearly 38% of CF patients require at least one surgical intervention for KSD, compared to 10–20% seen in the general population [69]. Surgical management and general anesthesia also carry risks for respiratory complications, particularly in patients with advanced CF lung disease. Within one year, KSD prevalence of 11% was reported in one adult CF center with almost equal distribution between first-time diagnosis versus recurrent KSD [72]. Dietary queries focusing on KSD risk revealed high intake of sodium, animal protein, caffeine and sweetened beverages, and suboptimal intake of calcium, potassium and water consumption, all of which predispose to nephrolithiasis [72].

KSD risk factors associated with CF are well-described and can be modifiable for prevention; these include diet, metabolic predispositions, and fat malabsorption as a sequela of PI. Calcium oxalate stones are the most common type of kidney stone found in PwCF. Urinary abnormalities that predispose to stone formation in PwCF include low urine output (i.e., dehydration), hyperoxaluria, hypocitraturia, and hypercalciuria [73,74]. PwCF are frequently prescribed antibiotics for pulmonary exacerbations; over time, frequent gut exposure to antibiotics can alter the GI flora. Antibiotics also decrease the breakdown of oxalate, causing hyperoxaluria. Reported mean age of onset for KSD in CF is early adulthood however stone recurrence is as high as 43% [69,73]. The CF community is seeing new emerging complexities in CF care in the era of HEMT and it is key that the CF care team has an increased awareness of new illnesses not commonly seen in PwCF in the

past. Increasing age of PwCF may be associated with increasing prevalence of KSD, and obesity may be on the rise as well. This highlights the need to address dietary preventative measures to reduce risk for KSD and its associated healthcare burden.

4. Nutritional CF Care in Unique Conditions

4.1. Nutrition in Pregnancy with Cystic Fibrosis

Advances in HEMT have led to an increased rate of pregnancies in PwCF, believed to be related to an improvement in cervical mucous viscosity and overall improved health and life expectancy. The CF community is just beginning to learn more about pregnancy courses in women with CF following the approval of HEMT. The MAYFLOWERS trial is following maternal and fetal outcomes in pregnant CF patients with the objective of monitoring lung function in women with CF during their pregnancy and two years postpartum while being on HEMT [75]. At this time, there are no existing clinical care guidelines specific to pregnancy in CF including nutritional care; thus, we refer to general guidelines related to nutritional health in pregnancy and make note of any unique circumstances relevant to CF.

Nutritional status during pregnancy is monitored using BMI, micronutrient and macronutrient levels, evaluating food intake through recall diaries for example, and completing a nutrient analysis by calculating daily intake of protein, carbohydrates, fats, sugars, fats, sodium and fiber, and routine follow ups with a registered dietitian. General weight gain recommendations are shown in Table 3 in order to maintain a healthy pregnancy and maternal and fetal weight gain. Impaired pre-pregnancy nutrition is a significant risk factor for suboptimal maternal and fetal outcomes, such as newborns with low birth weight, intrauterine growth restriction and reduced maternal nutrition supply to the fetus [76,77]. Women having a normal preconception bodyweight or BMI (18.5 to 24.9), have been associated with more favorable pregnancy outcomes, and reduced risk of requiring a caesarean section. When patients are underweight, there is an imbalance of hormones in the body, which causes the body to make less estrogen, and many underweight women cease to have periods due to the lack of estrogen [78].

Table 3. General weight-gain recommendations for pregnancy.

Pre-Pregnancy Weight Category	Body Mass Index	Recommended Range of Total Weight Gain throughout Pregnancy
Underweight	Less than 18.5	28–40
Normal weight	18.5–24.9	25–35
Overweight	25–29.9	15–25
Obese (all classes)	30 or greater	11–20

Nutritional screening as performed in general CF care should continue as it relates to assessment of micronutrient status, which is best carried out before conception. This requires an assessment of dietary intake and blood levels. PI leads to malabsorption of fat-soluble vitamins A, D, E, and K and requires supplementation. Goal dosing and fetal development relevance of vitamins and minerals in pregnancy are summarized in Table 4. Vitamin A supplements in deficient pregnant women reduces the incidence of maternal anemia and is associated with a reduced incidence of maternal infections. Vitamin D deficiency is associated with increased risk of gestational diabetes and preeclampsia. Vitamin E supplements should be adjusted based on blood levels, and vitamin K should be continued if needed prior to pregnancy. Continuous folic acid supplementation for at least 12 weeks and regular follow up with a CF dietitian is recommended [79]. Prenatal care will vary depending on the patient's symptoms and severity of their pregnancy. Patients are encouraged to follow up with their multidisciplinary CF team and obstetrician.

Table 4. Pregnancy vitamin and minerals recommendations.

Vitamins and Minerals	Fetal Development
Folic Acid (400–600 mcg/daily)	Supports the placenta and prevents spina bifida and other neural tube defects
Protein (75–100 g/daily)	Produces amino acids, and repairs cell
Iron (27 mg/daily)	Helps with hemoglobin production, prevents anemia
Calcium (1000 mg/daily)	Creates healthy bones, prevents blood clots
Vitamin A/ Beta Carotene (770 mcg, daily max 1000 mcg)	Promotes bone and teeth growth
Vitamin D (600 IU/daily)	Helps body use calcium and phosphorus to promote strong bones
Vitamin E (15 mg/daily)	Forms red blood cells and muscles
Vitamin C (85 mg/daily)	Protect against tissue damage, helps absorb iron
Docosahexaenoic acid—DHA (200 mg/daily)	Helps with brain development

4.2. Nutrition & Lung Transplantation

Following the approval of ETI, rates of lung transplantation (LTx) for CF have declined, although it remains a treatment option in patients with advanced CF lung disease, particularly if there is worsening nutritional status despite supplementation [80]. According to CFF consensus guidelines, all adults with CF should be referred when (1) FEV_1 is <30% predicted; (2) FEV_1 < 40% predicted accompanied by at least two pulmonary exacerbations and/or massive hemoptysis; (3) FEV1 is <50% predicted with a rapid decline of more than 20% FEV1 over one year, and/or sooner if other severity indicators are present, including a BMI < 18, and signs of advanced lung disease such as oxygen dependency, hypercapnia, and/or pulmonary hypertension [81,82].

Optimization of nutritional status before LTx is vital and a key reason why early referral is recommended, to allow for adequate time to address any malnutrition concerns. Malnutrition is a modifiable risk factor and a widespread problem in PwCF for all the reasons previously mentioned: increased metabolic demands associated with recurrent infections, chronic inflammation, and increased respiratory effort. Pre-LTx nutritional management includes adherence to a high-calorie/high-fat diet, PERT, vitamin and mineral replacement, calorie dense oral nutritional supplements, and enteral support if needed [83]. Lean body mass or fat-free muscle mass correlates with inspiratory muscle function [84]. This suggests that preserving fat-free mass stores may play an important role in diaphragmatic function in advanced lung disease, including in patients awaiting LTx. Depletion of fat-free mass is strongly associated with increased mortality while awaiting LTx and prolonged post-transplant intensive care unit stays [85]. While low BMI of <17 kg/m^2 or <18 kg/m^2 is commonly a contraindication for LTx for all lung diseases in many transplant centers, outcomes in CF transplant recipients with such low BMI may fare better than expected. In a study using the United Network for Organ Sharing Registry data between 2005–2015, post-transplant mortality among PwCF demonstrated a reasonable median post-transplant survival of 7 years in CF patients with pre-transplant BMI <17 kg/m^2 which is comparable to other advanced lung disease groups [81].

On the other end of the spectrum are patients with advanced lung disease and obesity; approximately one third of PwCF are overweight or obese [86]. A BMI >35 kg/m^2 is considered a relative contraindication to LTx by the International Society for Heart and Lung Transplantation as there is evidence of an increased risk of primary graft dysfunction and posttransplant mortality for obese recipients compared to normal or overweight candidates [87]. The prevalence of being overweight and obesity in the CF population follows the introduction of HEMT use. CF care teams and specifically dieticians will need

to remain vigilant in nutritional education and diet modifications tailored to patients' overall health. Predictors of posttransplant outcomes in CF lung recipients as they relate to nutritional status will surely need to be reevaluated in the era of HEMT and may influence the existing criteria thresholds for referral and listing [80].

5. Conclusions

People with CF are living longer, healthier lives following the approval of HEMT. The long-term effects of HEMT on nutritional status have yet to be established; more research is needed and further understanding will likely shape standard of care and clinical practices. The goal of current dietary recommendations for PwCF is to deliver more than 100% of estimated energy needs through a high-fat diet; however, studies are now showing reduced energy expenditure in the setting of HEMT use. Dietary modifications will likely be more variable going forward as some patients who are ineligible for HEMT or remain malnourished/underweight despite HEMT will continue to need the traditional CF diet on one side of the spectrum, while patients becoming overweight or obese (more likely on HEMT) will need less caloric diets on the opposite end of the spectrum. The traditional high-calorie, high-fat, salt-supplemented diet also poses a risk for cardio-metabolic disease in the general population and may in time become more apparent in the aging CF population [87,88]. In essence, guidelines will need to be adjusted on an individual basis. The evolution of the nutritional landscape in CF will be followed closely by the CF community.

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Review

Reproductive Counseling and Care in Cystic Fibrosis: A Multidisciplinary Approach for a New Therapeutic Era

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Abstract: With the advent of highly effective modulator therapies, many people with cystic fibrosis (CF) are living longer, healthier lives. Pregnancy rates for women with CF more than doubled between 2019 and 2021, reflecting increases in both planned and unplanned pregnancies. For men with CF, CF-associated infertility can be mitigated with assistive reproductive technology, yet patient knowledge of these challenges and options is variable. Preconception and prenatal counseling for individuals with CF and for parents of children with CF who wish to expand their families requires nuanced discussions to promote informed reproductive decisions, drawing from a combination of standard practice recommendations and CF-specific assessments. This review article synthesizes the current literature and practice recommendations regarding reproductive counseling and care in CF, outlining the role of genetic counseling, carrier screening, teratogen counseling, in vitro fertilization and pre-implantation genetic diagnosis, and careful assessment and management of cystic fibrosis-related diabetes when present. Via a multidisciplinary, patient-centered approach, clinicians can support adults with CF and parents of children with CF as they make informed reproductive decisions and embark on family planning.

Keywords: cystic fibrosis; family planning; pregnancy; fertility; genetic counseling; highly effective modulators

1. Background and Significance

Cystic fibrosis (CF) is an inherited condition that affects the lungs, pancreas, and other organs. Variants in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) disrupt the production of the CFTR protein, which functions as an ion channel in the apical membrane of epithelial cells [1]. Over 2000 *CFTR* variants have been described, and more than 700 have been characterized as CF-causing [2]. Advances in the treatment and management of CF have led to significant improvements in survival and quality of life for children and adults over the last 20 years. According to the United States (US) Cystic Fibrosis Foundation Patient Registry (CFFPR), the median predicted age of survival for people with CF in the United States has increased from approximately 32 years in 2000 to more than 50 years in 2021 [3]. Most people with CF are adults and receive care at accredited CF care centers [3]. With consistent access to specialty care and new therapies, all individuals with CF are now expected to live well into adulthood.

Ongoing research and development of new treatments inform our optimism that survival and the outcomes for people with CF will continue to improve. The availability of new medications, highly effective CFTR modulators, starting with ivacaftor (IVA; brand

name Kalydeco) in 2012 and elexacaftor/tezacaftor/ivacaftor (ETI; brand name Trikafta), in 2019, has led to improved lung function, attenuated disease progression, and improved nutrition. In the US, modulators are now available to over 90% of children and adults with CF over the age of 2 who have one or more responsive *CFTR* genetic variants, including the most common *CFTR* variant, F508del [4,5]; ivacaftor was recently approved for infants with responsive CFTR variants as young as one month of age [6]. However, despite widespread access in some countries, significant health disparities in CFTR modulator access exist, which can be attributed, in part, to the lower frequency of F508del in minoritized racial and ethnic groups [7]. Additionally, close attention to the benefits of nutritional support and management, such as the use of pancreatic enzyme replacement therapy and high-calorie, high-protein diets, have helped to prevent malnutrition and improve growth in people with CF [8]. These nutritional benefits begin right from birth, and the widespread implementation of CF newborn screening has enabled the early detection of infants with CF-related pancreatic insufficiency. Early detection has been especially critical because it spares children from developing failure to thrive and its developmental sequelae, which impacts pulmonary status as well [9].

With an increased life expectancy and improvements in the quality of years lived, a growing percentage of the adult CF population can envision a future [10] where they have families of their own, and the discourse with parents of children with CF is changing as the prognosis improves. The CFFPR tracks relationship status as a quality-of-life measure for all people with CF, as well as pregnancy data for women. In 2021, CF care centers reported that 44.8% of adults with CF over 18 years of age were married or living with a partner; an additional 5.5% were divorced, separated, or widowed [3] Figure 1. At this time, data about whether a person with CF has children and how—by natural (spontaneous) conception, assisted reproductive technology, gestational surrogacy, adoption, or foster care—is not collected in the CFFPR; this is an area for future consideration. Heightened attention to investigating the sexual and reproductive health of and gender-specific changes in CF symptoms among individuals with CF is evidenced by a growing body of literature, spearheaded by the prolific CF Foundation-sponsored Sexual Health, Reproduction and Gender Research Working Group [11–14] and informed by a grass-roots, patient-directed effort to improve interactions about reproductive and sexual health between women with CF and their healthcare teams [15].

Individuals with CF have expressed a desire for frank conversations with trusted CF care providers about their reproductive goals and options, integrating a shared decision-making model into their CF care plans [16]. Balancing the dual roles of parent and patient, navigating prognostic uncertainty, anticipating the impact of future health decline, and the risk of early mortality on children [17] are sources of concern for both prospective and current mothers and fathers with CF [18] that require consideration, support, and empathy from the CF care team [19]. Additional areas for meaningful discussion include uncertainty about starting a family, uncertainty about future health, concerns about treatment adherence during and after pregnancy, the impact of pregnancy and childbirth on future health [20] and the chances of having a child with CF. These discussions about fertility and family building are layered conversations that optimally start in adolescence [21]. It is important for adolescents with CF to have a supportive healthcare team, including CF physicians, nurses, genetic counselors, social workers, and psychologists, who can provide accurate information, address concerns, and promote a holistic approach to reproductive health and sexuality. Open communication between healthcare providers, adolescents, and their families is crucial to ensure that a patient's unique needs and questions are addressed in a respectful and age-appropriate manner [22].

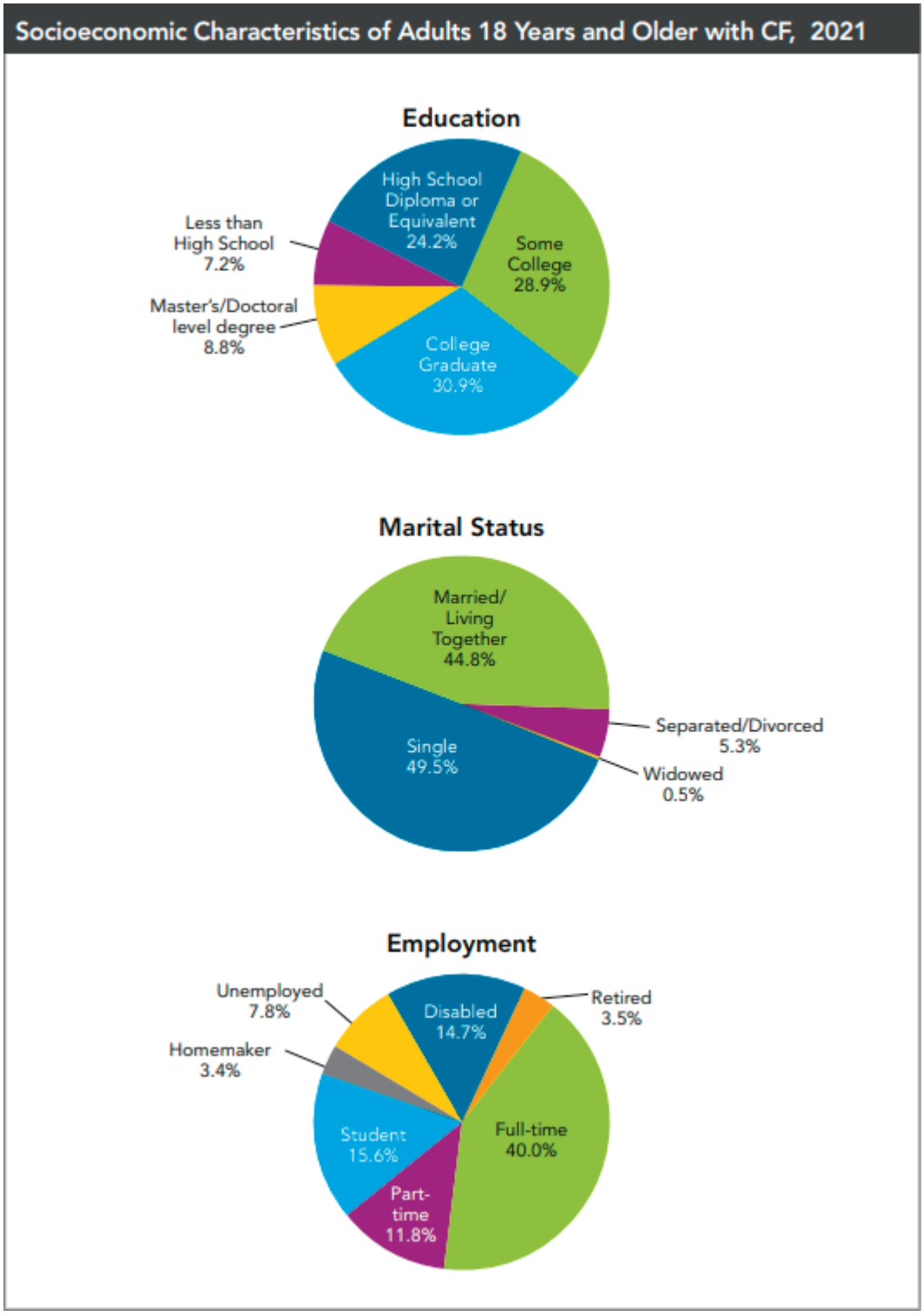


Figure 1. From the 2021 Cystic Fibrosis Foundation Patient Registry, reproduced with permission. In 2021, there were 32,100 individuals with CF in the Registry. Adults comprised 58.3 percent of the CF population (n = 18,714).

Despite the growing interest in reproductive decision-making in CF, gaps in patient knowledge regarding fertility and reproductive options have been documented [23]. As such, appropriate counseling and tailored guidance are needed for individuals with CF and parents of children with CF to foster opportunities for open dialogue about their options for family building and to promote informed decision-making that is consistent with the individual's and couple's goals and values.

2. Genetic Counseling

Genetic counseling is the process of educating patients and families about the inherited basis of disease, integrating family history, risk assessments, curated information, and counseling to promote adaptation to the risk or diagnosis of a condition [24]. A trained, certified and/or licensed genetic counselor provides personalized information and support to couples in their family planning journey, assessing and addressing the risks of genetic disorders in a non-judgmental, non-directive manner that respects the patient or couple's values and supports their decisions rather than persuading them to pursue a particular course of action [25]. Genetic counselors provide tailored information about genetics, inheritance patterns, and expected phenotypic spectrum for CF and other genetic conditions that are either present in the family history or commonly screened for in the preconception and prenatal period. Genetic counselors experienced in CF can help prospective parents appreciate the phenotypic spectrum of CF, ensuring that they understand that genotype is not entirely predictive of phenotype. Increasingly early access to CFTR modulator therapies in patients whose mutations and age render them eligible for treatment further substantiates that CF may impact future children differently from the family's experience with their first child. In addition, genetic counselors can discuss options for pursuing parenthood, as well as the available screening and diagnostic testing, appropriate monitoring of an at-risk pregnancy, and recommendations for birth planning and neonatal care [26]. For example, genetic counselors can provide anticipatory guidance that a fetus or newborn known to have CF, or who is at an increased risk to have CF based on family history, should be evaluated by a pediatric CF care center for additional monitoring, discussion of a treatment plan, and diagnostic assessment [27]. Providing emotional support and facilitating connections to additional family building, obstetric, and postnatal resources complements and enhances the role of the other members of the care team [28]. Genetic counseling can improve patient knowledge and facilitate decision-making about family-building options [29].

Preconception and prenatal counseling for adults with CF and parents of children with CF require consideration of the benefits, limitations, and risks of the available testing options specific to CF as well as the standard practice recommendations for the general population. These include discussions of genetic carrier screening, residual reproductive risks of genetic disease if the carrier testing is negative, and pre- and postnatal diagnostic testing options [30]. For women with CF, additional attention is paid to the risk-benefit analysis of medications that enable them to remain healthy while pregnant and any potential impact they have on pregnancy and fetal development [31]. For all individuals with CF and couples with a child with CF, a critical aspect of preconceptual genetic counseling is to educate families about the variability of CF, the shifting prognosis given CFTR modulator therapies, and the fact that, although genotype-phenotype data may provide some guidance about the pulmonary and non-pulmonary features that an individual with CF may experience, there is significant intra- and inter-familial variability [32] as well as considerations related to having multiple close family members with CF [33].

3. Reproductive Decision-Making in Adults with CF

Biological females with CF are not expected to have anatomical barriers to conceiving spontaneously. However, before ETI, many women with CF had difficulty conceiving due to thickened cervical mucus, poor nutritional status, and decreased lung function [34,35]. Preconception planning should include a gynecological evaluation of fertility status to

anticipate and explore options for conception, performed by a provider with experience with CF [36]. In a qualitative study of 25 women with CF who became parents, Bray et al. identified relationship status, health, finances, and personal preferences as key factors influencing both their decisions to pursue parenthood and to select a specific reproductive mechanism, such as natural/spontaneous conception, assisted reproduction, or surrogacy [37]. In 2019, prior to the availability of ETI, 310 pregnancies were reported to the CFFPR, reflective of an estimated five-fold increase in pregnancy rates among women with CF over the previous 20 years [38]. In 2021, more than double that number—675 pregnancies—were reported to CFFPR [3] (Figure 2). While this change may be attributable to an increase in the health and vitality of women with CF taking ETI, there was also a surprising increase in unplanned pregnancies, highlighting extrapulmonary implications of CFTR modulators [5] and the associated need for discussions about contraception and sexual activity in adolescents and women with CF [39]. This is especially important in light of the recent data regarding the increased medical risks for women with CF with unplanned pregnancies and their neonates [40].

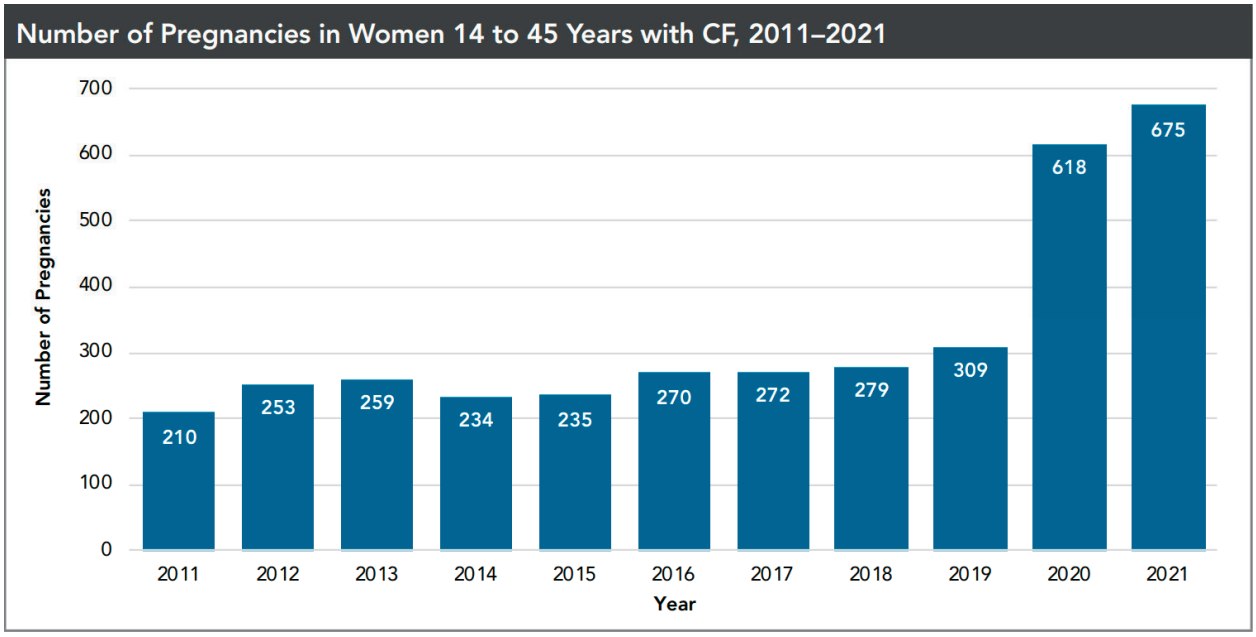


Figure 2. From the 2021 Cystic Fibrosis Foundation Patient Registry, reproduced with permission.

The physiological demands of pregnancy are well-known to impact lung function in women [41]. Ideally, women with CF work closely with their CF clinical team to optimize their lung function before and during pregnancy [42], as successful pregnancy outcomes are known to correlate with nutritional and pulmonary statuses at conception. Women with CF and unplanned pregnancies and/or poor health status at conception have an increased frequency of pulmonary exacerbations, hospitalizations, and illness-related visits [43,44]. However, pregnancy does not appear to impact longer-term outcomes for women with CF [45], including women with moderate to severe lung disease [46]. Data regarding higher rates of premature delivery (<37 weeks) and primary C-sections have been reported [44–46] but not consistently. Pre-gestational diabetes is more common in mothers with CF, yet even after controlling for the impact of diabetes on pregnancy outcomes, one study found an increased likelihood of cardiac anomalies in infants born to women with CF [47], underscoring the need for fetal monitoring.

The prevalence of infertility in men with CF is estimated as 98% [48], primarily a result of obstructive azoospermia secondary to congenital bilateral absence of the vas deferens (CBAVD) [49]. One common CF-causing *CFTR* variant, c.3718-2477C > T (legacy name 3849 + 10Kb C > T), has an estimated prevalence of 1.8% among people with CF [3] and has been associated with male fertility [50]. Men with CF and CBAVD are expected to have viable sperm that can be retrieved [51], either directly from the epididymis via percutaneous epididymal sperm aspiration (PESA), from a small piece of testicular tissue via testicular sperm extraction (TESE) or by using direct microscopic examination of the testes via microdissection testicular sperm extraction (micro-TESE) [52]. After sperm retrieval, the partner's egg (or donor egg) can be fertilized by using in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) [53]. In IVF with ICSI, a single sperm is injected directly into an egg, and the resulting embryo is then transferred to the partner's uterus for implantation. Prior to embryo transfer, pre-implantation genetic testing (PGT) can be used in combination with IVF to screen the embryos for aneuploidy and single-gene genetic disorders, including CF, if the reproductive partner or ovum donor has also been identified as a CF carrier [54].

Consideration of the reproductive goals and the potential desire for fertility preservation also require discussion with individuals facing organ transplantation, although CF care providers may not feel equipped to engage fully in these conversations [55]. Egg retrieval and sperm retrieval and storage prior to transplant maximize the reproductive options for post-transplant pregnancy, as well as the possibility of surrogacy [56]. Fertility preservation is also relevant in other contexts, such as pre-oncologic and pre-gender affirming treatments, which are explored elsewhere [57]. Regardless of the clinical impetus, discussions regarding fertility preservation require a multidisciplinary approach that is timely, educational, and appropriate to the clinical situation [56].

4. Carrier Screening

Genetic carrier screening identifies the individuals/couples at increased risk of having children with specific genetic conditions, facilitates informed reproductive decision-making, and optimizes pre- and postnatal care. Prenatal carrier screening recommendations for an individual or couple are influenced by many factors, including ethnicity, family history, prior pregnancy history, insurance coverage, personal preferences and values, and local practice. Both patients and parents of children with autosomal recessive conditions, including CF, have been found to be supportive of genetic screening in principle but may not choose to utilize it for themselves [58,59]. At present, the American College of Obstetrics and Gynecologists (ACOG) recommends that, at a minimum, carrier screening for CF, spinal muscular atrophy, and certain inherited forms of anemia be offered to all individuals who are pregnant or planning to become pregnant [30]. Offering carrier screening for additional conditions may be recommended for individuals/couples who are at higher risk of carrying genetic variants for certain genetic disorders based on their ethnicity or personal or family history of a genetic disorder. Taking a detailed family history and documentation of genetic ancestry can inform risk assessment [60]; however, self-reported ethnicity is not a reliable predictor of genetic ancestry [61] and basing test offerings on ethnicity may further compound health disparities. Accordingly, The American College of Medical Genetics recommends a consistent and equitable approach whereby all patients are offered tiered carrier screening options, including testing for a broader range of predominantly autosomal recessive conditions and a limited number of X-linked conditions, such as fragile X syndrome and dystrophinopathies [62–64].

As with most genetic conditions, genetic carrier screening for CF can detect a significant percentage of, but not all, disease-causing variants. Individuals in the general population who receive a negative result have reduced, but not eliminated, their chance to be a CF carrier. Residual carrier risks are based upon the estimated test sensitivity and the baseline or the *a priori* chance of being a carrier and are typically less than half of one percent, meaning the risk is greatly reduced but greater than zero [65]. It is recommended

that the labs offering CF carrier screenings assess a minimum panel of disease-causing *CFTR* variants; thus, when one member of a couple screens positive as a CF carrier or has CF, a limited genetic screening panel may be offered to the second partner, consistent with professional guidelines [30]. However, *CFTR* sequencing can provide additional carrier detection and permit a more refined reproductive risk assessment [66]. In counseling patients, it is critical to consider that, depending upon individual lab practices, genetic sequencing may also reveal variants of uncertain significance (VUS) and/or variants of varying clinical consequence (VCC), thereby complicating the discussion of reproductive risk with a broad phenotypic spectrum that includes mildly affected or asymptomatic individuals [67,68].

When both members of a couple are heterozygous carriers for an autosomal recessive condition, such as CF, with each pregnancy, there will be a 25% chance of having a child with the condition. If the partner of a person with CF is also a CF carrier, there is a one in two (50%) chance that each child will have CF. Targeted pre-implantation genetic testing (PGT) and prenatal testing are available for at-risk pregnancies in which the familial variants in the disease gene have been identified.

5. IVF with PGT

PGT is a screening test that can be performed on embryos created via IVF to genetically analyze the embryos for gene disorders and/or chromosome abnormalities prior to transfer into the uterus. After fertilizing the egg in a laboratory setting, one or more cells are removed from the trophectoderm and sent for genetic testing. Although PGT for CF is quite accurate and can significantly increase the chance of conceiving an unaffected pregnancy [69], embryos can be damaged in the process and may be unsuitable for transfer or fail to implant. Additionally, PGT may produce false-negative or false-positive results.

To reduce the error rate in PGT testing, it is recommended that original source DNA from a previously affected pregnancy or DNA from a carrier or affected parents be provided to the PGT lab [70]. Furthermore, while PGT is often pursued to avoid the risk of invasive prenatal diagnostic testing due to the chances of diagnostic error, confirmatory prenatal diagnosis is recommended after PGT [71]. As with all genetic tests, PGT does not detect all genetic abnormalities, only those tested. In the context of these limitations, PGT can help couples who are affected or carriers of genetic disorders, including CF, understand and refine or reduce the risk of passing on a genetic condition or chromosomal disorder to their children. Individuals and couples considering IVF with PGT should receive reproductive genetic counseling prior to embarking on this complex process.

Assisted reproductive technologies, with or without PGT, are expensive, and not all insurance plans cover the procedures, including most public insurance plans. Early referral to reproductive experts can help interested individuals/couples plan for the possibility of IVF with PGT by selecting an insurance plan that has IVF benefits, assisting with obtaining insurance pre-authorization, and/or identifying and accessing local resources, such as grants or other funding opportunities.

6. Prenatal Screening and Diagnostic Options

Once pregnancy is achieved, recommendations for prenatal genetic screening and diagnostic testing may again vary depending on factors that include the woman's age, medical history, their partner's medical history, and/or a family history of genetic disorders. Pregnant people are generally offered testing and screening options unrelated to cystic fibrosis, including nuchal translucency, serum marker, and/or cell-free DNA (cfDNA) screening to identify the risk of chromosomal abnormalities; second-trimester serum alpha-fetoprotein screening can be implemented to assess the risk for open neural tube defects, conducting ultrasounds to assess fetal growth and structural development, and prenatal diagnosis.

Prenatal diagnostic testing is available via chorionic villus sampling (CVS) or amniocentesis when both members of a couple are known to carry *CFTR* variants. In addition, emerging technologies, such as cell-free DNA screening for single-gene disorders, provide a non-invasive option for predicting the chance that a fetus will have CF [72]. However, cfDNA screening does not have the diagnostic accuracy afforded by CVS and amniocentesis, and false-positive and false-negative results can occur. Genetic counseling with a discussion of the benefits, limitations, and risks of the test options is recommended.

Preconception discussions with people with CF and parents of children with CF should maximize the ability of prospective parents to make informed decisions about their reproductive options and clearly convey that they will be supported by the medical team regardless of which approach they decide to pursue [25]. Decisions about having subsequent children after a child with CF are influenced by the existing family structure and parents' lived experiences with CF [73]. If diagnostic testing reveals that a fetus will have CF, the couple faces options regarding pregnancy termination or continuation with expectant management. When a pregnancy is known to have CF, additional medical monitoring can be implemented in the form of serial prenatal ultrasounds to evaluate for echogenic bowel and the risk of meconium ileus and to facilitate birth planning and postnatal care [74,75]. Similarly, parents who opt to forego PGT or invasive prenatal testing assessments should be offered monitoring to identify and plan for any neonatal care needs that arise. Coordination with state newborn screening programs can confirm CF status for siblings via a genetic diagnosis, as sweat test practices for siblings vary across centers [76].

Overall, goals and preferences regarding reproductive planning and family building should be routinely assessed and supported, noting that they may change over time [73,77]. Non-biological family planning modalities, such as foster parenting, adoption, step-parenting, conception via donor gametes and surrogacy, are options for individuals with CF as well as for parents of children with CF. Prior to the development of techniques for sperm retrieval in men with CF, these options were likely to be utilized by men with CF to build their families [78]. The cost of some of these approaches can be prohibitive. Learning about the range of options for family building can facilitate decision-making, with or without changing a planned course of action [29].

7. Medication Uses during Pregnancy

Counseling about the potential teratogenic impact of CF medications in pregnancy is influenced by dose, timing, medication interactions, and the potential for any detrimental effects of discontinuing the medication. Fat-soluble vitamin (ADEK) dosing of a pregnant woman requires particular attention in the setting of increased dosing due to maternal deficiency [79]. Should a pulmonary exacerbation during pregnancy prompt consideration of antibiotics, a multidisciplinary approach is necessary. Consultation among the CF and obstetrics physicians, a CF pharmacist, a genetic counselor with expertise in teratogen assessment and counseling, and the adult patient can facilitate shared decision-making that addresses the health needs of the mother and minimizes teratogenic risks to the fetus [80].

While modulators have played a key role in promoting pregnancy in CF patients, the safety of ETI during pregnancy is under ongoing investigation. The MAYFLOWERS study (NCT04828382) was launched in September 2021 to track the experiences of women with CF who are conceiving while on ETI, to monitor the health of the pregnant women, the pregnancy, and to assess neonatal and early childhood outcomes of infants born to women on ETI in at least the first two years of life [13]. The study is expected to be completed at the end of 2025.

Animal studies of the individual components of ETI have shown some evidence of developmental toxicity, such as reduced fetal weight and skeletal abnormalities, as well as juvenile cataracts [81]. These risks are weighed against reports of a rapid decline in women with CF following discontinuation of CFTR modulators [82], coupled with early findings of healthy babies born to women who continued to take CFTR modulator therapy

during pregnancy. More recent reports in the literature reveal cases of juvenile cataracts and elevated liver enzymes in babies exposed to ETI in utero and while breastfeeding [83].

In addition to these reported risks, two recent case reports suggest that ETI may be therapeutic when given prenatally in CF pregnancies. In one, an infant with CF whose mother remained on ETI throughout her pregnancy had a false-negative CF newborn screening result. Through coordinated postnatal communication and testing, the infant was subsequently diagnosed with CF and received appropriate treatment and monitoring [84]. A second case report involves a known CF carrier expecting her second child with CF. After dilated loops of bowel consistent with meconium ileus was noted on the fetal ultrasound, she began taking ETI. The resolution of the bowel distension was visualized after 27 days of treatment, and the baby was born free of intestinal obstruction [85]. The number of babies with CF born after experiencing in utero exposure to *CFTR* modulators may increase in the future as growing numbers of women with CF who are on modulators become pregnant. This nuanced and shifting landscape reinforces the importance of detailed discussions about the risks, benefits, limitations, and unknowns of remaining on ETI use during pregnancy [5]. Overall, it is critical that pregnancy in CF patients involves dedicated, specialized medical support so that individuals, in concert with their physicians, can make informed decisions about ETI during pregnancy.

8. CF-Related Diabetes

Cystic fibrosis-related diabetes (CFRD) is a common complication of CF and affects more than 50% of adults with CF. The Cystic Fibrosis Foundation guidelines include annual glucose and hemoglobin A1C levels and, starting at 10 years of age, an annual 2 h oral glucose tolerance testing [86]. Furthermore, as with type I or Type II diabetes, CFRD can have significant implications for pregnancy in women with CF and should be managed to assure good diabetic control in the months prior to conception and after completion of the pregnancy [87]. Additionally, women with CFRD may have more difficulty in controlling their blood sugar levels during pregnancy, which can lead to complications for both the mother and baby. In a French study, CFRD was associated with higher rates of assisted conception and increased risk of cesarean delivery [88], but not pre-eclampsia, preterm labor, or low birth weights, which has been reported elsewhere [89].

Regular discussion of family planning goals for women with CFRD is especially important to anticipate and address issues of glycemic control. Pre-conceptional supplementation with folic acid is recommended for all women but is especially critical for diabetic women to mitigate the increased risks of fetal neural tube defects associated with maternal diabetes [90]. Close monitoring of maternal and fetal health is needed to identify and manage any complications that may arise during pregnancy.

Pregnant women with CF are expected to remain under the care of their local cystic fibrosis center, endocrinologist, and a high-risk obstetrical service to jointly monitor the woman's pulmonary function, weight gain, glycemic control, blood pressure, and the fetus's growth. Fetal monitoring must attend to the possibility of cystic fibrosis in the fetus and the effects of maternal medical conditions and medications. When a fetus is known or highly likely to have CF, delivery at a tertiary CF Center hospital should be considered, ensuring the appropriate support of the mother and baby and the coordination of maternal and infant care after delivery. A strong therapeutic relationship between the CF center and high-risk obstetrical service ensures the dual monitoring of the mother and fetus. Figure 3 illustrates the need for an interconnected, multidisciplinary approach to the care of a woman with CF.



Figure 3. Clinical support and care team for a pregnant woman with CF. Pregnancy support system from CF team members and related specialties. Intersecting lines and arrows reflect the varying multidisciplinary care and communication needs.

9. Conclusions

The prognosis for individuals with cystic fibrosis has gradually evolved, with recent therapeutic advancements significantly improving overall health and outlook. Indeed, with the advent of highly effective modulator therapies, people with CF are living longer, healthier lives. Additionally, couples who are both CF carriers may now be more likely to continue CF-affected pregnancies, heartened by the availability of highly effective treatments. Preconception and prenatal counseling for adolescents and adults with CF, and for parents of children with CF who wish to expand their families, requires nuanced discussions. A timely, concerted discussion can promote informed reproductive decisions by drawing on a combination of standard practice recommendations and CF-specific assessments. These include genetic counseling regarding the risks, benefits, and limitations of carrier screening for CF and other available conditions, as well as the residual risks of having affected offspring after the screening. For women with CF, conversations to weigh the risks and benefits of medications that both enable them to remain healthy throughout the pregnancy and may potentially impact the pregnancy and fetal development are crucial. For men with CF, additional discussions of the etiology of male infertility and the options available to have biological children are recommended. Ideally, such discussions begin early, before reproductive concerns are pertinent and become more detailed in response to a patient's questions and expressed reproductive intentions. For parents of children with CF, who have a 25% chance with each subsequent pregnancy of having another child with CF, preconception counseling enables parents to consider all available screening and diagnostic options, thereby maximizing their ability to make informed family planning decisions. A multidisciplinary approach to ongoing care and counseling about family planning goals and decisions, which often change over time, can benefit young adults with CF and parents of children with CF.

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Review

Lung Transplantation in a New Era in the Field of Cystic Fibrosis

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Abstract: Lung transplantation for people with cystic fibrosis (PwCF) is a critical therapeutic option, in a disease without a cure to this day, and its overall success in this population is evident. The medical advancements in knowledge, treatment, and clinical care in the field of cystic fibrosis (CF) rapidly expanded and improved over the last several decades, starting from early pathology reports of CF organ involvement in 1938, to the identification of the CF gene in 1989. Lung transplantation for CF has been performed since 1983, and CF now accounts for about 17% of pre-transplantation diagnoses in lung transplantation recipients. Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have been the latest new therapeutic modality addressing the underlying CF protein defect with the first modulator, ivacaftor, approved in 2012. Fast forward to today, and we now have a growing CF population. More than half of PwCF are now adults, and younger patients face a better life expectancy than they ever did before. Unfortunately, CFTR modulator therapy is not effective in all patients, and efficacy varies among patients; it is not a cure, and CF remains a progressive disease that leads predominantly to respiratory failure. Lung transplantation remains a lifesaving treatment for this disease. Here, we reviewed the current knowledge of lung transplantation in PwCF, the challenges associated with its implementation, and the ongoing changes to the field as we enter a new era in the care of PwCF. Improved life expectancy in PwCF will surely influence the role of transplantation in patient care and may even lead to a change in the demographics of which people benefit most from transplantation.

Keywords: cystic fibrosis; lung allocation; lung transplantation; CFTR modulator therapy

1. Background

Cystic fibrosis (CF) is an autosomal recessive disease that results from inheriting two copies of a pathogenic mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, one from each parent [1]. The encoded CFTR protein is a cyclic adenosine monophosphate (cAMP)/protein kinase-regulated apical membrane anion channel expressed throughout numerous cells of the body, ranging from those of the lungs, intestines, kidneys, and pancreas [2] to lymphocytes and fibroblasts [3], and to cells of the central and peripheral nervous system [4]. Its most well-defined function is in epithelial cells where it typically facilitates flow of chloride ions across the cell membrane and allows for preservation of the balance between salt and water on several mucosal surfaces of the body [5]. CFTR total activity is related to its quantity and function, which are determined by the mutation encoding the defective protein [6]. For example, Class I CFTR mutations result from nonsense, frameshift or splicing mutations in which no functional protein is created. The most common CFTR mutation is F508del, a Class II mutation, which results

in protein misfolding, such that it is degraded before it reaches the cell surface. About 85% of the CF patient population carries at least one copy of F508del [7]. CFTR Class III mutations are characterized by defective chloride channel regulation and gating. Mutation Classes I through III are more commonly associated with more severe lung dysfunction and pancreatic insufficiency (PI). Class IV and V mutations are related to defects in channel conductance and stability, respectively; these result in proteins that have residual function and are usually associated with milder disease severity compared to Classes I, II, and III [8]. Absent or dysfunctional CFTR protein in the airways results in the accumulation of thickened secretions that are unable to be cleared by cilia, which leads to chronic airway infections, inflammation, and bronchiectasis. People with CF (PwCF) are known to also suffer from multiple comorbidities beyond respiratory illnesses, including pancreatitis, exocrine and endocrine PI, CF-related diabetes, biliary dysfunction, cirrhosis, intestinal obstruction, malabsorption, nutritional insufficiency, growth delay, nasal polyps, recurrent sinusitis, infertility, anxiety, depression, and many other pathologies [9]. Currently, more than 100,000 people worldwide suffer from the disease and about 1000 individuals are diagnosed each year [10].

Although the management of all conditions associated with CF is essential for the complete care of these patients, the pulmonary manifestations of CF have been estimated to account for greater than 90% of the morbidity and mortality of the disease [11]. In the respiratory epithelium, dysfunctional chloride channels lead to the formation of abnormal, viscous fluid on the surface of cells that is insufficiently mobilized by cilia. This leads to a vicious cycle of airway obstruction, infection, and inflammation that progresses to fibrosis resulting in bronchiectasis and ultimately, end-stage lung disease that can only be cured by lung transplantation [12]. Advancements in CF respiratory management include mucolytics, airway hydrators, chest physiotherapy devices, inhalational antimicrobials, and highly effective modulator therapy (HEMT) such as elexacaftor/tezacaftor/ivacaftor (ETI) that correct and improve the underlying protein defect [13]. Undeniably, this led to increased longevity for CF patients. The median life expectancy for CF patients born today now exceeds 50 years of age, whereas it was predicted to be less than 30 in the early 1990s [14]. However, there remains approximately 10% of the United States CF population that is still ineligible for HEMT and continues to have progressive disease [7]. Additionally, people with advanced CF lung disease despite HEMT may still have clinical features associated with worse mortality, warranting lung transplantation evaluation (see Figure 1). Thus, while advancements in CF therapeutics paved the way for an improved life expectancy, there is still no cure for CF, and lung transplantation remains a critical treatment modality.

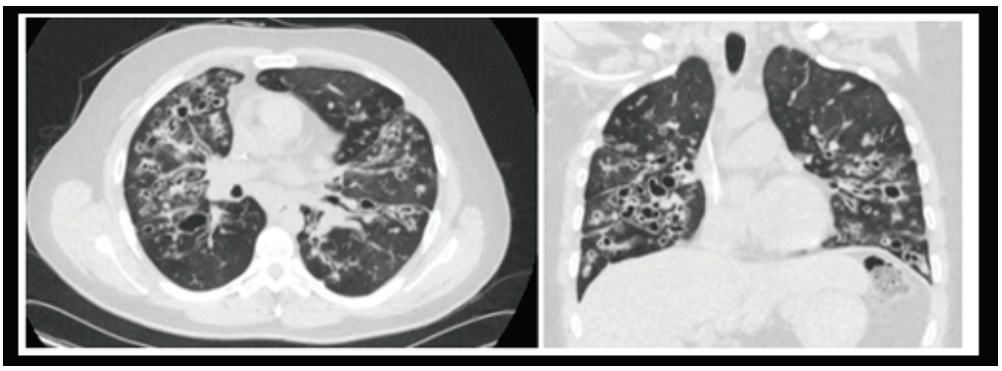


Figure 1. CT radiograph of a patient with advanced CF lung disease demonstrating extensive bronchiectasis and mucoid impaction. Patient was heterozygous for F508del with a forced expiratory volume in 1 s (FEV1) of 0.74 L/22% predicted, history of several pulmonary exacerbations per year, chronic hypoxemia and hypercapnia, and severe pulmonary hypertension. Highly effective modulator therapy, elexacaftor/tezacaftor/ivacaftor, had not yet been approved at the time when the patient required a bilateral lung transplantation.

2. Pre-Transplant

2.1. Lung Transplantation Referral Criteria in CF

Since the very first lung transplantation procedure was performed in 1963 [15], its implementation has grown dramatically, such that about 4500 lung transplantations are now performed annually across the world [16]. As per guidelines published by the International Society of Heart and Lung Transplantation (ISHLT) [17], ideal lung transplantation recipients have significant lung disease that progressed despite maximal therapy, causes symptoms that limit activities of daily living, and poses a high risk of death over the next two years in the absence of transplantation. However, these patients should not have other significant medical problems and are expected to have at least 80% survival for greater than five years after transplantation. Specifically, although exact contraindications may vary based upon the specializations of a particular transplantation center, individuals who are in shock, require life support, have a detectable HIV viral load, are greater than 70 years old, have limited functional status, are severely overweight (BMI greater than 35) or underweight (BMI less than 16), have had a recent stroke or myocardial infarction, or suffer from stage 3b or greater chronic kidney disease, advanced liver disease, or untreatable hematologic disorders generally are not considered for transplantation. It is strongly recommended to refer eligible patients for evaluation for transplantation early given the extended length of time necessary to complete the workup and to address any concerns before listing a patient. For example, early referral allows patients and caregivers ample time to gain education and to make an informed decision about whether or not to pursue transplantation and to establish a solid relationship with their lung transplant team. For PwCF, it eases transition of care between their CF and transplant care teams. Early referral also allows the transplant team to continuously assess adherence, optimize patients' overall health and functional status (e.g., weight concerns and deconditioning) and address psychosocial concerns [18].

Despite the fatal nature of end-stage lung disease being a shared likeness among many lung diseases, disease-specific indications for lung transplantation are necessary to reflect unique disease characteristics. For example, referral criteria for patients who suffer from chronic obstructive pulmonary disease (COPD) include having a BODE index [19], a composite score measuring body mass index, forced expiratory volume in one second (FEV1), dyspnea, and six-minute walk distance equal to five or six with other features of increased mortality such as an FEV1 that measures less than 25% predicted [17]. A typical referral for those who suffer from interstitial lung disease occurs when they have a forced vital capacity (FVC) that measures less than 80% predicted or a diffusing capacity for carbon monoxide (DLCO) that measures less than 40% predicted, or if they require supplemental oxygen.

Similar to other diseases, cystic fibrosis carries its own disease-specific indications for referral for lung transplantation (Table 1) long prior to development of terminal disease. According to the Cystic Fibrosis Foundation (CFF) consensus committee [18], specific indications for referral include–an FEV1 that measures less than 50% predicted associated with a greater than 20% relative decline in FEV1 over the previous year, an FEV1 that is less than 40% predicted and is associated with specific markers of shortened survival (greater than two pulmonary exacerbations per year, hemoptysis of greater than 240 mL, pneumothorax, BMI less than 18 despite aggressive attempts to gain weight), or an FEV1 less than 30% predicted even without associated complications. The disease course of CF can be very heterogeneous and, at times, carry an unpredictable nature. In some cases, even preceding lung transplantation referral, the CFF identifies individuals with advanced lung disease, who are at high risk of clinical decline, and, therefore, may benefit from early and robust mitigation of risk factors for disease progression [20]. Specifically, patients who have an FEV1 that measures less than 40% (even without an additional complication), have had a previous intensive care unit admission for respiratory failure, suffer from baseline hypercapnia (PaCO2 greater than 50 on stable blood gas), require oxygen use during the

day and at rest, have developed pulmonary hypertension, or cannot walk greater than 400 m would be classified as having advanced lung disease.

Table 1. Indications and Contraindications for Lung Transplantation and CF-Specific Factors [17,18,20].

General Indications	-Advanced lung disease that is refractory to medical therapy -Greater than 50% mortality from lung disease without transplantation over the next two years -Greater than 80% five-year survival post-transplantation
Absolute Contraindications	-Clinical shock, disseminated infection, or HIV infection with a detectable viral load -Malignancy with high risk of recurrence or cancer-related death -Lack of interest in transplantation, evidence of persistent non-adherence to treatment -Non-pulmonary organ dysfunction -GFR less than 40 -Stroke or acute coronary syndrome within the past thirty days -Acute liver failure or cirrhosis with synthetic dysfunction -Hematologic disorders not amenable to treatment -Active substance use or dependence
Relative Contraindications	-Age greater than 70 years -Severe coronary artery disease or cerebrovascular disease -BMI greater than 35 or less than 16 kg/m^2 -Severe esophageal dysmotility, chest wall deformity, or previous thoracic surgery expected to cause difficulty with post-transplant healing -Infection or colonization with highly resistant or virulent organism -Detectable hepatitis B or C viral load -Limited functional status or neurocognitive condition that may interfere with adherence to regimen after transplantation
CF-Specific Referral Criteria	-FEV1 < 50% predicted with a greater than 20% relative decline in FEV1 within one year -FEV1 < 40% predicted accompanied by: -More than two exacerbations per year requiring antibiotics -Massive hemoptysis of more than 240 mL that necessitates ICU admission or bronchial artery embolization -BMI less than 18 -FeV1 < 30% predicted -Six-minute walk test result < 400 m -Advanced CF lung disease: -Hypoxia (PaO2 < 55 mmHg) at rest or with exertion -Hypercapnia (PaCO2 > 50 mmHg) -Previous exacerbation requiring positive pressure ventilation -Pulmonary artery systolic pressure > 50 mmHg

2.2. Lung Allocation

The mechanism employed for distribution of lungs for transplantation in the United States changed over the years. Prior to 2005, lungs were distributed according to the duration of time spent on a waitlist. In essence, those people who had been listed for the longest time were those who preferentially received a transplantation. However, pre-transplant mortality was nearly 30% [21]. For that reason, the Lung Allocation Score (LAS) was adopted. It implements a measurement of anticipated survival (in number of days) prior to and following transplantation for each individual. The measurement of survival prior to transplantation is subtracted from that of survival following transplantation to yield a raw score that is normalized on a scale from 0 to 100—the final LAS [22]. Those with the highest LAS are assigned greatest priority for transplantation. In essence, those patients who are in greatest need of transplantation and have the longest anticipated duration of survival with transplantation are the ones who are assigned the greatest priority to receive a transplant, and this system is applied for all indications for lung transplantation. Following implementation of the LAS, the average time spent on waitlists decreased, the total number of transplantations increased, and the average LAS score of patients who underwent transplantation increased [23]. Later on, however, waitlist mortality increased

marginally. A suspicion is that, with advancements in treatment for all patients with lung disease, progressively sicker patients have been listed for transplantation, and overestimation of post-transplant mortality by the LAS may bias against transplantation for these people [24].

For PwCF in particular, a recent study showed that median transplant-free survival for patients whose FEV1 measured less than 30% predicted was only 6.6 years [25]. Unfortunately, the median time to transplantation was 8.3 years. Additionally, according to the annual report from the CFF [7], in 2021, 232 PwCF died, but only 54 PwCF underwent lung transplantation. One likely explanation is the insufficient supply of transplantable organs and inadequate referral of patients to transplantation. On average, the lung retrieval rate is lower (15–20%) when compared to other solid organs [26]. Donation after cardiocirculatory death (DCD) accounts for less than 5 percent of the donation pool but recent data showed short- and long-term outcomes after lung transplantation using controlled DCD donation yielded excellent results that were comparable to those of donation after brain death (DBD) [27]. The use of ex vivo lung perfusion (EVLP) as a method to improve organ quality and reverse any injury before transplantation helps to evaluate lungs from uncontrolled DCD and expand the pool of donor lungs available [27]. EVLP use slowly increased overtime, to almost 6% of deceased donor lungs recovered for transplant in the United States since 2015, and there is no evidence of a negative impact on one-year recipient survival [28]. Data are limited regarding the implementation of these tools for CF lung transplantation recipients; however, combined, these approaches are being carried out more frequently to combat an insufficient donor pool. Balancing the acceptable tolerability of donor organ function with transplant outcomes will also help to reduce waitlist mortality.

As the LAS does not consider factors specific to CF that are associated with increased mortality, such as frequency of CF pulmonary exacerbations, life-threatening hemoptysis, or recurrent pneumothoraces, waitlist mortality for PwCF remained greater than 10% even following implementation of the LAS [29]. Nolley et al. proposed modifications to the LAS to include CF-specific risk factors for mortality. While data are limited, and are not yet validated in an era where use of modulator therapy is more widespread, there is some literature to suggest that use of parameters specific to mortality in CF would increase the predictive value of the LAS for this demographic [30].

As of March 2023, a new Composite Allocation Score (CAS) has taken the place of the LAS. Analogous to the LAS, it includes estimates of the mortality prior to, and following, transplantation of a particular person. Unique to the CAS is information about the proximity of the organ donor and recipient to one another (in the interest of maximizing the survival of as many transplantable organs as possible), related to candidates who are of particular consideration for transplantation (patients younger than 18 years of age and those who previously served as organ donors), and concerning candidates who are hard to match with donor organs, such as those whose height or antibody panels are not compatible with most donor organs (in the interest of limiting the time that these people spend on transplant waitlists) [31]. Thus far, based upon data only from simulated patient scenarios, the lung CAS is expected to reduce waitlist deaths and decrease the average time spent on a waitlist while maintaining post-transplant survival [32]. The final new component is of particular relevance to PwCF. Many PwCF likely have suffered from lifelong nutritional deficiencies, intestinal malabsorption, and chronic inflammation, contributing to poor growth rates during childhood and reduced stature as compared to the general population. Although data are yet to come, the lung CAS is expected to lead to a shorter average waitlist time for these individuals.

3. Post-Transplant

In 2021, the CFF released a consensus statement [33] for the care of CF lung transplantation recipients (CFLTRs), as CFLTRs have unique comorbidities that require specialized care. The CFF organized a multidisciplinary committee that formulated 32 recommendations covering topics related to infectious diseases, endocrinology, gastroenterology,

pharmacology, mental health, and family planning. A main emphasis was on the close coordination of care between the lung transplantation team, the CF care center, and various sub-specialists with experience in the care of PwCF and lung transplantation recipients. The recommendations of the committee are listed in Table 2.

Table 2. CFF recommendations for the care of PwCF after lung transplantation.

Category	Recommendation	% Vote
General Care	CF Lung Transplant Recipients should follow up with a multidisciplinary CF care team within 6–12 months of transplant to resume extra-pulmonary CF care. Communication between the transplant and CF care teams is essential for coordination of care	100%
General Care	CF and Transplant programs should operationalize infection prevention and control policies across all services as indicated by the CF Foundation's Infection Prevention and Control Guidelines	95%
Infectious disease	Non–invasive CF-specific bacterial, fungal, and AFB respiratory cultures should be obtained by the transplant or CF center every 3 months in actively waitlisted transplant candidates, and clinicians should review prior pathogen history to guide the peri-operative antibiotic regimen	100%
Infectious disease	An intraoperative CF bacterial, fungal and AFB culture of the native lung should be obtained at the time of lung transplantation	100%
Infectious disease	In CF Lung Transplant Recipients with multidrug resistant pathogens, susceptibility-driven antimicrobials should be administered when the recipient has a susceptible antibiotic choice with acceptable toxicity. In the absence of a susceptibility-driven perioperative choice, previously effective regimens should be considered	100%
Infectious disease	For CF Lung Transplant Recipients, there are insufficient evidence to recommend for or against routine intraoperative pleural and tracheal irrigation with antimicrobial agents to decrease infections after transplant	100%
Infectious disease	Perioperative and/or early posttransplant inhaled antibiotics for bacterial pathogens isolated prior to transplant should be considered as a complement to systemic antimicrobials in CF Lung Transplant Recipients	100%
Infectious disease	There is insufficient evidence to recommend for or against the use of inhaled antibiotics for prevention of recolonization or chronic lung allograft dysfunction (CLAD)	100%
Infectious disease	There is insufficient evidence to recommend for or against the routine collection of sputum for bacterial, fungal or AFB cultures in asymptomatic CF Lung Transplant Recipients	95%
Sinus Disease	In individuals with CF and asymptomatic chronic rhinosinusitis (CRS), the CF Foundation recommends against pre-transplant prophylactic sinus surgery for the prevention of lung graft colonization.	100%
Sinus Disease	CF Lung Transplant Recipients should be screened for symptoms of CRS at least annually	100%
Sinus Disease	CF Lung Transplant Recipients with moderate or severe symptomatic CRS should be seen in consultation with an otolaryngologist experienced in CF for consideration of optimal topical therapies and endoscopic sinus surgery	100%
Nutrition and GI Complications	CF Lung Transplant Recipients should receive ongoing consultation with a dietitian with CF expertise in order to obtain individualized nutritional therapy to achieve an established BMI or weight-for-length goal	100%
Nutrition and GI Complications	In CF Lung Transplant Recipients, vitamin D supplementation should be continued, but a combination of vitamin A,D,E,K supplements should be discontinued after lung transplantation; fat soluble vitamin levels should be measured by 3 months after transplant, and levels should be repleted and followed individually as needed	100%
Nutrition and GI Complications	Symptoms should be assessed daily in hospitalized patients, particularly within the immediate post-operative period and with any opiate medication administration, for early signs of obstipation and obstruction that might herald emergence of distal intestinal obstruction syndrome (DIOS)	100%

Table 2. *Cont.*

Category	Recommendation	% Vote
Nutrition and GI Complications	In CF Lung Transplant Recipients who develop DIOS, early enteral lavage should be considered. Refractory DIOS should be managed in coordination with experts in CF gastrointestinal complications to reduce risk for prolonged obstruction and potential need for operative management	100%
Nutrition and GI Complications	For CF Lung Transplant Recipients who experience new or worsening symptoms of gastrointestinal dysmotility, a gastroenterologist and a dietitian with CF expertise should be consulted to guide the approach to symptom control and potential interventions	100%
Nutrition and GI Complications	CF Lung Transplant Recipients should undergo liver enzyme monitoring for CF Liver Disease (CFLD) at least annually, and when levels are elevated, patients should receive non-invasive imaging techniques for initial evaluation	
Diabetes and Bone Health	CF Lung Transplant Recipients who do not have Cystic Fibrosis-Related Diabetes (CFRD) should be screened with an oral glucose tolerance test (OGTT) at 3–6 months after transplant and then annually–following the recommended screening guidelines for CFRD	95%
Diabetes and Bone Health	CF Lung Transplant Recipients who have CFRD should be treated with insulin and should undergo intensive self-blood glucose monitoring (SBGM) and individualized close clinical follow-up in addition to lifestyle modifications. Furthermore, an endocrinologist with expertise in CF and transplant associated DM should be consulted when possible	
Diabetes and Bone Health	For CF Lung Transplant Recipients, bone density should be assessed with dual energy X-ray absorptiometry (DEXA) at 6–12 months after transplant	100%
Mental Health and Family Planning	CF Lung Transplant Recipients should have mental health screening and consultation for depression, anxiety, and post-traumatic stress disorder (PTSD) within *6* months of transplant and then should resume annual screening per the International Committee on Mental Health (ICMH) Depression and Anxiety Guidelines	100%
Mental Health and Family Planning	Caregivers of CF Lung Transplant Recipients should be screened for depression, anxiety, and PTSD within 6 months of transplant and should be referred for further assessment if necessary	90%
Mental Health and Family Planning	Females with CF who are post-lung transplant and are considering pregnancy should assess carefully their individual risks through shared decision making with maternal fetal medicine and transplant providers	100%
Mental Health and Family Planning	Females with CF who are post-lung transplant should avoid pregnancy for at least the first 2 years after transplantation because of the increased risk of acute rejection, accelerated chronic rejection, and death	100%
Pharmacology and Therapeutics	There is insufficient evidence to recommend for or against the use of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulators for CF Lung Transplant Recipients	100%
Pharmacology and Therapeutics	There is insufficient evidence to recommend for or against the use of induction immunosuppression for CF Lung Transplant Recipients	100%
Pharmacology and Therapeutics	CF Lung Transplant Recipients should have close monitoring of calcineurin inhibitor drug levels because of altered pharmacokinetics	100%
Pharmacology and Therapeutics	Reduced renal function is common in CF Lung Transplant Recipients, and serum creatinine is often a poor surrogate for renal function. Therefore, medications should be dosed carefully according to the estimated glomerular filtration rate (GFR) of the patient, and when available, therapeutic drug monitoring should be implemented	100%
Pharmacology and Therapeutics	There is insufficient evidence to recommend for or against the routine use of airway clearance, dornase alfa, or hypertonic saline among CF Lung Transplant Recipients	100%

Note. Reprinted from Table 1 in [34]. Creative Commons user license link: https://creativecommons.org/licenses/by/4.0/ accessed on 10 April 2023.

4. CF Pathogens

A common challenge in lung transplantation in PwCF is the various pathogens seen in the CF respiratory tract [35]—some of which portend worse outcomes (e.g., *Burkholderia cnocepacia*) post transplant. Persistent infections of the allograft lung impose a risk for

chronic lung allograft dysfunction (CLAD) [36]. There are two forms of CLAD: bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS). BOS is characterized by an obstructive pulmonary function with radiographic evidence of air trapping on a computed tomography (CT) scan; histopathology demonstrates obliterative bronchiolitis. In RAS, pulmonary function is restrictive, CT demonstrates pleural–parenchymal infiltrates, and pleuroparenchymal fibro-elastosis is seen on histopathology [37]. Certain pathogens such as *Pseudomonas aeruginosa* are significant risk factors for BOS. Thus, isolation of these pathogens from lung transplantation candidates and recipients are heavily considered in their clinical management. Here, we highlight CF pathogens that deserve particular attention during evaluation for, and following, lung transplantation.

4.1. Pseudomonas aeruginosa

Pseudomonas aeruginosa (PsA) is the most common organism colonizing the airways and sinuses in PwCF. It has been reported in up to 60%, although the prevalence appears to be decreasing since ETI was introduced as HEMT. It is yet to be determined if this is a direct effect of ETI or, more likely, a reflection of poor culture sampling due to diminished sputum production in the majority of PwCF taking ETI [7]. Oftentimes, these bacteria are multi-drug resistant (MDR) given the history of frequent antibiotic treatments that most CF patients receive over the course of their lives for management of pulmonary exacerbations [38]. The PsA strain in CF also tends to be mucoid alginate-producing, which is responsible for the production of a protective biofilm for the bacteria against airway clearance and antibiotic penetrance [39]. The mortality impact of MDR PsA compared to that of more susceptible PsA strains is unclear at this time in CF patients after lung transplantation [40,41]. There are two phases to PsA infection: acute and chronic [42]. Acute pulmonary exacerbations typically present with both pulmonary and systemic symptoms including increased sputum production, increased cough, malaise, and weight loss. Treatment for the acute phase typically includes airway clearance and antibiotics that target lower airway microbes in addition to those that target PsA. In contrast, chronic phase is more indolent and defined by Leed's criteria as having >50% sputum cultures positive for PsA in the preceding 12 months. Treatments for patients with chronic infections include cycling anti-PsA inhalational antibiotics such as inhaled tobramycin or aztreonam. Intermittent administration of oral and/or intravenous antibiotics is commonly used for disease control [36].

Researchers explored the relationship between post-transplantation bacteria and transplantation outcomes, and preliminary data suggested PsA infection or colonization was likely associated with increased BOS risk [43]. The exact mechanisms remain unknown, but studies in animal models and human lung allografts suggested that PsA colonization in lung allografts may promote activation and migration of lymphocytes into the lung allografts resulting in damages in the lung allograft epithelium [36]. Certain chemokine receptors such as CXCR1 and CXCR2 are integral membrane proteins that bind and respond to cytokines. Studies noted that in PwCF, CXCR1+ lymphocytes were found more frequently, in comparison to patients without CF, suggesting PsA may promote the expansion of CXCR1+ lymphocytes [44]. CXCR1/2 chemokine/ligand axis uses ELR+ chemokines (CXCL1, CXCL5, CXCL7, CXCL8), which are chemotactic for both neutrophils and lymphocytes expressing CXCR1 or CXCR2. Later studies would find that PsA infection, but not colonization, and elevated levels of CXCL1, increased the risk of BOS, with higher levels of CXCL1 furthering the risk [36].

4.2. Burkholderia cepacia Complex

Burkholderia cepacia complex (Bcc) poses a very high risk of reinfection after transplantation and is associated with high mortality rates. Similar to PsA, Bcc infection pre-transplantation persists after transplantation [45]. Bcc comprises over 24 different species. Bcc species most often found in PwCF are *B. cenocepacia*, *B. mutivorans*, *B. vietnamiensis*, *B. dolosa*, *B. cepacia*. While not a part of the Bcc, *B. gladioli* is another *Burkholderia* species of-

ten seen in CF [46,47]. Similar to pseudomonas, many strains of Bcc are MDR although Bcc species tend inherently to be resistant to many antibiotic classes. In a cohort of 216 PwCF who underwent lung transplantation, 22 had Bcc pre-transplantation, including 12 with *B cenocepacia*. Nine patients infected with *B. cenocepacia* died post-transplantation, and these deaths were attributed to Bcc sepsis [48]. Colonization with *B. cenocepacia* is commonly a contraindication to lung transplantation in many centers. Bcc carries a heterogeneous virulence; for instance, *B. multivorans* is considered less virulent than *B cenocepacia* and was not associated with a worse survival difference compared to non-infected patients post-transplantation [49].

The clinical course of an infection with a Bcc organism can be unpredictable, but the outcome is often related to the pathogenicity of the infecting strain [50]. Bcc typically establishes chronic infection in patients with CF, but can occasionally cause acute necrotizing pneumonia with respiratory failure and sepsis—termed cepacia syndrome. Bcc species tend inherently to be resistant to many antibiotic classes, and as a result, treatment of Bcc is challenging. To date, there are no randomized controlled trials of treatments for respiratory exacerbations in patients with CF chronically infected with Bcc. Thus, there are no optimal antibiotic regimens present [51]. At this time, clinicians must rely on susceptibility data, prior clinical responses and their experience until more data are available.

4.3. Mycobacterium abscessus

Mycobacterium abscessus (*M. abscessus*) and mycobacterium avium complex (MAC) are the most common nontuberculous mycobacteria (NTM) found in PwCF [52]. Infection prior to transplantation is associated with an increased risk of recurrence following surgery despite attempts at eradication. *Mycobacterium abscessus* is of particular interest in the field of lung transplantation due to its complication rate post-operatively [53]; for this reason, it may be considered a relative, and sometimes absolute, contraindication in certain transplantation centers [17,18]. Nonetheless, favorable outcomes have been reported in PwCF who are colonized with *Mycobacterium abscessus* and are lung transplantation recipients [54]. Treatment is challenging to tolerate and requires a multidrug treatment regimen. Data on treatment outcomes in PwCF and *Mycobacterium abscessus* lung infections remain limited despite its clinical significance. The American Thoracic Society and the Infectious Disease Society of America recommend a typical treatment schedule divided into two phases: an initial intensive phase and a continuation phase [55]. The intensive phase is composed of an oral macrolide and intravenous amikacin with one or more additional intravenous antibiotics, such as tigecycline, imipenem, or cefoxitin, for a 3–12 week period—depending on severity of infection, response to treatment, and tolerability of the regimen. The continuation phase continues the oral macrolide, and includes inhaled amikacin and two-to-three additional antibiotics such as minocycline, clofazimine, moxifloxacin, or linezolid. The choice of antibiotic should be guided, but not dictated, by drug susceptibility testing. Testing for drug toxicity is essential. Much of this crucial work is carried out through multidisciplinary care and coordination with experts in NTM treatment.

4.4. Aspergillus

Aspergillus is a common fungal infection occurring in up to 50% of patients awaiting lung transplantation. *Aspergillus* can demonstrate an array of disease states including asymptomatic colonization, allergic bronchopulmonary aspergillosis, tracheobronchitis, and invasive aspergillosis. Amongst PwCF who have undergone lung transplantation, studies demonstrated that there is a two-to-four-fold higher incidence of *Aspergillus* infections as compared to non-CF patients [56]. Factors that impact airway colonization/infections include direct exposure to the environment, impaired mucociliary clearance, immunosuppression, and donor-transmitted infections. Voriconazole is the drug of choice for invasive aspergillosis, as it was shown to improve outcomes in severely immunosuppressed patients [57]. Other therapies include amphotericin B, caspofungin, and posaconazole, but these treatments are often considered second-line or salvage therapy [58]. Following trans-

plantation, *Aspergillus* is associated with BOS, increases the risk of infections, and impairs oxygenation and ventilation. Due to the risk of BOS, most CF centers will often administer antifungal prophylaxis to their patients after lung transplantation [59]. However, more studies will be required to determine the optimal length of prophylactic therapy balancing the benefit of prophylaxis with the risks of drug toxicities.

4.5. COVID-19

Over the course of the past several years, the pandemic of COVID-19 infection undoubtedly devastated several patient populations across the entire world. Early in the pandemic, initial reports [60] and subsequent data [61] alike suggested that rates of infection among PwCF were not significantly higher than those of the general population. Although altered pulmonary anatomy and physiology might have mechanistically suggested a predisposition to infection, possible protective factors for PwCF include use of airway clearance medications and pre-existent knowledge about infection control practices and social distancing. Nonetheless, COVID-19 infection can predispose PwCF to pulmonary exacerbations and can lead to severe, life-threatening disease, particularly in lung transplant recipients who are immunosuppressed [61].

5. Noninfectious Complications

Noninfectious complications unique to CF patients after lung transplantation include endocrine, gastrointestinal, and oncologic pathologies. CF patients at baseline are uniquely susceptible to the development of insulin deficiency, and after transplant due to the use of corticosteroids and immunosuppressive therapies, the prevalence of diabetes mellitus increases [62]. Post-transplantation status and corticosteroid exposure also predispose to accelerated bone loss, pathological fractures, as well as osteonecrosis [63]. Epstein–Barr Virus mismatch, in the context of chronic immunosuppressive therapy, increases the risk for malignancy, particularly post-transplant lymphoproliferative disease (PTLD). Cancer screening is discussed in more detail in the article entitled "Future Comorbidities in an Aging Cystic Fibrosis Population".

Gastrointestinal complications include gastroesophageal reflux and intestinal dysmotility. These conditions are common in CF, and they may worsen after transplantation [64]. Due to inherent dysmotility, intestinal obstruction syndrome may occur and result in further malnutrition which is an important risk factor for poor outcomes after surgery [65]. Distal intestinal obstruction syndrome (DIOS) occurs in 15.9% of adults with CF and increased risk of gastrointestinal complications such as DIOS is well known after lung transplantation [64]. Differentiating DIOS from other causes of bowel obstruction, such as constipation, in CF may be difficult, but one of the more widely used definitions for DIOS is an acute complete, or incomplete, fecal obstruction in the ileocecum. Constipation, however, is defined as gradual fecal impaction of the total colon [66]. Studies have shown that the incidence of DIOS post lung transplantation is roughly 10% [67]. Factors that are theorized to contribute to this syndrome include pancreatic insufficiency, reduced intestinal water content, slow intestinal transit, and dehydration of the mucus layer due to altered intestinal secretion [68]. Additionally, pre-transplantation abdominal operation and prior meconium ileus were some of the strongest predictors of developing DIOS [67]. Preventive therapy for DIOS includes the implementation of oral laxatives, adequate hydration, and pancreatic enzyme supplementation; however, there is a lack of consensus regarding the best preventive measures [69].

6. Prognosis

The median survival of CF patients after lung transplant is 8.3 years, whereas it is 5.7 years after lung transplant for all indications [70]. Although lung transplant is the standard of care for CF patients with end-stage lung disease, and although predictors of mortality prior to transplant are well defined, factors that prognosticate mortality following transplant in CF are not as well established. Risk stratifications to determine prospective

lung transplant candidates who would benefit most from transplantation can be challenging [71]. A systematic review and meta-analysis evaluated risk factors of age, gender, FEV1, pulmonary hypertension, BMI, CF-related diabetes, and pre-existing infections with Bcc and/or PsA with post-transplant mortality in CF [72]. The single risk factor associated with increased mortality after transplantation was pre-existent Bcc infection. As discussed previously, the species of Bcc have varying virulence, and so, this must be taken into account; *B. cenocepacia* is associated with worse mortality. Thus, it is vital to identify species of microorganisms that are commonly identified within a complex. Surprisingly, factors that are known to be associated with waitlist mortality, such as low FEV1, pulmonary hypertension, low BMI, female gender, and CF-related diabetes, were not associated with post-transplant mortality. Therefore, the use of existing risk stratification tools may actually underestimate the survival benefit of transplantation in CF. The most likely reason that low FEV1 and pulmonary hypertension do not affect the mortality after transplant is that the surgery leads to the improvement of these two factors.

For some time, low BMI was believed to be associated with poorer outcomes in CF lung transplantation recipients. The theory was that worsening CF with advanced lung disease is correlated with reduced nutrition as patients will have dyspnea, anorexia, and increased basal energy expenditure from chronic respiratory infections. Early data showed that underweight status prior to lung transplantation was associated with mortality after transplantation [73]. Therefore, low BMI was frequently one of the absolute contraindications to lung transplantation [74]. Challenging this idea, recent data from the United Network for Organ Sharing (UNOS) dataset revealed that the median survival in patients with CF and BMI < 17 kg/m^2 was 7.0 years, which was comparable to subjects with other commonly transplanted diagnoses [75]. A likely reason for this disparity between theoretical risk and clinical outcomes data is that BMI does not differentiate between fat mass and fat-free body mass. In comparison, sarcopenia may be a more useful prognostic indicator. One study demonstrated that decreased muscle mass index, as measured by CT scan at the time of lung transplantation, was associated with decreased survival and increased hospital length of stay after transplantation [76]. Another study similarly showed that low muscle cross-sectional area, as measured by CT scan, was associated with increased duration of hospitalization after transplantation [77]. Although pre-transplantation FEV1 has not been associated with post-transplant mortality, as described previously, low FEV1 is a marker for severe disease. Thus, as recent data demonstrated that sarcopenia prior to transplantation is a predictor of poor outcomes subsequent to transplantation, it is understandable also that skeletal muscle mass index prior to transplantation is associated directly with FEV1 [78]. Another explanation for the lack of correlation between pre-transplant BMI and post-transplant outcome may be that nutritional status is reversible. Underweight patients with CF were shown to have higher risk of death without lung transplantation, highlighting the urgency for transplantation [75]. These new data challenge pre-existing beliefs and highlight that patients with CF and low BMI may be unjustifiably excluded from lung transplantation.

Although transplantation in patients with CF, as compared with transplantation in the context of other end-stage lung diseases, is associated with relatively favorable outcomes, CF patients tend to be quite young at the time of transplantation. For that reason, eventually, some CF patients will develop CLAD, but they may continue to have relatively limited medical comorbidities, so they may be eligible for an additional lung transplant. CLAD occurs in about 50% of recipients 5 years after lung transplantation [79]. Unfortunately, the survival rates of re-transplantation remain significantly worse, compared to those of primary transplantation despite advances in immunosuppression and post-operative management [80]. The risk factors associated with developing CLAD remain unclear. What has been shown is that patients who require repeat transplantation often experience a decline in clinical status across multiple organ systems [80]. The more notable organs include the renal system, with higher baseline creatinine, and the pulmonary system with increased oxygen requirements or lower FEV1 [80]. Due to these organ failures the patients

are often bridged with dialysis for their kidneys or with mechanical ventilation and/or extracorporeal membrane oxygenation (ECMO) for their pulmonary/circulatory failure. It is important to note, however, that, in general, patients who previously have undergone lung transplantation are at about a five-fold increased risk for all-cause mortality, as compared to the general population [81]. Additionally, these forms of external organ support were noted as predictors of worsening cardiopulmonary status and graft failure three years after transplantation [82] and, thus, are likely only further to exacerbate this known mortality risk. It is also important to note that, as compared to patients undergoing a primary lung transplantation, those who ultimately require a re-transplantation require a greater duration of mechanical ventilation and, on average, have a longer post-transplantation ICU stay [83]. Ultimately, these individuals have worse pulmonary function than those undergoing a first transplantation [83], and some single-center studies even noted greater mortality [84,85]. Therefore, earlier identification of patients who are at higher risk of needing another transplantation is imperative, as it may allow for earlier intervention and, thereby, alleviate the need to bridge these patients to transplantation with mechanical ventilation or ECMO. It also may allow for the optimization of patients such that they are in better condition prior to the re-transplantation surgery. In particular, preliminary data regarding the use of inhaled cyclosporine are promising. An initial study of 58 patients published in 2006 demonstrated that inhaled cyclosporine administered prophylactically to lung transplantation recipients did not decrease the incidence of acute rejection, but it did increase the duration of both survival and chronic, rejection-free survival [86]. A subsequent phase II study regarding the use of inhaled cyclosporine in patients who developed BOS demonstrated a decreased incidence of progression of BOS grade in the treatment arm [87]. Data from phase III randomized controlled trials of inhaled cyclosporine use in patients who suffer from BOS, both in the context of single lung transplantation [88] and double lung transplantation [89], are pending. Given these promising data that demonstrate potential for intervention to mitigate the need for re-transplantation, it is of utmost urgency to determine risk factors associated with the development, and worsening, of CLAD. The exact predisposing features associated with CLAD remain elusive, but some studies associated thymoglobuin use, number of infections treated, and certain infections with increased incidence of CLAD [37]. However, additional studies are needed to identify the specific risk factors associated with the development of CLAD.

7. The Changing Landscape of Lung Transplantation in CF

The average age of patients with CF undergoing lung transplantation has been rising—arguably as a result of implementation of modulator therapy and improved comprehensive care of these patients. The percentage of pediatric patients among those who received a lung transplant for CF decreased from 12.5% between 2005 and 2009 to 9.6% from 2010 to 2014, and the percentage of patients aged greater than 40 years increased 14.2% to 17.2% over the same time period [90]. According to pooled data from the UNOS from 1992 to 2016, of all PwCF who underwent lung transplantation, those patients who were older than 30 years had a significantly greater median survival as compared to those whose ages were between 18 and 29 years old [90]. Secondary analysis demonstrated that the younger group had higher allograft failure as well as higher incidence of malignancy. With the increasing age of CF lung transplant recipients, one could hypothesize that this could translate to improvement in survival post-transplantation. However, older aged patients may also carry additional age-related comorbidities. Further studies are needed to elucidate the underlying mechanisms of age-related disparity in survival, but there are a few hypotheses [91]. The older recipients may have had phenotypically milder CF; thus, they did not require a transplantation until they reached an older age. However, this study was performed over a relatively long period of time, over which the average age of lung transplantation recipients did increase dramatically–possibly due to external factors such as ongoing improvements in standardized care, thereby delaying the need for transplantation. Behavioral maturation, associated with better medication adherence and

psychosocial support, is another possibility for the observed difference in survival between the age groups. Furthermore, younger-aged patients typically had public insurance, in contrast to private insurance most commonly seen in older patients, which may have contributed to their level of access to care. Another observation is that cytomegalovirus (CMV) and Epstein–Barr virus (EBV) recipient sero-negative status was more likely to occur in younger patients, resulting in a higher chance for CMV and EBV mismatch if the donor was sero-positive, thereby increasing the risk of the development of post-transplant lymphoproliferative disorders.

8. HEMT Impact on Transplantation Landscape

The first FDA-approved modulator was ivacaftor, an oral agent which targeted a point mutation, G551D, in the CFTR. Mutations in G551D result in abnormal CFTR proteins that have an impaired ability to open. Ivacaftor allows the channel opening probability to increase and, therefore, is classified as a potentiator. Early studies conducted by Accurso and colleagues [92], which evaluated the safety of this medication, found in their secondary endpoints that there were also changes in predicted FEV1 and median sweat chloride levels. Later CFTR modulator studies would also measure these changes. Although there have been studies that demonstrated no correlation between reductions in FEV1 and mean sweat chloride level, with respect to an individual patient, in the context of ivacaftor use, when these studies were pooled together with respect to a population, sweat chloride level changes did appear to correlate with lung function changes [93].

Since the approval of ivacaftor, there have been other potentiators as well as correctors that were approved for CF. Further clinical trials demonstrated that a combination of potentiators and correctors are more efficacious than monotherapy alone [93]. The newest FDA-approved combination therapy is elexacaftor/tezacaftor/ivacaftor (ETI), the first triple combination therapy, for patients who are 12 years and older that have CF with at least one F508del mutation in the CFTR protein.

The development of HEMT such as ETI revolutionized the management not only of CF patients over the course of their lifespan but also of those with advanced lung disease. It is important to recognize that although CF patients no longer suffer from CF lung disease after transplantation, these patients continue to suffer from systemic complications of CF. Previously, it was theorized that patients who had undergone lung transplant would not benefit from HEMT because the primary end point for modulator therapy trials was commonly the change in FEV1% predicted. However, recent studies demonstrated that the use of HEMT, even after transplantation, helps to manage important non-pulmonary manifestations of CF, including nutritional and gastrointestinal outcomes, sinus disease, and persistent pseudomonal infections [94]. Other notable considerations in using ETI following transplantation include observational data demonstrating no impact on graft dysfunction [95] or on immunosuppression drug regimen/dosing [96]. As a result, the use of HEMT post-lung transplant may be of potential benefit with acceptable safety profiles. Nonetheless, clinicians must be vigilant and monitor for side effects and toxicities of HEMT including drug–drug [96] interactions, even though relative safety in combination with immunotherapy was observed. More research is needed before further recommendations can be made regarding the use of HEMT post lung transplant.

9. Conclusions

While CF is a rare disease, it has the potential to cause significant morbidity and mortality to those with advanced pathology, and the greatest suffering in CF is associated with pulmonary disease. The only cure for end-stage lung disease is pulmonary transplantation. Despite the hurdles specific to coordination of lung transplantation for CF patients, its success in this demographic is evident. The pathology central to CF was only first described in a case series in 1938 [97], and its gene was only identified in 1989 [98]. Nonetheless, lung transplantation for CF has been performed since 1983 [99], and CF now accounts for

about 17% of pre-transplant diagnoses [70]. The median survival of CF patients after lung transplant is 8.3 years, whereas it is 5.7 years after lung transplant for all indications [70].

Although HEMT continues to demonstrate promise in mitigating the symptoms of, and progression to, severe disease, it cannot be used to treat all illness-causing mutations, and some patients suffer from disease that already advanced greatly. Thus, it is likely that lung transplantation will remain a cornerstone of treatment of terminal pulmonary disease in CF for years to come.

However, there are still a lot of advances to be made to ensure that as many eligible patients as possible survive to the time of transplantation—particularly via modification of the lung allocation scoring system for CF patients. With ongoing advancements in care for CF patients, both before and after transplant, particularly due to the implementation of HEMT, pre- and post-transplant longevity of CF patients is likely to improve. For that reason, future research may focus on re-defining indications or contraindications for transplantation in this patient population. For instance, disease complications, such as suboptimal BMI, or infections with highly virulent organisms, which may previously have been deemed contraindications to transplantation, may represent stronger indications for transplantation in the future. Additionally, investigation might focus on longevity post transplantation, CLAD, and indications for re-transplantation, as the population of long-term survivors after transplantation grows.

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Review

Addressing Symptom Burden and Palliative Care Needs in Cystic Fibrosis: A Narrative Review of the Literature

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Abstract: Among people with cystic fibrosis (CF), illness burden is multifaceted, and symptoms may fluctuate in intensity across a lifespan. Caregivers of people with CF may also experience distressing symptoms. Recent developments in CF care, including the availability of highly effective modulator therapies (HEMTs) and new palliative care guidelines promoting palliative care screening may help alleviate symptoms. The objective of this review was to present a narrative view of the recent literature on symptom burden in CF, new screening approaches informed by the Cystic Fibrosis Foundation (CFF) palliative care guidelines, and early data from studies examining the impact of HEMTs on CF symptom burden. A review of the relevant literature was conducted using Google Scholar and PubMed. Included articles covered approaches to burden assessment in CF and other chronic illnesses, epidemiology of CF symptom burden, the impact of HEMTs on symptom burden, and the CFF palliative care guidelines. A primary palliative care model implementing the CFF guidelines was also described. Results of this review show that while recent developments in CF care have led to a reduction in physical symptoms, mental health symptoms remain prevalent. Ongoing screening and triage can ensure that physical symptoms, psychological symptoms, social needs, practical problems, and communication concerns are addressed by care teams.

Keywords: cystic fibrosis; symptom burden; palliative care; quality of life

1. Introduction

The illness burden experienced by individuals with cystic fibrosis (CF) and their caregivers can be characterized by problems in many domains (e.g., physical symptoms, psychological distress, and caregiver burden) [1]. The severity of this burden varies across subgroups with CF and may fluctuate across the lifespan and gradually worsen in the context of advanced illness. However, recent advances in novel CF treatments have helped to address symptoms and reduce burden among this population. In particular, the development of highly effective CF transmembrane conductance regulator (CFTR) modulator therapies (HEMTs) that correct the underlying molecular defect causing CF disease [2] has reduced physical symptom severity for many. The promulgation of recent palliative care screening guidelines [3] and recognition of the value of early and ongoing palliative care for CF [4,5] has helped care teams implement new approaches to identify sources of distress among patients and caregivers. Herein, we review the recent literature

on the epidemiology of symptom burden in CF, new screening approaches informed by the CF palliative care guidelines, and early data from national studies examining the impact of HEMTs on CF symptom burden.

The objective of this narrative review is to summarize the research literature examining symptom burden in adult and pediatric populations with CF, describe a screening approach informed by the CFF Palliative Care Guidelines, and highlight emerging data on the impact of HEMTs on symptom burden, including a growing body of literature that has focused on family caregivers of CF patients. We also describe the epidemiology of psychological distress among this population. For this narrative review, we included peer-reviewed journal articles and presentations with emerging/preliminary data from 2000–2023. This included research on the prevalence of physical and/or psychological symptoms and distress related to these symptoms and/or associations with symptom distress and other physical or psychological outcomes such as disease severity and quality of life among people with CF and their caregivers. Search terms included "physical symptom burden in cystic fibrosis", "physical symptom distress cystic fibrosis", "depression cystic fibrosis", and "anxiety cystic fibrosis". Summaries of selected studies can be found in Table 1.

Table 1. Summary of Selected Studies on CF Symptom Burden and Palliative Care Needs *.

Reference	Study Design	Sample	Outcome
Sawicki et al., 2008 [1]	Prospective longitudinal	303 adults with CF	Results show the presence of physical symptoms: respiratory (cough and shortness of breath), fatigue, and sleep disturbances and psychological symptoms: worrying, feeling irritable, and feeling sad. The most distressing symptoms were fatigue and irritability.
Stenekes et al., 2009 [6]	Cross-sectional	123 adults and children with CF	A total of 84% of participants reported pain (with headache and abdominal pain reported most frequently). A total of 83% reported cough, with 63% of participants with cough reporting that cough always or sometimes interfered with sleep. A total of 64% of participants reported breathlessness.
Goldbeck et al., 2010 [7]	Case-control study	670 German adolescents and adults with CF	CF patients showed high levels of anxiety (20.6%) and depression (9.6%). Adults with CF had more elevated anxiety levels than healthy controls.
Besier and Goldbeck, 2011 [8]	Dyadic-based survey	162 German adolescents with CF and associated parental caregivers	Caregivers reported significantly increased rates of symptoms of anxiety and depression (38.0%) compared to adolescent CF patients (8.0–12.0%).
Ploessl et al., 2014 [9]	Retrospective chart review	190 children with CF	A total of 9% of CF patients had a diagnosis of depression. CF patients with depression had lower FEV1% predicted, more hospitalizations, and more CF exacerbations requiring treatment each year than patients without depression.
Quittner et al., 2014 [10]	Cross-sectional	6088 adolescents and adults with CF and 4102 parents of children with CF from Europe and the U.S.	Overall, anxiety and depression symptoms were elevated in patients with CF and parents at a rate two to three times that of non-CF community samples.
Friedman et al., 2018 [11]	Prospective longitudinal (Note: article only reports on data from enrollment and baseline surveys)	41 adults and adolescents with CF	At baseline, patients reported numerous physical (cough, lack of energy, shortness of breath, pain, difficulty sleeping) and psychological symptoms (feeling sad, worrying, feeling nervous). Many psychological symptoms were rated as more distressing than physical symptoms. Anxiety and depression correlated with physical symptom distress and difficulty with CF self-management, but not with disease severity.

Table 1. *Cont.*

Reference	Study Design	Sample	Outcome
Dhingra et al., 2020 [5]	Prospective longitudinal	74 adults with CF	Participants completed a monthly online screening, in which two consecutive screenings revealing one or more "indicators of concern" triggered an attempted triage with follow-up by CF professionals. In total, 164 attempted triages resulted in 84 completed triages (51.2%), of which 39 (46.4%) required prompt follow-up.
Trandel et al., 2020 [12]	Cross-sectional	164 adults with CF	A total of 78% of participants reported $\geq$1 unmet palliative care need. Needs relating to physical and daily living (72%) and psychological domains (66%) were the most prevalent.
Schechter et al., 2021 [13]	Multi-center observational	1005 adolescents and adults with CF across nine countries	A total of 25.1% of participants had a positive screen for depression, and 33.6% had a positive screen for anxiety. A positive screening result for depression was associated with an increased risk of 5-year mortality among adults with CF.

* Ordered by year.

2. Symptom Burden and Approaches to Assessment in CF

Symptoms are inherently subjective. In populations with serious chronic illnesses such as CF, there is a very substantial variation in symptom experience and the extent to which one or multiple symptoms is associated with distress. Clinical observations suggest that distress from physical and psychological symptoms is highly prevalent and often undermines quality of life. This is known as symptom burden.

Work supported by the Cystic Fibrosis Foundation (CFF) addressing the quality-of-life concerns of people with CF and their families has long included a focus on symptom management. One important example has been the ongoing effort to develop consensus-based guidelines for pain management [14,15].

In clinical research, symptoms are often considered to be a patient-reported outcome, and studies usually measure symptom burden by the presence of varied symptoms and one or two characteristics, such as intensity or frequency. This measurement approach simplifies a clinical reality that is far more complex. Symptoms are multidimensional phenomena, and clinicians usually can elicit more nuanced information by evaluating each dimension separately [16]. For example, the history can assess *severity* (distinguishing intensity right now, on average, and at its worst); *temporal features* (describing rapidity of onset, course over time, overall duration, and episodic components); *quality* (pain, for example, may be aching, sharp, dull, or burning); *bodily areas affected*; and *factors that worsen or ameliorate the symptom experience* (Table 2).

Relatedly, each symptom can be characterized by the extent to which it adversely affects physical functioning, mood, and other aspects of psychological well-being, role functioning, and relationships with others, as well as overall quality of life. Symptoms also may be characterized by the meaning attributed to them; patients may describe a symptom in terms of organ damage, worsening illness, need for a change in therapy, or in terms of its impact on family burden.

This abundant clinical information about the symptoms experienced by people with CF often influences management approaches. Symptom assessment may guide the selection of treatments for symptom-focused therapy and may also illuminate an etiology or pathophysiology for the symptom that could be targeted for intervention.

Table 2. Approach to symptom assessment [17].

Symptom Characteristics	
Severity	Numeric scale ("0–10" where "0" is not intense at all, and "10" is the highest intensity; distinguishes "symptom severity right now", "symptom severity on average during the past week", and "worst severity during the past week")
	Verbal rating scale ("none", "moderate", "severe"; distinguish "symptom severity right now", symptom severity on average during the past week", and "worst severity during the past week")
Temporal features	Onset (rapid or gradual)
	Duration
	Course stable, improving, worsening, or fluctuating
	Presence of episodic flares
Location	Focal, multifocal, or generalized
	Referral pattern
Quality	Examples for pain include "aching", "throbbing", or "burning"
Exacerbating or relieving factors	Examples include coughing, eating, specific positions
Essential Historical Information	
Premorbid and comorbid medical and psychiatric conditions	
Prior evaluation for the symptom	
Prior treatments	

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3. Epidemiology of Symptom Burden in Community-Dwelling Populations with CF

The high prevalence and multidimensional nature of symptoms has been reported by numerous studies. Most of these studies have been conducted in seriously ill populations with cancer or other illnesses [18–23]. Although relatively few studies have evaluated the heterogeneous population with CF, the extant literature suggests that CF is like other chronic illnesses in terms of the burden and distress associated with numerous common symptoms [11,24]. These data suggest that adults with CF are more likely to report physical symptom burden than children or adolescents [4,6] and that women and older people have relatively higher symptom prevalence and severity [1,4,6]. The most prevalent physical symptoms are cough, shortness of breath, fatigue, sinus discharge, difficulty sleeping, and pain [1,5,11,12]. Cough, shortness of breath, sinus discharge, and fatigue often are rated as the most severe or distressing symptoms [1,5,11,25]. Additionally, initial data from one trial in 61 adults with CF found that a significant portion of participants reported cough and fatigue as highly distressing (i.e., "quite a bit" or "very much" distressing) at baseline, as well as at 3-, 6-, and 9-month follow-up assessments [26]. This study also showed that mean distress scores for physical symptoms commonly rated as highly distressing (i.e., fatigue, dyspnea, cough, sleep disturbance, and drowsiness) remained highly distressing over nine months [26]. Additionally, some symptoms with lower prevalence rates have been reported as severe or highly distressing (e.g., gastrointestinal symptoms) to patients [5,11].

Physical symptom burden is important to assess given its strong association with health-related quality of life (HRQOL). For example, one study with 87 children and adolescents receiving care at CF centers showed that sleep disturbance was associated with lower HRQOL scores [27]. Several studies of adolescents and adults treated in CF centers show that higher symptom burden and the negative effects of physical symptoms on function may also associate with worse pulmonary function (lower FEV1% predicted), which can lead to poorer HRQOL [1,27–29].

Psychological symptoms also are common and burdensome in the population with CF. Indeed, a recent investigation of 256 adults across five U.S. CF centers who completed the Integrated Palliative Care Outcome Scale [30] observed that feeling depressed and

anxious were more frequent sources of illness burden than most physical symptoms [4,31] suggesting the potentially larger impact of psychological symptoms on burden in the era of highly effective modulator therapy.

People with CF report higher levels of depression and anxiety than the general population [5,7–10,13]. In a large study of 6088 people with CF aged 12 and older across nine countries, 11% had mild to severe depression scores on the Hospital Anxiety and Depression Scale (HADS-D [32]; range 8–15%), and 27% had elevated depression scores on the Center for Epidemiologic Studies-Depression Scale (CES-D [33]; range 20–31%). An average of 30% endorsed mild to severe anxiety, as measured on the HADS-Anxiety subscale [34]. Other studies show that feeling irritable, sad, or worried—which are often symptoms of depression or anxiety—are among the most distressing symptoms reported among adults with CF [1,5,11]. Initial data from one longitudinal study examining the trajectory of symptoms in 61 adults with CF found that symptoms rated as highly distressing (i.e., "quite a bit" or "very much" distressing) by a significant portion of participants included irritability, sadness, and concentration problems. While these symptoms became less distressing for some adults over the course of nine months, they still remained highly distressing for a portion of adults [26]. This suggests that symptoms of depression and anxiety are commonly experienced by people with CF, and CF care teams can consider addressing the range of problems summarized in Table 3.

Table 3. Symptoms to consider in the assessment and management of anxiety and depression [35].

1. Core depression symptoms
Sad, depressed, or irritable mood
Lack of interest or pleasure (e.g., social withdrawal, disengagement from usual activities)
2. Additional depression symptoms
Trouble sleeping or sleeping too much
Decreased or increased appetite, weight change
Impaired concentration, low motivation, or decline in academic/work performance
Fatigue, low energy
Feelings of guilt, worthlessness, or being a burden on others
Low libido
Increased sensitivity to criticism or perceived rejection
Trouble making decisions, feeling helpless
Hopelessness, loss of meaning, emptiness
Thoughts of death or suicide
3. Core anxiety symptoms
Anxious, worried, fearful, or irritable mood
Avoidance of anxiety-provoking stimuli
4. Additional anxiety/traumatic stress symptoms
Generalized anxiety: Excessive, uncontrollable worry in multiple situations • Trouble sleeping, fatigue • Impaired concentration • Muscle tension, restlessness • Other physical symptoms: headache, abdominal pain, nausea, diarrhea

Table 3. *Cont.*

Panic attack: Intense fear with abrupt onset • Tachycardia, palpitations, chest pain • Dyspnea, choking sensation • Sweating, shaking, paresthesia, feeling dizzy, hot, or cold • GI distress, nausea • Feelings of unreality, fear of losing control or dying
Social anxiety: Excessive fear or panic in social situations • Fear of rejection, negative evaluation, humiliation • Reluctance to speak in public or to peers • Avoidance of social situations
Specific phobia: Excessive fear, panic, or distress related to a specific object or situation • Typical examples include animals (snakes), heights, enclosed spaces • In people with CF, some phobias may be better characterized as sequelae of medical traumatic stress (e.g., intense fears of medical settings or procedures, needles, hypoglycemia, hemoptysis)
Separation anxiety: Persistent anxiety with separation, excessive for developmental stage (e.g., in children or adolescents) • High distress when anticipating or during separation from attachment figure • Worry something bad will happen to loved ones • Refusal to leave home or to be alone (e.g., in part of the house, when sleeping)
Traumatic stress/Post-traumatic stress disorder (PTSD): Follows discrete or chronic traumatic stressor(s) • Intrusion—nightmares, flashbacks, distressing memories • Avoidance of related stimuli • Change in thoughts and feelings (e.g., feeling hopeless, detached, or emotionally numb)—can mimic depression or anxiety • Hyperarousal or reactivity (poor sleep, poor concentration, increased startle, hypervigilance) • Watch for physical symptoms, impaired self-regulation, risky behavior, or trouble maintaining relationships
Obsessive compulsive disorder (OCD): A pattern of specific intrusive thoughts causing anxiety (i.e., obsessions) and/or rituals aimed at reducing anxiety (i.e., compulsions) • Obsessions about germs, harm to others, order, unwanted religious or sexual thoughts • Rituals, such as excessive washing hands, counting, reciting, seeking excessive reassurance, checking, maintaining specific order
5. Emergent and urgent symptoms and signs
Requiring immediate attention • Wish to be dead, thoughts of self-harm or suicide, self-injurious behavior • Extreme irritability or anger; reckless behavior; aggression • Minimal oral intake, severe weight loss • Rapidly fluctuating mental status or autonomic changes—consider delirium, medication side effect, substance intoxication or withdrawal, serotonin syndrome • Psychotic symptoms (delusions, hallucinations) or catatonia (a state of abnormal behavior and movement resulting in stillness or extreme restlessness)
Requiring further evaluation • Rapid worsening from baseline • Psychomotor slowing (slow, quiet speech, limited activity or movement) • Psychomotor agitation (pacing, restlessness) • Poor self-care in daily activities or for medical needs • School refusal (children) or missing work (adults) • Comorbid alcohol or substance misuse • Rapidly shifting or elevated/irritable mood, increased energy, impulsivity—consider bipolar disorder, another disorder of emotion regulation (borderline personality disorder or other personality disorders), medication side effect, substance intoxication or withdrawal

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Depressive and anxiety symptoms have been found to associate with gastrointestinal and respiratory symptom distress [1] and with some measures of more severe disease severity, such as increased pulmonary exacerbations [36,37], poorer lung function [36,38], increased healthcare utilization [39], and increased mortality [13]. Other measures of

disease severity, including lower FEV1% predicted and lower body mass index, are not significantly associated with psychological distress [1,11].

In a recent qualitative study of 16 adults managed by three U.S. CF centers, psychological and physical symptoms were found to interact [40]. Specifically, patients perceived that poor mood undermined their treatment adherence, CF-related exacerbations initiated a sense of loss of control, and increased severity of one type of symptom (physical or psychological) compounded the burden of the other [40]. Other recent qualitative studies have identified psychological burden among specific subgroups of people with CF, including those with CF-related diabetes, those taking a HEMT, and those ineligible for modulator therapy. For example, people with CF-related diabetes reported experiencing negative feelings about the diagnosis of diabetes, such as difficulty coping and shock, and increased treatment burden from managing two chronic conditions [41]. People with CF who are ineligible for HEMT have reported feelings of sadness, disappointment, and hopelessness about ineligibility. Although those taking HEMT may experience lower psychological burden, they commonly report ongoing anxiety, fear, and grief [42].

Psychological distress is highly prevalent among family caregivers of people with CF, who also have higher rates of depression and anxiety compared to the general population [7–10]. Quittner and colleagues [34] found that 4102 caregivers of young children with CF often showed similar or higher levels of depressive symptomatology than adolescents and adults with CF. Additionally, they observed that anxiety symptoms were present in nearly half of mothers on average (48%) and over one-third of fathers (36%) across eight European countries and the U.S. [34].

In summary, epidemiologic findings highlight the need to assess both physical and psychological symptoms in both adults and children with CF and the family who are caregivers. Assessing symptoms as part of a broader evaluation of quality of life is a palliative care best practice and should be performed repeatedly to capture changes that occur over time [43].

4. The Impact of HEMTs on Symptom Burden in CF

Recent developments in CF treatments include the widespread availability of HEMTs—particularly the triple combination drug elexacaftor/tezacaftor/ivacaftor [44,45]—which may reduce symptom burden among people with CF. An unpublished cross-sectional pilot study (AHRQ R03HS026970) [46] that used the Cystic Fibrosis Questionnaire-Revised [47] (CFQ-R) to assess the impact of treatment adherence on quality of life among 28 adults with CF currently prescribed an HEMT found associations among the use of HEMTs, reduced symptom burden, and higher quality of life. The study found that while mean quality of life scores were moderate to high across all domains, mean scores were typically higher (i.e., better) among those domains associated with physical symptom burden, such as physical functioning, eating, respiratory symptoms and digestion as compared to those domains associated with psychological symptoms, perceived treatment burden, and perceived overall health. These initial findings could suggest that while HEMTs have led to decreased physical symptom burden, psychological and other non-physical symptoms continue to be a source of distress in the CF population.

The following description reflects the experience of symptom burden before and after starting the HEMT Trikafta from one individual with CF:

"Life before Trikafta and life after Trikafta. This surreal, mutually understood concept is one that I've often seen discussed amongst members of the CF community. Trikafta came into my life when I was at the lowest point that I had ever experienced. In 2017, I fell into a cycle of aggressive lung infections and sickness that I could not shake. For the first time, I experienced the fear of realizing that I had developed resistance to the IV antibiotic regimens and treatments that had worked for my lungs countless times before. I was scared and felt hopeless and I was unsure if my body could actually pull itself out of this worsening spiral. Every day I felt weak and tired and felt the very palpable effects of my failing health. Managing both the physical and mental symptoms of my progressing

disease was overwhelming. I wondered after living a seemingly "normal", active life filled with very little restrictions, if this was the chain of events that would negatively change the course of the rest of my life. I essentially had to put my entire life and future on hold and spent every day fighting to try to nurse my body and lungs back to health, spending hours daily on treatments and searching for something to pull me out of the trenches. After about a year of fighting through a time that felt like a terrible dream, I emerged as a different person. My baseline lung function had decreased approximately 15–20%, I lost a noticeable amount of weight, and I felt like a shell of my younger and healthier self both mentally and physically. For the first time in my life, I actually looked ill. I was unsure if the future I had always pictured myself building without question would even be possible or if I would be able to pursue my dream of working in medicine. Then my own personal miracle happened. In 2018, I was given the opportunity to be a phase 3 clinical trial participant for Trikafta- and the rest is history.

Five years later, life after Trikafta is a beautiful life that frankly at one point I did not think was in the cards for me. Within 24 h of my first dose, I entered a transformation period where my entire body started to change. Essentially every symptom that I experienced my entire life began to slowly disappear, including being freed from my classic chronic CF cough. I am healthier now than I have ever been. I am a version of myself far beyond what I could have ever imagined was possible for my body to achieve. I have been able to phase out almost all of my previous CF treatments and I have had no indication for IV antibiotics, sinus surgery, or hospitalization, when pre-Trikafta I would undergo these types of therapies every 6–12 months. I learned what it feels like to truly be able to breathe. I am active and can run, I can maintain a healthy body weight, my mental health and quality of life has vastly improved, and most significantly, I experienced approximately a 20% increase in FEV1 lung function. As I approach my 30th birthday I see a future filled with opportunity. I am preparing to graduate from graduate school and enter the field of medicine as a medical provider- a full circle milestone. Watching my body and life transform over these last five years has filled me with such gratitude for medical research. Trikafta is changing the face of CF and giving patients the opportunity to be the authors of their own futures, just like it did for me. I am eternally grateful to have been saved by science".

It should be noted that this piece reflects the perspective of one person with CF currently taking the HEMT Trikafta and may not reflect the experiences of all people with CF, particularly those not taking this HEMT.

5. Palliative Care in CF and the CFF Palliative Care Guidelines

Palliative care is a clinical approach provided by an interdisciplinary team to decrease symptom burden and improve the quality of life for patients with serious chronic illness and their families [42,48]. However, until recently, there were no recommendations to inform CF-specific palliative care. To address this gap, in 2017, the Cystic Fibrosis Foundation convened a committee to develop consensus-based guidelines for delivering palliative care to people with CF and their family members [3]. This included defining palliative care for CF. Using a modified Delphi methodology, the members developed the following definition: "Palliative care focuses on reducing physical and emotional symptoms and improving quality of life for people with CF throughout their lives. Palliative care occurs alongside usual treatments and is individualized according to the unique goals, hopes and values of each person with CF" [49]. The guidelines offer recommendations to CF care teams for repeated screening throughout the illness course for a range of unmet needs, including physical and psychological symptoms, communication issues, and practical concerns for people with CF, and also screening for burden among family caregivers.

The recommendations specify using the Integrated Palliative Care Outcome Scale [30] to screen for unmet needs among people with CF 12 years and older, the Brief Assessment Scale for Caregivers (BASC [50]) to assess caregiver needs and burden, and additional optional measures to further clarify identified concerns. The latter include the Patient

Health Questionnaire-9 item Scale (PHQ-9 [51]) and the Generalized Anxiety Disorder-7 item Scale (GAD-7 [52]) to measure depression and anxiety, respectively, among people with CF and caregivers. Other optional measures for individuals with CF include the Spiritual Needs Assessment for Patients (SNAP [53]) to assess the desire for help with unmet psychosocial, spiritual/existential, and religious needs, and one or more of the Cystic Fibrosis Questionnaire-Revised (CFQ-R [54]), subscales to assess HRQOL. An additional measure for caregivers is the Prolonged Grief Disorder (PG-13 [55]), which may be given to caregivers following the death of a loved one with CF, to screen for persistent complex bereavement disorder. For children with CF under age 12, the guidelines recommend using the Integrated Palliative Care Outcome Scale to guide conversations with children and their caregivers to identify unmet palliative care needs [3]. In summary, the CFF consensus guidelines define palliative care for CF and provide direction for CF care teams to integrate primary palliative care into their standard clinic care, with recommendations for referral to specialty palliative care when necessary.

The Improving Life with CF pragmatic implementation trial: Routine screening for unmet palliative care needs with subsequent triage by the CF care team (i.e., *primary palliative care*) may be one approach for addressing symptoms and distress throughout the life course of people with CF and their families. To implement and test such an approach, the *Improving Life with CF* study, a pragmatic implementation trial of a multicomponent primary palliative care intervention, is currently underway in five U.S. CF centers (Massachusetts General Hospital, Northwell Health North Shore, Northwell Health Lenox Hill, Stony Brook University, and Emory University; Principal Investigators: Anna Georgiopoulos and Lara Dhingra). The intervention model comprised (1) screening-and-triage care processes, (2) CF provider education, featuring a 6 h Train-the-Trainer curriculum and 11 accredited webinars, (3) seven best-practice treatment guides for addressing high frequency symptoms, (4) 10 patient educational handouts for self-managing common symptoms, and (5) a quality improvement toolkit; the model includes both foundational elements concordant with the CFF Models of Palliative Care Delivery guidelines [3] (e.g., routine screening for unmet needs in multiple domains using the IPOS [30] and the BASC [50,56]) and modifiable, CF center-specific elements (e.g., methods and processes for screening patients/caregivers can be customized based on available resources or patient/caregiver preference). As part of the model's national implementation trial, the "Assessing for Distressing Symptoms And Palliative Care Needs through Targeted Communication" (ADAPT-CF) guide has also been created by interdisciplinary pediatric, palliative care, and communication experts and piloted to assess illness burden in children with CF under age 12 [57]. The primary outcome analysis in this ongoing trial will evaluate the change in illness burden on the IPOS between baseline and the outcome assessment following 22 months of the intervention's implementation at each site (www.improvinglifewithcf.org (accessed on 1 May 2023)) [58].

6. Discussion

In this review, we have described the nature of symptom burden in the care of patients with a serious chronic illness like CF, reviewed state-of-the-art approaches for symptom assessment, discussed the epidemiology of symptoms in the CF population and the recent widespread use of HEMTs, as well as recent developments to inform CF care, including the CFF palliative care guidelines and a large ongoing national implementation trial. This latter work supports the need for ongoing screening-and-triage in CF centers to ensure that psychological symptoms, social needs, practical problems, and communication concerns are also addressed by care teams in addition to physical symptoms.

Prior studies highlighted that symptom distress may vary widely based on a patient's age, disease severity, and many other characteristics, including the current use of treatments. HEMTs are altering the prevalence and severity of CF symptoms [59], particularly among younger patients who have initiated HEMTs at earlier ages. For example, physical symptoms may be less severe in this latter group. By offering regular multidomain screening that is not solely limited to physical symptoms, but detects other types of problems and

clinical concerns, CF providers may identify sources of distress and offer effective, tailored interventions to address these issues and possibly improve quality of life.

At the present time, not all individuals living with CF are eligible for the use of HEMTs, as these therapies are not effective for altering the effects of all CF genetic variants [45]. Furthermore, there are no data on the long-term effects of HEMTs, and it is not known if symptoms may re-emerge over time. Efforts are needed to assess the long-term impact of routine palliative care and HEMT use on HRQOL and symptom burden and to determine the impact on CF-related treatment outcomes.

For example, studies have begun to explore the impact of reducing complex medication regimens as a means of alleviating treatment burden in CF [60]. Recently, the CF medication–withdrawal study SIMPLIFY-IP-19 ("study to evaluate stopping inhaled hypertonic saline or dornase alfa in people with CF who are taking Trikafta") looked at the effects of discontinuing hypertonic saline and pulmozyme while using HEMT [60]. Early results from this open-label study in 477 adolescents and adults randomly assigned to either continue or discontinue hypertonic saline showed that the discontinuation of the drugs did not have a meaningful effect on lung function at 6 weeks; in other words, it did not result in a reduction in lung function, suggesting that there is potential to reduce medication burden in CF patients taking HEMT. However, as this was not a long-term study, a longitudinal analysis of respiratory symptoms and lung function among a larger multi-age sample is ongoing.

Another area that warrants additional study is the long-term impact of HEMTs on mental health and coping. Further research is needed to better understand the complex connections between mental health and modulators, both for those using HEMT and those who are intolerant or ineligible, as well as how optimal delivery of CF primary palliative care is evolving in the modulator era [61–66].

7. Conclusions

While HEMTs may reduce physical symptom burden, their long-term impact on CF symptoms remains unknown, and CF continues to profoundly affect the lives of many people with CF and their families. Multidisciplinary CF teams can help to improve quality of life by systematically identifying unmet needs in multiple domains—physical, psychological, communication needs, spiritual/existential distress, and practical problems—and delivering primary palliative care interventions.

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Review

Diagnostic and Communication Challenges in Cystic Fibrosis Newborn Screening

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Abstract: As of December 2009, cystic fibrosis (CF) newborn screening (NBS) is performed in all 50 US states and the District of Columbia. Widespread implementation of CF newborn screening (CFNBS) in the US and internationally has brought about new and varied challenges. Immunoreactive trypsinogen (IRT) remains the first, albeit imperfect, biomarker used universally in the screening process. Advances in genetic testing have provided an opportunity for newborn screening programs to add CFTR sequencing tiers to their algorithms. This in turn will enable earlier identification of babies with CF and improve longer-term outcomes through prompt treatment and intervention. CFTR sequencing has led to the ability to identify infants with CF from diverse ethnic and racial backgrounds more equitably while also identifying an increasing proportion of infants with inconclusive diagnoses. Using the evolution of the New York State CF newborn screening program as a guide, this review outlines the basic steps in a universal CF newborn screening program, considers how to reduce bias, highlights challenges, offers guidance to address these challenges and provides recommendations for future consideration.

Keywords: cystic fibrosis; newborn screen; IRT; ethnic diversity; CF diagnostic challenges

1. Introduction

Newborn screening for cystic fibrosis (CF) has been available in all 50 US states and the District of Columbia since December 2009 [1]. CF newborn screening (NBS) programs refer screen-positive infants to CF care centers for diagnostic sweat chloride testing and evaluation of symptoms to promote the timely diagnosis of CF, enabling early access to clinical intervention and improving outcomes [2]. CFNBS programs are most successful when experienced CF care teams work in partnership with their state's laboratory to establish, evaluate and improve upon their CF newborn screening algorithm in accordance with the unique needs and challenges of their specific programs [1]. Using the New York State CFNBS program as our guide, we review the basic steps in all CF newborn screening programs and consider how to confront the challenges, including structural bias, that impede improved performance of screening protocols. We also discuss and provide recommendations for future areas of study.

2. Overview of IRT (Immunoreactive Trypsinogen)

In 1979, a breakthrough occurred in the early diagnosis of CF. It was noted that elevated levels of immunoreactive trypsinogen (IRT), a pancreatic enzyme precursor, could be measured using dried blood spots and were elevated in infants born with CF [3].

Despite lacking sensitivity and specificity as markers for CF, IRT is universally used as the initial test for CF in newborn screening programs [1]; the shortcoming of this imperfect biomarker presents both limitations and areas for improvement. Many factors can affect IRT measurements including birthweight, fetal distress, delayed testing, mishandling of specimens, storage and even seasonality [4]. The optimal IRT cut-off level for a positive result is controversial; an IRT level cut-off that is too high may miss cases and an IRT level cut-off that is too low can result in high rates of false-positive results (16) which can lead to unnecessary testing and undue stress for parents of children who test positive. Many states have adopted a floating IRT level cut-off that fluctuates based upon daily or weekly percentiles rather than a fixed IRT value [4] and some programs, typically those which routinely collected a second NBS sample from all infants, used a repeat IRT specimen as a second screening tier (IRT/IRT) [5]. Colorado was the first state in the US to undertake a CF newborn screening program in 1982 using an IRT/IRT algorithm [4]. Although IRT values can be misleading depending on the assay used by state programs, the floating IRT value cut-off has been associated with improved sensitivity and decreased missed cases of CF [6].

3. Brief Review of the History of Newborn Screening including Genetic Variant Panels

Given the limitations of IRT alone, the Wisconsin NBS program leveraged the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and modified its CF NBS algorithm in 1994, becoming the first US state to utilize a two-tier program that combined IRT measurements with DNA (deoxyribonucleic acid) testing for the most common CFTR genetic variant, F508del [7]. With improved sensitivity and specificity resulting from the addition of a DNA tier, the Wisconsin program reported marked success through improved identification of at-risk infants and confirmed nutritional benefits as a primary positive outcome measure [8]. The documented benefits of earlier diagnosis leading to initiation of pancreatic enzyme replacement therapy provided a clear rationale for universal CF screening of newborns and added urgency to the need for early diagnosis of those with classic CF [9]. Additional data suggest potential for improved survival in screened infants, with long-term epidemiological studies underway [10].

Other states followed Wisconsin's lead by redesigning their CFNBS programs. Massachusetts expanded the panel of CFTR variants included in two-tier testing, with resultant gains in sensitivity and the identification of more putative CF carriers [11]. California introduced a tailored panel combined with CFTR exon sequencing (IRT/DNA/SEQ), further improving sensitivity, particularly for non-white infants less likely to be identified by the standard panel [12]. Today, all 50 US states use an IRT/DNA or IRT/DNA/SEQ algorithm. In the California model, infants with a single CFTR variant after sequencing (likely carriers) were not considered screen-positive and were not referred for CF diagnostic evaluation. Additionally, California's results show that a considerable proportion of infants with two CFTR variants were screen-positive with negative sweat chloride test results [13]. In IRT/DNA algorithms, infants with an intermediate sweat chloride test result or, occasionally, infants with the R117H variant fall into a category of screen-positive with an inconclusive diagnosis. The utilization of genetic sequencing in CF newborn screening led to increased numbers of asymptomatic, screen-positive infants with inconclusive diagnostic test results, which highlighted a need for further guidance about how best to counsel and monitor them. Table 1 contains a summary of the three main types of CFNBS algorithms.

Table 1. CF newborn screening algorithms.

IRT/IRT	Initial measurement of immunoreactive trypsinogen (IRT); elevated IRT results are repeated on a separately collected specimen on a different day.
IRT/DNA	Single elevated IRT is followed by DNA analysis from the same blood spot specimen to detect the most common CF variant(s).
IRT/IRT/DNA	Second elevated IRT is followed by DNA analysis from the same blood spot specimen to detect the most common CF variant(s).
IRT/DNA/NGS	Single elevated IRT is followed by DNA analysis from the same blood spot specimen to detect the most common CF variant(s). If 2 CF variants are not identified, the specimen is reflexed to next-generation sequencing of CFTR.

Description of the methodologies utilized for newborn screening in the United States. Next-generation sequencing technology in New York State includes analysis of the CFTR coding region, intron/exon boundaries, specific intron variants and large exon deletions/duplications (del/dup) using bioinformatics. Minimum sequencing coverage depth was 10×. Standard or real-time PCR was used to confirm large del/dup. All possible del/dup and variants deep in introns or the promoter regions are not detected by these methods.

4. Quality Assurance

Universal CFNBS was instituted in all US states in 2009, but states continued to vary in the extent of CF DNA analysis, with some states screening only for the single most common CFTR variant, F508del, and others adopting a more extensive CF DNA variant panel. The infeasibility of a single standard was acknowledged by the European CF Society National Screening Working Group (ECFS) who reported that variations in culture and demographics, as well as regional and country-specific health economics, made a standard newborn screening protocol unworkable [14].

CF NBS programs have continued to examine ways of improving outcomes, such as decreasing the number of missed cases [15] and optimizing the communication between laboratories, specialty care centers (SCC), primary care providers (PCPs) and families about abnormal CF NBS results [16]. Another issue identified was the risk of missing newborns with meconium ileus, who may have low IRT levels due to intestinal blockage but have a high likelihood of having CF [17]. Kharrazi, et al. surveyed state newborn screening programs in the US with data from 29 states. They looked at factors associated with missed cases of cystic fibrosis due to IRT levels falling below the program's cut-off. False-negative cases had a median IRT of 39 ng/mL (range 10–100 ng/mL). Factors associated with a high false-negative result were black race, programs with high IRT cut-off, fixed IRT cut-offs and genotypes without two known CF-causing variants and meconium ileus. Factors associated with a lower false-negative IRT were older age at specimen collection, Saturday birth, hotter season on newborn dried blood collection, laboratory closure of $\geq$3 days, preterm birth and formula feeding newborns [4]. Babies with CF born with meconium ileus are readily diagnosed due to symptoms.

Counterbalancing concerns regarding missed cases is the goal of minimizing false-positive cases, which increases the medical burden to the health care system and emotional burden to families [18].

Diagnostic confirmation following a positive CF NBS result is typically performed by a CF specialty care center and includes a diagnostic sweat test according to the Clinical and Laboratory Standards Institute [19] and Cystic Fibrosis Foundation (CFF) guidelines. Sweat chloride results $\geq$ 60 mmol/L are considered diagnostic, 30–59 mmol/L are considered intermediate results and <30 mmol/L are negative [20]. Accuracy of sweat chloride testing is enhanced using a lab with specialized experience that takes part in regular quality monitoring and maintains a close partnership with the Cystic Fibrosis Center at their institution, as defined in the CF Foundation's accreditation process (CFF.org). For example, sweat samples that are insufficient for analysis, or Quantity Not Sufficient (QNS), are a source of emotional distress to families awaiting diagnostic resolution [21] and may delay initiation of treatment for infants with CF. Minimizing QNS rates to <10% in newborns haw been challenging [22], particularly for premature infants and infants with low birthweight [23]. A sweat test can be performed as early as 48 h of age [24]; however, QNS rates were found

to be higher in infants younger than 10 days old and premature infants [25]. The CFF recommends sweat test to be performed on an infant with a positive newborn screen or positive prenatal genetic testing by 4 weeks of age and not before 48 h old. In our shared experience, misperceptions of needing to wait until an infant is 6 or even 10 lbs. can lead to delayed diagnosis and treatment of babies with pancreatic insufficiency. Input from the CF care team regarding when to sweat test a particular baby might assist in a reduction in QNS rates. The size of the baby's arms must accommodate a macroduct electrode to ensure adequate contact with the skin. Evaluation first by the care center may provide guidance as to when to perform the test. The decision as to when is variable depending on the size of the baby.

5. The New York State CFNBS Experience

The New York State (NYS) CFNBS program began using an IRT/DNA algorithm in October 2002, assessing specimens from infants in the top 5% of IRT levels with an expanded CFTR panel (see Figure 1). A consortium consisting of NBS personnel from the Department of Health, CF center directors, center nurse coordinators and genetic counselors reviewed the algorithm regularly to improve sensitivity and specificity in the state's ethnically diverse population [26]. Although data showed poor predictive value for the group of infants who presented with very high IRT levels (VHIRT) (top 0.1% for the day) and no recognized CFTR variants from the expanded CFTRpanel, who comprised the largest category of CFNBS referrals in NYS at the time, the NYS program accepted a high level of false positives to avoid missing infants with CF in this diverse population. Since the standard 39-variant CFTR panel was more likely to miss CF-causing DNA variants in infants from minoritized groups, particularly those reported as Hispanic, the NYS consortium reached consensus that the NBS program should continue to refer infants in the VHIRT category [27], but eventually raised the cut-off from 0.2% to 0.1%, leading to a large decrease in referrals without missing pancreatic-insufficient infants with CF [26].

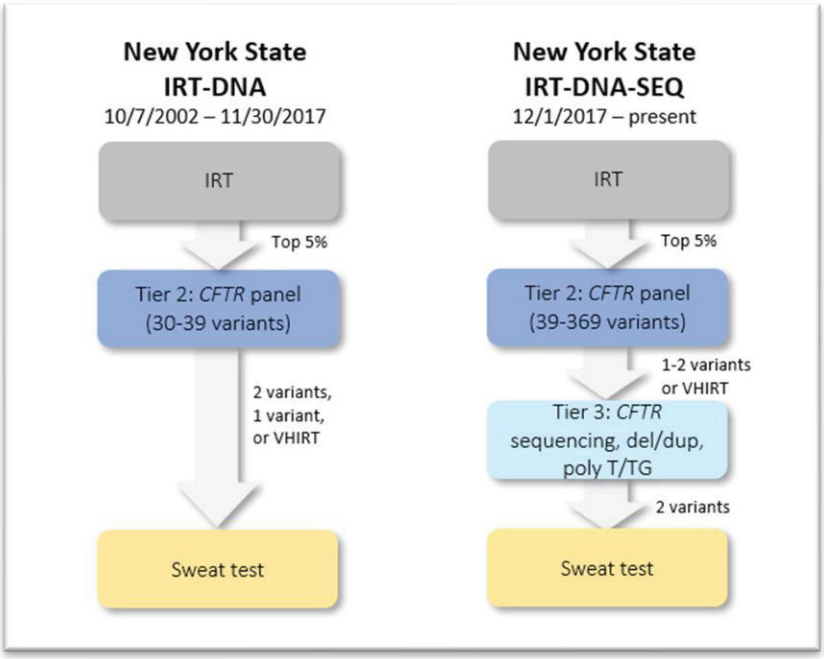


Figure 1. Evolution of the New York State cystic fibrosis newborn screening algorithm from inception until present. Courtesy of Denise Kay, NYSDOH (New York State Department of Health).

To address these issues, the NYS CFNBS program changed its algorithm in 2017, adding a third tier utilizing next-generation sequencing (NGS) for any infant with one CFTR variant on the second-tier screening or with VHIRT and no CFTR variants on second-tier screening [28] (Figure 1). This innovation was made possible by expanded technical

ability and reduced costs to the NBS program. By 2017, NGS technology made it possible to perform rapid, extensive CFTR testing in the state-run NBS lab. Eventually, the 39-variant second tier was expanded to be a targeted second-tier CFTR panel consisting of all CF-causing variants in the CFTR2 database [29]. In addition, the new testing platform was updatable as new pathogenic variants in CFTR were reported and confirmed as CF-causing.

The IRT/DNA/SEQ algorithm enables identification of infants with CF with rare CFTR variants, resulting in fewer missed cases, especially among underrepresented populations, who are likely to have less well-defined CFTR variants [30]. Additionally, in NY, we continue to note that many cases of meconium ileus fall below the floating IRT cut-off, reported to be as high as 18% in an Italian study [31]. Absolute rates of false-negative results for this population are not available at the time of publication. With the financial, programmatic and operational support of the New York State Department of Health, including the dedication of their Newborn Screening Director and staff and the commitment of all NY (New York) CF centers, the NYS algorithm has provided an approach to CFNBS that reduces delay in diagnosis and addresses disparities by more readily identifying rare variants within the diverse population served by the NYS NBS program [26].

6. Identification of CF Carriers

Improved detection of infants with CF has also identified more carriers who are not predicted to have CF [11]. Although these infants are not expected to require any medical intervention or monitoring for CF, carrier identification has its own set of implications. How to address genetic counseling needs for this population becomes important for the infants' parents and other relatives seeking to have children. This provides opportunity to offer genetic counseling to these individuals. It has potential implications to the infants' future reproductive planning, with the caveat of adding the possibility for increased lifetime health risks for CFTR-related conditions [32,33]. While these are not typically considered goals of newborn screening [34], identification of carriers has been postulated as both a benefit and liability of IRT/DNA algorithms [35].

6.1. "Diagnosing" Asymptomatic Newborns

The possibility of an inconclusive diagnosis exists within the framework of all CF newborn screening algorithms but is heightened when CFTR sequencing results are reported [36]. Diagnostic ambiguity after CF newborn screening may stem from an intermediate sweat chloride test result in an infant with at least two CF-causing CFTR variants, or identification of one or more variants of uncertain significance (VUS) and/or varying clinical consequence (VCC). Note that VCC is a term used to describe CFTR variants [37] but is not currently a formal variant classification as defined by the American College of Medical Genetics [38]. This diagnostic scenario is termed CFTR-Related Metabolic Syndrome (CRMS) in the US [39].

The European CF Society's Neonatal Screening Working Group further characterized the CRMS designation as Cystic Fibrosis Screen Positive Inconclusive Diagnosis (CFSPID). An international panel of experts proposed the term CFSPID in 2014 as a more descriptive and clinically correct designation for this large group of asymptomatic and genetically diverse patients [40]. The European Working Group spearheaded the development of guidelines for infants with inconclusive CF diagnosis after newborn screening. Representatives from 30 countries, including the US, reached consensus on proposed recommendations using Delphi methodology. Follow-up recommendations included evaluation by a physician from a CF specialty center or with CF expertise, repeated sweat chloride testing at 6–12 months, close contact with a local pediatrician, clear guidance to the family with need for follow-up given the increased risk for a future diagnosis of CF or CFTR-related conditions, and annual re-evaluation of the specific CFTR variants identified in each patient [41].

Additional international efforts published in 2017 provided a global harmonization of the CRMS and CFSPID designations [42]. The acronym CRMS continues to be used predominantly in the US while the more descriptive CFSPID is commonly used outside

the US. The term CRMS implies illness when these variants may never be clinically significant [14,43], whereas CFSPID implies a need for monitoring and follow-up to clarify the infant's proper designation over time, including the possibility of remaining healthy. The use of the term CRMS addresses the need for a unique diagnosis category for use by payors in the US. CRMS has a unique ICD-10 (E88.9) billing code on the platform used for reimbursement for US clinicians and hospitals. This diagnostic code enables providers to follow babies with inconclusive diagnosis after a positive CF NBS result, monitor them according to clinical management guidelines, and receive reimbursement for provision of these services.

Currently, such monitoring includes repeat sweat chloride testing, expanded genetic testing for the infant and parents, and other clinical tests such as fecal elastase, sputum cultures, chest X-rays and fat-soluble vitamin levels. Additional testing is decided based on the cystic fibrosis physician's clinical judgment [20]. Assessments of CFTR function via nasal potential difference and intestinal current measurement have demonstrated utility in reclassifying infants from CFSPID to CF and identifying those at risk for developing CFTR-related conditions [44] but are not widely used in children in the US.

6.2. CFTR *Variant Interpretation Challenges*

Incorporation of a CFTR sequencing tier into CF NBS programs has increased identification of variants not well characterized as CF-causing. Proper identification of the clinical significance of a novel or rare CFTR variant is still an evolving area of study in medicine, with guidelines for variant classification set by the American College of Medical Genetics and Genomics (ACMG) setting the standard [38]. In the context of a VUS or VCC, clinicians must interpret complex and uncertain genetic findings for the family of an asymptomatic infant.

Several public data repositories contain information about CFTR variants. The CFTR Mutation Database housed at the Hospital for Sick Kids in Toronto, Canada [45], maintains a database of the >2000 CFTR variants but reports limited clinical information. An invaluable repository of genetic and phenotypic information from individuals with a CF diagnosis is the CFTR2 database [29]. The French CF Association also maintains a searchable database that includes a wider phenotype than CFTR2, including CFTR-related conditions [46]; other sources of information can be found by searching for the variants in genomic databases such as Clinvar or gnomAd as well as PubMed or even web-based searches. Reviewing published reports about rare CFTR variants may be helpful when counseling families about the need for continued follow-up, recognizing that there may be variable phenotypes with the same or similar genotypes. Genetic counselors, who are trained to synthesize and communicate complex genetic information, are inconsistently associated with CF care centers for CF newborn screening follow-up [47].

6.3. Health Disparities in CF Newborn Screening

In our NY City experience, being in an urban area, we have found a multitude of challenges within our community, including the follow: 1. changes of address from the time of birth; 2. changes in surnames that are not readily noted on a newborn screening Guthrie card; 3. communication access to operational phones and computer networks for emails; 4. economic burden of lack of paid time off (PTO) in parental employment; 5. transportation difficulties to the CF center; 6. primary care setting within a clinic of multiple providers without ownership of the patient; and 7. lack of readily available interpretive phone service. These issues were heightened by the COVID-19 pandemic. It is important to create a plan as part of pandemic and disaster preparedness as such challenges are predicted to recur in the future.

The lack of inclusion of rare CFTR variants in newborn screening algorithms increases the likelihood that infants from minority groups (black/African American, Hispanic and other race) are missed disproportionately in the newborn period [48], resulting in a delay in diagnosis [49] that leads to worsened pulmonary, nutritional and cognitive outcomes [50].

This may further manifest in lower life expectancy as compared to those with earlier diagnosis and access to therapies. Individuals who have CF due to common CFTR variants such as F508del are more likely to be white and eligible for highly effective modulator therapies [34]. Children on CFTR modulators have an improved quality of life with reduced need for standard CF medications and respiratory treatments [51].

7. Longer-Term Implications for Access to Treatment of Rare, Modulator-Ineligible Variants

Newborn screening algorithms that include NGS identify individuals with less common CFTR variants. As ivacaftor is approved for infants as young as one month of age [52] and elexacaftor/tezacaftor/ivacaftor is approved for those at least 2 years of age, it is imperative to identify newborns who will benefit from early treatment. Individuals from minoritized groups are more likely to have rare variants, some of whom could respond to modulators for early treatment [46]. If a rare variant is not approved for treatment, one potential way to address the disparity is through theratyping [53]. The process of theratyping assesses whether a particular variant may be responsive to CFTR modulators by testing in vitro at designated research laboratories. Clinicians can use theratyping results to advocate for patients to trial modulators off-label or as an n-of-1 study. Positive outcomes including stabilization or improvement of pulmonary function and reduction in pulmonary exacerbations for patients with rare CFTR variants have been reported [54].

8. Implications for Primary Care Practitioners

Inconclusive CFNBS results lead to a significant challenge for primary care practitioners (PCPs), who are often the first to receive notification of abnormal screens. PCPs are integral to the process of referring babies with an abnormal CFNBS result for diagnostic resolution and particularly for monitoring babies with CFSPID, who may not exhibit any symptoms in the pediatric or adolescent age groups. The importance of providing education to PCPs about recognizing symptoms that prompt rapid referral to CF centers cannot be overstated. As CFNBS algorithms change, providing clear information to PCPs is important to avoid unnecessary referrals and sweat tests. Letters of communication with PCPs regarding positive newborn screen results should use language that is concise and leaves less interpretive errors. The work of Sontag, et al. highlights the importance of strong alliance and communication between primary care physicians (PCPs), State NBS programs and local CF care and research centers [1].

9. Psychosocial Considerations

As the newborn screening landscape continues to evolve with advanced genetic technologies and changing interpretations of CFTR variants, consideration of the psychological impact to parents and families, potential fiscal impacts and ethical concerns is important. In the summer of 2021, the UK National Screening Committee commissioned a task force to elicit input from a sample of the public who had no previous knowledge of cystic fibrosis, prior to the adoption of next-generation sequencing (NGS) in CFNBS. The project aimed to determine whether making a prompt diagnosis of cystic fibrosis was important. Even when faced with an increased number of missed newborns with a CF diagnosis, the public felt that avoiding the uncertainty of a CFSPID diagnosis was just as important a goal of a CFNBS program [53]. These findings reflect a central conflict in deciding to expand CFTR evaluation as part of the CFNBS as use of a limited CF genetic panel may not address the ethnic diversity within populations [27], while wider genomic screening can mitigate these disparities and shortcomings [51,52]. However, implementation of NGS will result in more families receiving an uncertain diagnosis. The waiting period for sweat testing and its results can be very distressing for parents. Cognitive understanding of carrier status and cystic fibrosis, the physician's approach to the parents and adjustment to the new infant may affect the level of distress [55].

The communication of specialty care teams with PCPs is critical in recommending the right language to convey newborn screen results and sweat testing results. This is

particularly challenging when the diagnostic status of the infant remains inconclusive after sweat testing, due to the presence of one or more VUS or VCC. Multi-center and long-term studies of the psychosocial impact of a positive CFNBS result, both with or without the additional complexity of an inconclusive diagnosis, have identified parental worry and anxiety as well as concerns about increased perceptions of child vulnerability [18]) associated with a positive CFNBS result. However, in an early study comparing parents of babies with CF, parents of babies with inconclusive diagnoses, and healthy controls, Perobelli et al. noted that parents of asymptomatic babies with inconclusive diagnoses viewed their babies as healthy, as did parents of healthy controls. Parents of inconclusive diagnoses and healthy controls experienced less distress than parents of babies with positive diagnosis of CF [56].

Tluczek, et al. published a systematic review in 2022 synthesizing findings from 92 evidence-based, peer-reviewed research reports about psychosocial issues associated with newborn screening. The expansion of genetic variant detection will affect minoritized groups at a larger scale, requiring added support in communicating positive results, whether it pertains a true CF diagnosis or CRMS/CFSPID [57]. The use of interpretative services may be needed and challenging. Access to certified personnel in medical interpretation is not always easily available but the use of tablets in CF clinics may offer a reasonable alternative to clinicians in a face-to-face encounter as wall-mounted phones lack the opportunity for this. The increased number of ethnicities identified in our NY experience highlights the importance of having the proper time, knowledge, sensitivity and communication skills to work with varying populations. Parents and families may require additional encounters to convey information and provide extra emotional support regarding NBS results. Parents are often sleep-deprived and are concerned about their baby when they come in to meet the CF team and receive the newborn screening results. Parents may not always be ready to receive the amount and type of information that we as the CF team may want to provide. Clinicians must remain cognizant of the varying needs of parents, the risk and/or presence of postpartum depression and the impact on other children in the family as these factors can impair their capacities to absorb and assimilate new information [57]. This process of CF newborn screening, diagnosis and communication of difficult and, at times, inconclusive results is a lengthy process including multiple team members, effective communication and ongoing follow-up.

10. Clarifying the Diagnostic Status: CFTR Phasing

Another diagnostic challenge follows identification of a baby with one or more rare CFTR variants, as parental phasing is needed to determine the inheritance pattern of the variants. The process of CFTR phasing is critical in differentiating a likely heterozygote, with all variants inherited in cis from the same parent, from an infant with variants on both copies of CFTR who needs ongoing monitoring for CFSPID/CRMS. A consequence of the phasing process might be the identification of parents who have either two variants or complex alleles themselves and who are asymptomatic or mildly symptomatic [58]. These results require additional time for explanation to the parents. It is appropriate that referral to an adult cystic fibrosis center for further evaluation and genetic counselling be offered. Of note, CFTR phasing also has utility for parents and families of infants with a clear CF diagnosis. Understanding the segregation of the variants can inform carrier screening options, genetic counseling and test selection.

11. Implications of Prenatal Modulator Exposure

Identification of CF in pregnancy raises added communication needs for interfacing with CFNBS programs [59]. A recently published case report [60] suggests that babies with CF born after prolonged prenatal exposure to CFTR modulators may have IRT values below the cut-off, leading to false-negative CFNBS results [61]. A second case report describes a heterozygous carrier mother who received ETI (elexacaftor/tezacaftor/ivacaftor) after her fetus was diagnosed with cystic fibrosis and bowel obstruction was noted on ultra-

sound [62]. Resolution of the bowel obstruction suggests that prenatal exposure to CFTR modulators may alter the clinical outcome of the disease. Ongoing communication between prenatal care providers, CF centers and NBS programs is crucial to avoid false-negative CF newborn screening results that cause delay in appropriate care and follow-up.

12. Conclusions

Since the institution of universal newborn screening in 2009, the evolution of diagnostic methods has improved diagnosis and care of CF newborns in the US and internationally. The addition of CFTR sequencing leads to more accurate diagnosis and many positive changes for children with CF by improving nutritional status and instituting earlier interventions at specialized care centers that ultimately increase life expectancy. Although advances in genetic testing methods allow for more expansive CFTR variant identification in newborn screening algorithms, the use of NGS leads to an unintended but expected increase in the diagnosis of CRMS/CFSPID cases. Examining long-term benefits and potential psychosocial consequences for this population is an area for continued research.

New York State has one of the most ethnically diverse populations in the US. The experience of the NYS CF NBS program highlighted challenges in early CF diagnosis, the expansion of the genetic variant panel, incorporation of CFTR sequencing, an increased number of inconclusive diagnoses and apparent disparities as it underwent major changes and evolved into the current IRT/DNA/SEQ algorithm. These challenges prompted quality improvement efforts and ongoing partnership between the state and specialty care centers. The continued reevaluation of our algorithm, ongoing collaboration with our state newborn screening program and commitment to the inclusion of all ethnicities may provide guidance in efforts to revise the CF newborn screening programs in other communities at the local level. At the state level, cystic fibrosis center teams are encouraged to meet with members of Department of Health newborn screening programs to continue to improve detection rates, decrease the number of false-positive tests and improve outcomes in their individual programs. In partnership with NBS laboratories, PCPs and CF care providers can aid in advocating for a more equitable CFNBS program. The implementation of third-tier sequencing, which has been successful in California and New York CFNBS models, is one approach. With highly effective modulator therapies now available in infancy, there is a new imperative for early identification and fair treatment of all babies with CF.

Our goal and our hope are that medical professionals, government bodies and other decision makers work toward a more uniform approach to the early diagnosis of cystic fibrosis newborns, promoting equity, reducing harm and avoiding delayed diagnosis in all babies with cystic fibrosis.

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Review

Advances in the Cystic Fibrosis Drug Development Pipeline

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Abstract: Cystic fibrosis is a genetic disease that results in progressive multi-organ manifestations with predominance in the respiratory and gastrointestinal systems. The significant morbidity and mortality seen in the CF population has been the driving force urging the CF research community to further advance treatments to slow disease progression and, in turn, prolong life expectancy. Enormous strides in medical advancements have translated to improvement in quality of life, symptom burden, and survival; however, there is still no cure. This review discusses the most current mainstay treatments and anticipated therapeutics in the CF drug development pipeline within the mechanisms of mucociliary clearance, anti-inflammatory and anti-infective therapies, restoration of the cystic fibrosis transmembrane conductance regulator (CFTR) protein (also known as highly effective modulator therapy (HEMT)), and genetic therapies. Ribonucleic acid (RNA) therapy, gene transfer, and gene editing are being explored in the hopes of developing a treatment and potential cure for people with CF, particularly for those not responsive to HEMT.

Keywords: cystic fibrosis (CF); cystic fibrosis transmembrane conductance regulator (CFTR); highly effective modulator therapy (HEMT); gene-based therapy; antibiotics; bacteriophage; anti-inflammatory; mucolytic therapy

1. Introduction

Cystic fibrosis represents one of the most striking examples of how the elucidation of the underlying pathogenetic mechanisms of an inherited disease has resulted in targeted therapeutics that have significantly altered the natural history of what, until recently, had been a relatively grim prognosis. CF is an autosomal recessive disorder which has been estimated to affect at least 100,000 individuals across the world [1]. The discovery of the culprit mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes the chloride ion channel responsible for transporting chloride and bicarbonate across the apical surface of secretory epithelia, has led to a remarkable improvement in the understanding of this multisystem illness. It is now estimated that, since the discovery of the original CFTR gene mutation in 1989, there have been over 2000 additional mutations characterized [1]. As is the hope with any disease process, with an improvement in the understanding of its pathophysiology there has been a resultant increase in the available therapies for people with CF (PwCF). While the foundation of treatment for CF will remain rooted in a team-based, multi-disciplinary approach that emphasizes thoughtful and selective use of anti-infective treatments, pulmonary hygiene, and adequate nutritional supplementation, among others, there is an ever-increasing array of therapies emerging which will not only enhance these well-established modalities, such as is the case with antibiotics, but will also introduce completely novel mechanisms of treatment. Although CF

is and will continue to be a multisystem disease with significant morbidity, the abundance of treatment options either available now or in development represents a dramatic and optimistic shift in the outlook of a previously devastating diagnosis.

The CF Drug Development Pipeline (Figure 1) has grown tremendously through the collaborative work between the Cystic Fibrosis Foundation (CFF), the medical and research community, and the pharmaceutical industry. The CFF's commitment to accelerate drug discovery through venture philanthropy has led to significant gains in the care of PwCF, but it is far from complete. Here, we review impactful therapies in the areas of mucociliary clearance, anti-inflammatory, anti-infective, and restoration of CFTR function through modulator and highly anticipated genetic based strategies. Nutritional care is not discussed in this review, but we refer readers to an updated nutritional review by Frantzen et al. [2].

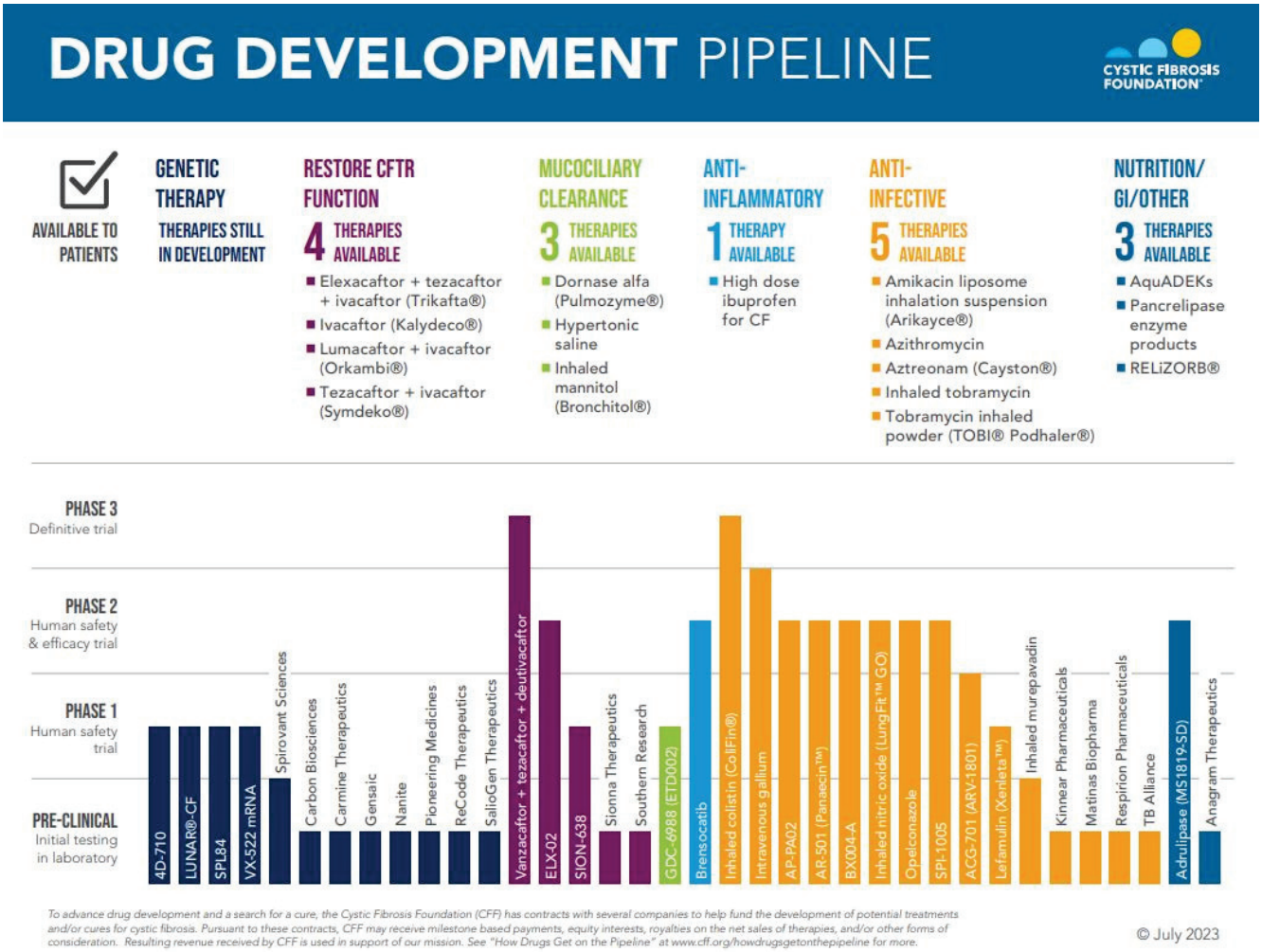


Figure 1. Reproduced with permission of the Cystic Fibrosis Foundation, Bethesda, Maryland. © July 2023.

2. Restoration of CFTR Function with CFTR Modulator Therapy

CFTR modulators comprise a group of small molecules, grouped as potentiators or correctors designed to target the protein defects resulting from CFTR mutations [3]. Potentiators function by increasing the probability of the CFTR channel opening at the cell surface, thus increasing chloride transport and reducing the viscosity of exocrine secretions [4,5]. Ivacaftor (IVA) is an example of a potentiator that has effectiveness in patients with CF carrying a gating mutation responsible for abnormal closing of the channel. Among the multitude of mutations identified, G551D is a comparatively common mutation (affecting approximately 4%) which affects this ATP-dependent conformational

change and, thus, limits CFTR mediated chloride transport. Although initial research confirmed the efficacy of IVA for this relatively common G551D mutation, subsequent work has established its efficacy in other gating mutations [4,5]. Unfortunately, gating mutations represent only one of five potential classes of CFTR mutations, and, thus, additional therapies have been developed to target this pathophysiologic diversity. The F508del mutation, which accounts for approximately 70% of CF mutations across the world, results in abnormal folding and trafficking of the CFTR gene to the cell surface and, thus, presents a problem not fully addressed with potentiators such as IVA [6]. IVA requires the presence of the CFTR protein at the cell surface in order to be effective. Thus, correctors were developed to target this deficiency in folding and to augment the amount of CFTR protein produced and available for further modulation. Correctors, when combined with a potentiator, result in clinically significant improvements in lung function in PwCF [7]. To date, commercially available combination therapies of corrector(s)/potentiator CFTR modulators include lumacaftor/ivacaftor (LUM/IVA), tezacaftor/ivacaftor (TEZ/IVA), and elexacaftor/tezacaftor/ivacaftor (ETI).

The efficacy of LUM/IVA was established in the TRAFFIC and TRANSPORT studies, two Phase 3 randomized controlled trials enrolling patients homozygous for the F508del mutation. Patients received the combination of LUM/IVA versus placebo for 24 weeks; the primary endpoint of the study was a change in forced expiratory volume in one second (FEV1) at the end of this period. In both studies, the patients who were randomized to receive LUM/IVA demonstrated improvements in their FEV1 versus placebo. Pulmonary exacerbations (Pexs) were also decreased in the treatment groups, as was the need for hospitalizations and intravenous (IV) antibiotics [7]. In a similar fashion, the EVOLVE study, a Phase 3 randomized controlled trial enrolling patients who were homozygous for the F508del mutation, demonstrated that TEZ/IVA improved FEV1 over a 24-week period in this population, as well as reduced rates of Pexs [8]. In addition to homozygous F508del mutations, a number of other mutations (A455E, D110E, E56K, etc.) have been determined to be responsive to these dual combination therapies, and possession of at least one of these mutations confers eligibility for treatment.

Following the dual combination therapies, triple therapy with ETI, or trade names Trikafta® or Kaftrio®, was developed, consisting of two correctors combined with the potentiator IVA. The effectiveness of ETI was demonstrated in a Phase 3 randomized controlled trial in which change in FEV1 from baseline was evaluated in patients who were heterozygous for the F508del mutation and a minimal function mutation over a 24-week treatment period. Minimal function mutations, such as nonsense and splice mutations, do not encode for translation of a CFTR protein. Patients in this trial not only experienced improvements in FEV1 but were less likely to experience Pexs, require IV antibiotics or hospitalization, and reported improved Cystic Fibrosis Questionnaire-Revised (CFQ-R) scores, indicating higher quality of life. Although the overall rates of adverse events were similar between the treatment and control groups, patients assigned to receive ETI had higher rates of elevations in aminotransferase levels and development of rashes. The authors also reported higher rates of creatine kinase level elevations, as well as mild blood pressure elevations in the treatment, neither of which resulted in discontinuation of the drug [9]. Although generally considered well tolerated, other common side effects include abdominal pain and diarrhea. Baseline and periodic opthalmological exams are recommended in pediatric patients, given concern that IVA may increase the risk of cataract development in pediatric patients. ETI is currently approved for patients who have at least one copy of the F508del mutation and other mutations demonstrated to be responsive to this combination. These impactful medications are referred to as highly effective modulator therapy (HEMT) and have contributed to improvement in lung function as measured by FEV1, body mass index (BMI), respiratory symptoms (based on the CFQ-R respiratory domains), and a reduction in Pexs. As a result of HEMT, life expectancy is dramatically improving; the 2021 United States Cystic Fibrosis Patient Registry estimated that the median predicted age of PwCF born in that year was 52 years [10].

Research continues for the development of new HEMT with comparable or better efficacy and acceptable safety profiles (Table 1). One such example is a Phase 2 trial studying the safety and efficacy of a novel potentiator, deuticaftor (D-IVA or VX-51), a deuterated form of IVA that gives it greater stability, thereby increasing its half-life and achieving greater plasma concentration compared to IVA [11]. A Phase 3 trial studying vanzacaftor (VX-121), a novel corrector, in combination with TEZ and D-IVA in CF patients who are heterozygous for F508del and a minimal function mutation, is also currently underway [12]. Phase 2 studies using triple combination navocaftor (ABBV-3067), a potentiator, with the corrector galicaftor (ABBV-2222) and the addition of either experimental drug ABBV-119 or ABBV-576 in patients who were heterozygous or homozygous for the F508 del mutation had begun; however, these studies were discontinued for reasons yet to be released by the study sponsor at the time of this manuscript writing [13].

Table 1. CFTR Targeted therapies. [1] = therapies are commercially available; [2] = therapies are in clinical trial phase; [3] = therapies are in pre-clinical phase; * Study was discontinued.

CFTR Modulators	mRNA-Based Therapies	Gene-Based Therapies
Ivacaftor [1]	Aminoglycoside read-through nonsense mutation (ELX-02) [2]	CFTR Gene Transfer Vectors: • Recombinant adeno-associated viral vectors (rAAV) (4D-710) [2] • Liposomal vector (pGM169/GL67A) [2] • Lentiviral vector [3]
Lumacaftor/Ivacaftor [1]	Depletion of termination factor, eRF1 (SRI-37240) [3]	
Tezacaftor/Ivacaftor [1]	Inhaled CFTR mRNA (MRT5005 [2] VX-522 [2] ARCT-032 [2] ReCode [3])	
Elexacaftor/Tezacaftor/Ivacaftor [1]	Short-interfering RNAs (siRNAs) [3]	Zinc-finger nucleases (ZFN) [3]
Deuticaftor (VX561) [2]		Transcription activator-like effector nucleases (TALENS) [3]
Vanzacaftor (VX-121)/tezacaftor/ Deuticaftor [2]		Clustered Regularly Interspersed Palindromic Repeats (CRISPR)/CRISPR-associated nuclease 9 (CAS9) [3]
Navocaftor (ABBV-3067), galicaftor (ABBV-2222) and ABBV-576 [2] *		

3. Genetic Therapies

3.1. RNA Based Therapies

In 2019, the CFF shared with the global CF community its research agenda, "Path to a Cure", which aims to develop treatments and a cure for every person living with CF, while prioritizing those who are nonresponsive to current therapies. The focused strategies encompass CFTR protein repair and CFTR gene correction [14]. Although the effectiveness of CFTR modulators is encouraging with regard to the improved prognosis of most patients, the great pathophysiologic diversity of CF remains unaddressed in about 10% of PwCF who carry either minimal function mutations that are not known to be responsive to HEMT or unknown genotypes. For example, premature stop mutations lead to truncated CFTR proteins, which cannot be rescued by CFTR modulator therapies and represent a significant gap in currently available therapy [4]. Nonsense suppression, or readthrough therapy, seeks to suppress this premature termination [15]. Aminoglycoside antibiotics have previously been shown to correct this defect by promoting "readthrough"—that is, the CFTR protein is translated in spite of the premature stop mutation, enabling production of the full protein. Previously available aminoglycosides not only come with a host of known toxicities but have also not been demonstrated to have significant clinical efficacy. However, novel non-antibiotic glycosides, such as the compound dubbed "ELX-02", have demonstrated the ability to promote readthrough of CFTR mRNA in the setting of nonsense mutations and their resultant premature stop codons, including the most common nonsense mutation, G542X, in human-derived "organoids" (three dimensional cellular structures derived from intestinal stem cells) (Figure 2) [16].

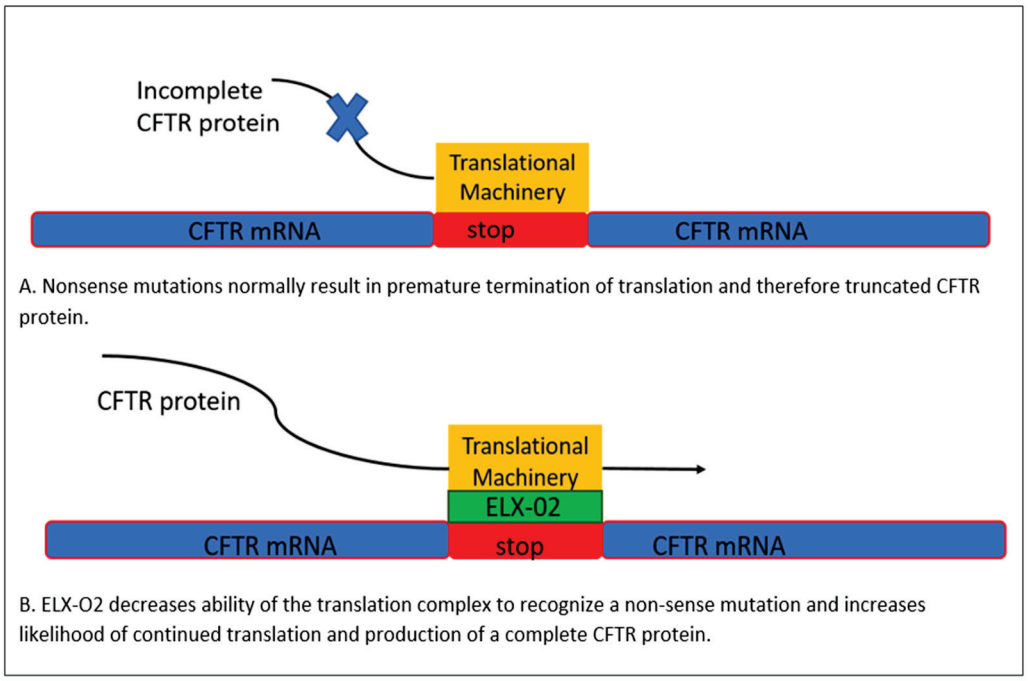


Figure 2. Under normal circumstances, translation is halted at stop or "nonsense codons" resulting in a truncated, non-functional CFTR protein. ELX-02 promotes continued translation in spite of such stop codons and increases the probability of full-length CFTR production [16].

Currently, a Phase 2 trial of subcutaneously administered ELX-02 in patients with CF with at least one G542X mutation is underway [17]. Current research is aimed at investigating the creation of novel aminoglycosides with more favorable side effect profiles and at novel strategies promoting CFTR translation. For example, recent research has identified another method with demonstrated ability to suppress CFTR nonsense mutations by promoting readthrough at premature termination codons via depletion of the termination factor, eRF1 [15]. Termination factors, as their name suggests, are proteins that mediate the termination of RNA transcription and may represent a novel target for future pharmacotherapy. Sharma et al., utilized CFTR nonsense mutation models derived from Fischer rat thyroid cells expressing human CFTR deoxyribonucleic acid (DNA) containing the G542X nonsense mutation and demonstrated that one identified compound, SRI-37240, not only possessed the ability to deplete eRF1 and increase mRNA production and CFTR function but also was selective for these nonsense mutations and did not interfere with suppression of normal termination codons (Figure 3) [15]. While compounds such as this are far from clinical application, they represent yet another promising method of restoring CFTR function and improving the lives of PwCF.

Aside from increasing CFTR mRNA with readthrough technology, another targeted therapy is introducing new, wild-type RNA into the cells to utilize in the production of complete CFTR protein. Research has encountered several challenges, which include ensuring adequate delivery of mRNA with effective penetration through the respiratory epithelium, as well as establishing an effective and practical dosing regimen to ensure sustained mRNA expression. RESTORE-CF, the first-in-human clinical trial of inhaled mRNA in PwCF, was a Phase 1/2 randomized, double-blind, placebo-controlled trial with the primary goal of evaluating the safety and tolerability of MRT5005, an mRNA delivered within a protective lipid nanoparticle aerosol [18]. The trial enrolled a total of 42 subjects with 2 class I and/or class II CFTR mutations over 3 parts: (A) single ascending doses (SAD), (B) weekly multiple ascending doses (MAD), and (C) daily dosing (DD) [18]. MRT5005 was, overall, well tolerated, with one serious adverse event being a pulmonary exacerbation. Similar to the observations of febrile reactions in mRNA vaccine trials against COVID-19, 14 mild or moderate febrile reactions were noted in 10 subjects, of whom 2 also

developed hypersensitivity reactions, including urticarial rash. Although not a primary outcome, FEV1 improved in the SAD group, but this was not replicated in the MAD and DD groups, raising concerns over the lack of efficacy signals. The precise reasons for the FEV1 discrepancies have not been elucidated, although there are several possibilities. Firstly, FEV1 was frequently measured over a short timeframe and the observed increases in FEV1 may not have reflected the varying measurements that may occur later in time; once corrected, CFTR proteins were made and mucous clearance of the airways took place. A possible reason for this may be the poor uptake of the lipid nanoparticles, resulting in inadequate expression of CFTR proteins. Bronchoscopy was not included in the trial design due to risk–benefit assessment; thus, adequate MRT5005 uptake could not be confirmed. The self-limited bioactivity of mRNA itself may be another area to address, in relation to frequency and duration of dosing. The small cohort sizes and the inclusion of patients taking ETI could have, additionally, played a role in diminishing any effects on FEV1 [18].

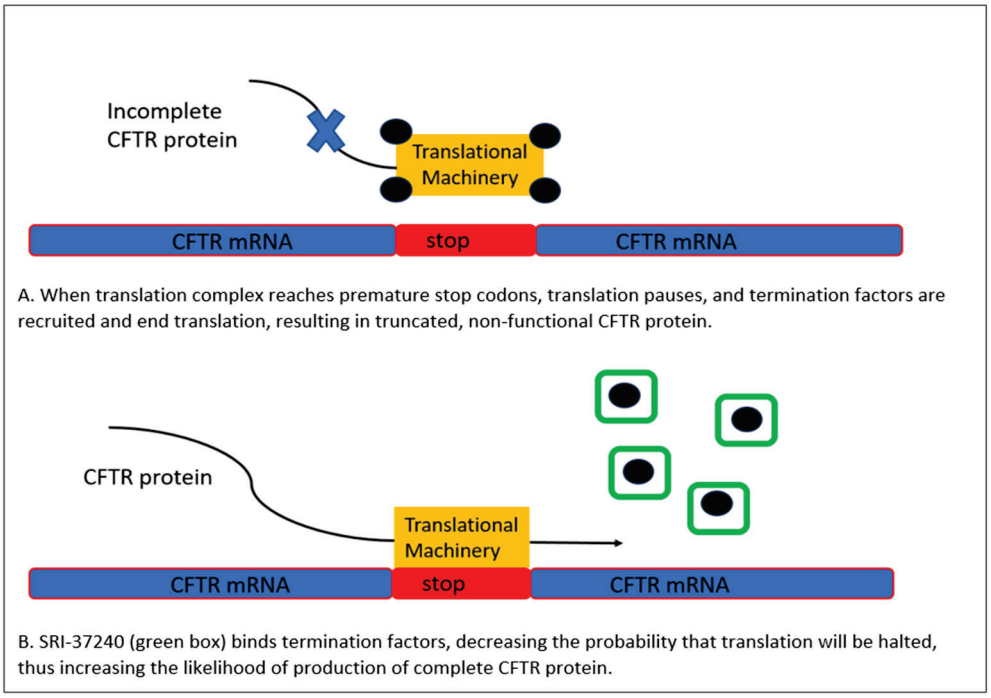


Figure 3. Mechanism of SRI-37240. By binding termination factors (black dots) and preventing their normal interaction with translational machinery at stop or "nonsense" codons, SRI-37240 (green boxes) increases the production of full length, and therefore fully functional, CFTR proteins [15].

Several other compounds in the pipeline also utilizing nebulization of CFTR mRNA encapsulated by a lipid nanoparticle include ARCT-032, ReCode, and VX-522 [19–22]. A Phase 1 trial investigating the safety and tolerability of VX-522 is underway in PwCF who have CFTR mutations not responsive to modulator therapy [23]. Preclinical data from the trials of ReCode and ARCT-032 therapies support advancement of the studies into the next research phase; ARCT-032 has recently been approved for first-in-human studies [20].

The epithelial sodium channel (eNAC) also plays a role in the pathophysiologic mechanisms of CF. In response to diminished and defective CFTR channels, eNACs are upregulated, which leads to increased absorption of sodium and fluid from airway surfaces and resultant thickened secretions, further impairing mucociliary clearance. Short-interfering RNAs (siRNAs) are RNA molecules that function as parts of RNA-induced silencing complexes that bind to and cleave mRNA, and they have been developed to target the mRNA responsible for the production of these eNAC channels [24]. In human CF bronchial epithelial cells and mouse lungs, siRNA delivery with nanocomplexes, which consist of cationic liposomes and peptides capable of self-assembly upon mixing with siRNA, led to reduced

levels of eNAC mRNA [25]. Most importantly, the aforementioned study also demonstrated normalization of ciliary function, restoration of airway surface liquid (normally decreased in CF), and decreased mucus protein concentration in the treated cell models. In addition to confirming that the effect of siRNA is sustainable such that it could offer a realistic modality of therapy, future research will also likely focus on enhancing its delivery across airway epithelium. For instance, lipid-polymer hybrid nanoparticles with PEGylated shells were analyzed as another delivery method of siRNA-laden nanoparticles. Despite the successful creation of hybrid lipid nanoparticles, penetration through mucus layers was limited in environments in which certain mucinous proteins found in the sputum of CF patients were present. Furthermore, the addition of the lipid shell was also found to prevent penetration of the nanoparticle across cell membranes.

Technical challenges encountered in the basic science setting have persisted into the clinical arena as well, as demonstrated in the RESTORE-CF trial [18]. Although promising in theory, the difficulties of the aforementioned studies highlight the challenge in the creation of RNA delivery systems: not only must these vectors penetrate the thick mucus layers characteristic of the vast majority of airways of PwCF, but, once through, the vectors must be able to enter the airway epithelial cells to be effective. Furthermore, they must be well tolerated by patients and result in clinically meaningful improvements in patient outcomes [26]. Other likely issues that will be faced by this therapeutic modality include the establishment of feasible dosing regimens and the optimization of feasible delivery systems such as nebulization. It is encouraging to note that, despite these challenges, several therapies with likely varying formulations of CFTR mRNA sequence composition, stability, and lipid nanoparticle vectors are being developed and studied in the pipeline.

Finally, anti-sense oligonucleotides, or nucleotides designed to bind to specific mRNA sequences, are being evaluated in a Phase 1/2 trial under the name SPL-84. This nebulized therapy aims to correct the 3849 +10 kb C to T splicing mutation, which normally results in the degradation of CFTR mRNA and the production of truncated non-functional protein that is not rescued by currently available modulator therapy. Antisense oligonucleotide application has led to FDA approved treatments for spinal muscular atrophy and muscular dystrophy, and SPL-84 represents yet another example of the development of mRNA therapy to treat CF [27,28]. It is promising to know that more novel therapies extending beyond CFTR modulation are in the therapeutic pipeline (Table 1). These innovative treatments represent yet another encouraging step towards providing a treatment for all PwCF.

3.2. Gene Therapy

Gene therapy, like RNA-based therapies (Table 1), are at the forefront of the next generation of new CF treatments. Gene therapy encompasses (1) gene transfer, in which the corrected CFTR DNA is delivered to cells, leading to synthesis of functional CFTR protein; and (2) gene editing, in which the CFTR mutation is repaired and the corrected copy of the gene is permanent [28]. Gene transfer vectors have been the target of focused research in this field. Adenoviral vectors has been investigated, but they are limited by inefficient transfer of genetic material, thought to be due to the absence of the cocksackie-adenovirus receptor in human epithelial cells lining the airways, among other reasons [29]. As has been recently demonstrated, recombinant adeno-associated viral vectors (rAAV) have the ability to correct the CF phenotype in nasal mucosa from mice and human intestinal tissue, as evidenced by an increase in fluid and chloride secretion in these models [30]. rAAV vectors are protein shells containing the transferring DNA of interest, and they are essentially protein-based nanoparticles without the immunogenicity and risk of triggering an inflammatory response following a "traditional" adenoviral infection. Currently, an rAAV vector called 4D-710 is being evaluated in a Phase 1/2 trial in CF patients with bi-allelic CFTR mutations or a single mutation who are ineligible or intolerant of available HEMT. Future research will likely emphasize the use or development of novel capsids to enhance the delivery of the wild type CFTR gene. For example, a hybrid vector utilizing the

viral capsid of the human Bocavirus-type 1, which has demonstrated tropism for human airway epithelial cells, and the genetic material of recombinant adeno-associated virus 2 can be used to infect the proximal airway cells of ferrets in order to deliver genetic material [31]. While this research is far removed from clinical application at this stage, it represents the successful creation of a model by which human Bocavirus-type 1 capsid-based vectors may be developed and studied with the goal of creating effective rAAV based therapies. Other vectors for the delivery of genetic material include lentiviral vectors and viral liposomal vectors. A Phase 2B trial in CF patients of various genotypes evaluating pGM169/GL67A, a nebulized liposomal vector carrying the CFTR gene, demonstrated an improvement in FEV1 compared to placebo by 3.7% predicted at 12-months follow-up [32]. While these results are not striking, they are supportive that there may indeed be a role for these gene therapies in CF. Challenges facing these potential therapies include developing effective vectors targeting airway epithelial cells, sustaining genetic expression in human hosts, and limiting detrimental immunogenic responses.

Gene editing mechanisms aim to correct native host DNA. To this end, genetic therapies with zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENS), and Clustered Regularly Interspersed Palindromic Repeats (CRISPR)/CRISPR-associated nuclease 9 (CAS9) operate under the principle that by creating double strand breaks into host chromosomes near the culprit mutation site, native repair mechanisms can then introduce and correct the functional genetic material initially absent (Figure 4). Crane et al. demonstrated that ZFN-mediated homology directed repair could be utilized to correct CFTR gene mutations in pluripotent stem cell lines derived from the skin fibroblasts of patients with CF who had one F508del mutation and one I507del mutation [33]. Restoration of proper CFTR expression and function through gene editing is an encouraging therapeutic approach toward curing CF; however, to target hundreds of pathogenic CF mutations is ambitious and not easily feasible. Furthermore, rarer mutations will take precedence due to the lack of any treatment targeting these genotypes. To address this issue, another study was able to successfully introduce a "super exon" into a cell line model with the F508del mutation, resulting in the transcription of "correct" CFTR mRNA [34]. Specifically, utilizing ZFNs, genetic sequences containing exons 11–27 of wild-type CFTR were inserted into the mutated cell line model, which was appropriately transcribed into functioning CFTR protein in the cells exposed to this therapy.

CRISPR/Cas 9 technology has also demonstrated the ability to introduce the correct F508del allele with restored CFTR function in adult intestinal stem cell lines with known F508del mutations [35]. Although these studies are promising given the potential of these various gene repair systems to restore native CFTR function in human models of CF, this therapy is based on introducing DNA strand breaks into native host DNA—an event that is, under normal circumstances, considered unwelcome and capable of significant collateral genetic damage. To circumvent this potential side effect, gene editing that does not depend on the action of nucleases has been investigated. For example, Mcneer et al. have shown that, in the absence of nucleases, peptic nucleic acids, which are synthetic oligonucleotide analogues, can be utilized to introduce donor DNA homologous to the CFTR F508del sequence in human bronchial epithelial cells and in the CF mouse model [36]. This confirmed that such a technique can restore wild type CFTR mRNA production and improve chloride ion efflux in treated human bronchial epithelial cells. Furthermore, histological and biochemical analysis did not reveal any increase in markers of inflammation within the mouse models, suggesting that this type of therapy may be of low immunogenic potential.

The current landscape of emerging therapy for CF is a dynamic and promising mix of strategies designed to target the disease at its most fundamental pathobiology. Established treatments of symptomatic consequences of defective CFTR function will remain a mainstay of care for patients with CF. However, the mechanisms described of restoring/augmenting CFTR function with small molecular modulators, amplifying functional CFTR expression with RNA based therapies, and genomic transfer and editing with restoration of "normal"

sequences all provide hope that discussion of a cure should no longer be regarded as a far-fetched dream but rather a realistic goal within reach with advancing medical technology.

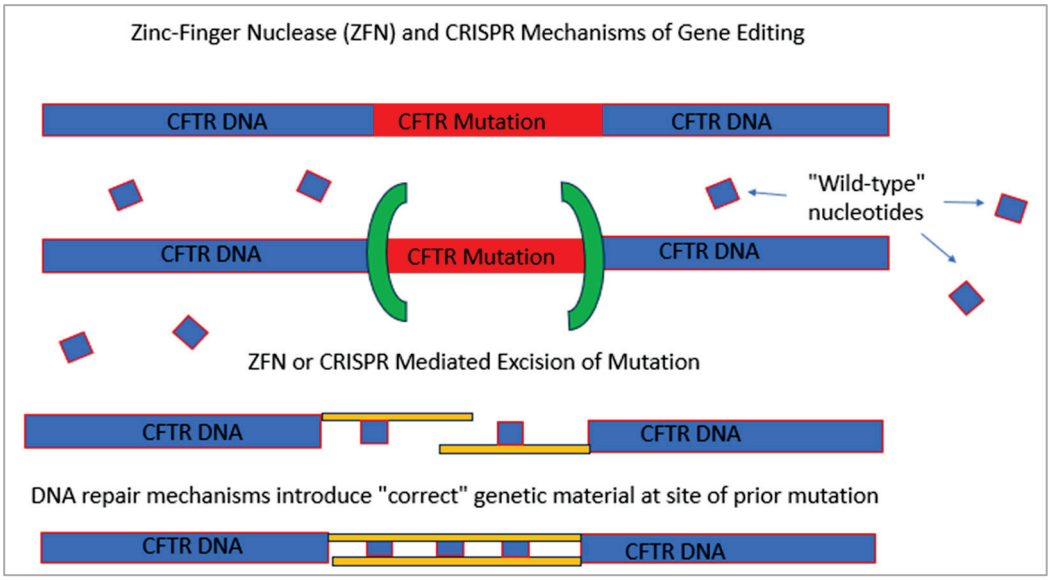


Figure 4. Simplified schematic of zinc finger nuclease (ZFN) and CRISPR/CAS9 technology: Both systems rely on the use of nucleases (green brackets) to remove mutations in the CFTR gene. Endogenous DNA repair pathways are then able to replace the removed genetic material with wild type nucleotides (small blue boxes).

4. Antimicrobial Therapies

PwCF are frequently infected with a diverse range of opportunistic microorganisms that are naturally occurring in the environment and that can be challenging to treat. Impaired respiratory mucociliary clearance in CF leads to respiratory and sinus colonization, which contributes to Pexs, lung function decline, and chronic symptoms that impact overall health and quality of life [37,38]. The CFF recommends routine sputum surveillance to monitor for respiratory pathogens that are known to contribute to increased morbidity and mortality, with a focus on eradication and chronic treatment. Data from the United States CF Patient Registry in 2021 showed that *Staphylococcus aeurus* (SA) was the most prevalent pathogen amongst PwCF, occurring in 63.8% of patients, with methicillin-sensitive SA (MSSA) noted in more than half (51.7%) of individuals who provided a respiratory sample [10]. The prevalence rate of *Pseudomonas aeruginosa* (PA) has decreased notably over the last few years, from 46.4% to 28.4% between 2016 and 2021; this may be secondary to less sputum sampling from patients benefitting from HEMT and no longer expectorating sputum as much as prior to HEMT [10]. *Methicillin-resistant staphylococcus aeurus* (MRSA) was noted to be the third most common pathogen (18%) in 2021, mostly occurring in PwCF ages 10 to 20 years old. Many of these pathogens are known to cause a more rapid decline in lung health in PwCF [39]. Treatment of these pathogens should be individualized, with consideration focused on infection severity and chronicity, pulmonary function response, and tolerability of treatments with respect to medication-induced side effects and toxicities, allergies, and drug delivery methods [39]. The demand for novel antimicrobial treatments is growing with the increasing prevalence of multi-drug resistant (MDR) bacteria in the CF patient population. As PwCF frequently experience acute or chronic respiratory and sinus infections, their lifetime exposures to antibiotics leads to significant antimicrobial resistance and treatment challenges.

4.1. Oral and Intravenous Antibiotics

Antibiotic usage in the CF patient population is known to be high, given its mainstay role in treating acute Pexs, chronic respiratory infections, and optimizing respiratory health. Antibiotic administration may be oral or IV depending on the targeted pathogen, its drug susceptibilities, the severity of the patient's lung disease, drug tolerance, and a multitude of other factors. Antibiotic choice is based on patient response and susceptibility testing, and recommended dosages are higher due to higher renal and non-renal clearance [40]. Newly established respiratory PA and MRSA infections are indications for eradication therapy [40]. Numerous studies have evaluated the efficacy of different regimens for PA eradication; the most commonly used drug is tobramycin inhalational therapy twice a day for 28 days [41]. The colonization of these organisms can lead to worsening lung function, progression of the patient's symptoms, and lung disease. Aminoglycosides, fluoroquinolones, cephalosporins, trimethoprim-sulfa, tetracyclines, penicillin, and azithromycin are among the commonly utilized regimens for acute Pexs [42]. Anti-infective and anti-inflammatory properties unique to azithromycin were demonstrated in CF patients colonized with PA and with at least moderately severe lung function; thus, three-times-weekly use as an anti-inflammatory drug is standard of care for select patients [43]. Quite often, PwCF have more than one pathogen and will likely require antibiotics of different classes to improve coverage for Gram negative, Gram positive, and atypical bacteria. These higher doses and multidrug regimens can lead to increased side effects and reduced tolerability. NTM infections are another complex pathogen with challenges related to its lengthy treatment duration with a multi-drug regimen requiring close monitoring for intolerances and toxicities; this topic is beyond the scope of this review. The use of IV antibiotics to treat Pexs is very common. The STOP2 trial was the first study that sought to determine the optimal duration of treatment of Pexs in PwCF; it concluded that 10 days of IV antibiotic therapy is not inferior to 14 days, and, for those with less improvement after one week, 21 days is not superior to 14 days [44]. The CFF is also supporting the development of the STOP360 trial, which will evaluate if the addition of an IV aminoglycoside and IV beta-lactam antibiotic treatment is superior to the use of a beta-lactam alone to treat Pexs. This study hopes to show that monotherapy with an IV beta-lactam regimen is not inferior to a two-drug IV regimen, which would alleviate the use of aminoglycosides and its associated side effects of ototoxicity and nephrotoxicity [45].

4.2. Inhaled Antibiotics

The utilization of inhaled antibiotics has become a standard of care for the management of chronic infections in PwCF. Colonization and persistent airway infection can lead to inflammation of the airways, frequent Pexs, and pulmonary decline [46]. Inhalational delivery of medications into the lungs achieves higher drug concentrations directly into the target site of the small airways while minimizing the systemic side effects associated with oral and IV administration. Inhaled antibiotics have been shown to decrease bacterial load and, subsequently, airway inflammation and lung damage; they are also associated with improved clinical outcomes of FEV1 and reduced Pexs [47]. The most successful use of this treatment strategy has been in PwCF; however, it is important to note that there are a limited number of commercially available inhalational antibiotics [48]. The following qualities must be considered when choosing an antibiotic for inhalation: stability and tolerability, suitable pH, retained activity in the airway environment, and limited systemic absorption [49].

4.2.1. Tobramycin inhalation solution

Tobramycin is an aminoglycoside antibiotic that is bactericidal against Gram-negative bacteria, including PA [39]. Tobramycin inhalation solution (TIS) is the best-studied aerosolized antibiotic for use in PwCF and chronic PA infections, and it is approved for people ages 6 and older [50]. TIS has been shown to be a safe and effective option for the treatment of PA, with improvement in FEV1 and a decrease in sputum pseudomonal density and hospitalizations [39]. Side effects of TIS include tinnitus, voice changes, and transient

increases in creatinine, although long-term therapy has been proven to be well tolerated by CF patients with no unexpected adverse events [46]. In an effort to reduce treatment burden, as nebulization is time-consuming, a dry powder version of tobramycin (TOBI podhaler®) was approved for use in 2013. Controlled clinical trials have demonstrated that the tobramycin dry powder formulation has a safety and efficacy profile comparable to TIS with greater treatment satisfaction and adherence rates [46]. While eradication of PA is important, a majority of patients are either unable to clear the infection or have recurrent infection that becomes chronic, which is managed with on/off cycling of inhaled antibiotics every 28 days [49]. PA strains in CF tends to be mucoid, producing a thick biofilm that is protective against clearance and antimicrobial treatment strategies. In order to grow and multiply, PA has an absolute requirement for iron. The combination of tobramycin with calcium ethylene diamine tetra-acetic acid (CaEDTA) is currently being studied to improve the penetrability of the bacterial biofilms of PA [51]. CaEDTA is a chelating agent with antimicrobial properties; it binds to a variety of divalent cations including iron, zinc, and magnesium, rendering them unavailable for use by microorganisms. This phenomenon, referred to as "nutritional immunity," has been successfully studied in vitro and in vivo in animals with chronic PA associated otitis, sinusitis, and endometriosis, and it weakens bacterial defense mechanisms, consequently increasing the bactericidal activity by the concomitant antibiotic [52]. Additionally, CaEDTA has been shown to disrupt the lipopolysaccharide layer in the outer membrane of PA, thus increasing the cell wall permeability and improving antibiotic penetration of the bacteria cell [52].

4.2.2. Aztreonam Lysine (Cayston)

Aztreonam is a monobactam antibiotic that inhibits cell wall synthesis and is active against aerobic Gram-negative bacteria. Aztreonam lysine for inhalation (AZLI) is an aerosolized formulation that was specifically designed for inhalation therapy and is approved for people ages 7 and older who culture PA [39,50]. The safety and efficacy of AZLI for the treatment of PA has been demonstrated in a randomized, double-blind, placebo-controlled study. AZLI improved mean respiratory function scores and reduced sputum density of PA in patients with CF after 28 days of treatment with TIS as compared to placebo. In addition, AZLI increased the median time between Pexs that required antibacterial agents (AZLI, 92 days vs. 71 days in placebo; $p = 0.007$) [53]. In practice, depending on the severity of lung disease, TIS may be prescribed alone or alternating with another agent, such as AZLI, every other month. When following an "on" cycle use of a single inhaled antibiotic, a decline in lung function may be observed during the "off" cycle month. Alternating use of inhaled therapy with inhaled tobramycin and aztreonam, for example, can be more efficacious in patients with persistent respiratory decline [46,50].

4.2.3. Liposomal Amikacin (Arikayce®)

Amikacin liposome inhalation suspension (ALIS) is currently approved for the treatment of refractory *Mycobacterium avium* complex (MAC). ALIS is therapeutically superior to amikacin alone, as the liposomes allow for the penetration and destruction of MAC biofilms, thereby achieving a higher therapeutic concentration [54]. Currently, ALIS is only approved by the U.S. Food and Drug Administration (FDA) for refractory MAC; however, it likely has utility in the treatment of other CF pathogens such as PA. Safety and tolerability of ALIS in PwCF who are colonized with PA has been studied and established globally [55]. In an open label randomized Phase 3 trial, cycling once daily ALIS proved to be noninferior to TIS for the treatment of chronic PA, as indicated by FEV1 improvements and reductions in PA sputum density. Patients also reported lower treatment burdens as measured by the Cystic Fibrosis Questionnaire-Revised (CFQ-R) [56].

4.2.4. Colistimethate Sodium (Colistin)

Colistimethate sodium, a derivative of polymyxin, acts by disrupting the integrity of the bacterial cell membrane and is commonly used in PA infections in IV and inhalational

forms [39,46]. Colistimethate sodium in dry powder formulation is approved in Europe and has been shown to be non-inferior to TIS for the treatment of chronic PA in patients with CF age 6 years and older [57].

4.2.5. Inhaled Vancomycin

Vancomycin is a glycopeptide antibiotic that exerts its bactericidal effects by inhibiting cell wall synthesis, with activity against MRSA and other susceptible Gram-positive organisms [58]. Currently, there is no consensus guideline or recommendation for the treatment of MRSA infections in PwCF [39]. Inhaled Vancomycin was well tolerated and achieved good absorption in the sputum and high minimal inhibitory concentrations for MRSA in a Phase 1 trial [59]. Unfortunately, in a Phase 3 trial of the study drug (Aerovanc®), the primary endpoint of change in FEV1 was not reached and the trial was discontinued [60]. Preventing MRSA colonization through eradication attempts early in the newly acquired stage plays an essential role in delaying Pexs associated with MRSA [61]. Current ongoing trials are looking at combined PO antibiotics with nasal and throat decontamination as treatment approaches for MRSA eradication. The Staph Aureus Resistance-Treat Or Observe (STAR-too) protocol was the first randomized trial of eradication treatment for early MRSA infection in CF. The treatment arm received trimethoprim-sulfamethoxazole (TMP-SMX) and rifampin, combined with nasal mupirocin and throat decolonization with chlorhexidine, for 5 days and antiseptic skin washes for 5 days. Subjects also performed intensified environmental cleaning in their homes for 3 weeks. The control group was a no-treatment arm rather than a placebo arm, with a primary end point of negative MRSA culture on day 28 [62]. The study yielded positive results; however, there were concerns over the feasibility of the demanding treatment regimen and DDI with rifampin, which led to a simpler regimen proposed by the Staph Aureus Resistance-Treat Early And Repeat (STAR-ter) clinical trial [63]. STAR-ter is still currently enrolling subjects and using the same inclusion and exclusion criteria as STAR-too. In the STAR-ter clinical trial, subjects will be treated with one single oral antibiotic (TMP-SMX) and topical (nare and throat) decolonization. Subjects with an allergy or intolerance to TMP-SMX will use minocycline as an alternative antibiotic, and the primary end point remains MRSA-negative culture at day 28 [64].

4.2.6. Inhaled Fluoroquinolones

Fluoroquinolones are bactericidal antibiotics that directly inhibit bacterial DNA synthesis [65]. They have been used extensively, as oral and IV formulations, to treat CF lung disease and Pexs. Fluoroquinolones have high potency and broad coverage, which makes them an attractive candidate for inhaled CF therapy [66]. Inhaled levofloxacin has been used for decades in the European Union and Canada for the treatment and management of PA [46]. It is well tolerated and associated with reduction in PA sputum load, FEV1 improvement, and decreased use of other anti-PA agents [66]. Although it did not meet its primary endpoint of reduction in Pex overall, it was still proven to be a safe and tolerable option for PwCF and PA infection of the airways [66].

4.3. Inhaled Murepavadin

Murepavadin is a pathogen-specific antimicrobial peptidomimetic that binds to the lipopolysaccharide transport protein in Gram-negative bacteria and causes cell death. It displays potent in vitro activity against PA in the IV formulation, and its application has recently been extended into inhaled therapies [67,68]. A Phase 1b/2a trial is planned to study inhaled murepavadin in adults with CF and is currently in the pre-clinical phase of investigation [69].

4.4. Phage Therapy

Research for new antimicrobials is a priority given the limitations in the currently available antibiotic treatments for PwCF, particularly in the setting of an aging patient

population and high prevalence of MDR pathogens. Phage therapy utilizes viruses to infect specific bacteria and replicate the viral genome in the bacteria, ultimately lysing the host bacteria and killing it [37,70]. Phages, also known as bacteriophages, have been studied for decades, but they recently gained momentum in the CF research community because they may serve as an alternative treatment option with potentially fewer side effects for complex bacterial infections in PwCF [71]. Phages occur naturally in the environment, and they are found mostly in wastewater, reserves, lakes, and mammalian serum or compost [70,71]. They are currently being investigated for a variety of pathogenic infections in humans, including, but not limited, to sepsis, urinary tract, respiratory, skin, and joint infections [42]. The "ESKAPE" organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species), along with other Gram-negative bacteria, have been designated priority pathogens by the World Health Organization against which to develop phages [42,71].

With limitations in the development of new antibiotics, phage therapy is currently being studied for patients with CF infected with PA, SA, *Achromobacter xylosoxidans*, and NTM, as seen in Table 2 [42,70]. In theory, phage therapy could be applicable for a multitude of pathogenic bacteria once an appropriate phage is identified, although mono-phage therapy may not be universally applicable to all patients with a particular pathogen. Bacterial resistance is ongoing, and phages will likely need to evolve as well [70]. Phage banks or libraries have been created and shared amongst scientists to expand access to and knowledge of existing phages [42,72]. Investigated phage delivery methods include oral, IV, and inhalational routes [42]. The AP-PA02 study utilized a phage cocktail against PA in vitro and demonstrated a reduction in over 80% of PA strains in patients with CF; this is currently under investigation in the SWARM-Pa study, a Phase 1b/2a trial for safety and tolerability [73]. In vitro and in vivo studies have demonstrated that phages also have the ability to adapt to bacterial mutations [70,72]. The BX004-A trial is a Phase 1b/2a double-blind placebo-controlled study investigating the safety and tolerability, as well as efficacy in reducing the PA bacterial load, of a nebulized bacteriophage for PwCF chronically colonized with PA (NCT05010577) [74]. Phages can penetrate the bacterial biofilms, which are not as easily penetrable by traditional antibiotics [71]. The use of single versus multiple bacteriophages in conjunction with antibiotics is also a strategy of interest, as this combination may have synergistic advantages in treatment [70].

Several published case reports have illustrated successful treatment with phage therapy in CF patients with infections from PA, SA, *Burkholderia dolosa,* and *M. abscessus* [37,75,76]. Reported outcomes following phage therapy included a reduction in sputum quantity and bacterial density, greater time between acute Pexs, and improvement in chest imaging, oxygenation, and weight gain [37,42,70]. A report of a pediatric CF patient with refractory respiratory symptoms and Pexs who was treated with inhaled pyophage, an inhalational preparation of five phages against PA, MSSA, *Streptococcus, Proteus*, and *E.coli*, demonstrated improvements clinically and in sputum bacterial density [75]. In another pediatric CF patient, nebulized pyophage against PA and MSSA was administered nine times at 4- to 6-week intervals with an interim addition of Sb-1 phage; this resulted in radiographic improvement and negative cultures [76].

Lung transplant CF recipients remain at risk for recurrent infections post-transplant, which poses an increased risk for chronic lung allograft dysfunction. Polypharmacy and drug toxicities in this patient population limits the use and duration of antibiotic regimens. Phage therapy has been used anecdotally in peri-transplant management of infections with promising outcomes [77,78]. In an adult CF patient with *B. dolosa* respiratory colonization following bilateral lung transplantation, recurrent pneumonias and postoperative complications led to the use of single lytic phage IV therapy for about 6 months in conjunction with IV antibiotics for 6 weeks [77]. Following phage therapy, clinical, radiographic and culture improvement was observed; however, due to liver failure, sepsis, and other complications, the patient died 11 months following transplant [77]. In a CF patient awaiting lung transplantation, a severe Pex led to endotracheal intubation, at which point a combination

treatment with IV antibiotics and an IV cocktail of four lytic phages against MDR PA was given [78]. This patient was successfully extubated and underwent transplant at a later stage. In the case of a CF transplant recipient with a history of *M. abscessus*, the post-transplant course was complicated by disseminated mycobacterial infection, which was treated with a three-phage therapy via IV and topical routes for 32 weeks, with resulting respiratory and overall clinical improvement, weight gain, and surgical and skin wound healing [79]. All of these patients were reported to have tolerated phage therapy with no significant adverse events or side effects.

While there is encouraging research in phage therapy for CF patients, the clinical utilization of phage therapy is currently limited, and there is much to be learned about the safety profiles, potential side effects, tolerability, dosing regimens, costs, and need for re-treatment. Thus far, the reported adverse events have been few and not serious; however, there is still concern for potential allergic reaction during and following phage administration [37,42,70,71]. Current phage investigations are in preclinical or early clinical trial phases. The future of phage therapy could potentially help CF patients suffering from MDR infections. In the age of HEMT, many patients have experienced great benefits, such as reduced sputum production, making sputum sampling difficult and limiting monitoring of pathogens. It remains to be seen how far phage therapy will develop in the drug pipeline; however, it is a crucial area of development in the treatment of challenging infections and may even serve as personalized therapy specific to unique patients.

4.5. Other Antimicrobial Therapies

Other novel antimicrobial agents in the CF therapeutic pipeline include: IV and inhalational gallium, inhaled nitric oxide, sodium fusidate, lefamulin, and inhaled glycopolymer SNSP113 (Table 2).

Gallium is an element similar to iron that disrupts iron dependent processes necessary for bacterial survival and reproduction, and it has been demonstrated to be a safe and tolerable agent with associated improvement in lung function [80]. A Phase 2 study is currently evaluating the efficacy of IV gallium in CF patients chronically infected with PA (IGNITE study; NCT02354859) [81]. Also targeting PA infections is an inhaled formulation of gallium, AR-501 (NCT03669614) [80,82]. The ABATE study is underway, testing the safety and efficacy of 5 days of continuous IV Gallium in PwCF and *Mycobacterium avium* and *abscessus* infections (NCT04294043) [80,83]. A potential advantage of gallium therapy is its seemingly broad application against multiple pathogens.

Nitric oxide (NO) is being studied as an antimicrobial for its ability to break down biofilms and kill bacteria [84]. There are several clinical trials using NO in CF related NTM infections with refractory *M. abscessus* in the United Kingdom (NOMAB; NCT05101915), as well as MAC and *M. abscessus* in CF and non-CF patients in Australia (LungFit^{TM} GO; Beyond Air Inc., NCT04685720) [85–87]. Preliminary data from the LungFit Go study supported good tolerability of high dose NO in the hospital and home settings, as well as anti-inflammatory and pulmonary vasodilatory effects [84].

Sodium fusidate, also known as fusidic acid, is a protein synthesis inhibitor that prevents bacterial replication and has been studied as an antimicrobial against MRSA and PA infections in bone, joints, and skin; it is available in topical, oral, and IV forms, and it has already been approved for use in Europe [88]. Sodium fusidate is being further investigated in the U.S. for treatment of pulmonary SA infections, as monotherapy and in combination with other antibiotics [88]. The REPRIEVE study is currently investigating the efficacy and safety of oral sodium fusidate (ACG701) in CF patients with acute Pexs and receiving optimized antibiotic therapy (NCT05641298) [89].

Lefamulin (Xenleta®) is a pleuromutilin antibacterial compound that interferes with the bacteria's ability to make protein during the translational process by binding to the bacterial ribosome and preventing the binding of transfer RNA for peptide transfer [90]. It is FDA-approved in oral and IV forms for the treatment of community acquired pneumonia caused by: *Streptococcus pneumoniae, MSSA, H. influenzae, Legionella pneumophila, Mycoplasma*

pneumoniae, and *Chlamydophila pneumonia* [90]. Lefamulin also has antibacterial properties against MRSA strains obtained from PwCF [90]. A Phase 1 open label trial will be assessing the pharmacokinetics and safety of Lefamulin in adult CF patients with SA infections (NCT05225805) [91].

Inhaled glycopolymer SNSP113, also known as Poly-N-glucosamine (PAAG), disrupts biofilm and has anti-inflammatory properties in vitro; it is being investigated as monotherapy and with antibiotics to treat infections with SA, Gram-negative bacteria, and NTM [92]. Evaluations of the safety and tolerability of inhaled SNSP113 have been completed in Europe, and dose escalation studies are pending.

Opelconazole, an inhaled antifungal therapy, is being studied for the treatment of *Aspergillus* in PwCF [93]. Opelconazole is a synthetic triazole that acts to prevent the formation of the fungi cell membrane, resulting in lysis, and it is administered via nebulization at high concentrations with the goal of limiting systemic absorption, which can reduce many of the unwanted side effects of systemic antifungal medications [93]. There is an active open label study in the U.S. assessing the use of inhaled Opeconazole for 12 weeks as a prophylaxis or pre-emptive treatment of patients who have undergone lung transplantation [94].

Table 2. Antimicrobial therapies available and under investigation. [1] = therapies are commercially available; [2] = therapies are in clinical trial phase; [3] = therapies are in pre-clinical phase; [4] = commercially available in Europe. MRSA = *Methicillin resistant Staphylcoccus aureus;* MAC = *Mycobacterium avium* complex. Refs. [37,39,46,50,58,65,67,70,80,87,88,90,92,93].

Antimicrobial Therapies	Mechanism of Action	Targeted Pathogen
Inhalation Antibiotics		
Tobramycin [1]	Aminoglycoside; inhibits protein synthesis of Gram-negative bacteria	*Pseudomonas aeruginosa*
Tobramycin dry powder [1]		
Aztreonam [1]	Monobactam; inhibits cell wall synthesis against aerobic Gram-negative bacteria	
Levofloxacin [1]	Flouroquinolone; directly inhibits bacterial DNA synthesis	
Vancomycin [1]	Glycopeptide; inhibits Gram-positive bacterial cell wall synthesis	MRSA
Colistimethate [4]	Polymyxin derivative; disrupts the integrity of the bacterial cell membrane of most Gram-negative bacteria	*Gram negative*
Murepavadin [3]	Antimicrobial peptidomimetic; binds to the lipopolysaccharide transport protein in Gram-negative bacteria and causes cell death	*Pseudomonas aeruginosa*
Phage Therapy Studied Targets		
Pseudomonas aeruginosa [2]	Viruses that infect specific bacteria and replicate the viral genome, ultimately lysing the host bacteria and killing it	As noted
Mycobacterium abscessus [3]		
Staphylococcal aureus [3]		
Achromobacter xylosoxidans [3]		
Methicillin resistant Staphylcoccus aureus [3]		
Other		
Galium [2]	Disrupts iron-dependent processes necessary for bacterial survival and reproduction	*Pseudomonas aeruginosa* MAC *Mycobacterium abscessus*
Nitric oxide [2]	Gas compound that breaks down biofilms and kills bacteria	MAC *Mycobacterium abscessus*

Table 2. *Cont.*

Antimicrobial Therapies	Mechanism of Action	Targeted Pathogen
Sodium fusidate [2]	Protein synthesis inhibitor that prevents bacterial replication	MRSA *Pseudomonas aeruginosa* *Staphylococcal aureus*
Lefamulin [2]	A pleuromutilin antibacterial compound that binds to bacterial ribosome, preventing the binding of transfer RNA and inhibiting the production of protein	MRSA *Staphylococcal aureus*
Glycopolymer SNSP113 [2]	Disrupts bacterial biofilms; has anti-inflammatory properties	*Staphylococcal aureus,* MAC *Mycobacterium abscessus*
Opelconazole [2]	Synthetic triazole; prevents the formation of the fungi cell membrane, resulting in lysis	*Aspergillus fumigatus*

5. Anti-Inflammatory Therapies

Chronic bacterial infections affecting PwCF trigger a chronic inflammatory state, and, while inflammation works to contain infection, it has harmful effects in the airways of PwCF [95]. In CF, the activation of toll-like receptor adaptor molecule (Trif)-dependent effectors are decreased, and this limited signaling interferes with the resolution of the inflammatory response, resulting in the inability to prevent the destruction of healthy lung tissue [96]. Studies conducted in infants with CF have shown evidence of inflammatory mediators present in the lungs before the detection of any signs of infection, suggesting that inflammation itself is intrinsic to the underlying pathophysiology in CF [97]. It is not entirely known why PwCF have chronic inflammation, but years of research has focused on some major contributors. Anion transport across CF airway epithelial cells is altered due to the defective CFTR protein [98]. As a result, there is a loss of chloride secretion leading to changes in osmotic pressure and electro-neutrality, which likely contribute to excessive sodium and water absorption [99]. The dehydrated airway surface liquid and mucus layer contributes to obstruction of the airway and chronic retention of pathogens, possibly serving as a trigger point for inflammation [98,99]. Additionally, there is an imbalance between omega-3 and omega-6 fatty acids, which may inhibit the capacity of cells to resolve inflammation [98]. Arachidonic acid (AA), derived from omega-6 fatty acids, is associated with the activation of an inflammatory response. In CF, AA levels are increased. Docosahexaenoic acid (DHA) is derived from omega-3 fatty acids and is associated with the termination of inflammation; in CF, DHA is reduced [98]. This relationship of higher AA levels relative to lower DHA levels in CF promotes the activation of inflammation. Furthermore, as part of the innate immune response, chemokines and chemoattractant molecules in the airways attract large quantities of neutrophils to clear the site of infection; however, neutrophilic phagocytosis is inefficient, particularly in excessive mucoid airways [100,101]. Neutrophils also release proteases, pro-inflammatory cytokines, reactive oxygen species, and neutrophil extracellular traps, thereby fueling further neutrophil influx and inflammation and ultimately causing lung damage [97,100,101]. Throughout time, clinical trials have been conducted to examine different therapies aimed at reducing inflammation in CF. Unfortunately, many anti-inflammatory trials have not produced a mainstay therapy to date.

The first anti-inflammatory drugs studied in CF were corticosteroids in the 1980s [95,99]. The first clinical trial of oral corticosteroids found that alternate-day prednisone dosing in children with CF ages 1–12 and mild to moderate lung disease was associated with better lung function, improved weight gain, and fewer hospital admissions [95,97,99]. Unsurprisingly, follow-up studies demonstrated increased adverse events with corticosteroid use, including glucose intolerance, growth impairment, and cataracts; thus, its use is not recommended for anti-inflammatory therapy in CF [97]. In 1994, a U.S. trial showed that twice-daily high dose ibuprofen of 200 mg was associated with slower lung function decline,

better preservation of body weight, fewer hospital admissions, and no increase in adverse events in CF patients [95,97–99,102]. Ibuprofen is a non-steroid anti-inflammatory drug (NSAID) and inhibits neutrophil influx into sites of inflammation [97,99]. Although the results of this study led to the recommendation of ibuprofen for chronic use in CF, its use has not been widely adapted due to the challenges of obtaining a pharmacokinetic study to determine appropriate dosing and concerns regarding safety [97,99].

The use of a monoclonal antibody, KB001, was studied for its anti-inflammatory effects in CF patients with chronic PA infections. Although KB001 decreased sputum neutrophil activity, it was not associated with clinically significant outcomes in the frequency of Pexs or antibiotic need [98]. Inhaled Alpha-1-proteinase inhibitor (Alpha-1 HC), a highly purified blood product, was evaluated for its properties of reducing neutrophil elastase burden; however, it did not progress in trial phases [98,103]. A Phase 2 clinical trial of lenabasum, a cannabinoid type 2 receptor agonist that acts as an anti-inflammatory and anti-fibrotic agent, was conducted in PwCF and found to be associated with reductions in inflammatory biomarkers, IL-8, and immunoglobulin G; however, there was no significant change in FEV1, and a larger scale study remains to be seen [98,104]. Investigation of acebilustat, a leukotriene A4 hydrolase inhibitor that works by preventing the formation of LTB_4, a neutrophil chemoattractant, also resulted in the lack of significant differences in lung function or risk of Pex when compared to placebo [98,105]. While many of the aforementioned study drugs showed evidence of reducing inflammation, this did not translate into meaningful clinical outcomes as generally measured by frequency of Pex and lung function. In the era of HEMT, anti-inflammatory related outcomes may be even more difficult to capture, given the effectiveness of HEMT. Nevertheless, inflammation is still a major contributing factor to patients' overall health.

New anti-inflammatory trials in the pipeline include several Phase 1 and 2 trials. A Phase 2 study using LAU-7b, a form of the retinoid-fenretinide, acts to increase DHA levels, thereby improving the AA/DHA imbalance found in CF and potentially reducing the inflammatory response in the lungs [98]. As mentioned, neutrophilic inflammation in CF is very characteristic of the inflammation in CF lungs and remains a crucial target for therapies. Brensocatib is an oral inhibitor of dipeptidyl peptidase 1, which is an activating enzyme of neutrophil serine proteases; brensocatib acts to inhibit the inflammatory cascade set off by neutrophils. Brensocatib was studied in non-CF bronchiectasis and found to delay time to Pex compared to placebo, and it may have applications in CF [106]. In Europe, a Phase 1 study is underway, evaluating the safety of inhaled lonodelestat (POL6014), which acts similarly to brensocatib by inhibiting neutrophil elastase [107]. Clinical development of anti-inflammatory drug therapies in the CF population is challenging due to a lack of validated and accepted biomarkers of drug efficacy and the need to study their effect on long-term clinical outcome measures [98]. Ongoing research that addresses these challenges is necessary in order to develop safe and effective anti-inflammatory drugs for CF.

6. Mucolytics and Mucociliary Clearance

Mucolytics and mucociliary clearance with accompanying use of airway clearance techniques has been essential in the care of CF patients, helping to clear the thick tenacious mucous that is a key factor of the disease. A major component of CF mucous is pus that is produced from viscous material derived from polymerized DNA from degraded neutrophils [108]. Finding effective mucolytics was among the first focuses of clinical trials supported by the CFF in attempts to break the cycle of pulmonary infection and inflammation from retained airway secretions. Dornase alfa (Pulmozyme®), the first drug targeted specifically for CF patients, was FDA approved for use in 1993 and remains a standard of care therapeutic in CF. Current CFF guidelines recommend the use of dornase alfa to improve lung function and reduce Pexs. Dornase alfa is inhaled via nebulizer once or twice daily and thins the mucous by acting like scissors to lyse through the sputum and by-products of inflammation in the airway. Sputum becomes easier to expectorate, airway clearance improves, and the risk of Pexs is reduced [109]. To date, three mucolytic agents

have completed phase three clinical trials, been proven to be safe and effective, and are now available for patient use in the United States [110]. The second mucolytic approved for inhalation is hypertonic saline. Hypertonic saline works by increasing salt concentrations in the airway through osmotic forces, increasing the volume of the airway surface liquid. Clinical trials showed a short-term increase in airway surface volume associated with improved lung function and respiratory symptoms over baseline values [111]. This sterile solution, available in a 3% or 7% concentration, is inhaled via a nebulizer once or twice daily before airway clearance. The third and most recently FDA approved mucolytic therapy for PwCF 18 years and older is inhaled mannitol (Bronchitol®). Inhaled mannitol is a muco-active hyperosmotic agent administered as a dry powder with a hand-held inhaler that was shown to be an effective add-on to standard of care therapy in adults with CF to improve mucociliary clearance. The effects of inhaling mannitol were shown to be transient, so following the medication with airway clearance techniques, such as high frequency chest wall oscillation, is important to clear airway secretions [112].

In the current CFF drug development pipeline, trials for two additional mucolytic therapies include OligoG (AlgiPharma and CFF) and GDC-6998 (formerly EDT002) (Roche-Genetech and CFF) in Phase 1 trials. OligoG is in Phase 2 development, and it is a dry powder drug that has been shown to make mucous in the lungs thinner through calcium chelation, which is required for mucin unfolding and may help clear the airways of PwCF [113]. It has also been shown to increase the efficacy of some antibiotics. The second compound in the CF drug development pipeline for mucolytic therapy is GDC-6998 (formerly EDT002). GDC-6998 has been found to affect a chloride channel similar to the CFTR channel on the surface of airway cells by increasing the activity of this channel, TMEM16A. This may increase liquid surface fluid and improve mucous clearance [114].

The future of CF care guidelines in the U.S. is certain to undergo changes in the post-HEMT era. Along with the voices of patients in directing future clinical trials (CF Community Voice), the focus of clinical trials is also shifting toward reducing polypharmacy and care burden and improving quality of life [115]. The SIMPLIFY study evaluated the impact of discontinuing hypertonic saline or dornase alfa for six weeks, in PwCF with well-preserved lung function, and taking ETI. The results reported that discontinuing daily hypertonic saline or dornase alfa for six weeks did not result in clinically meaningful differences in lung function as measured by FEV1 [116].

7. Conclusions

Historically, CF therapeutics relied heavily on treating the disease progression after it had set in, as seen in antimicrobial and mucociliary clearance therapies. The field of CF treatments is moving further upstream in the disease process, as evidenced by HEMT, and it is now moving into mRNA and genetic-based therapies, which will help to address the remaining 10% of the CF patient population not eligible for HEMT. Drug development is also seeing more innovative approaches to tackle today's challenges in CF disease. For instance, multi-drug resistant bacterial treatments are limited with today's antibiotic choices, and more antimicrobial agents, such as bacteriophages, are needed outside of the traditional antibiotics. Another challenge is selecting measurable and detectable outcomes in the design of interventional drug trials. For instance, measures of FEV1 may not easily distinguish a detectable difference when the patient population is becoming healthier in lung function and overall health, leading the research community to consider the use of the lung clearance index through multiple breath washout testing as an alternative and more sensitive clinical trial endpoint [117]. As we move forward with the next wave of novel therapeutics, trial designs will need to evolve alongside the CF population. The future of CF therapeutics will undoubtedly have exciting advancements to benefit the growing CF population.

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Calcitonin Gene-Related Peptide Level in Cystic Fibrosis Patients

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Abstract: Calcitonin gene-related peptide (CGRP) has long been implicated in both the physiology and pathophysiology of the respiratory tract. The objective of our study was to determine the serum concentration of alpha CGRP (αCGRP) in cystic fibrosis (CF) that arises from mutations in the gene responsible for encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Currently, there are not many data in the literature about the role of CGRP in CF. The serum level of αCGRP was estimated using the enzyme-linked immunosorbent assay among 64 patients with CF and 31 healthy controls. The αCGRP concentration in the CF group was 62.51 ± 15.45 pg/mL, while in the control group it was 47.43 ± 8.06 pg/mL ($p < 0.001$). We also compared the level of αCGRP in CF patients according to the type of CFTR mutation. Homozygotes for ΔF508 had higher αCGRP levels than heterozygotes (67.9 ± 10.2 vs. 54.5 ± 18.3 pg/mL, $p < 0.01$). The level of this neuropeptide was statistically higher in patients with severe disease than in those with mild CF ($p = 0.003$) when patients were divided into three groups by spirometry results. αCGRP concentration was not correlated with age, sex, clinical parameters, and pulmonary function test results in the study participants. The results of our study suggest a significant increase in the concentration of αCGRP in the serum of patients with CF compared to the control group. This observation opens interesting possibilities for understanding the role of αCGRP in the context of CF pathophysiology.

Keywords: calcitonin gene-related peptide; cystic fibrosis; CFTR mutation

1. Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide, featuring an N-terminal disulfide bond and a C-terminus that is amidated. It exhibits broad distribution within discrete regions of both the central and peripheral nervous systems, indicating potential involvement in cardiovascular, integrative, and gastrointestinal functions [1]. Furthermore, CGRP is a remarkably potent vasodilator and, to some extent, harbors protective mechanisms vital for both physiological and pathological conditions concerning the cardiovascular system and wound healing [2,3]. It is hypothesized that CGRP also plays a role in arthritis, skin conditions, diabetes, and obesity [4]. Moreover, CGRP is recognized as one of the fundamental factors in the pathophysiology of migraine [5]. Most neuropeptides, including CGRP, are also released from endocrine cells and can act on both neural and nonneural targets [2]. CGRP is present throughout the respiratory tract and CGRP receptors are expressed on many cells, including pulmonary artery cells, epithelial goblet cells, and innate lymphoid immune cells [6]. Cystic fibrosis (CF) is an autosomal recessive genetic disorder resulting from mutations in the CF transmembrane conductance regulator (CFTR) gene responsible for encoding a chloride channel. The deletion of phenylalanine at position 508 (ΔF508) represents the most prevalent mutation among CF patients. The functionality of this chloride channel plays a critical role in maintaining the osmotic balance of mucus and regulating its viscosity. The imbalance or lack of fluids and ions resulting from duct dysfunction causes the production of thick and

viscous mucus, leading to dysfunction of the exocrine glands [7]. Neural involvement in CF has been also proposed [8].

It is noteworthy that elevated levels of CGRP have been detected in the submucosal glands of the airways in humans with CF, as well as in CF animal models including mice, ferrets, and pigs [9]. This suggests that the increased expression of CGRP in CF submucosal glands may represent a compensatory mechanism aimed at restoring CFTR-dependent secretions [8,9]. Within the CGRP family, the two most closely related peptides are α-CGRP and β-CGRP, which are expressed from two genes located at different sites on chromosome 11 in humans. α-CGRP may be the predominant form secreted by neurons or endocrine cells, is well studied, and has important physiological functions [1–4].

Despite the significant implications of αCGRP in various physiological and pathological processes, there remains a notable gap in our understanding regarding its serum levels, specifically in patients diagnosed with CF. This lack of data underscores the need for comprehensive investigation to elucidate the role of αCGRP in the context of CF pathology. Thus, the primary objective of this study was to bridge this knowledge gap by quantifying and evaluating the serum concentration of αCGRP in individuals diagnosed with CF. Furthermore, we sought to explore the potential correlations between αCGRP levels and various clinical parameters characteristic of CF. These parameters may encompass but are not limited to pulmonary function tests, nutritional status, microbial colonization patterns, and disease severity indices. By examining these associations, we aimed to shed light on the potential clinical relevance of αCGRP in the context of CF pathophysiology and disease progression.

2. Materials and Methods

2.1. Ethical Issues

The study protocol, as outlined in resolution no. 2022/023 by the Bioethics Committee of Rzeszów, underwent thorough scrutiny and received official approval. In accordance with the principles set forth in the Declaration of Helsinki, the research was meticulously conducted, guided by the relevant guidelines and regulations governing scientific inquiry. Prior to commencement, all prospective participants, or their legal guardians in the case of minors or individuals unable to provide consent, were fully informed about the nature and objectives of the study.

2.2. Study Group

A cross-sectional study was conducted at a single center involving a sample of 64 CF patients and 31 healthy controls. Participants were recruited from the local CF clinic between February and October 2021. We recruited patients aged 9–39 with a confirmed diagnosis of CF based on current diagnostic criteria [10,11]. The next criteria for enrolling patients in the study were the following: forced expiratory volume in the first second (FEV_1) greater than 35% of predicted stable pulmonary disease as defined by both clinical impressions and no hospitalizations in the 1 month before recruitment to the study. Exclusion criteria were heart and liver failure, migraine, psychiatric disorder, diabetes mellitus, having undergone solid organ transplantation, disease exacerbation, chronic immunosuppressive and corticosteroids or antibiotic treatment. Furthermore, patients were excluded if they were unable to perform spirometry or declined to participate in the study. Consequently, six CF patients were excluded from the study.

All CF patients suffered from pancreatic insufficiency and received pancreatic enzyme replacement therapy, human DNase I recombinant, fat-soluble vitamins in the form of ADEK tablets, and inhalation of 3–7% sodium chloride 3–4 times daily as recommended [12]. It is worth adding that patients with CF were not treated with CFTR modulators because, at the time of the study, this treatment was not yet financed in Poland. On the day of study, we recorded and estimated the following clinical variables in all participants: sex, age, body mass index (BMI), type of CFTR mutations, blood morphology and serum analysis, and sputum microbiology. Data on the type of mutation and chronic bacterial infection were obtained from current hospital records. CF subjects were additionally stratified into

three groups according to the severity of their disease as determined by the results of FEV_1: mild disease (FEV_1 > 75% predicted), moderate disease (FEV_1 ranging from 45% to 75% predicted), and severe disease (FEV_1 < 45% predicted), as previously outlined in [13].

Healthy controls were recruited concurrently from the local clinic. The control cohort comprised volunteers matched for age and sex who were devoid of any medical history or findings indicating disease upon physical examination. These volunteers abstained from medication, including supplements, for a period of 30 days preceding the study. All individuals in the control group exhibited normal pulmonary function test results, along with standard findings in biochemical and hematological assays. Among the participants from the control group and patients with CF, there were no people with skin diseases, arthritis, other respiratory diseases such as asthma, central nervous system disorders, or wound healing disorders. The body mass index was calculated as a person's weight in kilograms divided by the square of height in meters (kg/m^2).

2.3. Spirometry

To ensure consistency and accuracy in assessing pulmonary function, spirometry was conducted for all subjects. Utilizing a standard spirometry device (Lungtest 1000, MES, Kraków, Poland), in accordance with established guidelines [14], pulmonary function was systematically evaluated. The mean value of FEV_1 over the last six months was calculated for each participant and expressed as a percentage of the predicted value based on age and sex. This approach enables normalization of lung function data, facilitating meaningful comparisons across individuals and groups while accounting for variations attributable to age and sex differences.

2.4. Blood Sampling

Following an overnight fasting period, peripheral blood samples were collected, with 5 mL drawn from each participant in the morning to minimize potential confounding factors. These blood samples were promptly transferred into specialized blood collection tubes. Subsequently, the collected blood samples underwent centrifugation at $1500\times$ *g* for 10 min at 4 °C. The obtained serum was then carefully aliquoted into individual storage vials. These aliquots were promptly stored at a temperature of −80 °C. Serum samples were not stored for more than one month to mitigate the effects of prolonged storage on sample quality. Furthermore, to prevent repeated freeze–thaw cycles that could compromise sample integrity, serum aliquots were thawed only once at room temperature immediately prior to analysis.

2.5. Blood Counts and Serum Analysis

Blood morphology was performed using a hematology analyzer (Siemens Healthineers, Erlangen, Germany). The concentration of C-reactive protein (CRP) was estimated using the dry chemistry immunological method on a VITROS 250 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson, Rochester, NY, USA). Immunoturbidimetric assays were used for the determination of IgG.

2.6. αCGRP

αCGRP serum concentrations were measured in duplicates using the enzyme-linked immunosorbent assay (ABclonal Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's protocol. The limit of detection for αCGRP was 5.35 pg/mL, and the within-assay and between-assay coefficients of variations were lower than 10% and lower than 12%, respectively. The absorbance was measured at the appropriate wavelength using a Tecan Infinite 200 PRO multimode reader (Tecan Group Ltd.; Männedorf, Switzerland). Serum levels of αCGRP were expressed in pg/mL.

2.7. Statistical Analysis

All statistical analyses were conducted using the STATISTICA software package (version 13.3, StatSoft Inc. 2017, Tulsa, OK, USA). Quantitative variables were reported as mean ± standard deviation (SD) and range. Qualitative variables were also reported as percentages. Comparisons of the groups were performed with the Mann–Whitney U test or Kruskal–Wallis test. Spearman's rank correlation coefficient analysis was used to estimate the associations between the CGRP and clinical parameters, assuming linear dependence. *p*-values of less than 0.05 were considered to be statistically significant.

3. Results

A total of 42 females (65.7%) and 22 males with CF and 20 healthy females (64.5%) and 11 healthy males were included in the study. The characteristics of the study groups are presented in Table 1.

Table 1. Baseline demographic and clinical data of the study participants *.

		CF	Healthy Controls	*p*
Sex (F/M)		42/22	20/11	
Age (years)	mean ± SD	18.02 ± 7.32	18.06 ± 5.26	0.631
	range	9–39	10–38	
BMI (kg/m^2)	mean ± SD	19.73 ± 2.17	22.34 ± 3.13	<0.001
	range	14.4–24.6	18.3–25.6	
Genotype				
Homozygous ΔF508, n (%)		38 (59.3)	-	-
Heterozygous ΔF508, n (%)		26 (40.7)	-	-
2184insA, n (%)		3 (11.5)	-	-
GLy480ASp, n (%)		5 (19)	-	-
1717-1G > A, n (%)		7 (26.9)	-	-
358G > C, n (%)		5 (19)	-	-
W1282x, n (%)		4 (15.7)	-	-
N1303K, n (%)		2 (7.9)	-	-
Clinical Laboratory Markers				
WBC (10^3/μL)	mean ± SD	9.97 ± 3.44	7.11 ± 2.88	0.068
	range	5.1–19.3	4.4–10.5	
NEU (%)	mean ± SD	58.2 ± 15.6	56.88 ± 9.4	0.928
	range	25.4–81.6	50.6–68.6	
CRP (mg/L)	mean ± SD	4.55 ± 3.8	2.13 ± 1.84	0.014
	range	0.5–22	0.3–4.0	
IgG (g/L)	mean ± SD	9.8 ± 2.1	9.3 ± 2.65	0.929
	range	5.7–16.3	7.8–12.3	
Pulmonary function				
FEV_1 (% predicted)	mean ± SD	88.7 ± 24.3	102.16 ± 9.12	0.011
	range	35–132	98–126	

* BMI—body mass index; WBC—white blood cells; NEU—neutrophils; CRP—C-reactive protein; FEV_1—forced expiratory volume in 1 s. Differences between means were analyzed using Mann–Whitney U tests.

There was no difference in the age of the study groups, but CF patients had significantly lower BMI ($p < 0.001$). Patients were classified into two groups by genotypes:

homozygous for ΔF508 (59.4%) and heterozygotes (40.7%). There was no difference in white blood cell and neutrophil counts, but CRP was significantly elevated in CF patients ($p < 0.05$). The FEV_1 values in patients with CF were significantly worse than in healthy controls ($p < 0.05$). In total, 18 (28.1%) CF patients were positive for *Pseudomonas aeruginosa* and 20 (31.25%) patients were infected with *Staphylococcus aureus*, while 26 (40.65%) were uninfected. Analysis of the results of spirometry allowed for the division of patients with CF according to the severity of the disease: mild (n = 25, 39.1%), moderate (n = 19, 29.7%), and severe (n = 20, 31.2%).

Figure 1 presents the concentration of αCGRP among patients with CF and healthy subjects.

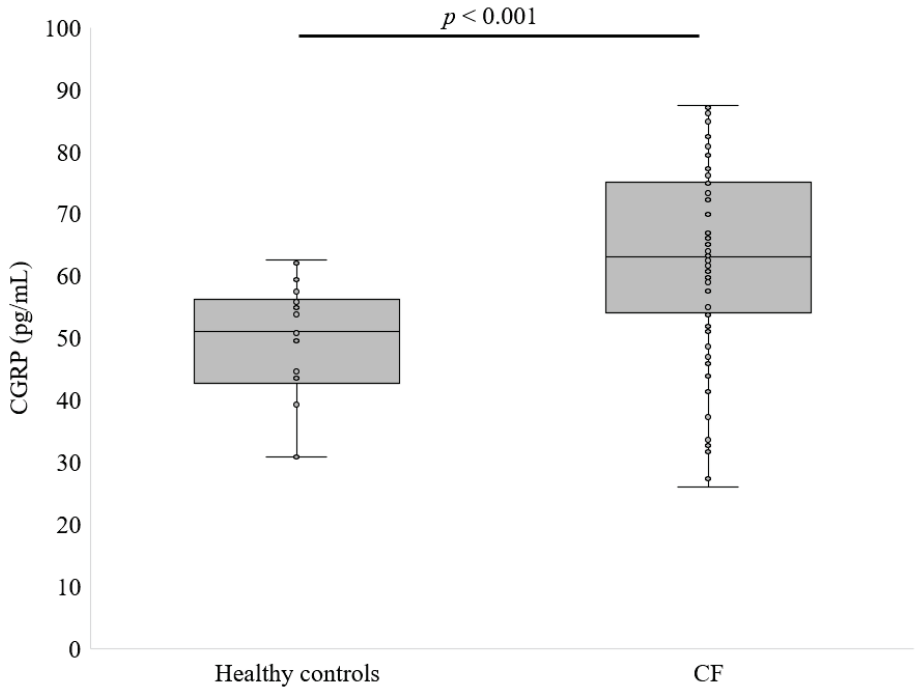


Figure 1. Level of CGRP in patients with CF compared to healthy participants (differences between means were analyzed using Mann–Whitney U tests).

The αCGRP concentration in the CF group was 62.51 ± 15.45 pg/mL, while in the control group it was 47.43 ± 8.06 pg/mL ($p < 0.001$, Figure 1). The next step of this study was to check whether the type of CFTR mutation and the sex of the patients had an impact on the level of CGRP. Homozygotes for ΔF508 had higher αCGRP levels than heterozygotes (67.9 ± 10.2 vs. 54.5 ± 18.3 pg/mL, $p < 0.01$, Figure 2). However, there was no statistical difference in αCGRP concentrations between females and males with CF (63.5 ± 14.8 vs. 60.6 ± 10.7 ng/mL, $p = 0.666$, differences between means were analyzed using Mann–Whitney U tests).

The type of chronic bacterial infection did not affect αCGRP levels (CF patients with *P. aeruginosa*: 64.22 ± 12.6 pg/mL; CF patients with *S. aureus*: 69.1 ± 11.6 pg/mL; uninfected group: 56.24 ± 17.2 pg/mL; $p = 0.063$; differences between means were analyzed using Kruskal–Wallis U tests), although participants infected with *P. aeruginosa* or *S. aureus* had slightly higher concentrations of this neuropeptide. The αCGRP level in patients with mild disease was 55.44 ± 14.81 pg/mL, in patients with moderate disease it was 61.37 ± 15.82 pg/mL, and in patients with severe disease it was 65.31 ± 11 pg/mL. The level of this neuropeptide was statistically higher in patients with severe disease than in those with mild CF ($p = 0.003$; differences between means were analyzed using Kruskal–Wallis U tests).

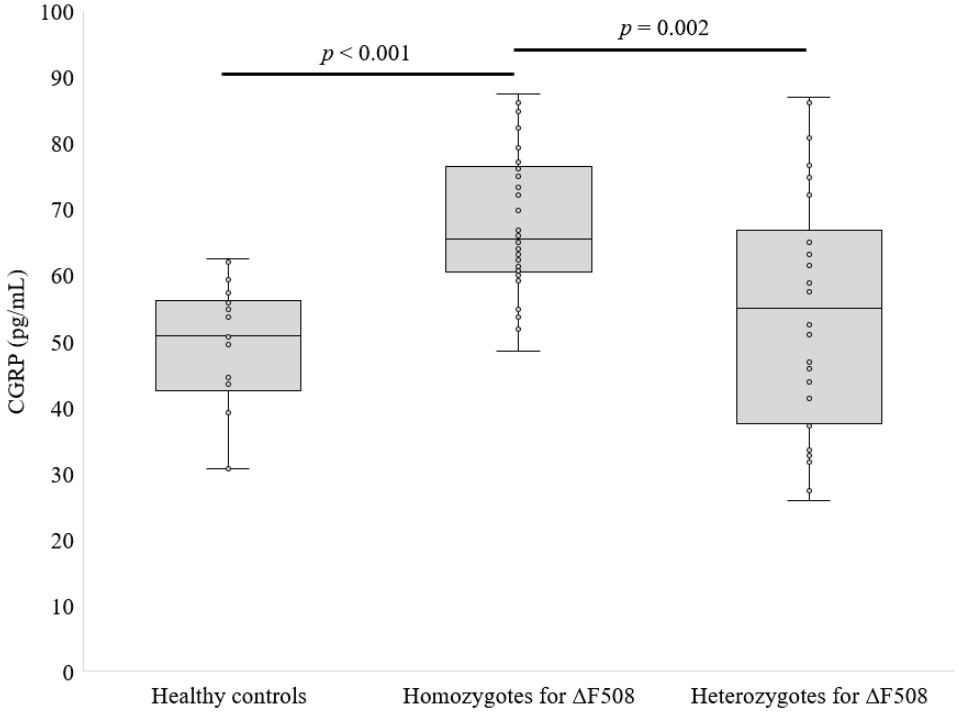


Figure 2. CGRP level by the type of *CFTR* mutations (differences between means were analyzed using Kruskal–Wallis U tests).

Another important aspect of our study was to assess whether patients' clinical parameters were correlated with αCGRP. Table 2 shows the correlation coefficients and the *p*-values estimated using Spearman correlation. αCGRP concentration was not correlated with BMI, CRP, IgG, and FEV_1. At the same time, the results of the correlation between CGRP and age, white blood cell count, and neutrophil count, although not reaching statistical significance, are close to $p = 0.05$, indicating that further analysis is required across a larger number of CF patients.

Table 2. Spearman's rank correlation coefficients (R) and *p*-values between CGRP concentrations and clinical features of studied patients *.

	Healthy Controls		**CF**	
	R	***p***	***R***	***p***
Age	−0.258	0.391	−0.258	0.065
BMI	−0.544	0.069	−0.147	0.299
WBC	−0.156	0.647	−0.244	0.081
NEU	−0.079	0.812	−0.261	0.061
CRP	0.354	0.301	−0.185	0.189
IgG	0.089	0.803	−0.019	0.902
FEV_1	0.094	0.824	0.128	0.364

* BMI—body mass index; WBC—white blood cells; NEU—neutrophils; CRP—C-reactive protein; FEV_1—forced expiratory volume in 1 s.

4. Discussion

The results from our study present significant differences in the concentration of αCGRP present within the serum of patients diagnosed with CF when juxtaposed against levels in the control group, underscoring the potential significance of this neuropeptide in the context of CF pathophysiology.

CGRP has long been suggested to participate in physiology and pathophysiology of the respiratory tract. A large number of studies in recent years have provided direct evidence for an important modulatory role of CGRP in airway functions such as bronchial smooth muscle tone or vasoregulation [15]. The most prominent effects of CGRP in the airways are vasodilation and, in a few instances, bronchoconstriction. Moreover, a further pulmonary effect of CGRP is the induction of eosinophil migration and the stimulation of β-integrin-mediated T cell adhesion to fibronectin at the site of inflammation [16]. By contrast, CGRP inhibits macrophage secretion and the capacity of macrophages to activate T cells, indicating a potential anti-inflammatory effect [17]. Since CGRP participates in goblet cell hyperplasia and muc5AC induction in other disease models, it is possible that this neuropeptide is also involved in CF exacerbations, in which elevated muc5AC has been noted [18,19]. Furthermore, CC motif chemokine ligand 17 stimulates the release of greater amounts of CGRP than other inflammatory cytokines through a CCR4-dependent mechanism that has been implicated in other respiratory diseases [20]. As a result, CGRP can limit the activation of immune cells and reduce the production of inflammatory mediators. In cases of hypoxia, airway stem cells respond by proliferating and differentiating into neuroendocrine cells that secrete CGRP [21]. Episodes of hypoxia occur frequently in patients with CF, which may also explain the increased concentration of this neuropeptide in sick people [22]. Moreover, a study by Chang et al. proved that CGRP may be important in the mechanism of chronic cough [23]. Cough is part of the daily life of patients with CF and its most common symptom, which may also be related to the increased concentration of this neuropeptide [24].

CGRP can be present in a variety of body tissues, where it plays tissue-specific activities. The main source of serum CGRP is believed to be from the perivascular nerve endings [3] and it is elevated in certain pathological states, such as sepsis [25]. A recent study showed that lower levels of CGRP should negatively impact the respiratory physiology of COVID-19 patients due to vasoconstriction, improper angiogenesis, less epithelial repair, and faulty immune response [17]. On the other hand, patients with pulmonary hypertension had higher plasma CGRP than patients with normal pressure [26]. There are not many data in the literature about the role of CGRP in CF. An interesting aspect is the αCGRP concentration in CF patients, which was 62.51 ± 15.45 pg/mL in our study and 346.54 ± 47.19 pg/mL in a recent study by Al-Keilani et al. [27]. This may be due to the specificity of the test that was used to determine αCGRP or differences in the studied groups. Furthermore, Al-Keilani et al. studied CF exacerbation, and this may be another reflection of how CGRP levels reflect disease severity.

Previous studies have shown increased levels of CGRP in the submucosal glands of the respiratory tract in humans with CF, as well as in mice, ferrets, and pigs with CF, indicating a potential role for CGRP as a compensatory mechanism to restore CFTR-dependent secretion [7,28]. As we observed higher αCGRP concentrations in participants with CF, our results also seem to support these observations.

One interesting aspect of our results is the difference in αCGRP levels between patients homozygous and heterozygous for the ΔF508 mutation. Homozygous individuals for the ΔF508 mutation had significantly higher αCGRP levels. This suggests that different types of CFTR mutations may affect αCGRP production. However, more detailed research is needed to decipher this relationship.

In our study, αCGRP levels were higher in patients infected with *P. aeruginosa* or *S. aureus* than in uninfected participants. Similarly, serum CGRP level was significantly associated with positive *S. aureus* microbiology tests ($p = 0.046$) and the type of antibiotic therapy ($p = 0.012$) in a recent study by Al-Keilani et al., who stated that serum levels of αCGRP may predict response to antibiotic therapy in CF patients with pulmonary exacerbations [27]. Moreover, El Karim et al. proved that CGRP displayed antimicrobial activity against several types of bacteria, including *P. aeruginosa* [29].

In our study, there were no correlations between αCGRP levels and sex, age, or other clinical parameters among CF patients. In healthy subjects, it has been shown that plasma

CGRP levels in women are significantly higher than in men [30,31], suggesting a direct relationship between estrogen levels and CGRP concentrations in blood. Due to the fact that our population included both children and adults, we did not observe this association.

The results of the correlation of CGRP with age, as well as with counts of white blood cells and neutrophils, are close to the assumed level of statistical significance, so the cohort is also too small to reject a correlation between CGRP and these parameters in CF. These results, although not statistically significant, may indicate the need for further studies in larger numbers of CF patients to clearly determine the associations between CGRP and age and hematological results. Specifically, a correlation between CGRP and age may reflect the association of CGRP with CF disease severity for CF disease progression. White blood cell count or neutrophil counts may correlate with CGRP in CF, but not in healthy controls. No correlation between CGRP and white blood cell and neutrophil counts was observed in hospitalized acute COVID-19 patients [32]. In addition to being synthesized by nerves, CGRP is also produced by immune cells, including lymphocytes, monocytes, and macrophages [16]. Furthermore, within the bone marrow, CGRP can act on hematopoietic progenitors [33].

CGRP can indeed be considered an inflammation marker, at least in certain aspects. While traditionally recognized for its role in vasodilation and nociception, emerging research suggests that CGRP is intricately involved in modulating inflammatory responses. Studies have shown that CGRP levels increase in response to various inflammatory stimuli. For example, in conditions such as rheumatoid arthritis, inflammatory bowel disease, and asthma, elevated levels of CGRP have been observed in affected tissues or systemic circulation [34,35]. This suggests that CGRP production is upregulated as part of the inflammatory response. Moreover, by dilating blood vessels, CGRP promotes increased blood flow to inflamed tissues, facilitating the delivery of immune cells and mediators to the site of inflammation. CGRP influences the function of immune cells involved in inflammation, including T cells, macrophages, and dendritic cells [15,36,37].

While our study provides valuable insights into the association between αCGRP and CF, it is imperative to acknowledge the inherent limitations that may impact the generalizability and depth of our findings. One such limitation pertains to the relatively modest sample size employed in this study. Although our study cohort was carefully selected and rigorously evaluated, the inclusion of a larger and more diverse patient population would strengthen the statistical power and robustness of our conclusions. By encompassing a broader spectrum of individuals with CF, including those with varying genetic backgrounds, future studies can offer a more comprehensive understanding of the role of αCGRP in CF pathogenesis. Additionally, the absence of long-term follow-up data represents another noteworthy limitation of our study. Longitudinal assessment over an extended duration would offer valuable insights into the dynamic changes and prognostic implications of αCGRP expression in CF progression.

5. Conclusions

A compelling association emerged between the severity of CF and αCGRP levels, with patients exhibiting severe manifestations of the disease demonstrating markedly elevated levels of this neuropeptide compared to those with milder forms of CF. This correlation underscores the potential role of αCGRP as a biomarker reflective of disease severity and progression, offering valuable prognostic implications for patient management and clinical decision making. This study opens new research perspectives and suggests potential clinical implications for the treatment of patients with CF.

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Institutional Review Board Statement: The study received approval from the Bioethics Committee of Rzeszów University (resolution no. 2022/023, date: 6 April 2022). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available on request from the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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One-Year Effect of Elexacaftor/Tezacaftor/Ivacaftor Therapy on HbA1c Levels and Insulin Requirement in Patients with Insulin-Dependent Cystic Fibrosis-Related Diabetes: A Retrospective Observational Study

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Abstract: Introduction: The impact of ETI therapy on pulmonary function and nutritional status has been widely studied; the literature on the possible outcomes on glycemic control and insulin requirement in patients affected by CFRD is controversial. Aim: The main objective of our study was to evaluate HbA1c levels in patients with cystic fibrosis-related diabetes (CFRD) after one year of therapy with elexacaftor/tezacaftor/ivacaftor (ETI). The secondary objective was to study the changes in the total daily insulin dose (TDD), pulmonary function and metabolism in this population. Materials and methods: A retrospective single-center observational study was conducted at the Regional Cystic Fibrosis Centre and Diabetology Centre of IRCCS Istituto Giannina Gaslini. The observation period was divided into four different time points: initiation (T0), 3 months (T_{3mo}), 6 months (T_{6mo}) and 12 months (T_{12mo}) of ETI therapy. Demographic and clinical data were collected. The results were then stratified by genotype (homozygous or heterozygous F508del). Results: Twenty-eight patients with CFRD undergoing insulin therapy were included. TDD (IU) significantly decreased at T_{3mo} and T_{6mo}, but not at T_{12mo}, whereas HbA1c decreased significantly at all three times. The number of hospitalizations and pulmonary exacerbations decreased significantly. Conclusion: We demonstrated both improvement in glycemic control (by means of HbA1c) and insulin requirement in insulin-dependent CFRD patients after one year of ETI treatment.

Keywords: CFRD; cystic fibrosis; diabetes; CFTR modulators; ETI treatment

1. Introduction

Cystic fibrosis-related diabetes (CFRD) is a specific extra-pulmonary complication associated with increased morbidity and mortality in the cystic fibrosis (CF) population. Among the extrapulmonary complications of patients with CF, CFRD is by far the most common [1]. CFRD is a unique form of diabetes, most frequently diagnosed in young adults and the prevalence increases with age [2]. The etiology of CFRD seems to be linked to the partial loss or dysfunction of pancreatic islets, leading to insulin deficiency and to insulin resistance [3]. The etiology of insulin secretion impairment is also linked to a direct effect of

the CF transmembrane conductance regulator (CFTR) protein [4]. A decline in the patient's pulmonary function and nutritional status begins about 2–4 years prior to the diagnosis of CFRD [5]. This deterioration is believed to be associated with protein catabolism due to insulin insufficiency; the worsening of pulmonary function is directly associated with the severity of insulin insufficiency [6]. As mentioned above, CFRD is strongly associated with a decline in pulmonary function, increased frequency of exacerbations and poor nutritional status, resulting in a worse prognosis for the patient [7,8].

CFTR modulators, a group of innovative drugs used in the treatment of CF, have revolutionized the prognosis of these patients; their role is to correct and potentiate defects in the channel function [9]. Various combinations of these modulators are available; the most recent formulation available is a triple combination therapy of elexacaftor/tezacaftor/ivacaftor (ETI) approved for patients with at least one copy of the F508del variation [10]. The impact of ETI therapy on pulmonary function and nutritional status has been widely studied and confirmed, while only a few studies have been published regarding the possible outcomes on glycemic control and insulin requirement in patients affected by CFRD.

Grancini et al. demonstrated a significant reduction in glycated hemoglobin A1c (HbA1c) values after 6 months of ETI therapy but unchanged insulin requirements [9]. In a large Danish study including 321 patients with CF, a HbA1c decline was reported over 12 months of ETI treatment, which was not associated with a change in insulin usage in the sub-cohort of 26 patients with CFRD [11]. Lurquin et al. showed a significant decrease in insulin requirement during follow-up in 17 patients undergoing CFTR modulator therapy with either ETI or tezacaftor/ivacaftor, despite an increase in HbA1c levels [12]. Recently, Cohen et al. demonstrated a decrease of 120 min in the Oral Glucose Tolerance Test (OGTT) value after the initiation of CFTR modulator therapy [13].

As stated by Salazar-Barragan et al.'s systematic review, most studies on this topic used small sample sizes, and therefore their power to demonstrate the effectiveness of ETI was limited [14]. Our goal was to evaluate whether ETI is effective for glycemic control and improvement in the total daily dose of insulin in a larger cohort of patients with CFRD all requiring insulin treatment.

The primary aim was to study how HbA1c levels change in adult and pediatric patients affected by cystic fibrosis-related diabetes after one year of elexacaftor/tezacaftor/ivacaftor therapy. The secondary aim was to study the improvement of total daily insulin dose, pulmonary function and metabolism in this population.

2. Materials and Methods

A retrospective single-center observational study was conducted at the Regional Cystic Fibrosis Centre and the Regional Diabetology Centre of IRCCS Istituto Giannina Gaslini. All patients with insulin-dependent CFRD who started ETI therapy were included. Patients who started insulin therapy after initiating ETI were excluded. The observation period was divided into four different time points: initiation (T0), 3 months (T_{3mo}), 6 months (T_{6mo}) and 12 months (T_{12mo}) of ETI therapy. The number of pulmonary exacerbations and hospitalizations, during the year before initiating ETI, was recorded, as well as previous use of CFTR modulators, six-minute walking test (6MWT) and chloride sweat test data during the 12 months prior to ETI initiation (pre-T0). Demographic (age, sex, ethnicity) and clinical data were collected. CFTR genotype, age of CFRD diagnosis, insulin therapy duration, age of ETI initiation and insulin regimen were collected at T0. At T0, T_{3mo}, T_{6mo} and $T_{12mo,}$ we compared weight, body mass index (BMI), forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), total daily dose of insulin (TDD), basal dose of insulin, bolus dose of insulin, TDD per kilogram and glycated hemoglobin (HbA1c) values. At T0 and $T_{12mo,}$ we compared the results of the cystic fibrosis questionnaire (CFQ-R) that measures the health quality of life of patients with CF (the higher the score, the better the mental status). At pre-T0 and $T_{6mo,}$ we compared 6MWT and chloride sweat test data; and at pre-T0 and $T_{12mo,}$ we compared the number of pulmonary exacerbations and

hospitalizations. The same data were consequently subanalyzed according to the different CFTR genotypes (Group 1—subjects F508del/F508del vs. Group 2—subjects F508del/any other CF-causing mutation).

Statistical Methods

Data are described as mean and standard deviation (SD) or median and range for continuous variables, and as absolute and relative frequencies for categorical variables. Comparisons between T0, T_{3mo}, T_{6mo} and T_{12mo} to examine continuous variables were performed using the paired Wilcoxon test. p values ≤ 0.05 were considered statistically significant, and all p values were based on two-tailed tests. Statistical analysis was performed using SPSS for Windows (SPSS 29-2022, Sept. SPSS Inc., Chicago, IL, USA). Data were extracted from electronic medical records.

This study was conducted in accordance with the Declaration of Helsinki. According to Italian legislation, the study did not need ethical approval, as it was a purely observational retrospective study on twenty-eight patients with anonymously collected data. Furthermore, it was not possible to request informed consent for participation in the study, given the nature of the study. In any case, consent to completely anonymous use of clinical data for research/epidemiological purposes was requested by the clinical team at the time of admission/diagnostic procedure.

3. Results

In total, 28 Caucasian patients (16 males vs. 12 females; 57.1% vs. 42.9%) affected by CF and diagnosed with CFRD undergoing insulin therapy followed at our regional CF centre were included, with a median age of 33 years (SD 13.26, minimum age 12 years, maximum age 56 years) at the start of ETI therapy. Only one patient was excluded due to the initiation of insulin therapy after beginning ETI treatment. Three pediatric patients (10.7% of the cohort) were included in the study (one patient aged 12, two aged 15). In total, 16 patients presented with genotype F508del with homozygosity and the remaining 12 patients had the genotype F508del with heterozygosity. The type of CFTR modulator therapy used before ETI was lumacaftor/ivacaftor in 12 patients, tezacaftor/ivacaftor in 2 patients; the remaining 14 patients had not taken any modulator therapy prior to ETI. We analyzed the insulin regimen of each patient: 16 patients used only basal insulin, whereas 12 patients followed the basal-bolus scheme. The demographic and clinical characteristics are shown in Table 1.

Table 1. Demographic and clinical characteristics.

Total = 28 Patients		
	Mean ± SD	**Median (min, max)**
Age (years)	33.5 ± 13.26	33.0 (12, 59)
Age at diagnosis CFRD (years)	24.9 ± 9.65	23.66 (9, 44)
Duration of insulin (years)	7.77 (±7.76)	4.67 (0.56, 32.19)
	Frequency (N)	**Percentage (%)**
Sex		
Female	12	42.9
Male	16	57.1
Genotype		
F508del/F508del (homozygous)	16	57.1
F508del/other (heterozygous)	12	42.9

Table 1. *Cont.*

Total = 28 Patients		
	Frequency (N)	**Percentage (%)**
Previous use of CFTR modulators		
None	14	50.0
Lumacaftor/ivacaftor	12	42.9
Tezacaftor/ivacaftor	2	7.1
Insulin regimen		
Once daily basal insulin	16	57.1
Basal-bolus	12	42.9

The main results are shown in detail in Table 2.

The TDD (IU) significantly decreased at T_{3mo} and T_{6mo}, but not at T_{12mo}, whereas HbA1c significantly decreased at all time points. The results of the chloride sweat test (mmol/L) also decreased significantly, as expected. The number of hospitalizations (n) and pulmonary exacerbations significantly decreased over the study period. All pulmonary function parameters improved significantly at 3, 6 and 12 months, particularly the parameter FEV1%. The patients' nutritional status significantly improved over the observational period; the BMI (kg/m^2) increased significantly. The psychological status and quality of life of our patients were assessed via CFQ-R and appeared to improve significantly in the first year of ETI treatment.

The results of the subanalysis based on diverse genotypes are illustrated in detail in the Supplementary Material.

The total daily dose of insulin (IU) in homozygous F508del patients only significantly decreased at T0 vs. T_{6mo} and HbA1c (%) only significantly decreased at T0 vs. T_{3mo}. In heterozygous F508del patients, HbA1c decreased significantly at T0 vs. T_{6mo} and T0 vs. T_{12mo}. The BMI improved significantly in both groups, except for T_{12mo} in the homozygous F508del group. As for pulmonary function, all spirometry parameters significantly increased at 3, 6 and 12 months. Patients with heterozygous F508del showed significantly higher FEV1 (mL) at 3 months and 6 months.

Table 2. Pulmonary function and metabolic changes in patients affected by cystic fibrosis-related diabetes before and after treatment with elexacaftor/tezacaftor/ivacaftor. Pre-T0: 1 year before; T0: treatment initiation; T_{3mo}: 3 months after; T_{6mo}: 6 months after; T_{12mo} 1 year after. FEV1: forced expiratory volume in the first second; FVC forced vital capacity; HBA1c: glycated hemoglobin; CFQR: cystic fibrosis questionnaire revised.

	N	Pre-T0	N	T0	T_{3mo}	T0 vs. T_{3mo}	N	T_{6mo}	T0 vs. T_{6mo}	N	T_{12mo}	T0 vs. T_{12mo}
Hospitalizations (n)	28	0.86 ± 0.89								28	0.32 ± 0.48	**0.003**
Pulmonary exacerbations (n)	28	1.64 ± 1.59								28	0.46 ± 0.79	**0.002**
Six-minute walking test (meters)	19	574.18 ± 68.04								19	572.75 ± 92.99	0.71
Chloride sweat test (mmol/L)	15	118.53 ± 22.21								15	49.93 ± 17.81	**0.001**
Body mass index (kg/m^2)			26	21.72 ± 2.88	22.53 ± 3.11	**0.0001**	26	22.48 ± 3.02	**0.0001**	25	22.47 ± 3.46	**0.006**
FEV1 (liters/1 s)			27	2420.0 ± 1038.4	2782.2 ± 946.6	**0.0001**	27	2774.8 ± 991.7	**0.0001**	25	2771.6 ± 967.1	**0.001**
FVC (liters)			27	3267.4 ± 1043.3	3640.0 ± 899.1	**0.001**	27	3694.8 ± 959.8	**0.0001**	25	3671.5 ± 938.7	**0.001**
FEV1%			27	75.74 ± 29.21	85.44 ± 23.70	**0.001**	27	85.37 ± 25.40	**0.002**	25	85.68 ± 23.12	**0.01**
Total daily insulin dose (UI/kg)			27	0.26 ± 0.22	0.18 ± 0.18	**0.01**	25	0.21 ± 0.20	**0.04**	23	0.21 ± 0.22	0.10
HbA1c (%)			23	6.17 ± 0.63	5.94 ± 0.58	**0.001**	26	5.89 ± 0.59	**0.004**	23	5.94 ± 0.60	**0.006**
CFQ-R (n)			26	64.63 ± 7.59			26	71.74 ± 4.12	**0.0001**	15	70.13 ± 4.84	**0.01**

4. Discussion

To the best of our knowledge, this is the first retrospective observational study to demonstrate both an improvement in glycemic control (by means of HbA1c) and reduction in insulin needs after ETI therapy in a cohort of pediatric and adult insulin-dependent patients with CFRD.

Our results regarding decreased HbA1c values are concordant with the only other study that demonstrates glycemic control improvement in patients with CFRD under ETI treatment [9]. Evidence supporting our findings in patients undergoing CFTR modulator therapy up until now has been contrasting or inconclusive, highlighting only favorable trends in glycemic control in patients with CFRD or impaired glucose intolerance [15,16] or no improvement at all [17]. Volkova et al. suggest that the prevalence of CFRD was lower in patients who received only ivacaftor, therefore underlining the efficacy of the sole use of a single CFTR modulator. This was also noted by Tsabari et al. who examined a cohort that was only administered ivacaftor [15,16]. Our results highlight the innovative amelioration in glycemic control while using the triple combined modulator therapy.

Many studies have demonstrated ETI's superiority compared to other modulators for various CF measures such as FEV1, sweat chloride levels, BMI and pulmonary exacerbations [18,19]. Clear glycemic and metabolic outcomes regarding ETI treatment are less studied and yet to be demonstrated. Scully et al. were among the first to demonstrate the potential improvements in glycemic control following ETI treatment, but chose a study period of 3–11 months for a mixed population (patients with and without CFRD) [7]. These results stand in contrast to those reported by Crow et al. who demonstrated no statistically significant changes in their measures of glycemia and HbA1c, or in basal insulin requirements after 6 months of ETI treatment [20]. This stands in contrast to our study in which both HbA1c and insulin requirements were statistically lower after ETI treatment. The physiopathological mechanism behind the amelioration of glycemic control is still unclear and the explanation is likely to be multifactorial, including better intestinal absorption and nutritional status, decreased inflammatory state, enhanced beta cell function and improved insulin sensitivity [9].

Compared to the baseline, we observed a reduction in insulin requirement at 3 months and 6 months, but not at 12 months of ETI therapy. Sustained reductions in the total daily insulin doses at 12 months may not have been evident due to the patients' increased appetite or improved adherence to CFRD treatment, leading to higher insulin doses per kg. No patients discontinued insulin therapy after initiating ETI treatment. More studies are needed to confirm the potentiated effect of ETI treatment in the first few months that could explain the reduction at the beginning of the treatment. HbA1c levels at all three times were significantly lower than at T0. Further studies and longer periods of observation are necessary to better understand this phenomenon.

The cohort's pulmonary function improved and all parameters assessing clinical and pulmonary improvements, such as spirometry and number of pulmonary exacerbations and hospitalizations, significantly improved in agreement with previously published studies, as well as nutritional status and BMI [21–26].

Because depression and anxiety are frequently diagnosed in patients with CF and associated with a lower quality of life and a lower adherence to airway clearance treatment, we concluded that patients' mental health status is an important outcome. Indeed, mental health appeared to improve significantly in our patients after beginning ETI treatment according to CFQ-R data, in agreement with other studies [27,28].

We subanalyzed our data based on two different genotypes, F508del with homozygosity and with heterozygosity. Pulmonary function improved constantly and significantly in patients who had F508del with homozygosity, while heterozygous F508del patients showed only a minor improvement. These data are likely explained by the fact that patients with heterozygous F508del have a better FEV1 and a smaller margin of improvement in our cohort of patients. The significant increase in BMI our results are in line with other studies in which weight gain appears to be higher in patients with F508del with heterozygos-

ity [29,30]. As for insulin requirements and glycemic control, our results appear contrasting between the two groups, where heterozygous F508del patients did not show any reduction in insulin requirements after one year of treatment, despite a significant reduction in HbA1c values at 6 and 12 months. On the other hand, homozygous F508del patients significantly decreased the amount of insulin required, which was only significant at T_{6mo}, accompanied by a smaller decrease in HbA1c values at T_{3mo}.

This study has some limitations including its retrospective nature and the lack of visits of some patients at 3–6–12 months post-ETI treatment initiation, as well as the small sample size with only a short follow-up period.

5. Conclusions

In conclusion, our study demonstrated that ETI therapy both significantly improved glycemic control and significantly decreased insulin requirement in a cohort of adult and pediatric patients with CFRD. Further studies with a larger population and a longer follow-up period are needed to better understand the trends in insulin requirements in patients with CFRD on ETI treatment and to appropriately adjust insulin therapy during their follow-up. In future studies, the use of continuous glucose monitoring in our patients affected by CFRD would allow us to consolidate our data.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/life14101309/s1; Table S1: Pulmonary function and metabolic changes in patients affected by cystic fibrosis-related diabetes with genotype F508del/F508del before and after treatment with elexacaftor/tezacaftor/ivacaftor. Pre-T0: 1 year before; T0: treatment initiation; T1: 3 months after; T2: 6 months after; T3 1 year after. FEV1: forced expiratory volume in the first second; FVC forced vital capacity; HBA1c: glycated hemoglobin. Table S2: Pulmonary function and metabolic changes in patients affected by cystic fibrosis-related diabetes with genotype F508del/any other CFTR mutation before and after treatment with elexacaftor/tezacaftor/ivacaftor. Pre-T0: 1 year before; T0: treatment initiation; T1: 3 months after; T2: 6 months after; T3 1 year after. FEV1: forced expiratory volume in the first second; FVC forced vital capacity; HBA1c: glycated hemoglobin.

Author Contributions: C.C., F.C., M.B., R.C., N.M. and G.d. were involved in the conceptualization, design, and performance of the study, M.F.S., M.S. and G.S. retrieved data, M.G.C. analyzed the data of the results, M.F.S. and M.B. wrote the first draft of the manuscript, N.M., M.M. and R.C. edited, reviewed, and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: This study was conducted in accordance with the Declaration of Helsinki. According to Italian legislation, the study did not need ethical approval, as it was a purely observational retrospective study on routinely collected anonymous data. Furthermore, it was not possible to request informed consent for participation in the study, given the nature of the study.

Data Availability Statement: The datasets generated during and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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Effect of the Complex Allele p.[Ile148Thr;Ile1023_Val1024del] in Cystic Fibrosis and Tracing of a Founder Effect in Mexican Families

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Abstract: Cystic fibrosis (CF) is a rare autosomal recessive disease most commonly affecting the Caucasian population. CF diagnosis can be a challenge due to the large spectrum of pathogenic variants in the CFTR gene and the effects of complex alleles. Next-generation sequencing has improved our understanding of the contribution of these complex alleles to the wide spectrum of CF clinical symptoms and to the response to medications. Herein, we studied nine CF patients from six unrelated families carrying the complex allele p.[Ile148Thr;Ile1023_Val1024del] with a frequency of 0.18%. All patients were from Central Mexico. This complex allele was found *in trans* with Class I and II pathogenic variants such as p.(Phe508del), and p.(Phe1078Profs*77)]. A targeted search of a dataset of 2217 exomes from healthy individuals revealed that eight individuals (0.18%) carried the p.(Ile148Thr) variant, but only one (0.022%), who was also born in Central Mexico, was a carrier of the complex allele. These findings show an enrichment of this p.[Ile148Thr;Ile1023_Val1024del] complex allele in Mexican CF patients in this region of Mexico. Finally, protein modeling revealed that this complex allele disrupts the secondary structure of the CFTR protein and might alter the ion flow.

Keywords: complex allele; CFTR gene; founder effect; Mexican families; p.(Ile148Thr); I148T; p.(Ile1023_Val1024del); 3199del6

1. Introduction

Cystic fibrosis (CF, OMIM #219700) is a rare autosomal recessive disorder. To date, 105,000 people live with CF in the world. Its incidence varies from 1/900 to 1/25,000 in different populations. In Mexico, its incidence is estimated at 1/8500 live births annually [1–3]. This disease arises from the presence of two pathogenic variants (PVs) *in trans* in the cystic fibrosis transmembrane conductance regulator (CFTR, OMIM #602421) gene, which is located on chromosome 7q31. The CFTR gene contains 27 exons encoding a glycoprotein (CFTR) of 1480 amino acids, composed of two transmembrane domains (TMD1 and TMD2), two cytosolic nucleotide-binding domains (NBD1 and NBD2), and a phosphorylation-dependent regulatory domain (R) [3]. CFTR dysfunction results in disrupted epithelial homeostasis of water, chloride, sodium, and bicarbonate across various organs and systems [4]. As a result of this, patients with CF present a variable clinical spectrum in which lung damage is the main cause of morbidity and mortality, due to the accumulation of abnormally thick and sticky mucus, leading to recurrent infections from *Sthapylococcus aureus* and *Pseudomonas aeruginosa* bacteria. Additionally, CF patients can present with pancreatic insufficiency (PI), malnutrition, meconium ileus (10–20%), and male infertility due to congenital bilateral absence of the vas deferens (CBAVD) [5]. The life expectancy of CF patients in developed countries is 50 years, while in Mexico it is 21.37 years [6].

In high-income countries, such as the USA, France, Italy, the UK, and Australia, newborn screening (NBS) has been quite successful for CF presymptomatic detection and diagnosis. For example, in populations with Northwestern European ancestry, NBS prognostic values are from 10 to 30% [7]. Despite this, in developing countries, CF diagnosis is traditionally established based on the family's clinical history and the classic characteristics of the disease, such as the presence of chronic obstructive pulmonary disease, IP, elevated sweat chloride $\geq$ 60 mmol/L, and molecular confirmation with the presence of 2 PVs in the CFTR gene [8]. In this sense, more than 2000 variants distributed throughout the CFTR gene have been reported, of which 1085 are PV or disease-causing, and 55 variants with variable clinical consequences (http://www.cftr2.org, accessed on 5 September 2024) [9]. The most common PV is p.(Phe508del) (F508del, c.1521_1523del, rs121909001), which is a deletion of the amino acid phenylalanine at position 508 of the protein. The frequency of this variant is similar in populations from the USA, Canada, and Australia since they share an origin with Northwestern European populations [7]. In fact, it is present in 100% of CF patients from the Faroe Islands of Denmark, but in other European countries, such as Italy, its frequency is 67.9%. In American populations, its frequency varies from 25 to 85.8% [7]. The particular genetic admixture of Latino populations has given rise to a wide spectrum of PVs, some of which are exclusive to some countries or even to some families, and Mexico is no exception, with a reported 95 PVs in patients with CF to date [2,6].

Usually, molecular diagnosis of CF has focused on identifying directly the 2 PVs inherited from each parent. However, with advances in next-generation sequencing (NGS) providing complete CFTR sequences, it is now possible to analyze additional variants in the gene, such as variants in the same allele (*in cis*) that can form complex alleles [10]. The study of these complex alleles will allow a deeper understanding of the disease and its broad spectrum of clinical manifestations.

Previous reports have shown that the combination of multiple variants, such as complex alleles, could worsen or mitigate the patient's clinical condition and potentially influence the structure and function of the CFTR protein, leading to differential responses to CFTR modulator treatment. An example of this was reported in 2020 by Chevalier's group with the p.[Arg74Trp;Val201Met;Asp1270Asn] (R74W;V201M;D1270N) (Table S1) complex allele. This allele produces a protein with a partial loss of exon 3 with a reduced function. A single (p.Asp1270Asn) variant, when present with another PV in a compound heterozygous state, is not enough to cause CFTR channel dysfunction. However, individuals with the p.[Arg74Trp;Asp1270Asn] combination in the same allele exhibit a minor functional defect, whereas p.[Arg74Trp;Val201Met;Asp1270Asn] (Table S1) leads to mild CF [10]. Similarly, the p.Leu997Phe (L997F) PV could be correlated with CFTR-related disorders (CFTR-RD), but

when it is presented as a complex allele with the variant p.(Arg117Leu) (R117L) (Table S1), this combination could produce a mild CF phenotype [11].

On another hand, the effect of *in cis* variants with p.Phe508del has also been reported. The p.[L467F;F508del] complex allele leads to a dysfunction of the CFTR protein with negative effects on the response to lumacaftor. Moreover, patients carrying the p.Phe508del/p.[Phe508del;Phe87Leu;Ile1027Thr] allele show no response to treatment with ivacaftor + lumacaftor; therefore, the triple combination (ivacaftor/tezacaftor/elexacaftor) is recommended [12]. Another variant studied was p.Ala238Val, *in cis* with p.Phe508del, which has been observed to be associated with more severe lung damage and resistance to targeted therapy in CF patients carrying these alleles compared to patients who only carry p.Phe508del [13].

In this study, we investigated the impact of the p.[Ile148Thr;Ile1023_Val1024del] (I148T;3199del6) (Table S1) complex allele, present in nine CF patients. Because these patients are originally from the central region of Mexico and considering the historical background of the migratory flow of the population, we suggest a possible founder effect of this complex allele. For the first time, we reported two homozygous patients carrying this complex allele, and we modeled the encoded protein to better understand the structural dysfunction that it causes.

2. Materials and Methods

We included nine Mexican patients with positive sweat chloride tests (>60 mmol/L) from six unrelated families. DNA extraction was whole venous blood with the QIAmp DNA Blood Maxi kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Previously, these patients were sequenced using Multiplicon CFTR Master DX (Agilent, Santa Clara, CA, USA) on the MiSeq system (Illumina, Inc., San Diego, CA, USA), which amplified all exons plus 30 bp of their 5′ and 3′ flanking sequences and UTRs regions. The analysis of CFTR sequencing was done using the MASTR Reporter software v.1.2.1 (Agilent, USA). All variants of the complex allele were validated in the patients through automated Sanger sequencing using the Big Dye Terminator (Applied Biosystems TM, Foster City, CA, USA), and the identification of the carrier status was screened in all their relatives by the same method. This study was conducted in accordance with the Helsinki Declaration and approved by the Ethics and Research Committees of the National Institute for Genomic Medicine (INMEGEN CEI 2015/10). Written informed consent and assent were obtained from all participating families.

The clinical characteristics of the patients were collected from their medical histories and the CF database of our laboratory. The birthplace of families was established through interviews, and family trees were constructed using the Progeny free online pedigree program (https://pedigree.progenygenetics.com (accessed on 10 June 2024)) [14].

The complex allele frequencies were obtained by searches in three different databases (CFTR [CFTR-France (https://cftr.iurc.montp.inserm.fr/cftr/ (accessed on 10 July 2024))] [15], gnomAD [https://gnomad.broadinstitute.org/ (accessed on 10 July 2024)] [16], and CFTR2 [http://www.cftr2.org/ (accessed on 10 July 2024)] [9] and from a dataset in our laboratory comprising 2217 exomes from unrelated adults (Mestizos and Mexican Amerindians) without a history of Mendelian diseases and recruited throughout the Mexican territory [17,18].

We used *in silico* methods to assess the impact of each variant and the complex allele on the CFTR protein. SWISS-MODEL (https://swissmodel.expasy.org/ (accessed on 10 July 2024)) [19] and I-TASSER (https://zhanggroup.org/I-TASSER/ (accessed on 10 July 2024)) [20] were employed to predict the structural effects of the complex allele on the CFTR protein. SIFT-Indel (https://sift.bii.a-star.edu.sg/ (accessed on 10 July 2024)) [21] and Mutation Taster (https://www.mutationtaster.org/ (accessed on 10 July 2024)) [22] were used to assess the functional consequences of p.(Ile1023_Val1024del) and p.(Ile148Thr). DynaMut (https://biosig.lab.uq.edu.au/dynamut/ (accessed on 10 July 2024)) [23] allowed us to analyze changes in protein stability and disruptions in interatomic interactions caused by both individual variants and the complex allele. Protein stability was assessed through ΔΔG estimation, which represents the difference in ΔG between the wild-type and

mutant proteins. The CFTR models were uploaded into PyMOL (https://www.pymol.org/ (accessed on 10 July 2024)) [24], where the align command was used to superimpose the mutant structures onto the wild-type structure (PDB ID: 5UAK). The domains of interest [NBD1, NBD2, R] along with residues 148, 1023, and 1024 of CFTR, were highlighted based on curated domain information from Uniprot and Swiss-Prot.

3. Results

A total of nine CF patients from six unrelated families carrying the complex allele p.[Ile148Thr;Ile1023_Val1024del] were studied. In all families, this complex allele was found *in trans* with the Class I and II PVs [p.(Phe508del) and, p.(Phe1078Profs*77)] (Figure 1a–c). In one family, the second PV could not be identified, possibly due to a deep intronic variant (Figure 1d). Families 5 and 6, declared as nonconsanguineous, carried the complex allele in a homozygous state (Figure 1e,f).

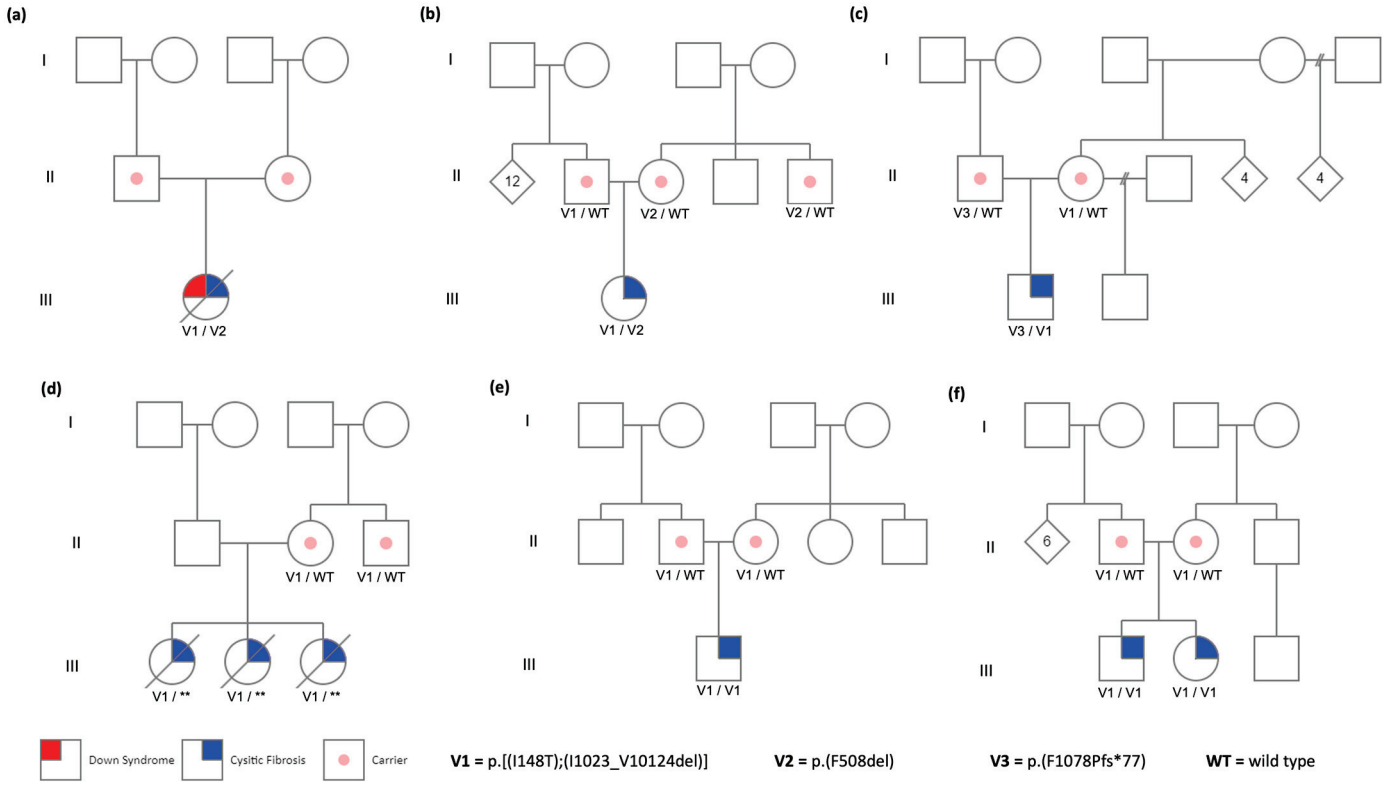


Figure 1. Three-generation pedigree of the six studied families from Central Mexico: (**a**) Family 1, (**b**) Family 2, (**c**) Family 3, (**d**) Family 4, (**e**) Family 5, and (**f**) Family 6. ** depicts that the second affected allele was not detected.

All patients presented severe clinical symptoms characterized by pancreatic insufficiency (PI) and elevated sweat chloride levels (≥85 mEq/L), with diagnosis at birth or less than 4 years of age. Clinical history revealed that all patients' ancestors were born in Mexico and the Guanajuato State (Central Mexico) (Figure 2).

To further investigate the frequency of variants forming the complex allele, we performed a targeted search in our laboratory dataset containing exome sequences from 2217 individuals. This revealed eight individuals (0.18%) carrying the p.(Ile148Thr) variant, but only one (0.022%), who was also born in Central Mexico (the San Luis Potosi state), carrying the complex allele (Figure 2).



Figure 2. Geographical distribution of the six unrelated families carrying the CFTR complex allele in the Mexican territory. Families are distributed in states from the central region of Mexico depicted by the red box: Guanajuato (green), San Luis Potosí (yellow), and the State of Mexico (blue).

To better our understanding of the structural impact of each variant and the complex allele on the CFTR protein, we performed an *in silico* analysis (Figure 3). Modeling of the p.(Ile148Thr) variant displayed a destabilized protein, with a predicted stability change (ΔΔG Stability wt to mut = −2.05 kcal/mol). MutationTaster also classified it as disease-causing (probability: 99.9%). Protein modeling of this variant revealed a significant structural disruption in CFTR (Figure 3b). The Ile148 is located at the junction between the second and third helixes of TMD1 near the interface between the membrane and the cytoplasm. The substitution of this isoleucine with threonine (148Thr) alters the interaction of helixes 1 and 2, and the cytoplasmic loops 1 and 2 in TMD1 in the protein. Additionally, the change to 148Thr induces changes in the cytoplasmic side of the protein compatible with a narrowing of the cytoplasmic side of channel pore (Figure 3b).

The SIFT-INDEL tool predicted a confidence score of 0.858 for the p.(Ile1023_Val1024del) variant, indicating a damaging effect. Additionally, MutationTaster classified this variant as disease-causing with a 71% probability. Furthermore, protein modeling revealed that this variant disrupts the secondary structure of CFTR. Specifically, it affects helix 10 of TMD2, which is crucial for its stabilization, configuration, opening and closing of the channel pore. This deletion also disrupts the helical structure of the helix 10, potentially destabilizing the entire transmembrane domain. The structural analysis also predicted a misconfiguration of the protein in the NBD1 and NBD2 domains (Figure 3c).

Finally, when both variants were modeled together, as a complex allele, the resulting protein conformation exhibited more pronounced changes, including a narrowing cytoplasmic face of the channel pore, rearrangement of the NBD1, and NBD2 domains, as well as the loss of part of the helix 10. In consequence, the combination of p.(Ile148Thr) and p.(Ile1023_Val1024del) variants might alter the ion flow (Figure 3d).

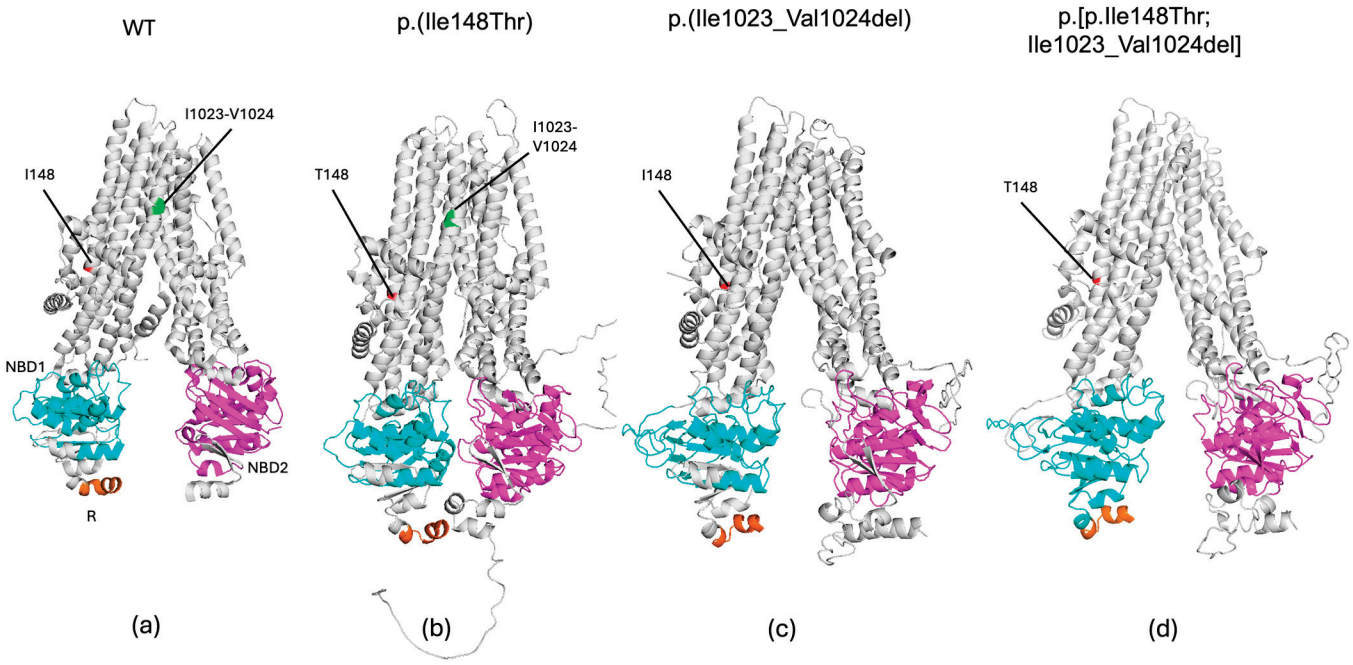


Figure 3. 3D structural models of the variants that form the complex allele, alone or in combination. TMD1 and TMD2 domains are depicted in gray, NBD1 and NBD2 domains are in blue and pink, respectively, and the R domain is indicated in orange. (**a**) Wild-type CFTR structure (5uak.pdb) with the position of the variants analyzed: p.(Ile148Thr) (I148T) in red and p.(Ile 1023_Val1024del) (3199del6) in green. Modeling of the resulting protein carrying the (**b**) p.(Ile148Thr) variant, (**c**) p.(Ile 1023_Val1024del), and (**d**) p.[p.Ile148Thr;Ile1023_Val1024del] complex allele.

4. Discussion

Traditionally, CF diagnosis has relied on identifying two PVs *in trans*, one inherited from each parent. Advances in sequencing technologies have enabled the detection of rare CFTR variants, including complex alleles. First described in 1991, complex CFTR alleles are characterized by the presence of two or more variants *in cis*, creating a significant challenge for CF diagnosis [4,10]. Such alleles may have significant implications for CFTR function and the clinical manifestation of the disease [25].

Previously, we screened 297 CF patients across Mexico [6]. Notably, the complex allele p.[Ile148Thr;Ile1023_Val1024del] was identified in nine patients from six different families, all of them born in the central region of Mexico. Here, we report for the first time two CF patients homozygous for that complex allele. All nine patients exhibited severe clinical symptoms.

Notably, p.(Ile1023_Val1024del) has been reported as a CF-causing variant, while p.(Ile148Thr) has been classified as a neutral, common CFTR variant that does not affect the pathogenicity of additional variants in complex alleles [26]. The classification of these variants is in line with the CFTR (http://www.cftr2.org/ (accessed on 10 July 2024) and https://cftr.iurc.montp.inserm.fr (accessed on 10 July 2024)) databases [9,15].

In this study, we documented the structural impact of a complex allele and each independent variant on the CFTR protein using an *in silico* analysis. The protein conformation derived from the complex allele showed more severe changes compared with the conformation associated with each independent variant. The p.(Ile1023_Val1024del) variant, which involves a deletion of the ATAGTG sequence in CFTR exon 19, results in the removal of isoleucine and valine at residues 1023 and 1024 in the TMD2 domain [27,28]. In addition, protein modeling revealed that this variant disrupts the secondary structure of CFTR, particularly affecting helix 10 of TMD2, which is essential for its stabilization. This analysis also showed misfolding affecting the NBD2 domain and the transmembrane domain connecting TMD2 and NBD2, suggesting that channel ion flow may be reduced.

Otherwise, modeling of the p.(Ile148Thr) variant indicates that it destabilizes the CFTR protein (ΔΔG Stability = −2.05 kcal/mol). Ile148 is located at the junction of the first and second helixes of TMD1 near the interface between the membrane and the cytoplasm. The substitution of Ile by Thr may significantly impact the protein's structure, stability, and interactions due to the significant differences in hydrophobicity and polarity between these two amino acids. In fact, we found that protein modeling with this amino acid substitution had a significant effect on the configuration of the TMD1 and NBD1 domains of CFTR. Furthermore, the MutationTaster tool classified this variant as disease-causing with a 99.9% probability. Supporting this, a previous functional study showed that nasal epithelial cells (NEC) from individuals carrying p.(Ile148Thr) exhibit a CFTR gating activity of 86–87.4% and induce in patients a variable spectrum of clinical characteristics with null or mild effects, displaying mild channel impairment [26]. Perhaps this dysfunction is not enough to cause CF manifestations, although it has been reported that in some patients, this variant *in trans* with a PV may result in CF-like symptoms [29] or result in a diagnosis of a CFTR-related disorder [27].

Finally, our modeling suggests that the p.(Ile148Thr) and p.[Ile1023_Val1024del] variants *in cis* synergistically affect protein conformation, narrowing the channel and thus altering the ion flow even more, as previously documented in individuals carrying the complex allele along with other Class I and II variants, who displayed a CFTR gating activity on NEC of 7.3% [26,29].

A growing body of evidence has demonstrated that patients carrying complex alleles could have altered CFTR biogenesis, inducing a variable spectrum of clinical characteristics which makes diagnosis more complicated. Furthermore, the presence of these alleles could induce a different response to the target drugs, such as elexacaftor/ivacaftor/tezacaftor, highlighting the importance of performing the CFTR-NGS in all CF patients for accurate diagnosis and for personalizing therapeutic strategies to improve patient outcomes [4,30].

To estimate the frequency of the complex allele in Mexico, we searched our laboratory's exome-sequencing dataset for carriers of the complex allele, as well as the individual variants p.(Ile148Thr) and p.(Ile1023_Val1024del). We identified no carriers of the p.(Ile1023_Val1024del), eight carriers of the p.(Ile148Thr), and the complex allele in only one individual, also of central Mexican origin, suggesting a possible enrichment of this variant in the central region of Mexico. The CFTR-France database shows that 23.53% of subjects carrying p.(IleI148Thr) are also carriers of the 3199del6 variant as a complex allele [15]. Moreover, it has been reported that this allele is present in 2.2% of CF patients from Quebec City, Canada [28]. In our patients, the p.[Ile148Thr;Ile1023_Val1024del] allele was present at a frequency of 0.18%. Notably, this complex allele has not been reported in other populations and is absent from the CFTR2 database, highlighting the importance of these results. Historical records indicate that French-Canadian immigrants settled in Mexico in the late 19th century, mainly in the cities of Central Mexico, driven by the growth of industries such as electrical infrastructure, mining, and railways [31,32]. This context, coupled with the geographic origin of the complex allele carriers, allows us to suggest that this allele may be of French origin and was introduced to America through the French-Canadian population, who much later brought it into the population of Central Mexico, supporting our hypothesis of a possible founder effect; however, studies of genetic markers are necessary to confirm this hypothesis.

5. Conclusions

Our study provides evidence that the p.[Ile148Thr;Ile1023_Val1024del] complex allele is enriched in the central region of Mexico, suggesting a possible founder effect in Mexican patients with CF. It also shows that the p.(Ile148Thr) variant has a functional effect, highlighting the importance of monitoring the clinical outcomes of patients carrying this variant to fully understand its implications. Additionally, our findings support the integration of NGS into CF diagnostics to ensure the identification of all CF alleles, including complex alleles such as p.[Ile148Thr;Ile1023_Val1024del], which are associated with severe

clinical manifestations, including early mortality. NGS diagnosis is crucial for developing personalized treatment strategies and improving patient quality of life.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life14111445/s1, Table S1: Nomenclature of the CFTR variants mentioned in the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

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Prevalence and Characteristics of Patients with Cystic Fibrosis-Related Diabetes in Croatia

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Abstract: This study investigated the prevalence of cystic fibrosis-related diabetes (CFRD) in the Croatian cystic fibrosis (CF) population, the age at diagnosis, insulin requirements, and the relationship between age at diagnosis and other clinical parameters. Medical records from 152 patients with genetically and laboratory-confirmed CF were reviewed through to 2025. The American Diabetes Association criteria were used to diagnose CFRD. Anthropometric and clinical data were collected from the latest medical records. A total of 17 out of 152 patients had CFRD, with a prevalence of 4.8% in the paediatric population (4/84) and 19.1% in adults (13/68). The median age of CFRD diagnosis was 14 years (range 9–22 years, SD = 3.95). Thirteen patients used insulin: one used bolus only, seven used basal-bolus multiple daily injections, and five used insulin pumps. The average total daily insulin (TDI) per kilogram (kg) body weight was 0.447 U/kg (SD = 0.429). The age at CFRD diagnosis was positively correlated with the body mass index (BMI) (p = 0.029). Patients requiring insulin by age 15 had higher TDI and were more likely to have CF liver disease (p = 0.027, p = 0.037, respectively). The prevalence of CFRD and age at diagnosis aligned with previous studies. Patients diagnosed at a younger age and requiring insulin earlier had lower BMIs, likely due to a faster decline in beta cell function and earlier onset of insulinopenia.

Keywords: cystic fibrosis; cystic fibrosis-related diabetes; prevalence; insulin requirements

1. Introduction

Cystic fibrosis (CF) is the most prevalent autosomal recessive genetic condition in patients of European descent and is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. CFTR dysfunction impairs the transport of chloride and bicarbonate in epithelial cells, leading to thick and viscous secretions in multiple organs including the lungs, pancreas, intestine, and liver [2]. Progressive respiratory impairment is the leading cause of morbidity and early mortality in patients with CF [3]. In the past few decades, advances in CF treatment—especially the introduction of CFTR modulators that partially restore CFTR function—have significantly increased the life expectancy of patients with CF. With this increased life expectancy, important non-pulmonary complications have emerged including cystic fibrosis-related diabetes (CFRD) [4].

The pathophysiology of CFRD is complex and not completely understood. CFRD shares some characteristics with both type 1 and type 2 diabetes but is a unique form of diabetes [5]. The primary cause is insulin deficiency, which is due to the dysfunction and destruction of pancreatic beta cells. However, unlike type 1 diabetes, there is no underlying autoimmune process. Instead, CFTR dysfunction causes thick, sticky secretions to accumulate in the pancreatic ducts, leading to exocrine pancreatic damage, inflammation, and fibrosis. This, in turn, damages the neighbouring pancreatic islets. In patients with CF, beta cells may also be directly affected by CFTR dysfunction. Since CFTR is expressed in beta cells, its dysfunction can impair exocytosis and glucose-stimulated insulin secretion. Thus, patients with CF exhibit a delay in first-phase insulin secretion, likely due to direct CFTR effects, and as ductal fibrosis progresses, a decrease in total insulin secretion. Like type 2 diabetes, there is also a degree of insulin resistance, driven by systemic inflammation, which periodically worsens during acute infections [6]. Hepatic insulin resistance is also seen in both CFRD and type 2 diabetes, and patients with CFRD have been shown to have an increased hepatic glucose production and a reduced suppression of hepatic glucose output by insulin [7]. Insulin resistance is further aggravated by glucocorticoids, which are sometimes used in acute lung exacerbations [8]. CFTR dysfunction may also make beta cells more susceptible to oxidative stress, resulting in impaired insulin secretion and beta cell apoptosis. Additional contributing factors include genetic predisposition related to type 2 diabetes and abnormalities in the incretin axis. Some research has suggested that patients with CFRD exhibit lower levels of glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) than those without diabetes and control groups [9], which may contribute to the postprandial hyperglycaemic excursions often seen in early CFRD [6].

Patients who develop CFRD have worse clinical outcomes including pulmonary and nutritional decline, increased pulmonary exacerbations, and increased mortality [8,10]. Nutritional decline is likely due to insulin deficiency-induced catabolism [11], while the increased susceptibility to infections can be explained by hyperglycaemia. Patients with CFRD have increased glucose levels on their airway surfaces, which can promote bacterial overgrowth and less effective inflammatory responses [12,13]. Thus, because CFRD is associated with worse clinical outcomes and its insidious onset, the American Diabetes Association (ADA) recommends annual CFRD screening with an oral glucose tolerance test (OGTT) in all patients with CF aged $\geq$10 years [14]. Screening is also recommended because timely treatment with insulin, currently the only approved therapy, improves respiratory function, body mass index (BMI), and survival for patients with CF [15–17].

Screening and early intervention have started to narrow the survival gap between patients with CFRD and those without it, but the risk factors of CFRD and optimal treatment strategies need more investigation [4]. Although the prevalence of CFRD has been reported in various European countries [18], most epidemiological studies were conducted before the widespread use of CFTR modulators, which may affect the onset of CFRD. Furthermore, the prevalence of CFRD in the Croatian CF population has not been reported. Thus, the primary aim of this study was to determine the prevalence of CFRD in the Croatian CF population, the age at CFRD diagnosis, insulin requirements to manage CFRD (total daily insulin/kg), and the insulin regimen used (basal only, prandial only, basal-bolus multiple daily injections, insulin pump therapy). Secondary aims were to explore the relationships between the age at CFRD diagnosis, the age of insulin introduction, and other clinical parameters.

2. Patients and Methods

This retrospective study was conducted at the Croatian State Referral Centre for Cystic Fibrosis in Children and Adults, which is accredited by the Ministry of Health and located at the University Hospital Centre Zagreb. This centre is the country's primary treatment facility for CF, which cares for 98% of the Croatian CF population. Patients at this centre are routinely seen at quarterly intervals and are managed by a multidisciplinary team including a pulmonologist, gastroenterologist, endocrinologist/diabetologist, and nutritionist.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the University Hospital Centre Zagreb (8.1-23/247-4) on 29 February 2024. All patients provided written informed consent for the entry of their data into the European CF registry, which was also used in this study.

Medical data from 152 patients with genetically and laboratory-confirmed CF were reviewed from 2019 to 2025. CFRD was diagnosed using the American Diabetes Association (ADA) criteria. CFRD was defined as fasting plasma glucose $\geq$ 7.0 mmol/L and/or 2 h glucose $\geq$ 11.1 mmol/L, and/or glycated haemoglobin (HbA1c) $\geq$ 6.5% [14]. Patients who fulfilled the ADA criteria for CFRD were classified as cases regardless of their insulin requirements at their last hospital visit. Patients with type 1 diabetes were excluded (positive autoantibodies).

Clinical and anthropometric measurements were recorded from the most recent medical records. Anthropometric measurements included weight, height, and BMI. Clinical data included age, sex, CFTR genotype, insulin requirements (total basal insulin, total bolus insulin, total daily insulin, and total daily insulin/kg) and regimen used (basal only, prandial insulin only, multiple daily injections, insulin pump), type and duration of CFTR modulator therapy, presence of pancreatic insufficiency (defined as use of pancreatic enzymes), presence of CF liver disease (CFLD), forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and whether they were lung transplant recipients. These categories were used to assess whether they were a risk factor for CFRD. Data on insulin doses (both basal and bolus) were collected from the latest diabetologist's report. For individuals using an insulin pump, the exact basal and bolus dosage data were extracted from the most recent 30-day report. For those on basal-bolus multiple daily injections, bolus dose estimates were based on anamnestic data from the most recent diabetologist visit including insulin-to-carb ratios and basal requirements. Patients who underwent lung transplants were excluded from the FEV1 analysis. The most recent glycated haemoglobin (HbA1c) levels were recorded.

The clinical characteristics of patients with CFRD, prevalence of CFRD, age of CFRD diagnosis, and insulin requirements were assessed using descriptive statistics. The Spearman's rank correlation coefficient was used to assess the relationships between age at CFRD diagnosis, age of insulin initiation, BMI, and HbA1c. The Mann–Whitney U test was used to assess the differences in insulin doses between patients who started insulin before and after age 15 as well as differences in the HbA1c cut-offs and insulin doses. The Fisher's exact test was used to assess the correlation between age of insulin introduction and the age of CFRD diagnosis—before and after 15 years—and the presence of CFLD. Values of $p < 0.05$ were considered statistically significant. Medcalc (version 23.1.6) was used for statistical analysis.

3. Results

3.1. Prevalence of CFRD Within the CF Population and Age at Diagnosis

Medical records from 152 patients (68 adult, 84 paediatric) with CF were reviewed. A total of 17 out of 152 patients (11.2%) were identified with CFRD. Of the 17 patients with CFRD, the majority (76%) were $\geq$18 years. The prevalence of CFRD was 4.8% (4/84, 95%

CI 1.313–11.746) in the paediatric CF population and 19.1% (13/68; 95% CI 10.591–30.470) in the adult CF population. The median age of CFRD diagnosis was 14.4 years (range 9–22 years, SD = 3.95, 95% CI 12.369–16.431), with the majority diagnosed between 9 and 15 years. More than half of the patients (53%, 95% CI 27.812–77.017) were diagnosed with CFRD by age 14, and 71% (95% CI 44.042–89.686) had their diagnosis by age 15 (Table 1).

Table 1. Frequencies of age at diagnosis of cystic fibrosis-related diabetes.

Age at CFRD Diagnosis	Counts	% of Total	Cumulative %
9	2	11.8%	11.8%
10	2	11.8%	23.5%
12	1	5.9%	29.4%
13	3	17.6%	47.1%
14	1	5.9%	52.9%
15	3	17.6%	70.6%
18	3	17.6%	88.2%
21	1	5.9%	94.1%
22	1	5.9%	100.0%

CFRD—cystic fibrosis-related diabetes.

3.2. Characteristics of Patients with CFRD

The characteristics of patients with CFRD are presented in Table 2 (continuous variables) and Table 3 (categorical variables). The median BMI of patients with CFRD was 23.0 kg/m^2 (range 13.5–30.5 kg/m^2, SD = 3.73), and the median age was 24 years (range 14–38 years, SD 6.64 years). Six patients (35.3%) had CFLD. All patients were using pancreatic enzymes for their pancreatic insufficiency, and four patients (23.5%) were using enteral nutrition. One patient was a transplant recipient and being treated with immunosuppressive therapy and corticosteroids. The majority of patients were treated with CFTR modulator therapy with an average of 36.6 months (SD = 4.27). At the last visit, only one patient was not receiving CFTR modulator therapy (transplant recipient). Only one patient was using CFTR modulator therapy at the time of CFRD diagnosis. The average FEV1 and FVC were 71.9% (SD = 19.8) and 85.4% (SD = 16.8), respectively. The average HbA1c was 6.45% (SD = 1.09). Thirteen out of seventeen patients (76.5%; 95% CI 50.101–93.189) had well-controlled diabetes (HbA1c < 7%). In terms of diabetic microvascular complications, only nine patients had a retinal screening appointment at least once, and no patients had evidence of retinopathy. As for albuminuria, only four patients were screened, two of whom had A2 albuminuria.

3.3. Insulin Regimens Used to Manage CFRD

The majority of patients with CFRD (76.4%; 13/17) were treated with insulin, while the remainder were managed conservatively with nutrition therapy and physical activity. No patients used non-insulin diabetes medications. Interestingly, 4 out of 17 patients were not using insulin at their last visit, despite previously requiring basal-bolus insulin therapy to manage their CFRD. In terms of the insulin regimen employed, one patient was using bolus insulin only, 41.2% (7/14 patients) were using basal-bolus multiple daily injections, and 29.4% (5/13) were using insulin pumps. The average total daily insulin/kilogram of body weight (TDI/kg) to treat diabetes was 0.854 U/kg (SD = 0.398), while the average TDI/kg in children was 0.817 (SD = 0.550) and 0.514 (SD = 0.347) in adults. When analysing the TDI/kg in all patients, including those not on insulin (assigned a value of '0'), the average TDI/kg was 0.447/kg (SD = 0.429).

Table 2. Characteristics of patients with cystic fibrosis-related diabetes continuous variables (N = 17).

	Mean	Median	Standard Deviation	Minimum	Maximum	95% Confidence Interval
Age	24.2	24	6.64	14	38	20.786–27.614
Height (cm)	165	161	10.7	147	182	159.5–170.5
Weight (kg)	62.7	63	14.7	35.3	95.4	55.142–70.258
BMI (kg/m^2)	22.9	23	3.73	13.5	30.5	20.982–24.818
Age at CFRD diagnosis	14.4	14	3.95	9	22	12.369–16.431
Duration of diabetes	9.82	9	6.15	2	23	6.658–12.982
Age at insulin introduction	14.6	14	4.05	9	22	12.518–16.682
Basal insulin (U)	18.6	10	13.5	0.0	44.0	11.659–25.541
Insulin bolus (U)	16.3	12	12.4	0.0	50.0	9.925–22.675
TDI	34.2	26	23.2	6.00	86.0	22.272–46.128
TDI/kg	0.447	0.327	0.429	0.00	1.38	0.226–0.668
HbA1c%	6.45	6.40	1.09	4.70	9.30	5.890–7.010
FEV1%	71.9	73.5	19.8	38	107	62.488–81.312
FVC%	85.4	88.5	16.8	52	113	76.762–94.038
Duration of modulatory therapy (months)	36.6	35.5	4.27	32	48	34.405–38.795

BMI—body mass index, CFRD—cystic fibrosis-related diabetes, TDI—total daily insulin, HbA1c—glycated haemoglobin, FEV1—forced expiratory volume in 1 s, FVC—forced vital capacity.

Table 3. Characteristics of patients with cystic fibrosis-related diabetes categorical variables (N = 17).

		F (%)	95% Confidence Interval/%
Sex	Male	6 (35.3)	14.210–61.672
	Female	11 (64.7)	38.328–85.790
Genotype	F508 del homozygous	14 (82.4)	56.568–96.201
	F508 del heterozygous	2 (11.8)	1.458–36.441
	Other	1 (5.9)	0.149–28.689
Patients treated with insulin	No	4 (23.5)	6.811–49.899
	Yes	13 (76.5)	50.101–93.189
Insulin regimen	No insulin	4 (23.5)	6.811–49.899
	Bolus only	1 (5.9)	0.149–28.689
	Basal only	0 (0.0)	0.000–19.506
	Basal-bolus multiple daily injections	7 (41.2)	18.444–67.075
	Insulin pump	5 (29.4)	10.314–55.958
Transplant recipient	No	16 (94.1)	71.311–99.851
	Yes	1 (5.9)	0.149–28.689
Pancreatic exocrine deficiency	No	0 (0.0)	0.000–19.506
	Yes	17 (100)	80.494–100.000
Cystic Fibrosis-Related Liver Disease	No	13 (76.5)	50.101–93.189
	Yes	4 (23.5)	6.811–49.899
Treatment with modulator therapy	Ivacafor, tezacaftor, elexacaftor + ivacaftor	15 (88.2)	63.559–98.542
	Ivacafor, tezacaftor, elexacaftor	1 (5.9)	0.149–28.689
	Ivacaftor	0 (0.0)	0.000–19.506
	None	1 (5.9)	0.149–28.689
Enteral nutrition	No	4 (23.5)	6.811–49.899
	Yes	13 (76.5)	50.101–93.189
Corticosteroids or immunosuppressive therapy	No	16 (94.1)	71.311–99.851
	Yes	1 (5.9)	0.149–28.689

3.4. Associations Between Various Clinical Parameters in Patients with CFRD

The association between various clinical parameters in patients with CFRD are shown in Table 4. The age at CFRD diagnosis and age at insulin introduction were positively correlated with BMI (Spearman's rank correlation: p = 0.029, p = 0.025, respectively). The total daily insulin, total daily insulin/kg, and total bolus doses were positively correlated with HbA1c (Spearman's rank correlation: p = 0.027, p = 0.033, p = 0.029, respectively).

When comparing groups based on age at insulin introduction—before and after age 15—using the Mann–Whitney U test, those who required insulin before age 15 needed a higher TDI/kg (p = 0.027), higher total daily insulin (p = 0.027), and higher basal insulin (p = 0.006). However, bolus dose requirements were not statistically significant between the groups (p = 0.119). The Fisher's exact test was used to assess the correlation between age of insulin introduction—before and after 15 years—and the presence of CFLD (the outcomes were 0 or 1 for the presence of CFLD), and an association was found (p = 0.037), with those having insulin introduced before age 15, who were more likely to have CFLD. Age at CFRD

diagnosis and presence of CFLD were not statistically different between the two groups ($p = 0.075$).

When comparing groups based on an HbA1c cut-off of 6%, using the Mann–Whitney U test, there was an association between TDI, TDI/kg, and total bolus insulin ($p = 0.022$, $p = 0.036$, $p = 0.013$, respectively), with those having an HbA1c > 6% requiring higher insulin doses.

Table 4. Correlation between age of cystic fibrosis-related diabetes diagnosis, age of insulin introduction, and clinical parameters.

Parameter I	**Parameter II**	**Spearman's Rank Correlation Coefficient**	
Age of CFRD diagnosis	BMI	$p = 0.029$	
Age of insulin introduction	BMI	$p = 0.025$	
Duration of diabetes	BMI	n.s.	
TDI	HbA1c	$p = 0.027$	
TDI/kg	HbA1c	$p = 0.033$	
Basal insulin-dose	HbA1c	n.s.	
Bolus insulin-dose	HbA1c	$p = 0.029$	
Group I	**Group II**	**Parameter**	**Mann–Whitney U Test**
HbA1c ≤ 6%	HbA1c ≥ 6.1%	TDI	$p = 0.022$
HbA1c ≤ 6%	HbA1c ≥ 6.1%	TDI/kg	$p = 0.036$
HbA1c ≤ 6%	HbA1c ≥ 6.1%	bolus insulin-dose	$p = 0.013$
HbA1c ≤ 6%	HbA1c ≥ 6.1%	Basal insulin-dose	n.s.
Age at insulin introduction ≤15 years	Age at insulin introduction ≥15.1 years	TDI	$p = 0.027$
Age at insulin introduction ≤15 years	Age at insulin introduction ≥15.1 years	TDI/kg	$p = 0.027$
Age at insulin introduction ≤15 years	Age at insulin introduction ≥15.1 years	Basal insulin-dose	$p = 0.006$
Age at insulin introduction ≤15 years	Age at insulin introduction ≥15.1 years	Bolus insulin-dose	n.s.
Group I	**Group II**	**Parameter**	**Fisher's Exact Test**
Age at insulin introduction ≤15 years	Age at insulin introduction ≥15.1 years	CFLD	$p = 0.037$
Age of CFRD diagnosis ≤15	Age of CFRD diagnosis ≥15.1	CFLD	$p = 0.075$

TDI—total daily insulin, HbA1c—glycated haemoglobin, CFRD—cystic fibrosis-related diabetes, CFLD—cystic fibrosis live disease, ns—not significant.

4. Discussion

This is the first study to report on the prevalence of CFRD in the Croatian CF population, with rates of 4.8% in the paediatric population and 19.1% in adults as well as an average age of CFRD diagnosis of 14 years. Furthermore, it provides valuable insights into the various insulin regimens used to manage CFRD and their associations with HbA1c. The study also explored the associations between the age at CFRD diagnosis, the age of insulin introduction, and other clinical parameters. The age at CFRD diagnosis and age at insulin introduction were positively correlated with the final BMI. Furthermore, patients who required insulin by age 15 had higher insulin requirements including TDI, TDI/kg, and total bolus doses. The average TDI/kg to treat diabetes was 0.447 U/kg, and patients who required insulin by age 15 were more likely to have CFLD. Insulin requirements (TDI, TDI/kg, and total bolus insulin) were positively correlated with HbA1c.

4.1. CFRD Prevalence

The prevalence of CFRD in the adult CF population was slightly lower than that previously reported by Moran et al., which found that 2% of children, 19% of adolescents, and 40–50% of adults were affected [19]. In their study, the prevalence of diabetes rose

steadily with age, and after the age of 40 years, 45–50% were affected. In a large European Cystic Fibrosis Foundation registry study, the prevalence of CFRD in 2015 was 0.8% in patients ≤10 years, 9.7% in those aged 10–19 years, 24.1% in those aged 20–29 years, and 32.7% in those ≥30 years [18]. Thus, the prevalence of CFRD in our population is consistent with the European Registry study, albeit slightly lower.

The lower prevalence of CFRD in our adult population may be attributed to the relatively younger demographic, as most patients at our centre are under 30, and CFRD prevalence is known to increase with age [19]. Additionally, prior epidemiological studies reported on the prevalence of CFRD before the widespread use of CFTR modulators, which may delay the onset of disease. Although our study did not assess the incidence of CFRD over time, registry studies from the United States and the United Kingdom reported lower CFRD rates in the 4–5 years following ivacaftor treatment [20].

The lower CFRD prevalence in our study could also be attributed to the use of different screening methods. Not all patients were diagnosed using the gold-standard OGTT. Some patients were diagnosed using extended glucose profiles during hospital visits, and others were diagnosed using HbA1c cut-offs for diabetes, which have a lower sensitivity compared with the OGTT. Variations in CFRD prevalence across studies may also be due to inconsistent screening rates. In a 5-year prospective study, the prevalence of CFRD at a single centre increased from 11% to 24% with annual screening [21]. Furthermore, registry studies show that CF centres with lower screening rates have faster rates of pulmonary decline prior to CFRD diagnosis [22]. Despite these findings, the screening rates remain low in many CF centres, and many barriers to testing exist [4,23].

Low screening rates may be due to competing medical priorities and the perceived burden of an OGTT, which requires overnight fasting and multiple venous blood draws. Additionally, poor gastric tolerability may further reduce test adherence [24]. Thus, some centres may choose to screen with HbA1c and continuous glucose monitoring (CGM) because it is perceived as more convenient [25]. Potential strategies to increase screening uptake may include educating both patients and providers about the importance of screening, implementing systematic reminders, and establishing processes for scheduling laboratory appointments [26].

4.2. Age of CFRD Onset

Our study supports the current recommendations for annual CFRD screening starting at age 10, as the median age at diagnosis was 14 years. We found that more than half of the patients (53%) were diagnosed with CFRD by age 14, and 71% had their diagnosis by age 15. In a European registry study of 3553 patients, 445 (13%) were diagnosed with CFRD, and the average age of CFRD diagnosis was 13 years [22], which aligns with our study. The European Cystic Fibrosis Society (ECFS) registry does not report the date at diagnosis of CFRD, but rather the age at CFRD diagnosis is calculated as the difference between the year in which the patient began insulin and the year of birth. As many patients have abnormal OGTTs before commencing insulin, the ECFS study may have overestimated the date of CFRD diagnosis. Furthermore, the ECFS registry defines cases of CFRD as those requiring insulin, and as seen in our study, not all patients may be using insulin but still fulfil the ADA criteria for CFRD. Thus, the ECFS registry may not capture all CFRD patients, and future studies should include all CFRD patients including those not on insulin.

4.3. CFRD Management

Insulin is currently the only recommended therapy for CFRD; therefore, the majority of our patients (78%) were treated with insulin at their final visit. Insulin treatment was individualised based on glycaemic patterns and nutritional needs. When CGM became

available, it was used to individualise treatment. Some patients with only postprandial glycaemic excursions were managed with bolus insulin alone, while those with fasting hyperglycaemia required basal insulin. Typically, the average TDI/kg in CFRD patients is in the range of 0.5–0.8 U/kg [27]. This aligns with our study, where the average TDI/kg to treat diabetes was 0.447 U/kg when considering all patients (including those assigned a value of '0' at their last visit), and 0.817 U/kg when analysing only those using insulin at their final visit (13/17 patients).

Multidisciplinary care and tailored interventions are needed to optimise the outcomes for patients with CFRD [28]. Best practices for managing CFRD include individualised insulin treatment, adjustments during acute illness, and care from a diabetes specialist experienced in diabetes technology and CFRD [14].

4.4. CFRD Remission

Interestingly, four patients (24%) who previously required insulin had de-escalation in dosing, eventually leading to the discontinuation of insulin altogether, and were considered to have clinical remission of CFRD. At their final visit, two out of these four patients had HbA1c values consistent with normal glucose tolerance, while two patients had values consistent with prediabetes.

Clinical remission of CFRD could be explained by the introduction of CFTR modulators at our centre in 2021, which likely improved pulmonary exacerbations and the hyperglycaemia associated with acute disease. There may also be a direct effect of CFTR dysfunction on beta cells [29,30], which may improve with CFTR modulators. This was reported in a retrospective study by Gaines et al., where one-third of patients experienced remission or near remission of their diabetes following CFTR modulator therapy [31]. Similarly, Cohen et al. reported that CFTR modulator therapy improved glucose metabolism, as assessed by OGTT, and some patients experienced CFRD remission, transitioning to normal or indeterminate glucose tolerance [32]. Further research is needed to determine the optimal management of patients with apparent clinical remission of CFRD, and whether these patients remain at risk of recurrence.

4.5. Association Between CFRD and Clinical Parameters

As seen in our study, CFRD can progress at different rates in different people. An earlier age at CFRD diagnosis and insulin initiation was associated with a lower final BMI, and patients who required insulin by age 15 had higher insulin requirements. Patients diagnosed with CFRD at a younger age likely had a more rapid decline in beta cell function, reduced endogenous insulin secretion, and insulinopenia, leading to a catabolic environment. Additionally, the TDI, TDI/kg, and total bolus doses were statistically significantly correlated with HbA1c, suggesting that patients requiring higher doses had more advanced CFRD.

There was also an association between the age of insulin introduction and the presence of CF liver disease. CF liver disease is a known risk factor for CFRD, but the specific mechanisms underlying this association have not been thoroughly studied [33]. Elevated circulating levels of inflammatory cytokines, including interleukin IL-6, IL-1, and TNF-α, can be found in liver disease. These pro-inflammatory cytokines may alter glucose metabolism by impairing insulin signalling, promoting lipolysis, and decreasing lipogenesis and glucose oxidation [34,35]. Reduced insulin sensitivity and impaired insulin secretion have been reported in CFLD patients with portal hypertension, and these factors may contribute to the increased risk [36].

Most patients were diagnosed around the age of 14, and when patients were grouped according to the age of diagnosis and age of insulin introduction—before or after age

15—some important associations emerged including the association with CFLD and higher total insulin doses. Thus, this age group may be more vulnerable and may require more frequent disease monitoring and timely CFRD screening.

4.6. CFRD Chronic Complications

Screening for diabetes complications in CFRD should begin annually, starting 5 years after the diagnosis [5,14]. Unfortunately, as is the case in many CF centres including ours, screening for diabetes complications is often inconsistent due to various competing medical priorities. In a study conducted at the All Wales Adult Cystic Fibrosis Service in the UK, only 64% of patients had retinal screening appointments in 2012, and 42% had evidence of retinopathy [37]. A review of 285 CFRD patients at the University of Minnesota found that 14% with fasting hyperglycaemia and a diabetes duration of 10 years or more had microalbuminuria, and 16% had retinopathy [38]. These findings highlight the importance of retinal and albuminuria screening, especially since patients with CF are now living longer, and microvascular complications will likely become more relevant in the ageing CFRD population.

4.7. Strengths and Limitations

Although this study presents several interesting findings, some limitations should be addressed. The main limitation was the retrospective design and the relatively small number of patients with CFRD compared with other countries with larger CF populations. Due to the retrospective design, not all patients were diagnosed and screened for CFRD using the gold standard OGTT. Additionally, although approximately 98% of CF patients in Croatia are followed at this centre, the results are not a true representation of the country's entire CF population. Nevertheless, this sample provides a good representation of the country's CF population. A strength of our study was the inclusion of all CFRD patients and not just those on insulin, thus capturing a wider range of CFRD patients. Furthermore, both the age of CFRD diagnosis and the age of insulin introduction were considered.

5. Conclusions

In conclusion, the prevalence of CFRD in Croatia, albeit slightly lower, is consistent with other studies. However, because the landscape of CFRD is evolving due to the widespread use of CFTR modulators, large prospective studies are needed to determine the effect of CFTR modulators on CFRD development and insulin requirements. Whether the recommendations for OGTT screening will remain in place in the new era of CFTR modulators will need to be addressed in future studies. Until such studies are available, CFRD screening recommendations should be followed, and patients should be managed at CF centres by multidisciplinary teams to ensure the highest standards of care.

Author Contributions: All authors were involved in the planning of the study. L.S.K.B. and K.S. were responsible for data entry. V.A. was responsible for statistical analysis. L.S.K.B. was responsible for drafting the manuscript. All authors discussed the results and contributed to the interpretation of results. All authors edited and approved the final manuscript. M.B. and V.A. supervised the work. All authors have read and agreed to the published version of the manuscript.

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Article

Adverse Events and Drug Interactions Associated with Elexacaftor/Tezacaftor/Ivacaftor Treatment: A Descriptive Study Across Australian, Canadian, and American Adverse Event Databases

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Abstract

People with cystic fibrosis may experience polypharmacy, which can increase the risk of drug induced complications such as adverse events and drug–drug interactions. This study aimed to examine the prevalence of adverse events and to identify potential drug–drug interactions associated with elexacaftor/tezacaftor/ivacaftor (ETI). Three databases, the Australian Therapeutic Goods Administration Database of Adverse Event Notification (TGA DAEN), the Canada Vigilance Adverse Reaction Online Database (CVAROD), and the USA Food and Drug Administration Adverse Event Reporting System (FAERS) Database were searched for spontaneous ETI adverse events between 2019 and 2024. Descriptive analysis of the data was undertaken. The FAERS database was analysed to identify adverse events of interest such as anxiety and depression and concomitant drugs prescribed with ETI. A total of 10,628 ETI associated adverse events were identified in all system organ classes. The incidence of psychiatric adverse events ranged from 7 to 15% across the three databases. Potential drug–drug interactions with CYP 3A4/5 strong inhibitors and strong inducers were identified from the FAERS database and azole antifungals were implicated in several ETI dose modifications. The prevalence and types of ETI adverse events were varied and use of concomitant drugs with potential drug interactions was significant, requiring more research to manage them.

Keywords: adverse events; drug–drug interactions; cystic fibrosis; elexacaftor/tezacaftor/ivacaftor

1. Introduction

Cystic fibrosis (CF) is a complex multiorgan autosomal recessive disease caused by genetic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This results in a decrease in the quantity and/or activity of the CFTR protein [1]. CFTR modulators are the mainstay treatment for CF where they improve the production, intracellular processing, and function of the CFTR protein [2]. The triple combination of CFTR modulators elexacaftor/tezacaftor/ivacaftor (ETI) was registered with the U.S. Food and Drug Administration (FDA) in 2019 for people with CF who carry at least one copy of the variant F508del. Clinical trials demonstrated that treatment with ETI significantly improved lung function, with percentage predicted forced expiratory volume in one second (ppFEV1) increasing by up to 14%, along with reductions in lung exacerbations and improvements in quality of life of people with CF [3,4].

People with CF often have multiple comorbidities, including diabetes (up to 35% of adults), anxiety (22–32%), and depression (10–19%) [5]. Therefore, polypharmacy, defined as five or more medications, is common in people with CF. This increases the risk of drug-induced complications, including adverse events and drug–drug interactions [6,7]. Advances in CF care have increased the life expectancy of people with CF to over 50 years of age, and this may increase further with extensive use and early introduction of CFTR modulators [8]. The increase in life expectancy for people with CF may correlate with co-morbidities that may increase medication burden. Thus, awareness of adverse events and drug–drug interactions is critical in preventing drug-induced toxicities in people with CF as they age [7].

An adverse event is an unexpected and unplanned response to a drug that occurs at normal doses. Clinical trials investigating ETI treatment have reported an acceptable safety profile. However, these trials are often not a true representation of patients seen in clinics, as they tend to be conducted in a small, selected group of participants observed over short study periods. As a result, clinical trials are often underpowered to detect adverse events, particularly those with delayed onset or lower frequency [9]. Several systematic reviews have described the efficacy and safety of CFTR modulators [10–12]. Systematic reviews are useful for identifying common and well-documented adverse events. However, they may not capture uncommon and rare adverse events. Small studies have described the wide range of adverse events observed with the use of CFTR modulators in clinical care, but these are in a selected population and maybe subject to reporting bias [13,14]. A recent study by Papadakis et al. reported heterogeneity of adverse events, highlighting the need for close monitoring and timely management of adverse events [15]. The study also found that mental health changes were one of the most reported adverse events, which were also identified in several other studies. Together, these studies highlighted the need for more research on the prevalence of psychiatric adverse events, in particular anxiety and depression, with ETI treatment, which are rarely reported during clinical trials [15–17].

Post-marketing surveillance is an important component of pharmacovigilance. International online databases have been used to investigate the prevalence of 'real world' adverse events. The Australian Therapeutic Goods Administration Database of Adverse Event Notification (TGA DAEN), the Canada Vigilance Adverse Reaction Online Database (CVAROD), and the United States Food and Drug Administration Adverse Event Reporting System (FAERS) Database are examples of databases that curate adverse drug events reported by healthcare professionals, consumers, and pharmaceutical companies within their respective countries [18–20]. Data from these international databases can be analysed to determine the prevalence, severity, risks, and trends of adverse events, as well as patient characteristics and treatment results. This information has been vital in gathering evidence to formulate strategies to mitigate adverse events [21].

Adverse events may result from drug–drug interactions, where one drug affects the efficacy or toxicity of another due to potentially significant changes to drug exposure. Previous studies have highlighted potential drug–drug interactions in individuals taking ETI; however, the clinical relevance of some of these interactions remains unclear [22]. Elexacaftor, tezacaftor, ivacaftor are extensively metabolised by enzymes of cytochrome P450 3A (CYP3A), including enzymes CYP3A4 and CYP3A5 [22,23]. Therefore, serum concentrations of these CFTR modulators can be potentially impacted by patients' metaboliser phenotype, as well as co-administration of CYP3A4/5 inducers and inhibitors. Ivacaftor is an inhibitor of CYP2C9 and drugs that are substrates of this enzyme (e.g., warfarin) may have their serum concentrations increased, potentially leading to increased rates and severity of adverse events [22]. Also, ETI are known to be substrates and/or inhibitors of certain transport proteins. However, the clinical impact of these interactions is still being elucidated and often less well characterised in comparison to metabolic interactions. Furthermore, the impact of metabolic drug interactions on patient outcomes has not been extensively described in the literature. Hence, analysis of concurrent medications taken by people with CF through these databases may provide some useful information on drug interactions and safety in a real-world setting.

2. Aim

The aims of this study were to examine the prevalence of adverse events and identify potential drug–drug interactions associated with ETI as reported in three international adverse events databases.

3. Ethics Approval

Ethical approval was not required for this study as it did not involve human participants, animals, or the collection of personal data. Exemption was granted by the ethics committee of RMIT University.

4. Methods

4.1. Study Design and Data Sources

The Australian, Canadian, and U.S. databases were searched for spontaneous adverse events reported over 4.5 years (last quarter of 2019 to the first quarter of 2024) as ETI was approved by FDA in the latter half of 2019. Adverse events have been classified by body system or organ class according to the Medical Dictionary for Regulatory Activities (MEDRA) which allowed for grouping of adverse events [24].

The U.S. FAERS database was also analysed for concomitant drugs that may be substrates, inhibitors, or inducers of CYP3A4/5 enzymes, and drugs that are substrates of CYP2C9 to investigate the potential for drug–drug interactions and the risk of adverse events.

4.2. Search Strategy

All three databases were searched for all adverse events reported for ETI. This included demographic data from the patient (e.g., gender and age). All three databases use the MEDRA System Organ Class (SOC) which enabled a comparison of the adverse events of SOC across the databases. Furthermore, a more detailed analysis of depression and anxiety was performed using the preferred term of the adverse events within the SOC.

Concomitant drugs were identified from the FAERS database because there were a significant number of reports in this database that included concomitant drugs. OpenVigil, which is a tool for the mining and analysis of FAERS database pharmacovigilance data,

was used to identify adverse events of interest, in particular anxiety and depression and the concomitant drugs used [25].

4.3. Data Analysis

The three datasets differed in the number of reports as the number of people with CF prescribed ETI differed across the three countries. The adverse events reports including patient demographics were exported to Microsoft Excel and descriptive analyses of the data were performed. The number of reports for each SOC was determined and analysed as a proportion of all adverse-event reports for each dataset. The findings utilising percentages were compared across the datasets to determine commonalities and differences. The SOCs with most reports were further analysed to determine the prevalence of specific adverse events. The top three adverse events were determined in the top 14 SOCs for each database.

The details of concomitant drugs, obtained from the FAERS database, were classified as inhibitors or inducers of CYP3A4/5 enzymes and substrates of CYP2C9 using the Flockhart CYP 450 drug–drug interaction table, which listed 40 CYP 3A4/5 inhibitors and 27 CYP 3A4/5 inducers that may have clinically relevant interactions [26]. These inhibitors and inducers were further classified as strong, moderate, or weak. The CYP3A4/5 inhibitors that interact with ETI included itraconazole, voriconazole, and clarithromycin, which are classified as strong inhibitors, while fluconazole and erythromycin are moderate inhibitors. The CYP 3A4/5 inducers which interact with ETI included rifampin and carbamazepine, which are strong inducers, while phenytoin is a moderate inducer. The Flockhart table also lists 33 CYP2C9 substrates, including warfarin and glipizide, which may interact with ivacaftor [26].

The current literature recommends ETI dose modifications for specific adverse events, e.g., anxiety and depression. The OpenVigil database was used to identify adverse events of anxiety and depression and investigated whether such dose modifications, if any, had been made. The OpenVigil database was also used to determine if dose modification of ETI was carried out for potential drug–drug interactions (CYP 3A 4/5 inhibitors and inducers) of interest.

5. Results

The 2022/2023 CF data registries reported that 3798 people with CF were enrolled in Australia (2023), 4445 people with CF in Canada (2022), and 33,288 people with CF in the U.S. (2023) [27–29]. Across the three countries, between 50% and 70% of people with CF were taking ETI, 67% (n = 2530) in Australia, 51% (n = 2272) in Canada and 70% (n = 23,350) in the U.S., respectively.

During the study period, 10,628 entries of adverse events associated with ETI use had been reported on the databases (U.S. = 10,503; Canada = 80; Australia = 45) (Table 1). Most adverse events were reported in people with CF aged 18 to 64 years in the three databases, with more adverse events reported in females in the U.S. and Canadian databases.

The adverse events were reported in all 27 SOCs, with the top ten being gastrointestinal, general disorders, injury, infections, investigations, nervous system disorders, psychiatric disorders, respiratory, skin and subcutaneous disorders, and surgical procedures (Table 2). The U.S. database reported more infections and investigations (11.4%) compared to Australia (5.6%) and Canada (7.1%). More psychiatric adverse events were reported in Australia (15.6%) and Canada (15.3%) compared to the U.S. (7.4%). The number of adverse events reported in each database was much greater than the actual number of reports, as most of the reports included more than one adverse event associated with ETI. The three most reported adverse events within the top 14 SOC are shown in Supplementary Materials.

Table 1. Patient demographics.

	Australia		**Canada**		**U.S.**	
Number of adverse events reports	*n* = 45		*n* = 80		*n* = 10,503	
Patient demographics						
Gender						
No. Males	18	(40%)	34	(43%)	4029	(38%)
No. Females	17	(38%)	41	(51%)	5333	(51%)
Unspecified	10	(22%)	5	(6%)	1141	(11%)
Age						
By age 0 to 11 yrs	7	(16%)	10	(12%)	429	(4%)
12 to 17 yrs	6	(13%)	12	(15%)	944	(9%)
18 to 64 yrs	19	(42%)	51	(64%)	3571	(34%)
65 to 85 yrs	1	(2%)	1	(1%)	134	(1%)
>85 yrs					1	
Unspecified	12	(27%)	6	(8%)	5424	(52%)

Table 2. Prevalence of adverse events in the three databases.

	Australia		**Canada**		**U.S.**	
Number of adverse events by SOC	*n* = 179		*n* = 254		*n* = 19,595	
Blood and lymphatic system disorders	2	(1.1%)	-		78	(0.4%)
Cardiac disorders	2	(1.1%)	1	(0.4%)	109	(0.6%)
Congenital, familial, and genetic disorders	-		2	(0.8%)	222	(1.1%)
Ear and labyrinth disorders	1	(0.6%)	-		89	(0.5%)
Endocrine disorders	-		-		27	(0.1%)
Eye disorders	2	(1.1%)	-		349	(1.8%)
Gastrointestinal disorders	24	(13.4%)	25	(9.8%)	2011	(10.3%)
General disorders and administration site conditions	16	(8.9%)	19	(7.5%)	1922	(9.8%)
Hepatobiliary disorders	3	(1.7%)	7	(2.8%)	403	(2.1%)
Immune system disorders	-		2	(0.8%)	149	(0.8%)
Infections and infestations	10	(5.6%)	18	(7.1%)	2242	(11.4%)
Injury, poisoning, and procedural complications	5	(2.8%)	11	(4.3%)	910	(4.6%)
Investigations	20	(11.2%)	45	(17.7%)	1803	(9.2%)
Metabolism and nutrition disorders	8	(4.5%)	5	(2.0%)	463	(2.4%)
Musculoskeletal and connective tissue disorders	4	(2.2%)	9	(3.5%)	466	(2.4%)
Neoplasms benign, malignant, and unspecified	1	(0.6%)	-		86	(0.4%)
Nervous system disorders	9	(5.0%)	12	(4.7%)	1511	(7.7%)
Pregnancy, puerperium, and perinatal conditions	3	(1.7%)	6	(2.4%)	176	(0.9%)
Product issues	-		-		32	(0.2%)
Psychiatric disorders	28	(15.6%)	39	(15.3%)	1451	(7.4%)
Renal and urinary disorders	2	(1.1%)	3	(1.2%)	211	(1.1%)
Reproductive system and breast disorders	-		1	(0.4%)	198	(1.0%)
Respiratory, thoracic, and mediastinal disorders	14	(7.8%)	7	(2.8%)	1955	(10
Skin and subcutaneous tissue disorders	17	(9.5%)	30	(11.8%)	1251	(6.4%)
Social circumstances	1	(0.6%)	-		79	(0.4%)
Surgical and medical procedures	5	(2.8%)	11	(4.3%)	1286	(6.6%)
Vascular disorders	2	(1.1%)	1	(0.4%)	116	(0.6%)

Note: some categories do not have any adverse events reported.

The OpenVigil database reported 429 incidents of anxiety and depression (Table 3). These included 188 reports of anxiety, 136 reports of depression, and 105 reports of combined anxiety and depression. Of these, 118 (28%) resulted in changes in the ETI dose, including switching morning and night doses and reducing the dose (for example, one ETI tablet in the morning and one ivacaftor tablet at night). Antidepressants were listed

as concomitant drugs in forty-three (10%) reports at the time they were submitted. Some of the reports of anxiety and depression also included other SOC adverse events such as respiratory and gastrointestinal.

Table 3. Prevalence of anxiety and depression in the OpenVigil database.

No. of Reports	Unknown Dose *	Dose Reduced/Switched ***	Standard Dose **	Dose Reduced/Switched ***
Anxiety (n = 188)	128 (68%)	30 (16%)	60 (32%)	23 (12%)
Depression (n = 136)	83 (61%)	17 (13%)	53 (39%)	15 (11%)
Anxiety and depression (n = 105)	57 (54%)	10 (10%)	48 (46%)	23 (22%)

* Dose of ETI used not specified in report. ** Standard dose of ETI specified in report. *** Dose modifications documented in reports with unknown or standard ETI dose.

Within the FAERS database, ciprofloxacin (n = 183) was the most common co-prescribed CYP3A4/5 inhibitor, followed by the azole antifungals (n = 105) (Table 4). The most common co-prescribed CYP3A4/5 inducer was prednisone/prednisolone (n = 241). The co-prescription of drug substrates of CYP2C9 varied between different drug classes, the most common being non-steroidal anti-inflammatory drugs (NSAIDs) (n = 190). Other CYP2C9 drug substrates included amitriptyline, irbesartan, losartan, fluoxetine, venlafaxine, rosuvastatin, and warfarin.

Table 4. Prevalence of CYP 3A 4/5 inhibitors and inducers, and CYP 2C9 substrates co-prescribed in people with CF in the FAERS database.

Drug—CYP 3A 4/5 Inhibitor	Inhibitor Strength Level *	No. of Reports	Drug—CYP 3A 4/5 Inducer	Inducer Strength Level *	No. of Reports	Drug-Substrates CYP 2C9	No. of Reports
Atomoxetine	Weak	6	Carbamazepine	Strong	2	Amitriptyline	38
Ciprofloxacin	Moderate	183	Prednisolone/ Prednisone	Weak	241	Celecoxib	24
Clarithromycin	Strong	37	Rifabutin	Weak	1	Diclofenac	17
Erythromycin	Moderate	17	Rifampin	Strong	7	Doxepin	2
Fluconazole	Moderate	41				Fluoxetine	86
Itraconazole	Strong	19				Glipizide	4
Ketoconazole	Strong	4				Ibuprofen	144
Posaconazole	Strong	24				Irbesartan	5
Voriconazole	Strong	17				Losartan	12
						Naproxen	29
						Rosuvastatin	15
						Torsemide	8
						Venlafaxine	31
						Warfarin	12

* Inhibitor/Inducer strength level classified by Flockhart drug interaction table or ETI product information.

Data from the OpenVigil database showed the outcome to ETI doses when combinations of ETI and CYP3A4/5 inhibitors were co-prescribed (Table 5). Of the 57 people with CF who took ETI with one of the azole antifungals, 34 (60%) had their ETI dose modified. It was the highest and lowest for posaconazole-ETI (69%) and fluconazole-ETI (50%) combinations, respectively.

Table 5. Modification of ETI dose with CYP 3A4/5 inhibitors from the OpenVigil database.

CYP 3A4/5 Inhibitor	No. of Reports Dose Modified		No. of Reports Dose not Changed		No. of Reports Dose Unknown	
Itraconazole (*n* = 16)	10	(63%)	1	(6%)	5	(31%)
Fluconazole (*n* = 14)	7	(50%)	1	(7%)	6	(43%)
Posaconazole (*n* = 16)	11	(69%)	3	(19%)	2	(12%)
Voriconazole (*n* = 11)	6	(55%)	1	(9%)	4	(36%)
Erythromycin (*n* = 4)	1	(25%)	1	(25%)	2	(50%)
Clarithromycin (*n* = 7)	1	(14%)	3	(43%)	3	(43%)

6. Discussion

This study was the first to compare spontaneous adverse events of ETI in three international databases of pharmacovigilance. Integrating and comparing adverse event reports from various databases enhances the analyses by expanding the sample size, diversifying data representation, and boosting the statistical power to identify associations.

The most common adverse events reported in the manufacturer's clinical trials according to SOC categories included the following: (1) infections and infestations, (2) nervous system disorders, (3) respiratory disorders, (4) gastrointestinal disorders, (5) skin and subcutaneous disorders, and (6) investigations [30]. Common adverse events, which were reported in the manufacturer's clinical trials and stated in the product information, were also reported in the pharmacovigilance databases. Other less common adverse events such as increase in blood pressure has also been stated in the product information and reported in the databases. However, the product information does not list all the uncommon, rare, and ultra rare adverse events that may have potentially been reported. Therefore, the real-world reporting of adverse events can highlight uncommon and rare adverse events. Our findings show a wide range of adverse events reported across all 27 SOCs, in comparison to what was reported in clinical trials and systematic reviews [10–12]. Notably, this study has highlighted several psychiatric adverse events (7 to 15% of all SOCs) reported in these databases.

Another key finding was the identification of commonly used concomitant drugs, not captured in clinical trials, which may have potentially contributed to drug–drug interactions. As people with CF's life expectancy increases, they will be at risk of developing comorbidities associated with ageing, adding to their drug therapy burden and increasing their risk of drug–drug interactions. This study has demonstrated that people with CF were co-prescribed a range of drugs with potential drug interactions. Therefore, it is important to be aware of this significant risk and develop strategies to mitigate the risk in people with CF experiencing polypharmacy.

As the use of ETI has increased since 2019, a growing number of adverse events have been reported in various SOCs. Real world data provides a broader view of drug safety across specific populations with comorbidities. The majority of adverse events were reported in the age group of 18 to 64 years, as ETI was initially available for people with CF aged 12 and older and uptake of ETI was rapid in those aged 18 and older. Furthermore, more female patients than males reported adverse events. This is consistent with what has been reported in the general population, with women often under-represented in clinical trials [9].

Although people with CF may have psychiatric comorbidities, the psychiatric adverse events which have been associated with ETI as reported in the real world is significant [16,17]. These psychiatric adverse events such as anxiety and depression can significantly impact quality of life. Analysis of the reports of anxiety and depression available in the OpenVigil database revealed that modifications in ETI doses had been made in most

instances. In some circumstances, more than one dose modification was made. However, it was not possible to determine when these dose modifications were implemented, nor whether they were prompted by the reported psychiatric adverse events. It was worth noting that several of these reports contained concomitant antidepressants (most common were selective serotonin reuptake inhibitors), but it was not possible to determine when these drugs were started or their respective doses. While not conclusive, our observation suggests that psychiatric adverse events may have played a role in some of the observed dosage adjustments which had been reported in two previous studies [31,32]. Both studies had demonstrated that some people with CF, who do not tolerate the full dose of ETI as a result of psychiatric adverse events, may require individualised dose adjustments to minimise adverse events to enable them to continue CFTR modulator therapy [31,32].

Several concomitant drugs with potential interactions with ETI were identified from the FAERS database. Ciprofloxacin was the most frequently prescribed moderate inhibitor of CYP 3A4/5. Prednisone and prednisolone, considered weak inducers, were also commonly co-prescribed with ETI in this database. However, these potential drug interactions were not considered significant. On the other hand, co-prescribing of strong CYP3A4/5 inhibitors such as azole antifungals (e.g., itraconazole and voriconazole) and strong inducers of CYP3A4/5, such as rifampin and carbamazepine, with ETI, warrants careful consideration, as these combinations may not produce the desired therapeutic benefit for people with CF. The OpenVigil database revealed a significant number of instances in which ETI doses were modified during co-administration with CYP3A4/5 inhibitors. However, it was unknown when these dose modifications were initiated. Of concern were cases where dose modifications were not made and whether this could have potentially contributed to the adverse event(s) reported. The identification of adverse events possibly involving drug interactions warrants further exploration; however, causality cannot be inferred from the available FAERS data due to inherent reporting limitations.

As a review of reported adverse events and drug interactions to regulatory authorities, there are several limitations to this study. Many reports included more than one adverse event which would have potentially confounded the analyses. The reports may have been completed by healthcare professionals and/or consumers, which affects the reliability of the data. The databases were limited by the amount of information provided and the level of detail provided for each adverse event varied. Some adverse events included extensive information, such as patient demographics and all concomitant drugs, while others only included information about the adverse event.

Another limitation is that this study may have included duplicate reports, as deduplication of the data was not possible due to different time points, potentially leading to an overestimation of some adverse events. Disproportionality analysis of the data was not able to be completed due to lack of deduplication of reports. Also, the lack of the total number of people with CF taking ETI over the study period made it difficult to determine the significance of the risks of the reported adverse events. Furthermore, data on patient co-morbidities, drug doses, when drugs were started or stopped, and if patients were taking the concomitant drugs at the time of the adverse event(s) being reported were not available in the databases. Consequently, it was not possible to determine whether the concomitant drugs may have contributed to the adverse events as a direct result of drug interactions. Generally, there is an underreporting of adverse events and a level of selective reporting of adverse events for new drugs. Therefore, it was not possible to draw firm conclusions on the incidence and causality of these adverse events of ETI, as reported in these databases. More studies are needed to determine the associations between adverse events and ETI, and the impact of potential drug–drug interactions.

7. Conclusions

This descriptive study examined the prevalence and types of adverse events associated with ETI in the real world, with a particular focus on potential drug–drug interactions. Adverse events were reported across all 27 system organ classes, reflecting a broad range of adverse outcomes. Psychiatric adverse events have been previously documented in the literature, and this study confirmed their prevalence. Furthermore, the co-prescribing of ETI with CYP3A/45 inhibitors and inducers, despite being well-known interaction risks, highlights a critical area for clinical vigilance. Our preliminary findings emphasise the need for further research to elucidate the underlying mechanisms of these adverse events and to develop evidence-based strategies for mitigating risks associated with ETI therapy and its concomitant use with interacting drugs. Ensuring safe and effective use of ETI is a priority in optimising health outcomes for people with CF.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life15081256/s1, Table S1: Types and number of adverse events in the top 14 SOCs. The top three adverse events within each SOC are also shown.

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CFTR Modulators Counteract F508del CFTR Functional Defects in a Pancreatic Epithelial Model of Cystic Fibrosis

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Abstract

Cystic fibrosis is a multisystem disorder caused by mutations in the *CFTR* gene that lead to impaired ion and fluid transport across secretory epithelia. Although the therapeutic impact of CFTR modulators has been extensively studied in airway epithelia, their efficacy in extra-pulmonary tissues, such as the pancreas, has been less explored. This study evaluated the effects of the CFTR modulators, VX770 (ivacaftor), VX661 (tezacaftor), and VX445 (elexacaftor), administered either individually or in combination, on CFPAC-1 cells, a pancreatic ductal epithelial cell line derived from a cystic fibrosis patient harboring the F508del CFTR mutation. The cells were cultured and differentiated onto porous supports, and a panel of functional parameters was assessed. These included transepithelial electrical conductance, fluid reabsorption, apical surface fluid pH, protein concentration, and microviscosity, the latter analyzed with multiple particle tracking. To simulate a pro-inflammatory microenvironment, the cells were preconditioned with lipopolysaccharide (LPS). Treatment with VX661 and VX445 resulted in significant improvement in epithelial function, with the triple combination producing the most pronounced rescue. Pro-inflammatory stimulation by LPS increased the production of cytokine IL6, IL-8, and IL-1β, as well as the protein content of the apical surface fluid. Despite the LPS pro-inflammatory stimulus, CFTR modulators preserved or slightly enhanced their efficacy in restoring CFTR-mediated ion and fluid transport. However, they did not reduce cytokine expression under pro-inflammatory conditions. Collectively, these findings show that CFTR modulators can restore critical aspects of cystic fibrosis pancreatic epithelial physiology in vitro, even under pro-inflammatory stress, supporting their potential relevance beyond the airway disease.

Keywords: cystic fibrosis; CFTR modulators; pancreatic duct epithelial model; inflammation; CFPAC-1 cells

1. Introduction

Cystic fibrosis (CF) is the most common fatal autosomal recessive disease in Caucasian populations, affecting approximately one in 2500 live births in Europe and North America [1]. It is caused by mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), which is a cAMP-activated chloride (Cl^-) and bicarbonate (HCO_3^-) channel located on the apical membrane of epithelial cells [2]. To date, more than 2000 CFTR mutations have been identified. The most common is the deletion of phenylalanine at position 508 (F508del), which is present in more than 90% of patients [3].

CF is a multisystem disease, with major clinical manifestations affecting the lungs, pancreas, gastrointestinal tract, and reproductive system [4]. In the airways, CFTR dys-

function leads to impaired mucociliary clearance, persistent bacterial infections, chronic inflammation, and progressive bronchiectasis, ultimately resulting in respiratory failure [5]. In the pancreas, CFTR dysfunction leads to the dehydration of pancreatic secretions, ductal obstruction, inflammation, fibrosis, and pancreatic exocrine insufficiency in almost 85% of patients. Patients categorized as pancreatic insufficient (PI) typically have a more severe disease phenotype than the remaining 15%, who are pancreatic sufficient (PS) [6,7].

The F508del mutation impairs the folding and trafficking of CFTR, leading to its retention in the endoplasmic reticulum and subsequent degradation via the ubiquitin-proteasome pathway [8]. However, if the F508del CFTR protein is properly trafficked to the apical membrane, it retains partial function [9,10]. This observation has guided the development of pharmacological strategies combining correctors, which improve CFTR folding and trafficking, with potentiators, which enhance channel activity. The most successful of these therapies is Kaftrio/Trikafta: a triple combination of the potentiator ivacaftor (VX770) with the correctors tezacaftor (VX661) and elexacaftor (VX445). This combination has demonstrated substantial clinical benefits for patients carrying at least one F508del allele, including improved lung function, reduced sweat chloride concentration, and enhanced quality of life [11,12].

CFTR is highly expressed in the pancreatic ducts, where it regulates the secretion of fluids and electrolytes, particularly bicarbonate (HCO_3^-) [13–15]. Bicarbonate secretion is crucial for solubilising mucins and digestive enzymes, as well as maintaining the optimal viscosity of pancreatic secretions [16,17]. As early proposed by Quinton [18,19], the lack of bicarbonate prevents the normal mucin unfolding due to inadequate Ca^{2+} and H^+ ion sequestration, resulting in abnormally dense and sticky mucus. More recent evidence has confirmed that bicarbonate-driven chelation of divalent cations is essential for the expansion and proper unfolding of secreted mucins, a process fundamental to mucus hydration and clearance in CF [20–23]. This pathological cascade may contribute to epithelial damage, inflammation, progressive fibrosis, and, ultimately, pancreatic injury [24].

Although inflammation is a hallmark of CF pathology, the response of the CF pancreas to pro-inflammatory stimuli remains comparatively understudied. Unlike in the lungs, where neutrophilic inflammation and excessive cytokine release drive progressive tissue damage, pancreatic inflammation in CF is thought to involve more subtle, yet chronic, epithelial activation, innate immune signaling, and cellular stress responses [25,26]. Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), and inflammasome components have been identified in pancreatic ductal cells. Here, they respond to pro-inflammatory stimuli and contribute to the production of cytokines, including IL-6, IL-8, IL-1β, TNF-γ, and TNF-α [27]. These cytokines may contribute to exacerbating epithelial dysfunction, ultimately worsening exocrine pancreatic insufficiency [28,29].

Notably, stimulation with lipopolysaccharide (LPS), a component of Gram-negative bacterial membranes and a known ligand of TLR4, induces the release of pro-inflammatory cytokines in various epithelial models, including airway and intestinal epithelia [30–32]. Consequently, LPS exposure is widely used to reproduce epithelial inflammatory stress in vitro. Although the pancreas is generally considered a sterile organ under physiological conditions, in the present study, we employed this approach to investigate the interplay between inflammation and CFTR modulator efficacy in a pancreatic epithelial context, without aiming to replicate bacterial infection per se.

We investigated the effects of the CFTR modulators VX770, VX661, and VX445, administered alone or in combination, on pancreatic epithelial cell physiology, using CFPAC-1 cells, a human pancreatic ductal cell line that endogenously expresses the F508del mutation [33]. CFPAC-1 cells retain key morphological and functional characteristics of the pancreatic ductal epithelium and have already been used as an in vitro model to study

CF-related pancreatic dysfunction [34–37]. To promote epithelial polarization and differentiation in a manner that mimics the physiological pancreatic microenvironment, the cells were cultured at either liquid-liquid interface (LLI) or air-liquid interface (ALI). We used a multidisciplinary approach, combining measurements of transepithelial electrical conductance and fluid reabsorption, as well as assessment of apical surface fluid (ASF) pH and viscoelastic properties, to comprehensively evaluate the impact of CFTR modulators on pancreatic epithelial function under both basal and pro-inflammatory conditions. Our results demonstrate that, in CFPAC-1 epithelial layers, VX661 and VX445, particularly when used in combination with VX770, enhance CFTR-dependent ion and fluid transport, increase ASF pH, and reduce mucus microviscosity. Notably, the efficacy of these modulators was maintained, and in some cases, even slightly enhanced, under pro-inflammatory conditions, supporting their therapeutic potential in addressing pancreatic dysfunction in CF. Notably, the CFPAC-1 cell line provides a valuable and widely accepted model for investigating CFTR-related defects in a pancreatic context; however, its tumor origin and potential differentiation-related limitations should be taken into account when extrapolating findings to native ductal epithelium. Complementary studies using primary pancreatic cells will be important to validate the physiological relevance of the obtained results.

2. Materials and Methods

2.1. Chemicals

Ivacaftor (VX770, N-(2,4-ditert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxamide), tezacaftor (VX661, 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(2-hydroxy-1,1-dimethylethyl)-1H-indol-5-yl]-cyclopropanecarboxamide), and elexacaftor (VX445, (S)-N-((1,3-dimethyl-1H-pyrazol-4-yl)sulfonyl)-6-(3-(3,3,3-trifluoro-2,2-dimethylpropoxy)-1H-pyrazol-1-yl)-2-(2,2,4-trimethylpyrrolidin-1-yl)nicotinamide) were purchased from Selleck Chemicals (Munich, Germany). The CFTR inhibitor PPQ102 (12,14-dimethyl-9-(5-methylfuran-2-yl)-17-phenyl 1,8,12,14tetrazatetracyclo [8.7.0.02,7.011,16]heptadeca-2,4,6,10,16-pentaene-13,15-dione) and forskolin (Fsk, 3R,4aR,5S,6S,6aS,10S,10aR,10bS-3-ethenyl-6,10,10b-trihydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-5,6,6a,8,9,10-hexahydro-2H-benzo[f]chromen-5-yl] acetate) were obtained from Merck (Milan, Italy). Unless otherwise specified, all other reagents and chemicals were obtained from Merck (Milan, Italy).

2.2. Cell Culture

CFPAC-1 cells, immortalized pancreatic ductal epithelial cells expressing the F508del mutation in the CFTR gene, originally obtained from a patient with CF [33], were seeded at a high density (2.5×10^5 cells per well) onto permeable polycarbonate Snapwell supports (0.4 μm pore size, 1.12 cm diameter; Corning, New York, NY, USA), which had been precoated with rat tail collagen type I. The cells were cultured under liquid–liquid interface (LLI) conditions in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin-streptomycin, and 2 mM L-glutamine. The medium was replaced every 48 h until a stable, confluent epithelial layer was established, which typically took 7–10 days. Epithelial integrity was verified by monitoring transepithelial electrical resistance (TEER) using a voltohmmeter (EVOM2, World Precision Instruments, Sarasota, FL, USA).

For selected experiments (e.g., fluid reabsorption and MPT assays), the cells were transitioned to air–liquid interface (ALI) conditions to promote apical surface exposure and mucus secretion. For ALI culture, the medium on the apical surface was removed, and epithelial preparations were maintained for a further 5–7 days with only the basolateral medium. Morphological integrity, mucus presence, and TEER stability were verified prior to experimental procedures.

For the experiments aimed at evaluating the mucin secretion, HEK-293 (human Embryonic Kidney) and Calu-3 (human bronchial epithelial) cells were grown in Dulbecco's modified Eagle's medium (DMEM) or DMEM:F-12, both supplemented with 10% fetal bovine serum, 100 UI/mL penicillin, 100 μg/mL streptomycin, 20 mM L-glutamine, and 1% *v*/*v* non-essential amino acids, respectively. The Calu-3 cells were seeded onto permeable polycarbonate Snapwell supports and grown under the ALI condition, as previously described.

2.3. Pharmacological Treatments and Inflammatory Stimulation

To assess functional rescue of F508del CFTR, mature CFPAC-1 epithelial preparations were treated with CFTR modulators. Tezacaftor (VX661, 5 μM), elexacaftor (VX445, 5 μM), or their combination (VX661, 5 μM + VX445, 5 μM) were dissolved in 0.1% (*v*/*v*) DMSO and applied to the basolateral compartment for 24 h to allow the correctors sufficient time to promote proper folding, trafficking, and apical localization of the F508del CFTR protein. When combined, VX661 and VX445 were applied simultaneously at 5 μM each, for a total final concentration of 10 μM.

DMSO (0.1% *v*/*v*) alone was used as a vehicle control.

To induce an inflammatory response, lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 (Merck) was added to the basolateral medium at a final concentration of 10 μg/mL and maintained for 24 h [38]. LPS exposure was continued during CFTR corrector treatment [39–44].

Ten minutes prior to functional measurements, cells (except the DMSO control) were acutely stimulated with forskolin (Fsk, 20 μM), applied to both apical and basolateral compartments to activate cAMP-dependent CFTR signaling. Ivacaftor (VX770, 1 μM) was co-applied with FSK to assess its potentiating effect on the gating function of CFTR channels already localized at the apical membrane.

In selected experiments, CFTR inhibition was achieved by applying PPQ102 (30 μM) to both apical and basolateral compartments 10 min after Fsk + VX770 stimulation.

2.4. Transepithelial Electrical Resistance (TEER) Measurements

Transepithelial electrical resistance (TEER) was measured using the EVOM2 voltohmmeter (World precision Instrument, Sarasota, FL, USA) with STX2 chopstick electrodes. Measurements were performed at 37 °C, and data were normalized to the surface area of the insert (1.12 cm^2). TEER values were converted to transepithelial conductance (TEC, in $\mu S/cm^2$) using the formula TEC (G) = 1/TEER. Baseline values (G_pre) were recorded immediately before stimulation. Final values (G_post) were obtained 10 min after Fsk + VX770 treatment. In conditions with PPQ102, a third measurement was recorded 10 min after inhibitor addition. CFTR-dependent ion transport was evaluated by measuring the change in transepithelial conductance (ΔG = G_post − G_pre) before and after stimulation. All experiments were performed in replicate ($n \geq 8$ per condition), and results were reported as mean ± standard error of the mean (SEM).

2.5. Measurement of Fluid Absorption (Jw) and Total Protein Content of the Apical Surface Fluid (ASF)

To assess epithelial fluid absorption, 150 μL of isotonic buffer solution (NaCl 150 mM, HEPES 10 mM, pH 7.4) was applied to the apical surface of ALI-cultured epithelia, either with or without LPS-induced pro-inflammatory stimulation. After 24 h of incubation at 37 °C and 5% CO_2 in the presence of CFTR modulators, the residual apical fluid was col-

lected and weighed in pre-weighed tubes. The final volume was calculated gravimetrically, assuming a density of 1 g/mL. Net fluid absorption rate (Jw) was calculated as follows:

$$Jw = (Vi - Vf)/(A \times t), \quad (1)$$

where Vi = 150 µL, Vf = final volume, A = 0.33 cm^2, and t = 24 h.

Cell-free blank inserts were used to control for evaporation and pipetting variability. Results were expressed in $\mu L \cdot h^{-1} \cdot cm^{-2}$ as mean ± SEM ($n \geq 6$). The method was adapted from previous studies employing gravimetric quantification of fluid absorption [45–47].

Total protein concentration in the collected apical surface fluid was measured with Bradford assay at 595 nm (Jenway 6705 UV/Vis, Cole-Parmer, Vernon Hills, IL, USA) using BSA as standard. Results were expressed in µg/µL as mean ± SEM ($n \geq 4$). Samples not immediately analyzed were stored at −80 °C for up to three weeks.

2.6. pH Measurement in Apical Surface Fluid (ASF)

After 24 h of treatment with CFTR modulators, the ASF was collected from ALI-cultured CFPAC-1 epithelia and its pH was measured using a microelectrode (SevenCompact, Mettler Toledo, Novate Milanese, Italy). To ensure pH stability, measurements were performed immediately after collection, and the time between sampling and analysis did not exceed 2 min.

The instrument was calibrated using standard buffer solutions at pH 7.00 and pH 4.00. Each condition was tested in at least 6 replicates, and results are reported as mean ± SEM.

2.7. Western Blot and Dot Blot Analyses

Western blot analysis was performed on whole cell lysates and basolateral fluids of CFPAC-1 epithelial preparations in order to examine the expression of the F508del CFTR and the pro-inflammatory cytokines IL-6, IL-8, and IL-1β, respectively. Briefly, the CFPAC-1 layers were rinsed with PBS and lysed in RIPA buffer containing protease and phosphatase inhibitors. To detect secreted cytokines, the basolateral media were concentrated using Amicon Ultra-0.5 mL centrifugal filter units with a 3 kDa cut-off (MWCO) at 14,000× *g* for 20 min at 4 °C.

Protein concentration (µg/µL) in both whole cell lysates and concentrated basolateral fluid was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA), according to the manufacturer's protocol. Aliquots corresponding to 30 µg of total protein were separated on 12% SDS-PAGE gels and transferred to PVDF membranes (MilliporeSigma, Burlington, MA, USA). After blocking in 5% milk in PBS containing 0.1% Tween-20 (PBS-T), membranes were incubated overnight at 4 °C with primary antibodies: CFTR (mouse monoclonal, 1:200, clone MM13-4; MilliporeSigma), IL-6 (rabbit polyclonal, 1:100; Thermo Fisher Scientific, Waltham, MA, USA), IL-8 (rabbit monoclonal, 1:100; Abcam, Cambridge, UK), or IL-1β (rabbit polyclonal, 1:100; Thermo Fisher Scientific). After washing, HRP-conjugated secondary antibodies (goat anti-mouse or anti-rabbit, 1:2000; Santa Cruz Biotechnology, Dallas, TX, USA) were applied for 1 h. Detection was performed using Amersham ECL Plus (GE Healthcare Europe GmbH, Milan, Italy), and signals were visualized with Amersham Hyperfilm ECL. For secreted cytokines, Coomassie Blue staining (R250, Biorad) of the SDS-PAGE gels was performed prior to the immunodetection procedure to verify uniform protein loading and to enable semi-quantitative comparisons. For CFTR expression, membranes were stripped (62.5 mM Tris–HCl pH 6.8, 10% SDS, 1% β-mercaptoethanol, 55 °C for 30 min) and re-probed with anti-actin antibody (polyclonal, 1:2000; Merck) to ensure equal protein loading. For CFTR quantification, band intensities were normalised to actin from the same lane on the re-probed membranes. For secreted cytokines, the band intensities were normalized to the corresponding Coomassie-stained lanes. Densitometric analysis

was performed using ImageJ software (version 1.53t, NIH, Bethesda, MD, USA) [48]. All values were expressed relative to the Fsk-treated LPS-unstimulated sample, which was set to 1. At least four biological replicates were analyzed per condition.

As part of the phenotypic characterization of the CFPAC-1 model grown under air–liquid interface (ALI) conditions, dot blot analysis was performed to assess mucin secretion. The protein concentration of ASF was measured using the Bradford assay. Control untreated ASF was concentrated using Amicon Ultra-0.5 mL centrifugal filter units, and an aliquot of 10 μL (approximately 20 μg of total protein) was spotted directly onto nitrocellulose membranes (Amersham Protran, GE Healthcare). The membranes were air-dried at room temperature and incubated overnight at 4 °C with primary antibodies against MUC1 (mouse monoclonal, 1:500, Thermo Fisher Scientific, Waltham, MA, USA) and MUC5B (rabbit polyclonal, 1:200, Thermo Fisher Scientific) in PBS-T. After washing with PBS-T, the membranes were incubated with HRP-conjugated anti-mouse or anti-rabbit secondary antibodies (1:2500) for 1 h at room temperature. Membranes were processed using standard immunodetection procedures. To verify the specificity of the antibodies, dot blot assay was also performed using 10 μL of concentrated (2 μg/μL) apical surface liquid (ASL) collected from Calu-3 cells, cultured under the ALI condition, while 10 μL of concentrated (2 μg/μL) culture medium collected from HEK-293 cells cultured under standard conditions was used as a negative control due to the lack of mucus production in these cells.

2.8. Multiple Particle Tracking (MPT) Analysis

To evaluate the microrheological properties of the apical surface fluid (ASF), the MPT assay was performed on ASF samples collected from ALI-cultured CFPAC-1 cell layers [45–47,49,50]. A 25 μL sample of ASF was mixed with 1 μL of yellow-green fluorescent carboxylated polystyrene beads (200 nm diameter, λ_exc = 488 nm; λ_em = 505–515 nm, Life Technologies, Monza, Italy). A total of 8 μL of the mixture was sealed between glass coverslips to prevent evaporation and equilibrated for 20 min at room temperature. All MPT measurements were performed at room temperature (approximately 22–24 °C), with consistent conditions applied to all samples to ensure comparability across treatments. The beads were then imaged at mid-height using a 60× oil-immersion objective (NA 1.42) connected to a CCD camera. Videos (1280 × 960 pixels) were acquired at 5 frames/s. Brownian trajectories were analyzed using the Multitracker plug-in of the ImageJ software [48], tracking ~400 beads from 4 to 8 fields per sample. The movement of the fluorescent beads within the ASF in a given time interval, τ, is described by its mean squared displacement, <msd>, from which it is possible to compute the diffusion coefficient D_0, according to the following equation:

$$\langle \mathrm{msd}(\tau) \rangle = 4\, D_0 \tau^{\alpha}, \quad (2)$$

where τ and α represent the time interval and the elastic contribution of the fluid ($0 < \alpha \leq 1$), respectively.

The microviscosity (η) was calculated via the Stokes–Einstein equation:

$$\eta = K_B\, T / 6\pi\, D_0\, r, \quad (3)$$

where K_B is Boltzmann's constant, T is absolute temperature, and r is the bead radius. Microviscosity values were expressed in centipoise (cP), where 1 cP = 0.001 Pa·s. This unit reflects a model-derived estimation of fluid microviscosity based on the thermal diffusion behavior of the probe particles.

2.9. Statistics

Initial ANOVA testing was performed to assess variability within and between groups. Once homogeneity had been confirmed, data from 4–12 replicates per condition were pooled. Each experiment was independently repeated using at least three distinct cell culture passages to ensure biological reproducibility. Sample sizes were chosen to provide sufficient resolution for detecting treatment-dependent differences while maintaining technical feasibility within the constraints of in vitro experimentation. The results are presented as mean ± standard error of the mean (SEM). All statistical and MPT analyses were performed using IgorPro 9 software (WaveMetrics, Lake Oswego, OR, USA). Multiple comparisons were performed using the Kruskal–Wallis non-parametric ANOVA, followed by Dunn's post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. CFTR Modulators Enhance Transepithelial Conductance (TEC) in CFPAC-1 Epithelial Preparations

To evaluate the ability of CFTR modulators to restore CFTR functional expression in CFPAC-1 epithelial preparations, transepithelial electrical conductance (TEC) was measured before and after treatment with the potentiator VX770 and the correctors VX661 and VX445, administered either individually or in combination. With the exception of the DMSO control condition, the cAMP activator forskolin (Fsk) was included in all other experimental conditions. The specificity of the CFTR-mediated response was verified using the CFTR inhibitor PPQ102. Pro-inflammatory conditions were modelled by preconditioning the epithelial preparations with lipopolysaccharide (LPS).

CFPAC-1 cells cultured on permeable supports formed confluent layers, exhibiting a baseline TEC of 3033 ± 29.51 $\mu S/cm^2$ (n = 72). This corresponded to a transepithelial electrical resistance (TEER) of approximately 330 ± 3.2 $\Omega \cdot cm^2$, a value consistent with those reported by other research groups under comparable experimental conditions [35].

In the absence of LPS, Fsk alone induced a modest increase in conductance (ΔTEC = 78.19 ± 27.25 $\mu S/cm^2$, n = 8) with respect to DMSO (ΔTEC = 30.68 ± 13.45 $\mu S/cm^2$, n = 12). Similarly, treatment with Fsk + VX770 (hereafter referred to as VX770) produced a comparable, though non-significant, increase in conductance (ΔTEC = 199.42 ± 20.40 $\mu S/cm^2$, n = 12). In contrast, dual combinations of CFTR potentiator with either VX661 or VX445 (hereafter referred to as VX661 and VX445, respectively) led to significantly greater increases in TEC (ΔTEC = 498.64 ± 40.97 $\mu S/cm^2$, n =11 and ΔTEC = 544.36 ± 28.26 $\mu S/cm^2$, n = 10, respectively). The triple combination VX661 + VX445 + VX770 (hereafter referred to as VX661 + VX445) produced the greatest increase in epithelial conductance (ΔTEC = 844.42 ± 39.4 $\mu S/cm^2$, n = 12). As expected, inhibition of CFTR function with PPQ102 significantly decreased TEC (ΔTEC = −298.05 ± 42.12 $\mu S/cm^2$, n = 8). Figure 1 and Table S1 summarize the obtained results, with Table S1 reporting the statistical comparisons performed between each CFTR modulator treatment and the DMSO-treated, LPS-unstimulated control group, together with the corresponding *p*-values.

Under the pro-inflammatory conditions induced by LPS, modulator responses were generally preserved or modestly enhanced. Forskolin alone increased conductance to a similar extent (ΔTEC = 98.23 ± 25.16 $\mu S/cm^2$, n = 12) as VX770 (ΔTEC = 188.41 ± 34.65 $\mu S/cm^2$, n = 12), with no statistically significant difference compared with the non-inflamed forskolin response. By contrast, VX661 (ΔTEC = 635.21 ± 26.2 $\mu S/cm^2$, n = 12), VX445 (ΔTEC = 673.59 ± 27.68 $\mu S/cm^2$, n = 12), and VX661 + VX445 (ΔTEC = 877.87 ± 30.43 $\mu S/cm^2$, n = 12) promoted significantly enhanced conductance values compared with forskolin alone under non-inflamed conditions. Notably, PPQ102 retained its inhibitory effect under pro-

inflammatory stress, leading to a marked decrease in conductance (−383.24 ± 33.37 μS/cm^2, n = 12, Figure 1 and Table S1).

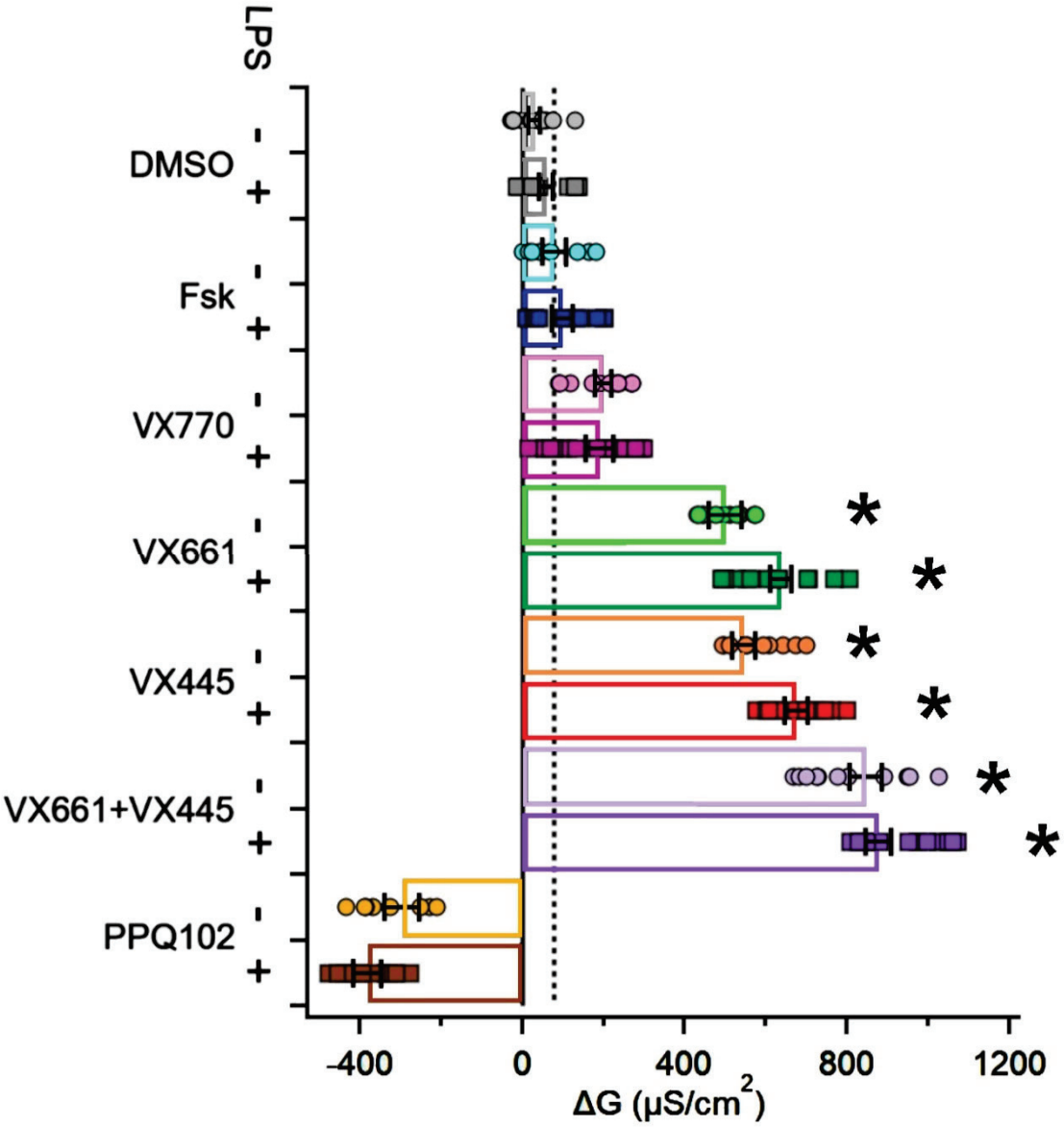
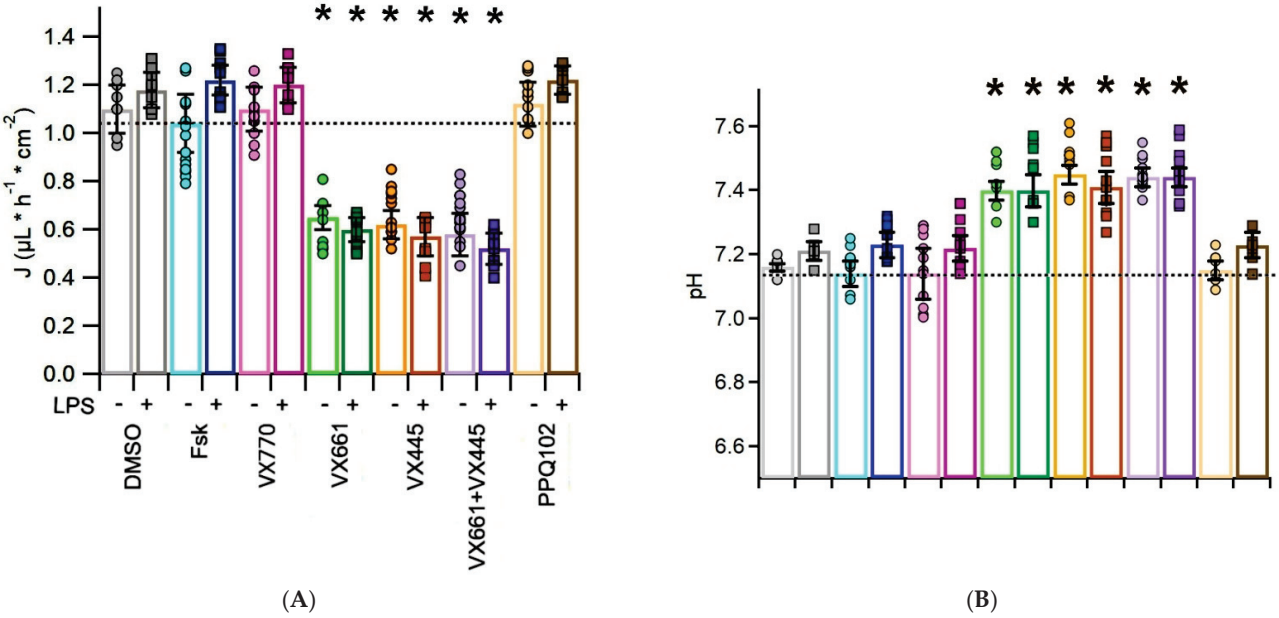


Figure 1. The effect of CFTR modulators on transepithelial electrical conductance (ΔTEC) in CFPAC−1 epithelial preparations, both with and without LPS stimulation. The Bar graph shows the changes in TEC (ΔG, μS/cm^2) measured in CFPAC−1 epithelial preparations after treatment with vehicle (0.1% DMSO), forskolin (Fsk, 20 μM), VX770 (1 μM), VX661 (5 μM), VX445 (5 μM), VX661 (5 μM) + VX445 (5 μM) or the CFTR inhibitor PPQ102 (30 μM). Each condition was tested in the absence (−) or presence (+) of lipopolysaccharide preconditioning (LPS, 10 μg/mL, 24 h). Bars represent the mean ± standard error of the mean (SEM) of at least n = 8 independent experiments. Circles (−LPS) and squares (+LPS) indicate individual data points. Statistical comparisons were performed using the Kruskal–Wallis ANOVA followed by Dunn's post hoc test. Comparisons were made versus the Fsk−treated, LPS-unstimulated group, whose mean value is represented by the black dashed line. Asterisks (*) indicate statistically significant differences (* $p < 0.05$).

3.2. CFTR Modulators Reduce Apical Surface Fluid Reabsorption in CFPAC-1 Monolayers

To evaluate the effects of CFTR modulators on apical fluid dynamics, the rate of apical fluid reabsorption (J, expressed in μL·h^{-1}·cm^{-2}) was measured in CFPAC-1 epithelial preparations following treatment with CFTR modulators, in both the presence and absence

of the pro-inflammatory stimulus LPS. In this experimental model, CFTR activation leads to chloride and fluid secretion toward the apical compartment, resulting in a reduction of net fluid reabsorption; therefore, lower J values indicate improved CFTR function. The results are summarized in Figure 2A and Table S2, with Table S2 also providing the statistical comparisons between each CFTR modulator treatment and the DMSO-treated, LPS-unstimulated control group, together with the associated *p*-values.



(**A**) (**B**)

Figure 2. CFTR modulators improve fluid reabsorption and alkalinize the pH of the apical surface fluid (ASF) of CFPAC−1 epithelial layers. Bar graphs showing (**A**) Fluid reabsorption rate (J) and (**B**) the pH of the ASF pH in CFPAC−1 epithelial layers treated with vehicle (0.1% DMSO), Fsk (20 µM), VX770 (1 µM), VX661 (5 µM), VX445 (5 µM), their combination (5 µM VX661 + 5 µM VX445), or the CFTR inhibitor PPQ102 (30 µM). Experiments were conducted in the absence (−) or presence (+) of lipopolysaccharide (LPS, 10 µg/mL, 24 h). Fluid reabsorption was measured gravimetrically while the pH was assessed using a microelectrode. Bars represent the mean ± standard error of the mean (SEM) of at least n = 6 replicates. Circles (−LPS) and squares (+LPS) indicate individual data points. Statistical comparisons were performed using the Kruskal–Wallis ANOVA followed by Dunn's post hoc test. Comparisons were made versus the Fsk−treated, LPS−unstimulated group, whose mean value is represented by the black dashed line. Asterisks (*) indicate statistical significance (* $p < 0.05$).

Treatment with VX770 yielded a mean absorption rate of 1.10 ± 0.09 µL·h^{-1}·cm^{-2} (n = 10), which was comparable to the rates observed with Fsk (1.04 ± 0.12 µL·h^{-1}·cm^{-2}, n = 8) and DMSO (1.1 ± 0.10 µL·h^{-1}·cm^{-2}, n = 9). This indicates that VX770 alone did not significantly stimulate CFTR-mediated reabsorption in CFPAC-1 cells, consistent with the absence of corrected CFTR at the plasma membrane. In contrast, the addition of CFTR correctors significantly reduced fluid reabsorption. Cells treated with VX661 or VX445 displayed markedly lower J values (0.65 ± 0.05 µL·h^{-1}·cm^{-2}, n = 8 and 0.62 ± 0.06 µL·h^{-1}·cm^{-2}, n = 10, respectively), while the combination VX445 + VX661 had the most pronounced reduction in fluid reabsorption (0.58 ± 0.09 µL·h^{-1}·cm^{-2}, n = 12), reflecting enhanced CFTR activity.

In the presence of LPS, the baseline absorption (Fsk + LPS) increased to 1.22 ± 0.06 µL·h^{-1}·cm^{-2} (n = 10), suggesting that the inflammatory environment may alter epithelial transport dynamics subtly, possibly favoring increased apical fluid accumulation. Similarly, the response to DMSO and VX770 was not improved by LPS, with J values of 1.18 ± 0.07 µL·h^{-1}·cm^{-2} (n = 10) and 1.20 ± 0.07 µL·h^{-1}·cm^{-2} (n = 10), respectively.

Notably, LPS preconditioning resulted in significant reductions in J in all corrector-treated conditions, reaching 0.60 ± 0.05 $\mu L \cdot h^{-1} \cdot cm^{-2}$ (n = 12) with VX661, 0.57 ± 0.08 $\mu L \cdot h^{-1} \cdot cm^{-2}$ (n = 12) with VX445, and 0.52 ± 0.06 $\mu L \cdot h^{-1} \cdot cm^{-2}$ (n = 12) with VX661 + VX445. Finally, the CFTR inhibitor PPQ102 reversed the fluid absorption effect when administered after Fsk + VX770 stimulation, both in the absence and presence of LPS. Indeed, it yielded J values comparable to those of Fsk-treated, LPS unstimulated samples: 1.12 ± 0.09 $\mu L \cdot h^{-1} \cdot cm^{-2}$ (n = 6) and 1.22 ± 0.06 $\mu L \cdot h^{-1} \cdot cm^{-2}$ (n = 9), respectively.

3.3. CFTR Modulators Increase the pH of the Apical Surface Fluid in CFPAC-1 Cells, with Additive Effects in the Presence of LPS

To investigate ion transport and bicarbonate secretion in CFPAC-1 cell layers, the ASF was carefully collected and its pH recorded immediately using a microelectrode [46,47].

In the absence of LPS, the pH of DMSO-treated epithelia was 7.16 ± 0.01 (n = 8). Fsk and VX770 treatment did not significantly modify the pH (7.14 ± 0.04, n = 8 and 7.14 ± 0.08, n = 12, respectively). Conversely, treatment with VX661 and VX445 increased the ASL pH to 7.4 ± 0.03 (n = 8) and 7.45 ± 0.04 (n = 12), respectively. The combination VX661 + VX445 further increased the pH to 7.44 ± 0.03 (n = 11). PPQ102 slightly reduced the ASL pH to 7.15 ± 0.03 (n = 7) (see Figure 2B and Table S3).

Following LPS preconditioning, the pH of the DMSO-treated fluid samples increased modestly to 7.21 ± 0.03 (n = 8) compared with the unstimulated LPS condition. Fsk and VX770 produced slight, non-significant increases in pH (7.23 ± 0.04, n = 12 and 7.22 ± 0.04, n = 11, respectively). VX661 (7.4 ± 0.05, n = 8), VX445 (7.41 ± 0.05, n = 12), and VX661 + VX445 (7.44 ± 0.03, n = 12) significantly increased the alkalinity of the ASF. Finally, under LPS stimulation, ASF samples treated with PPQ102 displayed a slightly higher pH (7.23 ± 0.04, n = 6) than under non-inflamed conditions (7.15 ± 0.03, n = 7), which may indicate LPS-induced modulation of pH-regulating pathways independently of CFTR. The results are summarized in Figure 2B and Table S3, which also reports the statistical comparisons performed between each CFTR modulator treatment and the DMSO-treated, LPS-unstimulated control group, together with the corresponding *p*-values.

Overall, these findings demonstrate that CFTR modulators enhance bicarbonate secretion and make the ASF more alkaline in F508del CFTR-expressing CFPAC-1 epithelial layers, and that a pro-inflammatory stimulus, such as LPS, further enhances this response.

3.4. CFTR Modulators Do Not Affect Total Protein Concentration in the Apical Surface Fluid (ASF) of CF-PAC1 Epithelial Layers

The total protein concentration in the ASF was quantified using the Bradford assay and expressed in $\mu g/\mu L$. To avoid procedures requiring destructive sampling, which would compromise the integrity of ALI-cultured epithelial layers, normalization of the protein content to cell number or surface area was not performed. Therefore, protein values were reported relative to apical fluid volume only. In the absence of LPS, apical fluid collected from Fsk-treated CFPAC-1 layers displayed an average protein concentration of 1.36 ± 20.21 $\mu g/\mu L$ (n = 5). No significant differences were observed among the different treatment groups, indicating that CFTR modulators did not affect overall protein secretion under basal conditions (see Table 1).

Following LPS preconditioning, a significant increase in apical protein concentration was observed in all experimental groups compared to the Fsk-treated, LPS-unstimulated group. Specifically, the average protein concentration in the ASF of Fsk-treated layers exposed to LPS was 1.68 ± 22.53 $\mu g/\mu L$ (n = 6). However, no significant differences were observed among the different treatments within the LPS-stimulated conditions, suggesting that the modulators do not differentially impact protein secretion during inflammation.

Table 1. Total protein concentration in the ASF of CFPAC-1 epithelial layers following treatment with CFTR modulators, in the absence or presence of LPS. Total protein concentration in the ASF was measured using the Bradford assay and expressed in μg/μL. The CFPAC-1 layers were treated for 24 h with the following: 0.1% DMSO (vehicle control), 20 μM Fsk, 1 μM VX770, 5 μM VX661, 5 μM VX445, a combination of 5 μM VX661 + 5 μM VX445, or the CFTR inhibitor PPQ102 (30 μM). This was done in the absence (−LPS) or presence (+LPS) of lipopolysaccharide (LPS, 10 μg/mL, for 24 h). Data are reported as mean ± SEM, with the number of independent replicates indicated in parentheses. Statistical comparisons were performed using the Kruskal–Wallis ANOVA followed by Dunn's post hoc test. Comparisons were made versus the Fsk-treated, LPS-unstimulated group. Asterisks (*) denote statistically significant differences ($p < 0.05$).

	DMSO	Fsk	VX770	VX661	VX445	VX661 + VX445	PPQ102
−LPS	1.29 ± 29.79 (5)	1.36 ± 20.21 (5)	1.40 ± 42.26 (5)	1.28 ± 37.38 (5)	1.28 ± 39.63 (4)	1.44 ± 29.50 (5)	1.31 ± 39.65 (5)
+LPS	1.78 ± 59.96 (4) *	1.68 ± 22.53 (6) *	1.83 ± 44.23 (4) *	1.78 ± 31.22 (4) *	1.97 ± 40.29 (4) *	1.87 ± 19.60 (5) *	1.72 ± 27.83 (4) *

3.5. CFTR Modulators Enhance Endogenous F508del CFTR Levels in CFPAC-1 Cells but Do Not Reduce Pro−Inflammatory Cytokine Expression

Western blot analysis was performed to evaluate the expression levels of endogenous F508del CFTR protein in CFPAC-1 whole cell lysates from CFPAC-1 epithelial preparations, as well as of IL-6, IL-8 and IL-1β, pro-inflammatory cytokines secreted into the basolateral medium [28,51–55].

The F508del CFTR isoform was detected by the monoclonal antibody MM13-4, directed against the CFTR N-terminus, as two electrophoretic bands: band B (~160 kDa), corresponding to the immature, core-glycosylated form retained in the endoplasmic reticulum (ER), and band C (~180 kDa), corresponding to the mature, fully glycosylated isoform processed through the Golgi apparatus. As expected, CFPAC-1 cells predominantly expressed the immature CFTR band B, consistent with the severe folding and trafficking defect associated with the F508del mutation (see first lane of the upper panel in Figure 3A,B). Densitometric analysis revealed that treatment with Fsk or VX770 did not significantly modify the total CFTR expression (sum of bands B + C) or maturation rate (ratio of band C to the sum of bands B + C). In contrast, treatment with VX661 and VX445, and particularly their combination (VX661 + VX445), significantly increased both the total expression and maturation rate of F508del CFTR (see Figure 3C,D). Notably, the effect of correctors also included a marked increase in the immature CFTR band B, suggesting that treatment may enhance overall CFTR protein expression, potentially through effects on protein synthesis and/or stability (see Figure 3A,B).

Although the effects of CFTR modulators in LPS-preconditioned samples did not lead to a statistically significant increase in CFTR total protein expression or maturation rate compared with LPS non-preconditioned samples, the modulators remained effective under inflammatory conditions, indicating that their corrective activity is preserved in a pro-inflammatory epithelial environment. Specifically, under LPS preconditioning, Fsk or VX770 alone had no effect on CFTR expression or processing. Treatments with VX661 and VX445 produced comparable levels of CFTR rescue, whereas their combination exhibited the most robust effect, increasing total CFTR expression by approximately threefold compared with Fsk-treated, non-LPS-stimulated samples (see Figure 3C,D). Quantitative data

for CFTR total expression and maturation rate, normalized to actin (used as a housekeeping protein), and referred to the Fsk-treated LPS-unstimulated whole cell lysates (set to 1), are reported in Table S4, which also provides the statistical comparisons between each CFTR modulator treatment and the DMSO-treated, LPS-unstimulated control group, together with the associated *p*-values.

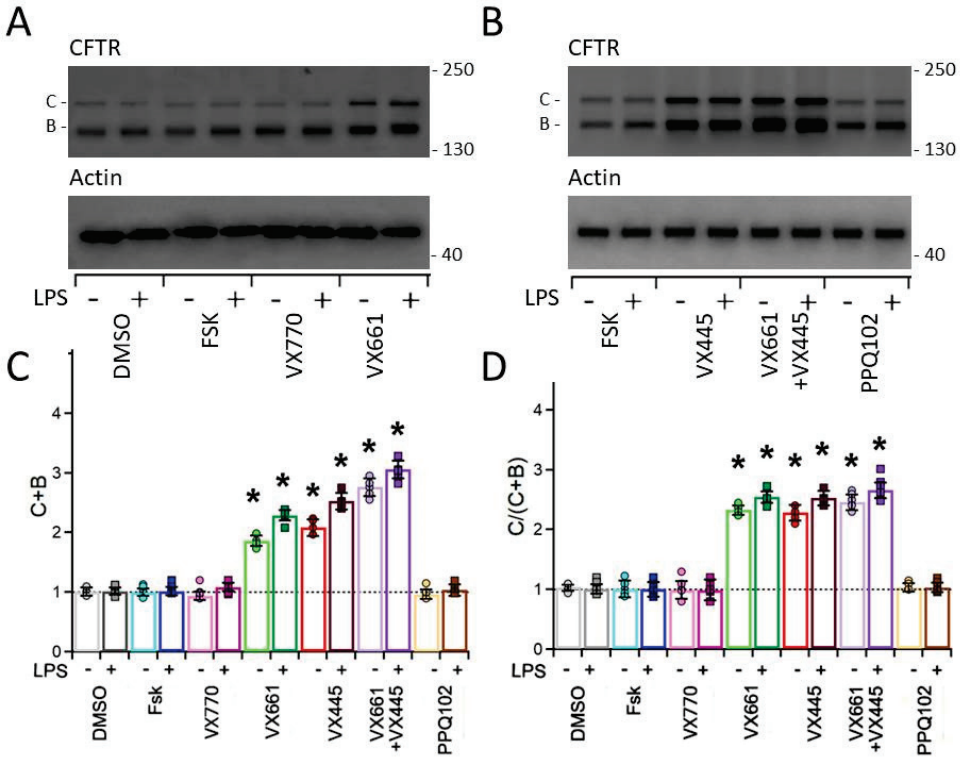


Figure 3. Detection and quantification of F508del CFTR protein expression in CFPAC−1 epithelial layers by Western blot analysis. (**A**,**B**) Representative Western blots showing F508del CFTR expression in whole-cell lysates from CFPAC-1 epithelial layers treated with vehicle control (0.1% DMSO), forskolin (Fsk, 20 μM), VX770 (1 μM), VX661 (5 μM), VX445 (5 μM), VX661 (5 μM) + VX445 (5 μM) combination, and PPQ102 (30 μM). The upper panels show the F508del CFTR protein detected using the monoclonal antibody MM13-4, which is directed against the CFTR N-terminus. The lower panels illustrate the immunoblotting of actin, which was used as a loading control. Molecular weight markers (in kDa) are indicated to the left of each blot. The positions of the immature, core-glycosylated ER-resident form (band B) and the mature, fully glycosylated form (band C) of CFTR are also indicated on the right of each blot. (**C**,**D**) Bar graphs showing the densitometric quantification of total CFTR expression (the sum of bands B + C) and the CFTR maturation rate (the ratio of the mature band C to the total CFTR bands C/(C + B)). Band intensities were normalised to actin and expressed relative to Fsk-treated, LPS-unstimulated samples, whose mean was set at 1 (black dashed line). Bars represent the mean ± SEM from at least four independent experiments. Individual data points are indicated by circles (−LPS preconditioning) and squares (+LPS preconditioning). Statistical comparisons were performed using the Kruskal–Wallis non-parametric analysis of variance followed by Dunn's post hoc test. Comparisons were made versus the Fsk-treated, LPS-unstimulated group, whose mean value is represented by the black dashed line. Asterisks (*) indicate statistically significant differences (* $p < 0.05$).

Under basal conditions (DMSO and Fsk treatments), the levels of IL-6, IL-8, and IL-1β detected in the concentrated basolateral media were relatively low, indicating minimal spontaneous inflammatory activity. Cytokine expression in samples treated with CFTR modulators (VX770, VX661, and VX445, either individually or in combination) did not differ significantly from those treated with DMSO and Fsk (see Figure 4A–F). Following stimulation with LPS, CFPAC-1 epithelial layers treated with DMSO and Fsk exhibited significantly higher levels of IL-6, IL-8, and IL-1β secreted in the basolateral medium com-

pared with non-stimulated controls (see Figure 4A–F). Notably, cytokine levels remained elevated in samples treated with CFTR modulators (VX770, VX661, VX445, either individually or in combination) and were comparable to those observed in samples treated with DMSO- and Fsk- samples stimulated with LPS (see Figure 4A–F). These findings confirm that LPS effectively induces a pro-inflammatory response in CFPAC-1 epithelia. Furthermore, under these specific conditions of pro-inflammatory stimulation (10 μg/mL LPS, 24 h), our data demonstrate that CFTR modulators did not exert detectable anti-inflammatory effects on IL-6, IL-8, and IL-1β cytokine production in CFPAC-1 epithelial layers. Semi-quantitative data for interleukins, normalized to the Coomassie-stained gels of concentrated basolateral medium from CFPAC-1 cultures and expressed relative to the Fsk-treated, LPS-unstimulated whole-cell lysates (set to 1), are reported in Table S5. The table also includes the statistical comparisons between each CFTR modulator treatment and the DMSO-treated, LPS-unstimulated control group, together with the corresponding *p*-values. A representative Coomassie Blue staining of the gels used for protein normalization is shown in Figure S1, illustrating the quality and uniformity of protein loading across the samples.

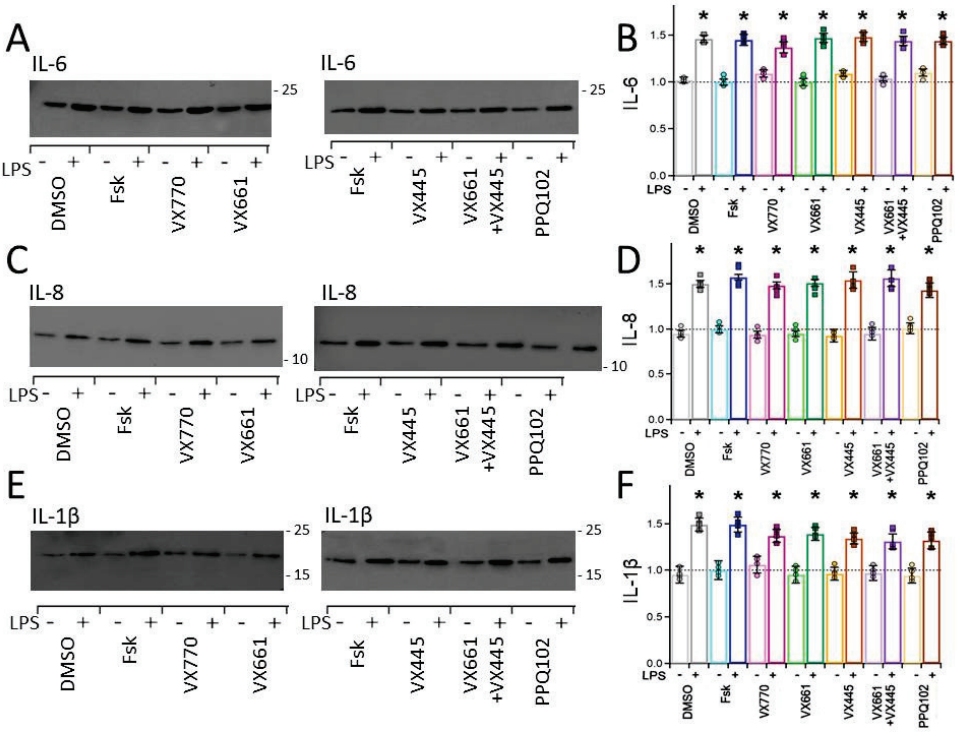


Figure 4. Detection of interleukins secreted by ALI−cultured CFPAC−1 epithelial layers. Representative Western blots demonstrating the expression of (**A**) IL−6, (**C**) IL−8, and (**E**) IL−1β in concentrated basolateral medium from CFPAC−1 epithelial layers that were treated with vehicle (0.1% DMSO), forskolin (Fsk, 20 μM), VX770 (1 μM), VX661 (5 μM), VX445 (5 μM), VX661 (5 μM)+ VX445 (5 μM) combination, and PPQ102 (30 μM). Molecular weight markers (in kDa) are indicated to the left of each blot. Densitometric quantification of (**B**) IL−6, (**D**) IL−8, and IL−1β (**F**) expression levels. Band intensities were normalised to the Coomassie signal of the corresponding SDS−PAGE gels and expressed relative to Fsk−treated LPS−unstimulated samples, whose mean was set at 1 and indicated by the black dashed line. Bars represent the mean ± SEM from at least four independent experiments. Individual data points are shown as circles for −LPS preconditioning and squares for +LPS preconditioning. Statistical analysis was assessed using Kruskal–Wallis non−parametric analysis of variance followed by Dunn's post hoc test. Comparisons were made versus the Fsk−treated, LPS−unstimulated group. Asterisks (*) indicate statistically significant differences (* $p < 0.05$).

3.6. CFTR Modulators Promote Mucin Secretion in the Apical Surface Fluid (ASF) of CFPAC-1 Epithelial Preparations

To verify the secretory phenotype of CFPAC-1 epithelial layers grown under ALI conditions, dot blot analyses were performed on concentrated ASF collected from DMSO-treated control cultures. Dot blot analysis revealed the presence of mucin MUC1 but not of MUC5B in the ASF collected from CFPAC-1 epithelia cultured under the ALI conditions (see the first spots in the upper and lower blots of Figure S2), confirming that CFPAC-1 cells can produce and secrete organ-specific mucins at the apical surface under ALI conditions [56–60]. To validate the reliability of mucin detection, parallel dot blot analyses were performed using the apical fluid collected from ALI-cultured Calu-3 bronchial epithelial cells, a well-established mucus-producing cell line [61,62]. As expected, strong signals for MUC1 (second spot in the upper blot of Figure S2, [63–65]) and MUC5B (second spot in the lower blot of Figure S2) were detected in the ASL samples from Calu-3 cells, confirming the specificity of the antibodies used and the reliability of the assay [63,64,66]. Conversely, no mucin signals were detected in the culture medium collected from HEK-293 cells (see the third spot of the upper and lower blots of Figure S2), which served as a negative control due to their non-mucus-producing phenotype. This further validated the specificity of the mucin detection protocol.

3.7. CFTR Modulators Decrease the Viscosity of the Apical Surface Fluid (ASF) in CFPAC-1 Epithelial Layers

The rheological properties of the ASF secreted by CFPAC-1 epithelial layers were evaluated using the multiple particle tracking (MPT) technique [37–42]. In samples treated with DMSO or Fsk, the recorded microviscosity values were markedly elevated (2.75 ± 0.03, n = 4 and 2.62 ± 0.06, n = 5, respectively), which is indicative of a dense and poorly hydrated fluid layer covering the apical surface. Figure 5 and Table S6 report the quantitative data of ASF microviscosity under the different treatment conditions, with Table S6 also presenting the statistical comparisons versus the DMSO-treated, LPS-unstimulated control group, together with the corresponding *p*-values. Treatment with the CFTR potentiator VX770 alone did not significantly alter microviscosity (2.61 ± 0.01, n = 5), suggesting that enhancement of channel gating alone is insufficient to restore fluid homeostasis in the absence of prior correction of CFTR trafficking to the apical membrane (see Figure 5 and Table S6). In contrast, treatment with CFTR correctors VX661 and VX445, administered either individually or in combination, led to a significant reduction in ASF microviscosity (Figure 5 and Table S6). This effect reflects improved hydration of the epithelial surface, likely due to enhanced CFTR rescue and function. This facilitates more effective apical surface fluid clearance. Among the treatments tested, the VX661 + VX445 combination elicited the most significant decrease in microviscosity, indicating a synergistic corrective effect (1.83 ± 0.02, n = 5, 1.69 ± 0.02, n = 4 and 1.45 ± 0.03, n = 4, for VX661, VX445 and VX661 + VX445, respectively). Notably, the beneficial effects of CFTR correctors on ASF microviscosity were preserved under inflammatory conditions induced by LPS preconditioning. This suggests that the efficacy of CFTR modulators is not substantially impaired by the pro-inflammatory environment (see Figure 5 and Table S5). In contrast, treatment with the selective CFTR inhibitor PPQ102 significantly increased ASF microviscosity under both basal and LPS-stimulated conditions (2.89 ± 0.11, n = 5 and 2.73 ± 0.14, n = 5, respectively), restoring values to levels comparable to or even higher than those observed in DMSO controls (see Figure 5 and Table S6). These findings confirm that the rheological improvements of ASF observed following modulator treatment are directly attributable to restored CFTR activity.

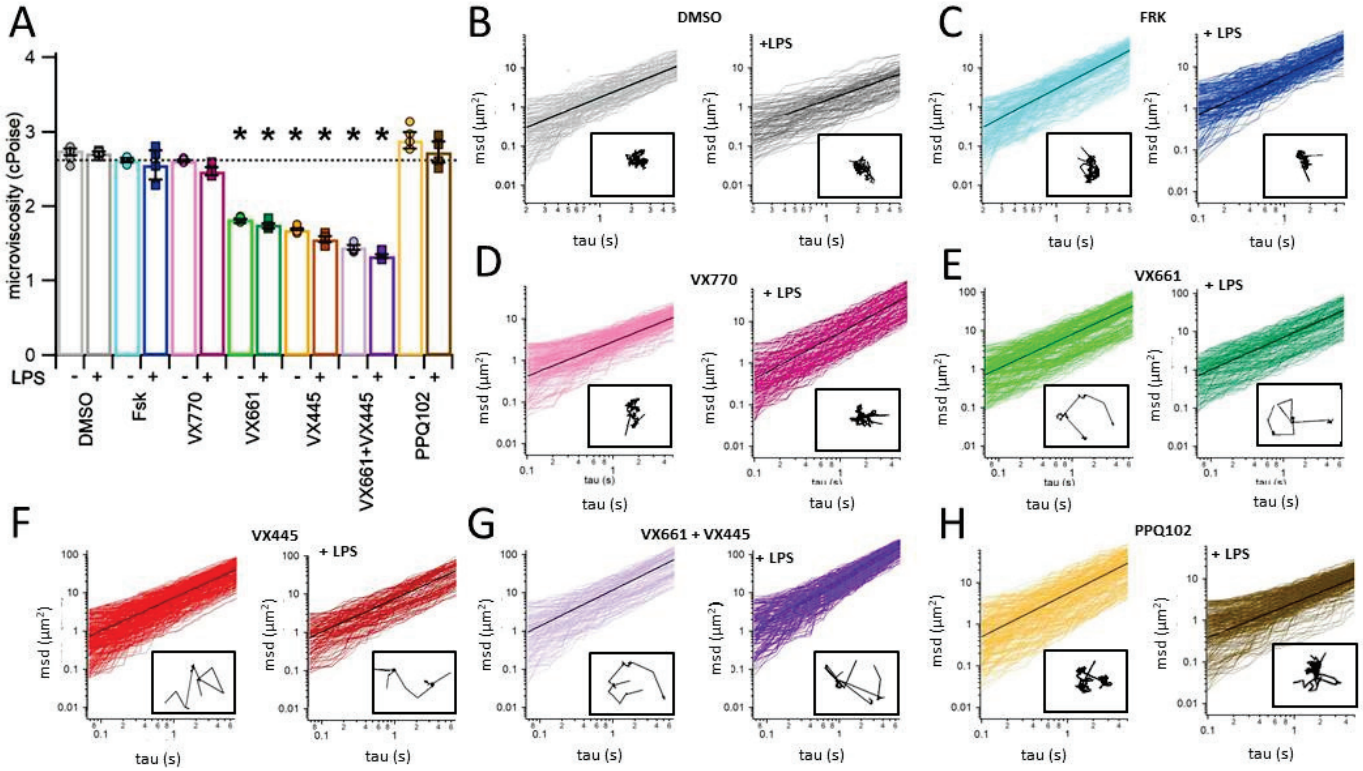


Figure 5. Microviscosity analysis of the apical surface fluid (ASF) secreted by ALI−cultured CFPAC−1 epithelial layers, as measured using multiple particle tracking (MPT). (**A**) Bar graph showing the microviscosity of ASF collected from the apical surface of CFPAC−1 layers treated with vehicle (0.1% DMSO), forskolin (Fsk, 20 µM), the CFTR potentiator VX770 (1 µM), correctors VX661 (5 µM) and VX445 (5 µM), their combination (VX661, 5 µM + VX445, 5 µM), or the CFTR inhibitor PPQ102 (30 µM). Treatments were performed in the absence (−LPS) or presence (+LPS) of lipopolysaccharide (10 µg/mL, 24 h) to mimic pro−inflammatory conditions. Bars represent the mean ± standard error of the mean (SEM) of at least n = 6 replicates, with individual data points indicated by circles (−LPS) and squares (+LPS). Statistical analysis was assessed using Kruskal–Wallis non−parametric analysis of variance followed by Dunn's post hoc test. Comparisons were made versus the Fsk−treated, LPS−unstimulated group, whose mean value is represented by the black dashed line. Asterisks (*) indicate statistically significant differences (* $p < 0.05$). (**B**–**H**) Representative plots showing the mean square displacement (msd) versus time interval (tau) for fluorescent beads embedded in ASF samples under each experimental condition: 0.1% DMSO (**B**), 20 µM Fsk (**C**), 1 µM VX770 (**D**), 5 µM VX661 (**E**), 5 µM VX445 (**F**), 5 µM VX661 + 5 µM VX445 (**G**), and 30 µM PPQ102 (**H**). Black lines represent the average msd traces. For each treatment, the plots on the left illustrate experiments performed without LPS, and the plots on the right represent conditions with LPS stimulation (+LPS). The insets show the trajectory of a bead acquired during the MPT analysis.

4. Discussion

Cystic fibrosis (CF) is a multisystemic disorder caused by mutations in the CFTR gene, leading to defective chloride and bicarbonate transport across epithelial surfaces. CFTR defects result in dehydrated, viscous secretions and progressive injury in different organs [1–5]. While lung disease is the leading cause of morbidity and mortality, the pancreas is the second most severely affected organ in CF. Ductal obstruction, fibrosis, and eventual fibrotic degeneration frequently culminate in exocrine pancreatic insufficiency, affecting the majority of individuals with CF [13,14]. Despite its clinical relevance, pancreatic involvement in CF has received comparatively less attention in in vitro studies than airway pathology.

The advent of CFTR modulators, small molecules that enhance CFTR protein folding, trafficking, and function, has revolutionized the management of CF [67]. In particular, the

triple combination therapy comprising the potentiator ivacaftor (VX770) and the correctors tezacaftor (VX661) and elexacaftor (VX445), commercially known as Trikafta in the US and Kaftrio in Europe, has led to substantial clinical benefits. These include marked improvements in lung function, nutritional status, and quality of life, especially in individuals carrying at least one copy of the F508del mutation [10,68,69]. Despite these advances, the majority of preclinical research on these transformative therapies has concentrated on airway-derived epithelial models, leaving their effects on extra-pulmonary tissues, such as the pancreas, less thoroughly explored.

To address this knowledge gap, we used a multidisciplinary approach to investigate the effects of CFTR modulators VX770, VX661, and VX445 in an in vitro model of the pancreatic duct epithelium. We utilized CFPAC-1 cells, a human pancreatic ductal cell line derived from a CF patient that endogenously expresses the F508del CFTR mutation [33]. These cells retain several key characteristics of pancreatic ductal epithelium, including polarization [34,35], active ion and fluid transport [34–37], and mucin secretion [52]. Our primary aim was to determine whether the treatment with the CFTR modulators could restore critical physiological parameters in this CF pancreatic epithelial model, including transepithelial conductance (ΔTEC), fluid absorption (Jw), apical surface fluid pH, protein concentration, and microviscosity. Additionally, we aimed to evaluate whether a pro-inflammatory micro-environment could influence the efficacy of these modulators. In line with their distinct mechanisms of action, Fsk and VX770 were applied acutely, 10 min before measurements, to both the apical and basolateral compartments, whereas the correctors VX661 and VX445 (alone or in combination) were administered for 24 h prior to functional assays. This protocol reflects an established in vitro experimental approach whereby correctors, which act on CFTR folding and trafficking, require prolonged exposure, whereas the potentiator VX770, which exerts its effect on the gating properties of already membrane-localized CFTR, is effective following short-term application. Although this regimen does not directly mirror the chronic systemic administration of the triple therapy in patients, it is widely adopted in in vitro models to dissect individual drug effects on CFTR function [70,71].

Consistent with its role as a potentiator, VX770 alone produced minimal functional effects in the absence of prior CFTR correction. In contrast, the correctors VX661 and VX445 significantly improved CFTR-dependent ion transport, fluid reabsorption, apical surface fluid pH and microviscosity (see Figures 1–5). Notably, the combination of VX661, VX445, and the acutely applied VX770 in the presence of Fsk yielded the most pronounced functional rescue across all measured parameters. However, it is important to clarify that these maximal effects likely reflect the full potentiating capacity of CFTR under the specific in vitro conditions employed, wherein both VX770 and Fsk were applied acutely. As such, this experimental design does not allow for a precise dissection of the individual contributions of Trikafta components and forskolin to the observed improvements. Further studies will be required to address this limitation. Nevertheless, the present results demonstrate that mutant CFTR expressed in pancreatic epithelial layers is pharmacologically responsive to modulators, similarly to what has been reported in airway models [47,72–75].

To simulate the chronic inflammatory environment typically found in the lungs of CF patients [76–78], human bronchial epithelial (HBE) in vitro models have been exposed to different stimuli, including lipopolysaccharide (LPS) [79], IFN-γ [80], TNF-α [81], interleukin-17 (IL-17) [82,83] bronchoalveolar lavage fluid (BALF) from pediatric CF patients, and supernatant from mucopurulent material (SMM) collected from explanted CF lungs [84,85]. Under these conditions, epithelial integrity and transepithelial electrical resistance are maintained, thus validating the models' suitability for pharmacological testing. Notably,

inflammatory conditioning has been shown to consistently enhance the efficacy of CFTR modulator combinations in restoring F508del-CFTR function [68,84–86].

Although the pancreas is traditionally considered a sterile organ, there is increasing evidence that CF-related ductal obstruction and epithelial injury can trigger localized inflammation, even in the absence of infection [87,88]. In pancreatic-sufficient (PS) patients, who account for approximately 10–15% of the CF population, episodes of acute or recurrent pancreatitis have been reported. By contrast, patients with pancreatic insufficiency (PI) tend to develop early and irreversible ductal fibrosis, which limits overt inflammatory manifestations [13,14,17,89]. Nevertheless, innate immune signaling likely contributes to disease progression in both subtypes [17,24–26,89]. To model the inflammatory aspect of CF pancreatic pathology in vitro, we exposed CFPAC-1 pancreatic ductal epithelial cell layers to 10 μg/mL LPS [30–32,90–92]. This concentration was selected based on preliminary experiments and on previous studies in epithelial and other mammalian cell models, demonstrating that 10 μg/mL LPS effectively induces a measurable inflammatory response, as evidenced by increased cytokine expression, without compromising cell viability [39–44].

Although LPS stimulation did not replicate infection per se, it served as a valuable strategy for simulating the pro-inflammatory microenvironment that may emerge in the CF pancreas in vitro.

Preconditioning with LPS did not reduce the therapeutic responses to VX770, VX661, or VX445. Conversely, we observed a trend towards improved outcomes, including increased ion transport, enhanced fluid absorption, a greater apical surface fluid pH alkalinization, and reduced ASF microviscosity (see Figures 1–5). These results mirror those observed in HBE models, in which inflammation enhances the functional rescue of F508del-CFTR, suggesting that pro-inflammatory stress may improve modulator efficacy in the pancreatic context [68,84,85,93–95]. Importantly, the observed enhancement in modulator efficacy under pro-inflammatory conditions may be mechanistically linked to changes in the cellular proteostasis network induced by LPS exposure. Indeed, LPS preconditioning in CFPAC-1 cells could facilitate CFTR folding and trafficking via inflammation-induced modulation of the ER environment, which would consequently improve the response to correctors' therapy.

Western blot analysis revealed that treating CFPAC-1 epithelial layers with CFTR correctors significantly increased the total expression and maturation rate of F508del CFTR (see Figure 3). These findings, particularly evident with the VX661 + VX445 combination, are consistent with those reported in HBE cells bearing the F508del mutation, in which corrector therapy leads to robust CFTR rescue and functional chloride channel activity at the apical membrane [10,73,96–98]. Interestingly, treatment with VX661 and VX445, especially in combination, resulted in an increased intensity of the immature band B, which contributes to the enhancement in total CFTR protein level. This may indicate a modulatory effect of correctors not only on CFTR processing and trafficking but also on its synthesis and/or post-translational stability. While this phenomenon has been rarely reported in airway epithelial models [71,73], where the primary action of correctors is generally limited to trafficking enhancement, our findings raise the possibility of a distinct modulatory response in pancreatic ductal cells. This implies that CFTR modulators may exert cell-type-specific effects in their mechanism of action. Further mechanistic studies will be required to clarify whether the observed increase in immature CFTR reflects enhanced translation, reduced proteasomal degradation, or other stabilizing mechanisms.

Importantly, pro-inflammatory preconditioning with LPS did not reduce the efficacy of the modulator treatment in rescuing F508del CFTR expression. On the contrary, we observed a slight yet consistent increase in total protein and maturation rate of mutant CFTR protein with VX661 and VX445, and an even greater enhancement with their combination.

These results are consistent with earlier studies in F508del HBE epithelia, where exposure to inflammatory stimuli, such as supernatant from mucopurulent material (SMM), was shown to increase the endoplasmic reticulum (ER) protein folding capacity and promote CFTR processing [99,100]. It has been proposed that inflammation-associated ER expansion and up-regulation of molecular chaperones in airway epithelial cells may create a more favorable environment for the folding of conformationally unstable proteins such as F508del-CFTR. Furthermore, exposure to a pro-inflammatory stimulus such as LPS has been shown to enhance the activity of the ER quality control system, contributing to an accelerated degradation and recycling of mutant CFTR in epithelial cells. By extending these observations to a pancreatic epithelial model, our results suggest that similar mechanisms may operate beyond the airways. LPS preconditioning in CFPAC-1 cells could facilitate CFTR folding and trafficking via inflammation-induced modulation of the ER environment, which would consequently improve the response to correctors' therapy.

TLR4, the canonical receptor for LPS, is expressed in the pancreatic ductal epithelium and activation of this pathway can trigger the secretion of cytokines [51,52,90–92]. In our study, LPS treatment resulted in a moderate, yet consistent, increase in the expression levels of pro-inflammatory cytokines IL-6, IL-8, and IL-1β (~1.5-fold, see Figure 4A–F). Although the responses observed in our model are not as robust as those seen in co-culture or airway-derived systems, the increase in IL-6, IL-8, and IL-1β expression confirms the activation of the pro-inflammatory pathways and validates the use of LPS as a stimulus for studying inflammation-modulated epithelial physiology in the pancreatic context [29–31].

In our experimental conditions, involving pre-exposure to 10 μg/mL LPS for 24 h, treatment with CFTR modulators, whether as single agents or in combination, did not reduce cytokine expression, suggesting that these drugs lack direct anti-inflammatory activity (see Figure 4A–F). These findings are consistent with those of previous studies that used primary human bronchial epithelial (HBE) cells expressing the F508del-CFTR, in which correctors and potentiators, including VX770/VX809 and VX770/VX661/VX445, failed to decrease cytokine levels following LPS or other inflammatory stimuli [101,102]. A few studies have reported partial reductions in the expression of pro-inflammatory cytokines, such as IL-8 or TNF-α, following CFTR modulator treatment [103]. However, such effects were often modest, and their reproducibility was limited, likely reflecting the influence of several experimental variables, including the timing and nature of the inflammatory challenge, the presence or absence of immune cells, and the cellular origin of the epithelial model employed. We acknowledge that our results should be interpreted in the context of a pre-established pro-inflammatory stimulation. Further studies exploring co-treatment protocols, different stages of inflammatory activation, LPS concentrations, and exposure durations will be instrumental in more precisely evaluating the immunomodulatory potential of CFTR modulators in pancreatic epithelial cells. Moreover, the absence of a measurable anti-inflammatory response in our study underscores the complexity of CF-related inflammation and supports the notion that restoring CFTR function alone may be insufficient to suppress epithelial cytokine production in the absence of immune-epithelial interactions. With all due limitations, our findings may have potential clinical relevance, as they suggest that adjunctive anti-inflammatory therapies could still be required to fully address the inflammatory burden in extra-pulmonary tissues such as the pancreas.

In summary, our findings reinforce the notion that CFTR modulators primarily act by rescuing defective F508del CFTR protein functional expression and restoring CFTR-mediated ion transport. Under the current in vitro conditions, which lack immune or stromal cell components, no measurable intrinsic anti-inflammatory activity was detected. Further studies are warranted to determine whether CFTR correction may indirectly in-

fluence inflammatory responses through crosstalk with immune cells in more complex co-culture systems or in vivo models.

Dot blot analysis was used to explore the mucin expression profile of CFPAC-1 epithelial preparations cultured in ALI under basal conditions (i.e., in the absence of pharmacological CFTR treatment or pro-inflammatory LPS stimulation). Although this approach was inherently qualitative and did not allow for rigorous quantification, it served to phenotypically characterize the secretory output of the epithelium. Among the mucins tested, MUC1, but not MUC5B, was detected in ASF samples (see Figure S2). This result is consistent with the established expression patterns of these macromolecules: MUC1 is a transmembrane mucin highly expressed in pancreatic ductal epithelium, whereas MUC5B is predominantly secreted by goblet cells in the respiratory and salivary glands [104]. This finding supports the physiological relevance of the CFPAC-1 system beyond CFTR expression alone and provides the basis for investigating mucus-related aspects of CF pathophysiology in the pancreatic context. Moreover, future studies assessing whether MUC1 secretion is modulated by CFTR correction could further elucidate the interplay between CFTR function and the secretory behavior CFPAC-1 epithelial preparations. This would be particularly relevant in light of our MPT data, which demonstrated that treatment with CFTR modulators reduced ASF microviscosity, potentially reflecting improved mucin unfolding and surface hydration. Such mechanisms are consistent with the known role of CFTR-mediated chloride and bicarbonate secretion in regulating mucus rheology and could help explain how CFTR correction contributes to restoring the biophysical properties of epithelial secretions.

As mentioned above, we employed multiple particle tracking (MPT) to assess how the pharmacological modulation of CFTR affects the rheological behavior of the ASF [45–47,49,50]. Our results demonstrated that treatment with CFTR correctors reduced ASF microviscosity, indicating improved fluid hydration and decreased viscoelastic resistance. Among the tested conditions, the combination of VX661 and VX445 yielded the most pronounced improvement, suggesting a synergistic effect in restoring surface fluid properties. Notably, these rheological improvements were sustained or slightly enhanced under inflammatory conditions.

These findings underscore the capacity of CFTR modulators to not only restore ion transport but also improve the physical characteristics of epithelial secretions. This dual action is particularly relevant for preventing mucus stasis and ductal obstruction in the pancreatic context of CF. Although the present study did not quantify mucin secretion or hydration status directly, the observed changes in ASF viscosity likely reflect enhanced CFTR-mediated water and bicarbonate transport. Future comparative analyses with other pancreatic epithelial models and complementary assessments of mucin abundance and fluid content would undoubtedly strengthen the mechanistic understanding of this phenomenon.

5. Conclusions

This study demonstrates the effectiveness of VX661 and VX445, both individually and in combination, in addressing multiple aspects of F508del CFTR dysfunction in CFPAC-1 pancreatic epithelial cells. Inflammatory preconditioning with LPS does not reduce the efficacy of these CFTR modulators and may even amplify their functional impact. The results obtained support the use of Trikafta/Kaftrio also in the treatment of pancreatic symptoms of CF and confirm that ALI/LLI-cultured CFPAC-1 cells are a reliable and flexible in vitro model for the study of CFTR rescue and epithelial physiology in the context of the pancreas. Importantly, further validation in primary pancreatic tissues and in vivo systems will be necessary to confirm the physiological relevance and translational applicability of the obtained results and to better define the strengths and limitations of CFPAC-1 cells as a preclinical model for CF-related pancreatic disease.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/life15081315/s1, Table S1. ΔTEC (ΔG, μS/cm^2) measurements under basal and pro-inflammatory conditions in CFPAC-1 epithelial layers. Table S2. Fluid secretion rate (J, μL·h-1·cm^{-2}) in CFPAC-1 epithelial layers treated with CFTR modulators under basal and pro-inflammatory conditions. Table S3. pH of the airway surface fluid (ASF) in CFPAC-1 epithelial preparations treated with CFTR modulators under basal and pro-inflammatory conditions. Table S4. Evaluation of endogenous F508del CFTR protein expression in whole-cell lysates from CFPAC-1 epithelial layers. Table S5. Evaluation of IL-6, and IL-8 and IL-1β expression in the basolateral medium of CFPAC-1 epithelial layers. Table S6. Evaluation of the microviscosity of the apical surface fluid in CFPAC-1 epithelial layers under CFTR modulator treatment and pro-inflammatory conditions. Figure S1. Representative Coomassie blue-stained SDS-PAGE gels from concentrated basolateral medium of ALI-cultured CFPAC 1 epithelial layers. Figure S2. Representative dot blot assays showing detection of MUC1 (upper panel) and MUC5B (lower panel) in apical fluid collected from ALI-cultured CFPAC-1 epithelial layers (spots on the left), ALI-cultured Calu-3 epithelia (middle spots), and culture medium from HEK-293 cells (spots on the right).

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Review

Review of Disordered Eating Behaviors in Cystic Fibrosis

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Abstract

Background: CF transmembrane conductance regulator (CFTR) modulators are available for 90% of people with cystic fibrosis (PWCF), which has contributed to substantial nutritional changes. PWCF identify differences in their relationship with food, as well as alterations in body size and image when taking CFTR modulators. This has led to increasing risks relating to issues with body image, disordered eating behaviors (DEBs), and eating disorders (EDs). DEBs can be an early indication of an ED. CF care has traditionally emphasized body mass index and weight gain, which may have heightened the critical focus of body habitus. Prior to CFTR modulators, the "legacy diet" was often promoted and after years of encouragement to eat high volumes of calorically dense foods, PWCF on modulators have shared that the subsequent body changes have been challenging. Given the body changes that PWCF may have experienced, CF care team nutritional guidelines are evolving. The prevalence and etiology of EDs is largely unknown. Therefore, interventions designed to reduce risk factors for EDs and enhance protective factors against the development of DEBs need to be prioritized. To date, there are no reliable and validated screening tools in the United States to identify DEBs for PWCF. The purpose of this paper is to (1) review eating behaviors and disordered eating in PWCF, and (2) discuss important future directions for the assessment and treatment of DEBs to improve quality of life for PWCF.

Keywords: nutrition; chronic disease; appetite; screening; assessment; eating disorders; food choices

1. Introduction

Cystic fibrosis (CF) is a genetic disorder that occurs in all races and ethnicities. CF impacts multiple organ systems of the body and is considered a diet-related chronic health condition (DR-CHC). Chronic health conditions (CHCs) are defined as health conditions with a biological, psychological, or cognitive basis; they are expected to last for at least one year, with symptoms that may limit function and activities, and require medical care or related services. DR-CHCs (Figure 1) include CF, inflammatory bowel disease, type 1 diabetes mellitus, celiac disease, and irritable bowel syndrome and are CHCs with prescribed dietary regimens [1,2]. CF is associated with a spectrum of diseases that can

cause progressive bronchiectasis, pancreatic insufficiency, and liver and gastrointestinal dysfunction. There are an estimated 40,000 diagnosed persons living with CF (PWCF) in the United States (U.S.) [3]. With recent advances in treatments for CF, poor nutritional status and low body mass index (BMI) are less common than in the past. Historically, malabsorption and high-caloric expenditure, in part due to the increased effort of breathing, contributed to poor nutritional status. Now that CF transmembrane conductance regulator (CFTR) modulators are available for approximately 90% of PWCF (based on age and mutation eligibility), the nutritional landscape and outcomes have changed [4].

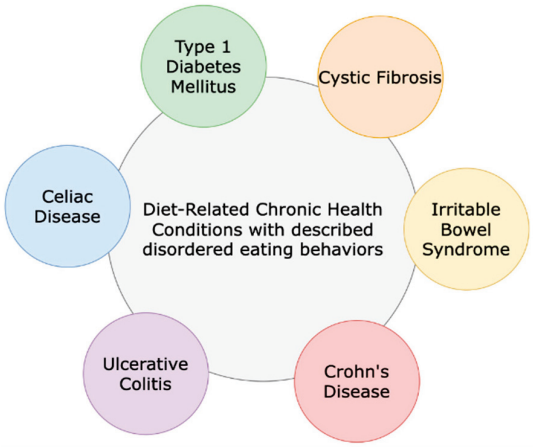


Figure 1. Cystic fibrosis and other diet-related chronic health conditions that include dietary regimen recommendations for treatment and management.

Many PWCF who take CFTR modulators have experienced significant weight gain, and recent estimates in a U.S. sample show that more than 30% of adults living with CF are in the overweight or obese (OW/OB) range [5]. Estimates in Canada are lower, but adults with CF who can be categorized in the OW/OB range exhibited worse cardio-metabolic profiles compared to adults with CF in the normal weight range [6]. In 2022, the CF Foundation Patient Registry identified that 65% of adults >20 years of age were meeting BMI goals for PWCF, while 13% are now considered in the obese BMI category, as defined by a BMI > 30 kg/m^2 [7]. Adults with CF in a normal BMI range can exhibit a normal weight obesity, which is significantly higher than the typical body fat percentage and is associated with poorer lung function [4,8]. Adults with CF are also at a greater risk for increased visceral adipose tissue, which has been shown to be associated with elevated fasting glucose levels [9].

While often grateful for personal health improvements, many PWCF identify substantial differences in their relationship with food, as well as significant alterations in their body size and body image when taking CFTR modulators [10]. Many PWCF living with a chronic disease that focuses on food choices, nutritional status, and body size have noticed a considerable change in conversations with care teams in the era of CFTR modulators [10]. In addition, there is very little data on body image in children with CF. This new context has led to the possibility of an increased risk for problems with body image, relationships with food, disordered eating behaviors (DEBs), and, in some cases, an eating disorder (ED) [11]. The purpose of this paper is (1) to review eating behaviors and disordered eating in PWCF and (2) to discuss important future directions for the assessment and treatment of DEBs/EDs to improve the quality of life for PWCF.

As a narrative review, the authors offer a broad overview of disordered eating in PWCF to identify key concepts and current gaps within the field. This narrative review sought to identify English-language articles reporting disordered eating and cystic fibrosis. Published journal articles and articles in press were included. The PubMed/MEDLINE

database was initially searched in March 2024, as well as being searched again in January 2025 and July 2025. The search concepts included a variety of keywords to describe disordered eating, cystic fibrosis, and chronic diseases including cystic fibrosis, disordered eating behaviors, maladaptive eating, nutrition, eating disorders, chronic disease, appetite, screening, assessment, and food choices. The authors carefully reviewed the titles of articles and abstract data to extract the most pertinent studies to include in this narrative review. To be included, articles needed to be available in English and be peer-reviewed published academic articles.

1.1. Physiology of Appetite

PWCF historically had a different physiology to persons living without a chronic disease. PWCF commonly experience altered absorption and metabolism, along with an increased effort to breathe, often resulting in a caloric deficit, weight loss, and poor nutritional status [11]. The drive to eat, also referred to as appetite, is foundational for life; the complementary state is referred to as satiety and denotes feeling satiated or "full" [12]. The energy balance equation refers to caloric intake and caloric expenditure; it states that the rate of change in energy storage in the body, such as fat mass and fat-free mass, is equal to the rate of energy intake minus the rate of energy expenditure [13]. While this equation may on the surface appear simple, there are numerous factors at play, some of which have been well-characterized, while others have yet to be fully elucidated [13].

Foundational research from over 50 years ago has identified a non-linear relationship between energy intake, energy expenditure, and energy storage or body weight [13]. Physical activity, including intentional exercise, significantly impacts appetite. In 2015, Blundell et al. published a review discussing the impact of physical activity and exercise on hunger, appetite, and satiety [14]. Appetite and satiety feelings are a result of very complex interactions between the brain and gastrointestinal hormones [15]. Several well-characterized factors influencing appetite are hormones including leptin and ghrelin. Ghrelin, often referred to as the "hunger hormone," promotes the storage of adipose/fat. Leptin, on the other hand, decreases appetite and helps to regulate body weight. Based upon a review on the underlying neural mechanisms of appetite, Andermann and Lowell described that hormones like leptin and ghrelin communicate with the brain—specifically an area called the hypothalamus [16]. The hypothalamus is responsible for regulating not only appetite but also sleep–wake cycles, mood, and sex drive. If there is an imbalance in leptin or ghrelin, problematic eating behaviors can result, including anorexia or excessive eating [15] (Figure 2).

In 2017, Zanchi et al. published a comprehensive review of the physiology and neuroscientific underpinnings of appetite; they describe "satiety stimulators," which are hormones such as glucagon-like peptide-1, peptide tyrosine, and cholecystokinin that signal the brain to decrease the drive to eat and cease food intake [17–19]. Insulin, a pancreatic hormone, also plays a large role in human metabolism and eating behavior; it is altered for PWCF and in CF-related diabetes [20]. Cortisol is another hormone involved in appetite and there is evidence that higher cortisol levels may be associated with cravings for calorically dense foods [21]. All these hormones interact with the brain; however, other areas outside the hypothalamus are also involved in our appetite and decisions to eat.

The newer field of cognitive neuroscience has advanced the mechanistic understanding of appetite and food motivation. Advances in neuroimaging techniques including functional magnetic resonance imaging have allowed for a better understanding of the brain's role in appetite and eating decisions. A meta-analysis from 2022 demonstrated that the brain regions responsible for pleasure, reward, taste, homeostasis, deliberate decision-making, and self-regulation are integral for responding to food cues and for making food

decisions [22]. In this meta-analysis, the brain regions responsible for regulating appetite were found to be the insula, amygdala, hippocampus, and orbitofrontal cortex [22]. The brain regions responsible for helping to regulate satiety (feeling of fullness) were the hypothalamus, thalamus, caudate, putamen, and anterior cingulate cortex. This review was of healthy adults; there is extremely limited data examining the neurohormonal mechanisms of appetite and satiety among PWCF.

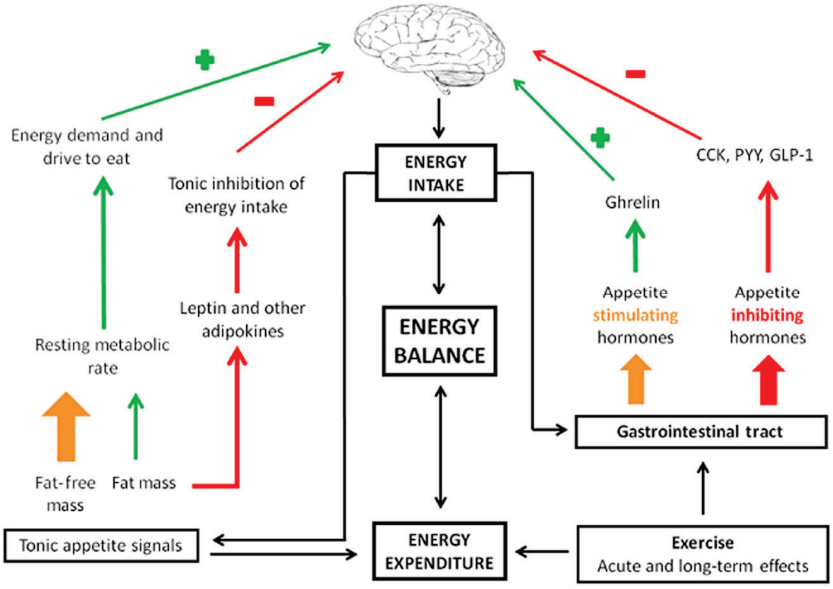


Figure 2. Appetite control. CKK: cholecystokinin; PYY: peptide YY; GLP-1: glucagon-like peptide-1. Reproduced with permission from Blundell et al. [14].

1.2. Eating Behaviors and Food Decision-Making

While hormonal levels and brain activations are at play "under the surface," there are also other factors that influence when, what, and how much we eat. The process of making choices about what to eat and drink is complex and as described above, involves both internal and external influences [23]. Extensive research has demonstrated a host of influences on food choices, including genetic, familial, hormonal, environmental, and physiological determinants [23]. Adults make countless food decisions during just a single day. Across days, weeks, years, and decades, the cumulative impact of patterns of food choices is substantial.

Internal factors include previously mentioned hormones influencing hunger and satiety, the interaction with relevant brain regions, and another important concept called interoception. Interoception refers to a person's awareness of bodily states including heart rate, body temperature, pain, and hunger [24]. The Embodied Predictive Interoception Coding (EPIC) model purports that, in fact, the anticipation of sensations has an important impact on how we perceive the world. Interoception plays a very important role in a person's sensations of hunger (appetite) and fullness (satiety), and therefore in eating behaviors and food choices.

Pleasant, delicious foods are referred to as "comfort foods" because of the emotionally appealing aspects of consumption [25]. Mostly in adults, research has shown how important the pleasure centers in the brain, namely the ventral striatum, respond to gustatory stimuli, including sucrose solution and chocolate milkshakes [26]. Eating in the absence of hunger is a related construct used to describe eating past the point of satiety [27]. While there are likely many reasons that people eat in the absence of hunger, a strong body of research has emerged showing that brain-mediated emotional rewards may play a particularly important role in overconsumption [28]. Historically, PWCF may have been instructed to

ignore internal cues of satiety and eat past the point of fullness to gain enough calories to maintain or gain weight. Overriding one's satiety cues can have longstanding and important implications, which may be made more complex as modulators have made the absorption of nutrients and calories easier [29].

Some of the important external influences on feeding and eating behaviors include the following: chest feeding versus formula feeding, parenting, school, peer influence, food marketing, food security, and socioeconomic status [30]. When things go awry in a systematic way and for a sustained period, a person can be considered to have disordered eating behaviors [23].

1.3. Historical Nutrition Recommendations for Cystic Fibrosis

The well-known "legacy diet" for PWCF promoted calorically dense, high-fat foods with a high salt content because nutritional status impacted pulmonary function and survival prior to the development of CFTR modulators [31–33]. Guidelines encouraged PWCF to maintain a BMI > 50% for children, as well as $\geq$22 kg/m^2 and $\geq$23 kg/m^2 for adult females and males, respectively. These nutritional benchmarks were shown to be associated with improved pulmonary outcomes [34]. Historically, care teams promoted the legacy diet to the majority of PWCF, especially in the setting of frequent pulmonary exacerbations or declining lung health [3,35]. Many PWCF struggled to even *maintain* their weight prior to CFTR modulators [35].

Given the often substantial changes in their bodies that PWCF may have recently experienced with the ongoing use of CFTR modulators, CF care team nutritional guidelines are evolving; instead of an emphasis on quantitative BMI cut offs, recommendations are being tailored to promote optimal nutritional health to maintain weight and consume, for example, a fiber-rich diet with lean protein in order to prevent chronic conditions associated with aging such as heart disease [11].

1.4. Disordered Eating Behaviors and Eating Disorders

Disordered eating behaviors (DEBs), actions that may pose harm or lead to adverse long-term outcomes, can be an early indication of the development of an eating disorder (ED) [36]. PWCF have unique opportunities for DEBs [11]. Bowel regimens may be misused or overused. In PWCF who are pancreatic insufficient, which is approximately 85% of PWCF, the malabsorption of macronutrients can result from omitting pancreatic enzyme replacement therapy. In fact, pancreatic exocrine insufficiency increases the risk of nutritional abnormalities including fat-soluble vitamin deficiencies, slowed body habitus development, and a low BMI [37–39]. CFTR modulators may be restricted, or taken without fat-containing food, as is prescribed for best absorption. PWCF who also have CF-related diabetes and are insulin-dependent may restrict insulin intentionally, which is classified as a diabetes-specific eating disorder (ED-DMT1), colloquially known as diabulimia [40]. Additionally, PWCF may engage in "classic" DEBs such as avoiding/restricting foods or food groups, binge eating, skipping meals, and/or medication misuse [41]. Combining the potential for CF-specific disordered eating, with the more classic behaviors seen in other patient populations, it is evident that there are a multitude of potential actions that can pose harm both acutely and chronically in PWCF [11,41].

Eating disorders (EDs) are serious, complex medical conditions that negatively affect a person's physical and mental health due to prolonged alterations in eating behaviors. EDs are the second most fatal mental illness, second only to opioid use disorder in the general population [42]. The prevalence of EDs in PWCF is largely unknown. For the general population, at least one in ten people in the U.S. will be diagnosed with an ED in their lifetime, and 22% of children and adolescents worldwide exhibit DEBs [5,43]. In

addition, most individuals living with an ED also meet the criteria for another psychological disorder, most commonly anxiety and depression [35,44]. Of women hospitalized for an ED, two-thirds meet the criteria for an anxiety disorder and 90% meet the criteria for depression [6].

ED treatment is essential in reducing morbidity and mortality. Unfortunately, access to treatment is challenging due to treatment cost and a lack of identification of ED symptoms. Identifying individuals with EDs can be difficult, as only 6% of individuals with EDs are in the medically underweight category [45]. In fact, people who are overweight/obese are at higher risk of developing an ED [46,47]. Overall, there is limited identification of EDs, limited insurance coverage of treatment for EDs, and limited access to experts in relation to the treatment of EDs.

1.5. Body Image and Weight Stigma

Body image is defined as a multidimensional construct that involves an individual's internalized view of their body including perceptions, thoughts, feelings, and attitudes to the body's physical appearance [48]. These thoughts and feelings can comprise physical aspects of weight, figure, thinness, build, strength, sexual attractiveness, physical ability, and aging [48]. The concept of body image is broad; however, research has focused upon how self-perceptions, thoughts, feelings, and attitudes of physical appearance can impact negative psychological consequences [48]. Body dissatisfaction is defined as the negative conceptualization of body image, especially appearance, and is present in all genders and ages. It most frequently develops during adolescence and is more prevalent in females [48,49]. Body dissatisfaction may be further complicated for PWCF due to some visible effects on the body including digital clubbing, scars, altered posture, delayed puberty, and short stature [50]. CF care has traditionally emphasized BMI and weight gain, which may also inadvertently heighten the critical focus of body habitus [31,51].

At one time, undernutrition was a common diagnosis in children with CF and the struggle to maintain adequate weight continued into adulthood [52]. The correlation between improved lung function, survival, and quality of life in PWCF with better nutritional status led to a central focus on body weight, weight gain, and BMI, along with the promotion of the legacy diet that emphasized energy intake over nutrient density [53]. Despite consuming this diet, many PWCF struggled to gain and maintain weight even with additional calories from enteral feeding through bolus or overnight infusions. Notably, PWCF receiving supplemental enteral feeding are generally less satisfied with their body [54]. Gender differences toward body image have been identified for young adults with CF, with females overestimating body size and males underestimating their body size and weight [10,55].

Much of the literature regarding body image and DEBs in PWCF occurred prior to the widespread use of CFTR modulators. The introduction of CFTR modulators may give rise to positive effects on lung and gastrointestinal function, enabling PWCF who had previously struggled to gain and maintain weight to readily gain weight for the first time in their lives [11]. The response to this weight gain in PWCF on CFTR modulators regarding body image has been varied [5]. Some PWCF may feel a combination of perceptions and feelings about their bodies that may be neutral, negative, positive or a mixture of these on any given day, time, or situation. These feelings may range from body dissatisfaction with gradual acceptance and appreciation to body image disturbance, increasing the risk for DEBs and EDs. Notably, the introduction of elexacaftor/tezacaftor/ivacaftor occurred just prior to the COVID-19 pandemic when there was a generalized increase in the occurrence of DEBs and body image disturbances in the general population [56].

A mixed-method study examined the perspectives of adolescents and young adults with CF and their healthcare professionals (HCPs) on body image communication [51]. Although 85% of PWCF reported never discussing body image with a HCP, 74% of HCPs reported discussing this topic. Both PWCF and HCPs agreed that body image is important and should be discussed comfortably and supportively. Kass et al. distributed a one-time web-based survey to a multidiscipline group of CF HCPs in the U.S. via CF Foundation email list management software programs [43]. The survey investigated HCPs' understanding and perceived importance of DEBs and body image disturbance in adolescent and young adults with CF, along with current screening practices. Most HCPs recognized the importance of screening for DEBs and body image disturbance, but fewer than a third felt comfortable with this process. HCPs reported that assessment tools (86%), standardized partnerships with ED specialists (80%), and CF Foundation or national guidelines (79%) would be helpful to improve screening and counseling [43].

Currently, no evidenced-based treatment for body image disturbances exists. While research is underway, existing approaches include the promotion of body acceptance through body positivity or neutrality and media literacy. To assist PWCF in navigating this evolving landscape, utilizing assessment tools and language that promote positive images of healthy weight gain by using appropriate language like "growing taller, stronger, healthier, well-nourished" (https://uconnruddcenter.org/research/weight-bias-stigma/healthcare-providers/, most recently accessed on 17 August 2025) and examining personal implicit biases toward weight are beneficial. When interacting with PWCF, the focus should be on interventions to improve health rather than weight. Additional resources to support HCPs can be found through the Association for Size Diversity and Health (Table 1), at https://cfreshc.org/SRH-Guide/body-image/, most recently accessed on 17 August 2025), and through the "Tips for CF Care Teams: Conversations about Body Image" from the CF Foundation Mental Health Advisory Committee [57]. Furthermore, caregivers can be encouraged to promote and model apositive body image. Research indicates that early positive body image experiences can have a protective effect against false images from external sources. Caregivers modeling flexible eating, limiting food rules, and demonstrating appreciation of their own body, as well as different sizes and shapes of bodies, can help youth have a healthy body image [58].

Table 1. The Association for Size Diversity and Health.

Weight inclusivity is the ability to accept and respect the inherent diversity of body shapes and sizes and reject the idealizing or pathologizing of specific weights.
Health enhancement is defined as the ability to support health policies that improve and equalize access to information and services and personal practices that improve human well-being, including attention to individual physical, economic, social, spiritual, emotional, and other needs.
Eating for well-being is the capacity to promote flexible, individualized eating based on hunger, satiety, nutritional needs, and pleasure rather than any externally regulated eating plan focused on weight control.
Respectful care acknowledges our biases and works to end weight discrimination, weight stigma, and weight bias. Respectful care provides information and services from an understanding that socioeconomic status, race, gender, sexual orientation, age, and other identities impact weight stigma, as well as support environments that address these inequities.
Life-enhancing movement supports physical activities that allow people of all sizes, abilities, and interests to engage in enjoyable movement, to the degree that they choose.

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1.6. Existing DEB Screening Tools

The Sick-Control-One stone-Fat-Food (SCOFF) questionnaire is a simple screening tool used to identify potential EDs (Table 2) [59]. It consists of five questions, each addressing different behaviors or attitudes related to eating and body image. A score of 2 or more affirmative responses suggests a possible ED that warrants further evaluation. It is a quick way to identify people who might need more support for DEBs. The SCOFF questionnaire has been found to be a valid and reliable screening tool for identifying individuals at risk of EDs in the general population. Research indicates that it effectively distinguishes between those who may have an ED and those who do not. Here are some key points about its validity—the SCOFF has a good sensitivity and specificity; it is brief (5 questions); it has been validated in different populations, which supports versatility across cultural contexts; and a positive result does not confirm an ED, rather a comprehensive assessment by a trained professional is necessary for a formal diagnosis.

Table 2. Sick-Control-One stone-Fat-Food (SCOFF) questionnaire used in the general population.

SCOFF Questionnaire
S: Do you make yourself Sick (vomit) because you feel uncomfortably full?
C: Do you worry you have lost Control over how much you eat?
O: Have you recently lost more than One stone (14 pounds) in a 3-month period?
F: Do you believe yourself to be Fat when others say you are too thin?
F: Would you say that Food dominates your life?

The Eating Attitudes Test (EAT-26) is another screening measure designed to identify EDs in the general population that focuses on the following three subscales: dieting, bulimia and food pre-occupation and oral control (Figure 3) [60]. It consists of 26 statements that the respondent indicates a degree of agreement or disagreement with based upon a Likert scale. The EAT-26 also includes five additional behavioral questions regarding maladaptive eating behaviors. The original 26-item version is highly reliable and valid but alone does not yield the diagnosis of an ED. Individuals who score 20 or more on the test should be evaluated by a qualified professional to determine if they meet diagnostic criteria for an ED, as it indicates a high level of concern about dieting, body weight, or maladaptive eating behaviors. The EAT-26 has been especially helpful in assessing ED risk in high school and college general populations, as well as in athletes. While the above two measures have shown promising validity and reliability, they do not include issues that are often relevant for PWCF, making them less-ideal screening tools when applied to PWCF.

The CF-specific screening tool for disturbed eating attitudes or behaviors (CFEAB) questionnaire is a 21-item self-report measure to highlight areas of eating disturbance in PWCF (Table 3). It was administered to 155 PWCF (11–62 years) at Great Ormond Street Hospital in the United Kingdon (U.K.) before the advent of highly effective modulator therapies (HEMTs); it revealed a three-factor structure with good internal consistencies for (1) desire for thinness and weight loss, (2) DEBs, and (3) appetite [61]. Participants respond to the statements in the CFEAB table. Further work to establish construct validity and clarify subscale interpretation was not conducted. Also, this was used prior to the era of HEMTs and may no longer identify maladaptive attitudes towards food, body weight/image, or eating behaviors in PWCF.

Eating Attitudes Test (EAT-26)©

Instructions: This is a screening measure to help you determine whether you might have an eating disorder that needs professional attention. This screening measure is not designed to make a diagnosis of an eating disorder or take the place of a professional consultation. Please fill out the below form as accurately, honestly and completely as possible. There are no right or wrong answers. All of your responses are confidential.

Part A: Complete the following questions:

1) Birth Date	Month:		Day:		Year:	2) Gender:	Male ☐ Female ☐
3) Height	Feet :		Inches:				
4) Current Weight (lbs.):		5) Highest Weight (excluding pregnancy):					
6) Lowest Adult Weight:		7: Ideal Weight:					

Part B: Check a response for each of the following statements:		Always	Usually	Often	Some times	Rarely	Never
1.	Am terrified about being overweight.	☐	☐	☐	☐	☐	☐
2.	Avoid eating when I am hungry.	☐	☐	☐	☐	☐	☐
3.	Find myself preoccupied with food.	☐	☐	☐	☐	☐	☐
4.	Have gone on eating binges where I feel that I may not be able to stop.	☐	☐	☐	☐	☐	☐
5.	Cut my food into small pieces.	☐	☐	☐	☐	☐	☐
6.	Aware of the calorie content of foods that I eat.	☐	☐	☐	☐	☐	☐
7.	Particularly avoid food with a high carbohydrate content (i.e. bread, rice, potatoes, etc.)	☐	☐	☐	☐	☐	☐
8.	Feel that others would prefer if I ate more.	☐	☐	☐	☐	☐	☐
9.	Vomit after I have eaten.	☐	☐	☐	☐	☐	☐
10.	Feel extremely guilty after eating.	☐	☐	☐	☐	☐	☐
11.	Am preoccupied with a desire to be thinner.	☐	☐	☐	☐	☐	☐
12.	Think about burning up calories when I exercise.	☐	☐	☐	☐	☐	☐
13.	Other people think that I am too thin.	☐	☐	☐	☐	☐	☐
14.	Am preoccupied with the thought of having fat on my body.	☐	☐	☐	☐	☐	☐
15.	Take longer than others to eat my meals.	☐	☐	☐	☐	☐	☐
16.	Avoid foods with sugar in them.	☐	☐	☐	☐	☐	☐
17.	Eat diet foods.	☐	☐	☐	☐	☐	☐
18.	Feel that food controls my life.	☐	☐	☐	☐	☐	☐
19.	Display self-control around food.	☐	☐	☐	☐	☐	☐
20.	Feel that others pressure me to eat.	☐	☐	☐	☐	☐	☐
21.	Give too much time and thought to food.	☐	☐	☐	☐	☐	☐
22.	Feel uncomfortable after eating sweets.	☐	☐	☐	☐	☐	☐
23.	Engage in dieting behavior.	☐	☐	☐	☐	☐	☐
24.	Like my stomach to be empty.	☐	☐	☐	☐	☐	☐
25.	Have the impulse to vomit after meals.	☐	☐	☐	☐	☐	☐
26.	Enjoy trying new rich foods.	☐	☐	☐	☐	☐	☐

Part C: Behavioral Questions: In the past 6 months have you:		Never	Once a month or less	2-3 times a month	Once a week	2-6 times a week	Once a day or more
A	Gone on eating binges where you feel that you may not be able to stop? *	☐	☐	☐	☐	☐	☐
B	Ever made yourself sick (vomited) to control your weight or shape?	☐	☐	☐	☐	☐	☐
C	Ever used laxatives, diet pills or diuretics (water pills) to control your weight or shape?	☐	☐	☐	☐	☐	☐
D	Exercised more than 60 minutes a day to lose or to control your weight?	☐	☐	☐	☐	☐	☐
E	Lost 20 pounds or more in the past 6 months	Yes ☐		No ☐			

* Defined as eating much more than most people would under the same circumstances and feeling that eating is out of control

Figure 3. Eating Attitudes Test (EAT-26) questionnaire used in the general population. Reproduced with permission from Garner et al. [60].

Table 3. Cystic Fibrosis Eating Attitudes and Behaviors (CFEAB) questionnaire.

CFEAB Questionnaire
I want to be thinner.
I cut down on food to lose weight.
I am afraid of becoming fat.
I would like to eat less to lose weight.
I spend time wishing I weighed more.
I feel I am too fat.
I exercise as a way to lose weight.
I feel I need to be thin to be happy with myself.
Gaining weight makes me feel happy.
I eat low-fat or low-sugar foods so I won't gain weight.
The thought of eating food makes me feel worried.
So I won't gain weight, I deliberately don't take my enzymes.
So I won't gain weight, I deliberately don't take my insulin.
So I won't gain weight, I deliberately don't take my extra feeds or supplements.
I make myself vomit (sick) after I eat to control my weight.
I am put off eating because my CF makes me feel sick.
I pretend to others that I have eaten.
I feel guilty after eating.
I enjoy eating.
I feel full quickly.
I have a good appetite for food.

1.7. Research Gaps and Future Directions

Given the changing landscape of nutrition since the advent of HEMTs, we need a screening tool that is specific to PWCF in the era of HEMTs. We need to survey CF HCPs to hear their voices about what is needed to better assess DEBs and support PWCF experiencing DEBs and EDs. Given that the prevalence and etiology of EDs is largely un- known, interventions designed to reduce known risk factors for EDs and enhance protective factors against the development of DEBs needs to be prioritized. Prior to the widespread use of CFTR modulators, a CF-specific screening tool for assessing DEBs had been developed with promising psychometrics in detecting EDs in the U.K. [61]. To date, there are no reliable and validated screening tools in the U.S. to identify DEBs for PWCF. This contributes to limitations in assessing EDs or DEBs in the CF population. For people with a chronic illness, general ED screening tools have the possibility to miscalculate ED risk [7,10]. Table 4 further characterizes the clinical needs identified for DEBs within the CF population. It is crucial to use a validated screening tool for PWCF to better identify DEBs and body image disturbances to improve accurate identification in PWCF. Currently, questions like, "Do you change your food choices based on your weight?" and "Do you alter your consumption of pancreatic enzymes based on your weight?" can help with screening while a standardized approach is explored. Once identified, a systematic approach to identifying DEBs in PWCF is needed and would likely improve interventions and referral to ED programs when deemed appropriate. CF care team members should be encouraged

and supported to seek and complete ED-specific training to enhance their knowledge of the signs, symptoms, and available treatments for EDs.

Table 4. Clinical needs for disturbed eating in cystic fibrosis.

Clinical Needs	Suggested Approach
Limited understanding of the development of an eating disorder (ED)	Conduct focus groups with CF healthcare providers; conduct focus groups with PWCF
Lack of CF-specific ED screening tools	Development of CF-specific screening tools that address eating disturbances and negative body images; frequent use of screening tools in CF clinics for consistent assessment and early identification
Limited training for healthcare professionals (HCPs) regarding CF-related EDs	Provide education to healthcare providers regarding identifying, addressing, and managing eating disorders; development of resources to provide additional support and education to healthcare providers
Lack of ED prevention programs	Development of a structured prevention program for HCPs to refer patients of concern; collaboration between CF physician, nutritionist, social worker, and psychologist to address patients of concern
Poorly developed ED intervention programs	Development of a structured intervention program that occurs over multiple sessions; treatment approaches that reduce individual, social–familial, and environmental barriers that interfere with self-management

Reproduced with permission from Darukhanavala et al. [10].

Parent conversations focused on weight and size are associated with an increased risk of adolescent DEBs [62]; a 2024 systematic review completed by Levinson et al. found a similar correlation in adults [63]. Instead of focusing on weight, the promotion of healthy eating behaviors is protective against DEBs [62]. Healthy eating behaviors include eating regularly throughout the day, consuming a wide variety of foods, and internally regulating eating, which incorporates tuning into hunger and satiety cues.

The focus on healthy eating behaviors promotes a flexible eating style without forbidden foods and avoids dividing foods into "good" versus "bad" categories, which can prevent cognitive distortions linked to EDs [63]. Other behavior interventions such as the promotion of enjoyable physical activity, a reduction in sedentary time, stress management, and body image support may help decrease DEBs. Self-weighing facilitates clinically significant weight loss in those without EDs [63]. It is suggested for individuals with EDs that weekly weighing replaces too-frequent weighing or the avoidance of weighing, as the latter can perpetuate DEBs. While these suggestions exist and may be useful in some cases, it is important to honor and listen to the individual wishes of PWCF. Some PWCF will prefer not to be weighed, nor to discuss their body size at CF clinic encounters. It should also not be assumed that individual PWCF desire to lose weight. HCPs should aim to provide compassionate, patient-centered care, while listening to the individual goals of PWCF [7].

2. Limitations and Future Directions

There are some limitations of the current project, one of which is the limited scope of research to draw from. There are a limited number of high-quality studies specifically examining disordered eating in CF populations, with many studies having small sample sizes that may limit generalizability. In addition, the variety and heterogeneity of screening and assessment measures used makes a more-direct comparison across studies quite challenging. As in many areas of research, cross-sectional studies dominate, which can limit the understanding of any causal relationships. There is no standardized screening protocol for disordered eating behaviors in CF care settings and there is an underrepresentation of

certain demographic groups (ethnic minorities, adults with CF, those with severe disease progression). Finally, most of the research has been conducted in Western healthcare systems, limiting cross-cultural understanding.

The development and validation of CF-specific disordered eating screening tools and training for healthcare professionals to recognize and address eating concerns in PWCF are vital next steps. Eventually, the design and testing of integrated treatment approaches that address both CF management and eating disorder symptoms will be paramount for improving quality of life for PWCF.

3. Conclusions

In this paper, the current information on eating behaviors and disordered eating in PWCF was reviewed and important future directions for the assessment and treatment of disordered eating behaviors to improve quality of life for PWCF were examined. The advent of highly effective CFTR modulators has contributed to weight gain and an evolving nutritional landscape for PWCF [64]. This new and rapidly evolving landscape encompasses an increased risk for issues with body image, relationship with food, DEBs, and, in some cases, EDs. Until more research and standardized interventions can be established, CF HCPs can leverage the multidisciplinary team expertise to utilize more weight-neutral conversations to assess and help address DEBs. In 2018, the CF Foundation Food Security Committee conducted a qualitative survey of the CF community, who identified social workers as a trusted care team member to discuss food insecurity with at that time. This highlights the importance of the multidisciplinary care team approach for discussing complex chronic illnesses that encompass physical and mental health. Registered dietitians, social workers, psychologists, and other care team members are likely already discussing concerns regarding body image, relationship with food, and DEBs with PWCF and their families; therefore, the development of resources and interventions to support care teams and PWCF navigating these challenges with DEBs, body image, and the changing landscape of nutritional recommendations need to be prioritized. To date, there are no reliable and validated screening tools in the United States to identify DEBs for PWCF.

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Improvement of Cystic Fibrosis-Associated Liver Disease in Adults on Long-Term Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Modulators

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Abstract

Cystic fibrosis (CF) transmembrane conductance regulator (CFTR) modulators have been reported to improve lung function and reduce CF exacerbations. We aimed to investigate the efficacy of CFTR-modulators in CF-associated liver disease (CFLD) during long-term treatment. Longitudinal data were collected from genetically confirmed adult CF patients receiving CFTR-modulators. CFLD was diagnosed using conventional criteria combined with liver stiffness measurement (LSM). A total of 57 patients [56.1% male; median age at baseline (T0), 26 years (interquartile range [IQR], 23–35)] were included. Patients received lumacaftor/ivacaftor and/or elexacaftor/tezacaftor/ivacaftor for a median of 43 months (range, 15–123) until last assessment (T2). The prevalence of CFLD decreased from 15 (26.3%) at T0 to 8 (14.0%) at T2 ($p = 0.016$), and no new cases of CFLD were observed. Median LSM decreased from 6.2 (IQR 4.9–8.0) to 5.0 kPa (IQR 4.1–6.2) in the overall cohort ($p < 0.001$) and from 10.2 (IQR 6.8–13) to 6.2 kPa (IQR 5.0–12.4) in the CFLD subgroup ($p = 0.025$). Mild, transient fluctuations in liver enzymes occurred in 26.3% of patients. In conclusion, adults with CF receiving long-term CFTR modulators, showed improvement of liver disease assessed by ultrasonography and transient elastography. At the last assessment, half of the patients no longer met the criteria for CFLD.

Keywords: cystic fibrosis; cystic fibrosis-related liver disease; liver stiffness; CFTR modulators; lumacaftor/ivacaftor; elexacaftor/tezacaftor/ivacaftor; adults

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disorder with high prevalence in Caucasian populations [1]. Mutations in the gene encoding CF transmembrane conductance regulator (CFTR), a chloride-conducting transmembrane channel, result in impaired transport of bicarbonate and chloride anions and diminished mucociliary clearance of the airways leading in mucus plugs, chronic infection, and local airway inflammation [2,3]. CF affects several organ systems, including the liver and bile ducts (Cystic Fibrosis-associated Liver Disease—CFLD) [1]. Hepatobiliary involvement increases with age and accounts for about 32% by age 25 [4].

Liver disease in CF may be related to the underlying CFTR defect itself but also to etiologies that are indirectly related to CF, like drug hepatotoxicity, bacterial infections of the liver and/or biliary tract, liver steatosis associated with diabetes mellitus and liver congestion as a cardio-pulmonary complication [5]. CFTR defect in hepatic endothelium and cholangiocytes induces abnormal flow of anions, high viscosity, and biliary obstruction [6]. CFLD includes a broad pattern of liver and biliary disorders which may overlap, i.e., multinodular cirrhosis, focal biliary fibrosis, liver steatosis, porto-sinusoidal vascular disease, sclerosing cholangitis with focal or multiple biliary stenosis, disorder of the gallbladder, etc. [6]. Usually, it is difficult to discriminate if the liver disorder is related to the underlying CFTR defect or is secondary due to extrahepatic or iatrogenic complications [7].

It has been reported that CFLD is the third leading cause of mortality in CF after respiratory failure and complications of transplantation, but most data are on pediatrics [8,9]. The diagnosis of CFLD is important for outcome prediction but is difficult to be documented in its early stages, as it progresses insidiously until advanced, when life-threatening manifestations appear. A combination of diagnostic modalities including physical examination, liver enzymes, and imaging was utilized in the conventional Debray criteria [10]. Liver biopsy is the gold standard for the diagnosis and staging of liver diseases [11,12] but is not widely used in CF population mainly due to patchy pattern of liver lesions and the invasive nature of the procedure. Thus, non-invasive liver fibrosis tests (NITs), mainly liver stiffness measurement (LSM) evaluated by transient elastography (TE), are valuable tools for the identification of CFLD [6,13,14].

Ursodeoxycholic acid has been proposed to improve bile flow and bicarbonate secretion but no clinical benefit was reported to justify its routine use in CFLD [6,7]. Innovative therapies have been approved starting with lumacaftor in combination with ivacaftor (LI) [15,16] and more recently the triple elexacaftor/tezacaftor/ivacaftor (ETI) for patients harboring at least one copy of the DF508del mutation in the CFTR gene [17,18]. These CFTR modulators were reported to improve lung function, decrease CF pulmonary exacerbations, and have extrapulmonary benefits [19]. Lumacaftor, elexacaftor, and tezacaftor act as correctors, helping the misfolded CFTR protein to be processed correctly and reach the cell surface, while ivacaftor acts as a potentiator, enabling the channels to open and chloride ions to flow through the defective channels [6,7]. However, the drug efficacy and tolerability of adults with CFLD need further investigation. In the current study, we explored the impact of LI and/or ETI on adults with CFLD by evaluating longitudinal changes in liver enzymes, ultrasonography, and LSM.

2. Materials and Methods

2.1. Design of the Study

This is a single center, non-interventional, mixed retrospective—prospective real-world study. Longitudinal data were collected from July 2017 to April 2025. Only patients who were receiving CFTR modulators, either LI and/or ETI were included in the study. Patients with hepatitis B, C, or alcohol consumption more than 30 gr/day for men or

more than 20 gr/day for women were excluded. No patient was obese. No patient with decompensated liver disease received CFTR modulators. Of 66 patients who were first evaluated, 9 were excluded. Seven patients were excluded due to ineligible mutations, one discontinued treatment and another had decompensated liver disease with ascites. Thus, 57 patients with CF diagnosed with sweat chloride concentration exceeding 60 mmol/L and confirmed by a genetic test were included.

The study had a retrospective followed by a sequential prospective component. Existing data from medical records stored in the center or databases were recorded (retrospective component). The same subjects were followed forward, and additional data and outcomes were collected (prospective component). The first recording of the study (baseline) was the initiation of a CFTR-modulator (baseline) (T0) and the last recording, the time of last assessment (T2). The time of prospective study onset is defined as T1. The median duration of the retrospective component was 15 months (IQR 6–44), while that of prospective component was 25 months (24–27). Demographic (age, gender, body mass index [BMI]), clinical examination, laboratory (hemoglobin, white blood cell and platelet counts, AST, ALT, ALP, gammaGT), ultrasound of the upper abdomen, Doppler imaging of splenoportal axis and LSM data were retrospectively (T0–T1) and then prospectively (T1–T2) collected in all patients. The time elapsed between laboratory and LS evaluation was not longer than 3 months. FibroScan® Echosens (Paris, France) was used for LSM. During the prospective part of the study, liver enzymes were tested every 6 months. Nineteen (33.3%) patients started with LI and then changed without interruption to ETI (LI + ETI group), 37 (65%) started with ETI alone (ETI group), and 1 patient continued to LI alone (1.7%) until the end of follow-up. A total of 48 (84.2%) patients who had at least one F508del mutation were first started CFTR modulators while the rest (15.8%) received ETI in the beginning of the treatment as off label use after legal approval by the Greek Pharmaceutical Regulatory Agency. The study protocol was approved by the Hospital Ethical Committee. All patients signed a written informed consent before their inclusion in the study.

2.2. CFLD Patients

All patients (N = 57) were evaluated for CFLD by liver enzymes, clinical examination, ultrasound of the upper abdomen, LSM, and other NIT, like FIB-4 and APRI at least three times; at T0, T1, and T2. The CFLD diagnosis, according to the literature [9], was based on Debray criteria [10] at first and then on NITs. Liver ultrasound was considered a key examination for the diagnosis of CFLD in present study, according to Dana et al. [7]. However, abnormal liver enzymes and NIT, especially liver elastography, was also used according to cutoffs recommended in adults by Koh et al. [13]. More specifically, it was designed so that those who had liver stiffness >6.8 kPa and/or APRI > 0.5 and/or FIB-4 > 1.5 could be included in CFLD group [13]. Our diagnostic approach for liver involvement is defined by the algorithm described in the review by Dana et al. [7]. All patients with CFLD were treated with ursodeoxycholic acid until T0. Longitudinal changes in liver enzymes, ultrasonography abnormalities, and LSM are exhibited.

2.3. Statistical Analysis

Categorical variables were expressed as count (percentage) and differences over time were compared using the McNemar test. Quantitative variables were expressed as median and interquartile ranges (IQR).

The Wilcoxon signed-rank test for two related samples was used to evaluate changes in variables in the same subjects over two time points (T0 and T2 or T1 and T2). All statistical analyses were performed by the statistical package SPSS (version 21; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Total Patients

Fifty-seven patents with CF [56.1% male, median age at diagnosis 6 months [Interquartile range (IQR) 1.5–24] months, median age at baseline (T0) 26 (23–35) years] were included. All but two patients (14 and 16 years old) were adults at T0. Forty-eight patients (84.2%) have at least one copy of the F508del mutation. A total of 19 carried two (33.3%) and 29 (50.9%) one copy (Table 1).

Table 1. Demographics, genotypic, and treatment characteristics at baseline in all patients (N = 57).

Characteristic	
Age at initiation of treatment (years)	26 (23–35)
Gender (Male%)	32 (56.1)
Median Age of CF diagnosis (months)	6 (1.5–24)
Genotype (N%)	
DF508/DF508	19 (33.3)
DF508/0ther	29 (50.9)
Other	9 (15.8)
Chloride sweat test ≥60 mmol/L(N%)	57 (100)
Type of treatment (N of patients)	**Treatment duration in months [median (IQR) (range)]**
LI+ETI (N = 19) *	80 (IQR 63–102) (range 37–123)
LI only (N = 1)	29
ETI only (N = 37)	39 (IQR 31–43.5) (range15–69)
Total duration of CFTR modulators treatment	43 (IQR 35–67) (range 15–123)

LI, lumacaftor/ivacaftor; ETI, elexacaftor/tezacaftor/ivacaftor; * the 19 patients who received LI before ETI had a median LI administration of 44 months (IQR 29.5–60) (range 10–83).

The median (IQR) period of drug administration was 43 (35–67) months in total, 44 (29.5–60) for LI, 39 (31–43.5) for ETI and 80 (63–102) for LI+ETI (Table 1). All patients had received LI and/or ETI for at least 15 months until the last assessment (T2). Forty-eight patients had received treatment before T1 and the remaining nine around that time point. Body mass index and hemoglobin values showed a statistically significant increase in T2 compared to T0 (Table 2). Platelet count showed a statistically significant decrease and total bilirubin an increase in T2 compared to T0 but the values were within normal limits. No statistically significant differences were observed in aminotransferases, ALP or gammaGT values between T0 and T2.

Table 2. Comparison of Body Mass Index, laboratory values, and characteristics of liver disease at initiation of treatment and last assessment in all patients (N = 57).

	Onset of CFT Modulators (T0)	**Last Assessment (T2)**	***p* Value**
Body mass index (BMI) (kg/m^2)	22.4 (19.6–24.1)	23.3 (20.9–26.4)	<0.001
Laboratory values			
Hemoglobin (gr/dL)	14.0 (12.8–15.1)	14.4 (12.95–15.1)	0.033

Table 2. *Cont.*

	Onset of CFT Modulators (T0)	Last Assessment (T2)	*p* Value
White blood cell count($\times 10^9$/L)	8.12 (6.71–9.26)	6.95 (5.88–8.51)	0.065
Platelet count ($\times 10^9$/L)	292 (244–345)	273 (223–311)	0.002
AST (IU/L)	20 (17–26)	21 (18–26.5)	0.301
ALT (IU/L)	23 (16–35)	23 (17–29)	0.786
ALP (Value/ULN)	0.82 (0.64–1.02)	0.82 (0.63–1.02)	0.245
gammaGT (IU/L)	14 (10–24.5)	14 (10–19.5)	0.42
Total Bilirubin (mg/dL)	0.48 (0.31–0.67)	0.63 (0.43–0.95)	<0.001
Non-invasive Fibrosis markers			
Liver Stiffness (kPa)	6.2 (4.9–8.0)	5.0 (4.1–6.2)	<0.001
APRI > 0.5 (N%)	2 (3.5%)	0 (0%)	NS
CFLD rate over time			
CFLD (N%)	15 (26.3%)	8 (14.0%)	0.016

CFLD, cystic fibrosis liver disease; the BMI, laboratory and liver stiffness values are expressed in median (IQR).

Median LSM decreased from 6.2 kPa (IQR 4.9–8.0) at T0 to 5.0 kPa (IQR 4.1–6.2) at T2 ($p < 0.001$) (Table 2, Figure 1A). It is important that Liver Stiffness (LS) continued to decline during the prospective period of the study, long after initiation of treatment [from 5.4 (4.6–6.6) at T1 to 5.0 (4.1–6.2) at T2 ($p = 0.009$)]. AST to Platelet Ratio Index (APRI) values were more than 0.5 in 2 (both included in CFLD group) at T0 and in nobody at T2. Fibrosis-4 Index (FIB-4) did not help to diagnose CFLD, as it never reached a value of more than 1.5 at any time of the study.

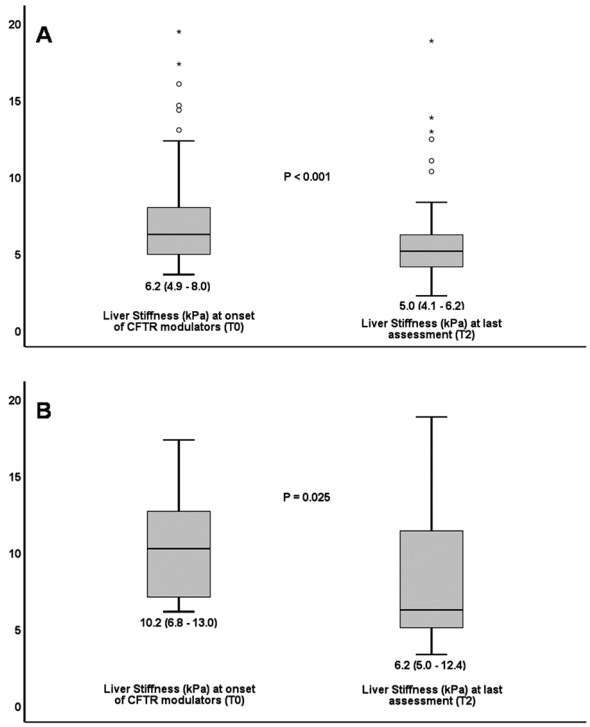


Figure 1. Decline in liver stiffness from treatment onset to last assessment in all patients (**A**). Decline in liver stiffness from treatment onset to last assessment in patients with cystic fibrosis liver disease (**B**). Circles (o) denote mild outliers i.e., values between 1.5 and 3 times above the upper quartile (Q3). Asterisks (*) denote far outliers i.e., values more than 3 times above Q3.

Fifteen (26.3%) patients at T0, and 8 (14.0%) at T2 were characterized as CFLD ($p = 0.016$) (Table 3). Seven out of fifteen demonstrated mild or normal ultrasonography findings (Table 4) and low LSM < 6.85 kPa (Table 3) at the last assessment (T2) and were declassified from CFLD group. No new patient developed CFLD and no patient had liver-related complications during the total study period.

Table 3. Demographic, genotypic, and treatment characteristics of patients with CFLD. Liver stiffness evaluation in different time points of the study and classification according to CFLD at the onset of treatment (T0) and last assessment (T1).

Pt #	Genotype	Age at Treatment Onset (years)	Gender	Total Treatment Duration (months)	ETI Duration (months)	LSM (kPa) at Treatment Onset (T0)	LSM (kPa) at Initiation of Prospective Period (T1)	LSM (kPa) at Last Assessment (T2)	CFLD at Treatment Onset	CFLD at Last Assessment
1	DF508/DF508	14	M	116	40	10.4	19.7	18.8	YES	YES
2	DF508/DF508	16	M	72	36	7.3	6.1	4.8	YES	NO
3	DF508/G542X	19	F	53	53	7.9	7.5	5.1	YES	NO
4	DF508/DF508	23	M	93	40	9.0	7.6	6.1	YES	NO
5	DF508/DF508	24	M	80	40	6.2	6.6	6.8	YES	NO
6	DF508/621+1G>T	25	M	39	39	6.8	5.0	5.2	YES	NO
7	DF508/C524x	26	M	39	39	6.1	6.1	4.9	YES	NO
8	DF508/W496X	26	M	61	61	6.8	6.8	3.3	YES	NO
9	DF508/1677delTA	27	F	34	34	10.2	5.4	5.0	YES	YES
10	DF508/DF508	29	M	72	42	17.3	6.6	6.2	YES	YES
11	DF508/DF508	30	F	101	44	11.4	10.5	10.3	YES	YES
12	G542x/621+1G>T	31	M	15	15	13.0	13.0	12.4	YES	YES
13	DF508del/621+1G>T	37	M	34	34	16.0	16.0	12.9	YES	YES
14	DF508/G542X	38	M	29	29	12.3	12.3	13.8	YES	YES
15	DF508/N1303K	33	M	41	41	14.6	7.9	7.9	YES	ϒES

CFLD, cystic fibrosis liver disease; ETI, elexacaftor/tezacaftor/ivacaftor; Two of the patients (#1 and #12) both at T0 and T2 had clinically significant portal hypertension with oesophageal varices diagnosed at the upper GI endoscopy; Patient #9 had persistently elevated liver enzymes × 3–5 times the upper normal limit from baseline to last assessment and was classified as CFLD.

Table 4. Findings of ultrasound of the upper abdomen at treatment onset and at the end of follow-up in 15 patients with cystic fibrosis liver disease.

Patient #	(T0) Treatment Onset	(T2) Last Assessment
1	Macronodular margin, coarse liver echotexture. Reduced size of right lobe. Enlargement of caudate lobe. Portal vein diameter 1 cm. Portosystemic collaterals: recanalization of umbilical vein, gastroesophageal varices, left gastric vein varices along lesser omentum, splenorenal shunts. Splenomegaly, spleen diameter 17.2 cm.	Liver enlarged, caudate lobe hypertrophy. Diffuse heterogeneous echotexture. Lobulated parenchymal margin. Splenomegaly, spleen diameter 17.4 cm.
2	Hepatomegaly, heterogeneous echotexture with features of fibrosis, increased echogenicity. Lobulated margin. Small collateral varices at splenic hilum. Splenomegaly, spleen size 15 cm.	Liver homogeneous. Increased echogenicity, consistent with mild fatty infiltration. Dimensions within normal limits.
3	Liver mildly enlarged. Diffuse increased parenchymal echogenicity with heterogeneous echotexture. Gallbladder with anechoic content.	Liver normal size. Mildly increased echogenicity.
4	Hepatic heterogeneous echotexture. Portal vein diameter 1.2–1.4 cm, flattened flow waveform. Gallbladder with cholelithiasis. Borderline splenomegaly, spleen 13 cm. Splenic vein diameter 1.2 cm with flattened flow waveform, consistent with portal hypertension.	Liver normal size, 14.2 cm. No focal lesions. Gallbladder with cholelithiasis. Spleen 12.4 cm. Splenic vein 0.8 cm, normal Doppler waveform. Portal vein diameter 1.1 cm, normal phasic Doppler waveform.

Table 4. *Cont.*

Patient #	(T0) Treatment Onset	(T2) Last Assessment
5	Diffuse increased liver echogenicity with heterogeneous echotexture. Mild splenomegaly, spleen 13.1 cm. Portal vein maximum diameter 1.0 cm, normal lumen and normal waveforms. Splenic vein diameter 0.7 cm with normal velocities.	Liver with mild heterogeneous echotexture. Spleen normal size, 11.7 cm. Splenoportal axis normal on triplex.
6	Hepatomegaly, liver size 17 cm. Diffuse increased parenchymal echogenicity with heterogeneous echotexture. Splenomegaly, spleen size 15.8 cm.	Liver normal size, no focal lesions. Spleen of normal size.
7	Liver enlarged, 16 cm. Heterogeneous echotexture due to multiple scattered nodular lesions, largest 2.2 cm. Findings may represent imaging features of focal biliary fibrosis. Multiple microlithiasis in gallbladder.	Liver normal size and echogenicity. Multiple microlithiasis and sludge in gallbladder.
8	Diffuse increased parenchymal echogenicity, fatty infiltration. No focal lesions. Borderline splenomegaly, spleen 13 cm. Splenic vein dilated, maximum lumen diameter 1.7 cm at splenic hilum, normal Doppler waveform.	Liver normal size (11 cm) and echogenicity, no focal lesion.
9	Normal findings	Normal findings
10	Liver enlarged, 15.7 cm. Significant heterogeneity and coarse liver echotexture, granular margin, no focal lesions. Borderline splenomegaly, 14.2 cm.	Liver enlarged, 17.1 cm, mild heterogeneous echotexture. Gallbladder sludge with wall aggregates. Focal anterior wall thickening, 1.32 cm, suggestive of chronic cholecystitis. Spleen enlarged, 15.4 cm.
11	Liver 15 cm, heterogeneous echotexture, mild fatty liver. Splenomegaly, spleen 14.8 cm.	Normal findings, spleen 11 cm.
12	Slightly increased liver echogenicity and coarse granular heterogeneous echotexture with mild focal lobulation of margin, findings suggestive of early cirrhotic changes. Contracted gallbladder. Splenomegaly, 15.6 cm.	Significant hepatic heterogeneous echotexture. Splenomegaly, 14.2 cm. Spectral Doppler: flattened waveform of portal vein, absence of respiratory alterations, findings consistent with portal hypertension.
13	Coarse hepatic echotexture, macrolobulated margin. Portal vein diameter 1.5 cm, periportal echogenic thickening. Gallbladder filled with echogenic material, Spleen 13.1 cm.	Cirrhotic liver with lobulated margin, mild hypertrophy of left and caudate lobes. Mild portal vein dilatation. Borderline splenomegaly, 13.5 cm.
14	Liver heterogeneous echotexture with few small hypoechoic elements in right lobe. Fatty liver infiltration. Hepatomegaly with inferior margin extended by 3 cm. Spleen, 13 cm.	Increased hepatic echogenicity consistent with fatty infiltration. Liver size within normal limits. Gallbladder with sludge. Mild splenomegaly, spleen 13.6 cm.
15	Focal increased liver echogenicity, consistent with fatty infiltration. Contracted gallbladder.	Heterogeneous hepatic echotexture.

Two of the patients (#1 and #12) both at T0 and T2 had clinically significant portal hypertension with oesophageal varices diagnosed at the upper GI endoscopy; Patient #9 had persistently elevated liver enzymes × 3–5 times the upper normal limit from baseline to last assessment and was classified as CFLD.

Mild fluctuations in liver function tests (2–3 times the upper limit of normal) occurred in 15 patients (26.3%) in the beginning of ETI treatment but did not require discontinuation of therapy. Only one patient with CFLD received the drug initially in a reduced dose and then returned to the recommended dose.

3.2. CFLD Patients

A total of 11 out of 15 (73.3%) who were classified as CFLD at baseline were male. From those with CFLD, 14 had at least one copy of the F508del mutation (Table 3). Liver, biliary, and spleen imaging modalities at T0 and T2 are shown in Table 4. One patient (#9) classified as CFLD from baseline to the end of the follow-up despite normal liver ultrasonography, LSM, or APRI at T2. However, she had persistently elevated liver enzymes × 3–5 times the upper normal limit.

Based on findings in ultrasonography and LS values, eight patients (#1, #4, #10, #11, #12, #13, #14, #15) had advanced chronic liver disease and/or portal hypertension at T0 and seven of them remained CFLD at T2 (#1, #10, #11, #12, #13, #14, #15) with LSM >6.85 and/or ultrasonography findings consistent with liver disease. The remaining (#4) showed a decrease in LSM less than 6.85 and normalization of ultrasonography findings

(Tables 3 and 4). Two of patients with advanced chronic liver disease (#1 and #12) both at T0 and T2 had clinically significant portal hypertension with oesophageal varices diagnosed at the upper GI endoscopy.

LSM in CFLD patients decreased from 10.2 kPa (6.8–13) to 6.2 (5.0–12.4) kPa at T0 and T2, respectively ($p = 0.025$) (Figure 1B). No patient experienced ascites, variceal hemorrhage or died due to liver complications during the total study period.

4. Discussion

In this study, we found that adult patients with CF under treatment with CTFR modulators for 15 to 123 months showed a significant regression in liver fibrosis throughout the total study period. Moreover, 26.3% of patients were diagnosed with CFLD in the initiation of treatment but only 14.0% in the last assessment while no patient developed de novo CFLD during treatment.

Liver involvement in CF is challenging to diagnose, as most patients are asymptomatic and may present with normal liver enzymes until advanced disease develops (Σφάλμα! Δεν έχει οριστεί σελιδοδείκτης, [7,19]). Hence, liver disease may be underdiagnosed and underestimated. The use of liver biopsy is considered essential to differentiate cirrhotic from non-cirrhotic portal hypertension and to diagnose the liver disease pattern, stage, and progression. However, it did not reach sufficient consensus due to its invasive nature and to sampling errors in the setting of focal lesions of CFLD [6]. Therefore, some investigators recommended dual-pass percutaneous liver biopsy to avoid sampling errors [12,20]. Non-invasive biomarkers were added to the conventional assessment to identify all patients with liver disease [12,21]. Transient or ShearWave elastography (SWE) are considered the most valuable tools for estimating liver fibrosis and have the advantage of repeated measurements during liver disease course to assess fibrosis progression or regression [22]. However, LSM may misjudge liver disorders like steatosis, cholangiopathy and porto-sinusoidal vascular disease common in CFLD. Regarding liver steatosis, controlled attenuation parameters or magnetic resonance proton density fat fraction can be used to identify it. Injury of intra-hepatic ducts manifesting as single stenosis or multiple stenoses are difficult to diagnose, and magnetic resonance cholangiography is needed. Finally, porto-sinusoidal vascular disease is usually underestimated by all the above methods and liver histology is required to provide the evaluation of that entity [6,7].

Other biomarkers such as APRI or FIB-4 have been widely studied in various forms of liver disease but their usefulness in CFLD is less established [6,7].

In the current study, LSM confirmed CFLD at baseline and demonstrated significant regression of fibrosis at the end of follow-up after long-term CFTR modulator therapy. FIB-4 did not help to diagnose CFLD, as it never reached a value more than 1.5. On the other hand, the conventional criteria should not be overlooked as four (26.6%) patients with findings consistent with CFLD in ultrasonography had LSM less than 6.85 kPa at baseline. It seems, therefore, that diagnostic methods should be used in parallel and in a complementary way and it is wise for the clinician to use a variety of diagnostic methods to accurately identify all patients with CFLD.

CFTR modulators act in extra-pulmonary epithelium by improving its function. Regarding gastrointestinal involvement, an improvement in pancreatic disease by improving glycaemic control [23] and a modest improvement in gastrointestinal symptoms and markers of inflammation after a short period of 6 months of ETI administration was demonstrated [24]. Theoretically, CFTR modulators could improve abnormal CFTR in cholangiocytes and liver endothelium by enhancing bicarbonate secretion and stimulating bile flow [25]. However, in clinical practice, the results are controversial regarding CFLD. In the United States CF Foundation Patient Registry, no difference in aminotransferase values

were reported in a mean duration of 20 months of ETI treatment [26]. In another study of 6 month-duration including children and young adults, no difference in liver fibrosis assessed by SWE was observed [27] while in another, in adolescents or young adults, where liver fibrosis was assessed by transient elastography, reduction in liver stiffness was reported [28]. However, most data are on pediatrics and the effect of CFTR modulators on adults with CFLD is scarce. No changes in liver fibrosis outcomes apart from APRI were seen in a study (mean duration of 21 months) of 74 adults [29]. It is reasonable that adults have more frequently advanced liver fibrosis compared to children, resulting in irreversible liver damage. In that case, treatment focuses on stopping or slowing its progression and preventing complications.

In this study, we have evaluated longitudinal liver-related outcomes for a long period of time, i.e., 15–123 months of LI+ETI or ETI alone in adults. We incorporated a variety of methods to capture liver disease progression or regression over time including laboratory, imaging modalities, and surrogate markers of fibrosis. We found both an essential reduction in LSM in total cohort as well as in those with CFLD. Ultrasound abnormalities and liver fibrosis markers consistent with CFLD, when present at treatment initiation, normalized or significantly improved at the last evaluation in almost half CFLD patients (7 out of 15) in a way that they could not be classified as CFLD. The most important finding was that no new patient developed CFLD during the treatment period. In addition, no CFLD patient developed any liver-related complications during the long study period. However, seven out of eight patients who remained CFLD at last assessment (despite the improvement in LSM) may have long-term chronic liver disease and/or portal hypertension at baseline and two of them had documented clinically significant portal hypertension with oesophageal varices diagnosed by upper GI endoscopy. Our findings support the view that individuals with chronically established liver disease may not completely respond to CFTR modulators even after long-term treatment. Consequently, treatment may be most beneficial if it is administered at an early liver disease stage. Liver enzymes did not show a reduction longitudinally. It is evident from the literature that liver biochemistry tests fail to accurately evaluate severity of liver disease or predict liver-related outcomes [6].

Importantly, in our study, no patient discontinued treatment due to liver enzyme abnormalities. Mild elevations of liver enzymes were frequent but returned to normal with ongoing treatment and no patient developed severe hepatic complications. This is explained by the fact that our study population were adults at ETI onset and liver enzyme abnormalities caused by CFTR modulators are more frequent in young children [30].

Our study acknowledges strengths. It is a real-word study on adults where the data in the literature are rare. It is a long-lasting study with treatment administration from one to ten years. Moreover, many diagnostic modalities for the evaluation of liver disease have been used longitudinally and no patient was lost from follow-up.

The limitations of the study are related to its retrospective nature as the retrospective period of the study was long without frequent evaluations. For example, during this period, confounding factors (like nutrition, BMI, and inflammation) may have improved overall clinical status. Furthermore, there is a lack of a control group not receiving CFTR modulators. Finally, the study has limited generalizability due to single-center and small CFLD subset.

5. Conclusions

In conclusion, this long-term, real-world study of adults receiving CFTR modulators showed improvement of liver disease assessed by ultrasonography and transient elastography. At the last assessment, about half of the patients no longer met the criteria for CFLD. No liver-related complications were observed even in those who remained with

liver involvement until the end of follow-up. No new cases of CFLD emerged during the period of the study. Well-designed, long-term, prospective studies are needed to reach safe and accurate conclusions about the impact of CFTR modulators on the natural history of CFLD.

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Abbreviations

The following abbreviations are used in this manuscript:

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APRI	AST to Platelet Ratio Index
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CF	Cystic Fibrosis
CFLD	Cystic Fibrosis-associated Liver Disease
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator modulators
ETI	Elexacaftor/Tezacaftor/Ivacaftor
FIB-4	Fibrosis-4 Index
gammaGT	Gamma-Glutamyl Transferase
GI	Gastrointestinal
Hb	Hemoglobin
INR	International Normalized Ratio
IQR	Interquartile Range
LI	Lumacaftor/Ivacaftor
LS	Liver Stiffness
LSM	Liver Stiffness Measurement
NITs	Non-Invasive Liver Fibrosis Tests
SWE	ShearWave Elastography
TE	Transient Elastography

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Long-Term Effects of Elexacaftor/Tezacaftor/Ivacaftor on Nocturnal Cardiorespiratory Polygraphy Parameters in Patients with Cystic Fibrosis: A Prospective Study

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Abstract

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CFTR gene, leading to multi-system impairment. Sleep respiratory disorders (SRDs) are frequent in individuals with CF—even in those with normal or mildly impaired lung function—and may adversely affect overall health. The triple combination of elexacaftor, tezacaftor, and ivacaftor (ETI) has markedly improved clinical outcomes in CF; however, its long-term impact on SRDs remains unclear. This study aimed to assess the effects of ETI on nocturnal cardiorespiratory parameters in individuals with CF over a two-year period. Thirty-five clinically stable patients aged ≥13 years, eligible for ETI therapy, were enrolled. Nocturnal cardiorespiratory polygraphy and spirometry were performed at baseline (T0), one year (T1), and two years (T2) after ETI initiation. After one year, significant improvements were observed in mean oxygen saturation ($mSpO_2$), time with $SpO_2 \leq 90\%$ ($t \leq 90\%$), and respiratory rate. Spirometric indices (FEV_1, FVC, FEF) also significantly increased ($p < 0.05$). Correlation analysis revealed positive associations between $mSpO_2$ and FEV_1 ($\rho = 0.515$, $p = 0.002$) and between FEV_1 and FVC ($\rho = 0.894$, $p < 0.001$), while $t \leq 90\%$ negatively correlated with FEV_1 ($\rho = -0.404$, $p = 0.016$). No additional significant changes were found at T2. ETI therapy resulted in sustained improvements in nocturnal oxygenation and lung function, supporting the importance of nocturnal respiratory monitoring during follow-up.

Keywords: cystic fibrosis; elexacaftor/tezacaftor/ivacaftor; sleep respiratory disorders; lung function; cardiorespiratory polygraphy

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an epithelial ion channel responsible for chloride and bicarbonate transport across cell membranes [1]. Mutations in this gene impair ion exchange, leading to thickened secretions, chronic respiratory infections, pancreatic insufficiency, and progressive lung disease. In recent years, CF care has advanced markedly with the introduction of CFTR modulators that enhance protein function by improving chloride ion transport or promoting correct protein folding and trafficking to the cell membrane [2,3]. A highly effective CFTR modulator, the triple combination of elexacaftor, tezacaftor, and ivacaftor (ETI), is approved for individuals with at least one F508del mutation

and widely prescribed to patients aged 6 and older [2,4]. ETI has shown exceptional clinical effectiveness, altering disease progression with improvements in lung function, fewer pulmonary exacerbations, lower sweat chloride levels, reduced hospitalizations, higher body mass index, enhanced quality of life, and decreased infections and mortality. Furthermore, ETI has shown a favorable safety and tolerability profile [5–11]. However, the impact of ETI on sleep respiratory disorders (SRDs) has yet to be thoroughly evaluated.

SRDs are a significant yet often overlooked comorbidity in people with CF, even in those who are clinically stable and have normal to mildly impaired lung function [12–15]. These include obstructive sleep apnea, nocturnal hypoxemia, and nocturnal hypoventilation, which may arise from chronic lung disease, nocturnal cough, sinusitis, obesity, and gastrointestinal disorders [16–18]. These disturbances often arise early and can worsen pulmonary, cardiovascular, metabolic, and cognitive health, underscoring the need for effective screening and treatment strategies [17]. Interest in sleep studies for people with CF has grown, with an increasing number of studies highlighting the importance of nocturnal polysomnography or pulse oximetry as routine investigations in CF [15,19–21]. Although several studies have explored the effects of ETI on sleep in people with CF, objective assessments using instrumental methods remain limited, and the available evidence is largely based on short-term observations and subjective reports [22–28]. In this prospective study, we aimed to expand the investigation of ETI therapy by evaluating its effects in a larger and heterogeneous cohort of clinically stable patients, assessing not only its initial benefits but also their long-term persistence.

2. Materials and Methods

2.1. Patients and Study Design

This was a prospective, single-center, non-profit study conducted between January 2022 and July 2024 at the Pediatric Pulmonology and Cystic Fibrosis Unit of San Marco Hospital, University of Catania, Italy. We included CF patients eligible for ETI, aged over 13 years, to ensure the application of uniform diagnostic criteria for sleep disorders [29]. Only individuals who were clinically stable at the time of follow-up were enrolled. Clinical stability was defined as the absence of any recent deterioration in respiratory symptoms—such as increased dyspnea, cough, or sputum production—together with no new pathological findings on chest examination and no decline in pulmonary function compared with previous assessments. Patients with ongoing or recent pulmonary exacerbations were therefore excluded, and all evaluations were performed during a clinically stable period of at least three months. Additional exclusion criteria included chronic oxygen therapy and previous lung transplantation. Patients using nocturnal non-invasive ventilation (NIV), such as CPAP or BiPAP, were included if they did not require continuous oxygen supplementation and met the stability criteria. Given the small number of NIV users (n = 5) and their clinical stability at baseline, their data were pooled with those of non-NIV users for all analyses.

No formal a priori power calculation was performed, as the study was exploratory and the sample reflected the full eligible population available during the study period.

At baseline (T0), all patients initiated ETI therapy, consisting of two morning tablets containing 75 mg ivacaftor (IVA), 50 mg tezacaftor (TEZA), and 100 mg elexacaftor (ELX), and one evening tablet containing 150 mg IVA. ETI was administered in addition to the patients' usual standard of care, including airway clearance techniques, inhaled antibiotics or mucolytics, and pancreatic enzyme replacement when indicated.

We collected data on overnight monitoring and pulmonary function tests from each patient at baseline (T0), after one year (T1) and after two years (T2).

The study complied with the Declaration of Helsinki [30], approved by the ethical committee (Ethical Committee n. 278/2021, date of approval 20 December 2021). Informed consent was obtained from all enrolled patients and the parents or legal guardians of the minors involved.

The primary objective of the study was to evaluate longitudinal changes in nocturnal cardiorespiratory polygraphy parameters during ETI therapy compared with baseline, with the aim of determining whether CFTR modulation results in measurable and sustained improvements in sleep-related respiratory function.

2.2. Data Collection

Lung function was evaluated by standard spirometry (Cosmed srl, Rome, Italy), according to the European Respiratory Society (ERS) and American Thoracic Society (ATS) criteria [31]. Values of forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and forced expiratory flow at 25% (FEF-25), 50% (FEF-50), 75% (FEF-75) were expressed as a percentage of the predicted normal values adjusted for age, sex, height, ethnicity and weight.

Patients underwent nocturnal cardiorespiratory polygraphy at the hospital using VitalNight Plus® (software v.5.19b, VitalAire, Paris, France), while maintaining their usual respiratory support. Each study recorded the following parameters: apnea-hypopnea index (AHI), defined as the number of apnea and/or hypopnea episodes per hour of sleep; oxygen desaturation index (ODI), which represents the number of $\geq$3% arterial oxygen desaturation events per hour of sleep; mean oxygen saturation ($mSpO_2$); time spent with SpO_2 below 90% ($t \leq 90\%$); and mean overnight respiratory rate (RR). Polygraphy was scored according to the American Academy of Sleep Medicine (AASM) adult guidelines [29], with AHI $\geq$ 5.0 events/hour indicating sleep apnea. Desaturation events was defined as $\geq$3% decreases in SpO_2. Each recording lasted at least 6 h.

Demographic and anthropometric data—including sex, age, age at ETI initiation, and BMI—were also collected for each participant.

2.3. Statistical Analysis

All statistical analyses were performed using SPSS (IBM SPSS Statistic for Windows, version 26.0, IBM Corp., Armonk, NY, USA). Descriptive analyses are presented as medians (25th–75th percentiles) or means $\pm$ standard deviation, based on the distribution assessed with the Shapiro–Wilk test. Categorical variables are presented as numbers and percentages. Pre- and post-treatment comparisons were conducted using the Wilcoxon signed-rank test. Effect sizes were calculated using the correlation coefficient r, derived from the formula $r = z/\sqrt{N}$ (Rosenthal, 1991). Interpretation followed Cohen's (1988) established thresholds: small ($\approx$0.10), medium ($\approx$0.30), and large ($\approx$0.50). Given the exploratory design and limited sample size, more complex longitudinal approaches (such as mixed-effects models) were not applied, as they would not be statistically reliable in this context. Spearman correlation coefficients were computed for T2 variables. The significance level was set at $\alpha = 0.05$, and *p*-values < 0.05 were considered statistically significant.

3. Results

Thirty-five White CF individuals were included (mean age at ETI initiation was 28.6 $\pm$ 13.6 SD). Eighteen were female (51.4%) and seventeen male (48.6%). Before treatment, five patients were receiving nocturnal NIV without continuous oxygen supplementation. NIV was discontinued within 12 months of starting ETI due to clinical improvement.

Patient characteristics are summarized in Table S1.

Comparing the sleep study results baseline (T0) to one year after treatment (T1), we observed significant improvement in average oxygen saturation, t ≤ 90%, and overnight respiratory rate (RR). In contrast, no significant changes were detected in ODI or AHI values between T0 and T1. Specifically, the median value of $mSpO_2$ at T1 was 97.0% (96.0–98.0) versus 97.0% (94.7–98.0) at T0 ($p = 0.007$, r = 0.45). Similarly, t ≤ 90% decreased significantly from T0 to T1, with the median value decreasing from 0.0 (0.0–0.3) at T0 to 0.0 (0.0–0.0) at T1 ($p = 0.005$, r = 0.47). Mean RR declined from 23.0 (22.0–25.0) at T0 to 22.0 (22.0–23.0) at T1 ($p = 0.049$, r = 0.33).

Spirometry also showed significant improvements after 12 months of therapy, with changes in FEV1, FVC, FEF-25, FEF-50, and FEF-75 being statistically significant ($p < 0.05$). For instance, median FEV1 at T1 was 87.8% (68.8–107.0) versus 68.9% (50.0–94.7) at T0 ($p < 0.001$, r = 0.78). BMI likewise increased significantly at T1. The relevant statistical data are presented in Table 1.

Table 1. Comparison between T0 and T1.

	T0	T1	z	*p*	r
AHI	3.30 (1.00–3.70)	2.20 (1.80–3.80)	−0.080	0.936	0.001
ODI	2.50 (1.20–3.32)	3.20 (1.60–4.20)	−1.132	0.258	0.19
$mSpO_2$	97.0 (94.7–98.0)	97.0 (96.0–98.0)	−2.679	0.007	0.45
t ≤ 90%	0.00 (0.00–0.32)	0.00 (0.00–0.00)	−2.805	0.005	0.47
overnight RR	23.0 (22.0–25.0)	22.0 (22.0–23.0)	−1.973	0.049	0.33
FEV1	68.9 (50.0–94.7)	87.8 (68.8–107.0)	−4.619	<0.001	0.78
FVC	91.6 (66.0–101.2)	93.7 (82.1–110.4)	−4.234	<0.001	0.71
FEF-25	58.5 (38.9–93.3)	78.6 (46.3–104.5)	−3.861	<0.001	0.65
FEF-50	45.2 (21.8–68.9)	60.5 (35.6–93.8)	−4.210	<0.001	0.71
FEF-75	30.6 (14.3–48.4)	36.4 (24.1–67.1)	−3.669	<0.001	0.11
BMI	21.5 (19.0–24.4)	22.4 (20.1–24.7)	−3.906	<0.001	0.66

Legend: $mSpO_2$: mean nocturnal oxygen saturation; t ≤ 90%: time spent with SpO_2 at or below 90%; RR: mean overnight respiratory rate; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; BMI: body mass index; Data are expressed as median and interquartile range. Effect sizes are reported as r, calculated from the z-value ($r = z/\sqrt{N}$). Significance level was set at $p < 0.05$.

When comparing the results between one year (T1) and two years (T2) of therapy using the same statistical approach, the improvements observed in $mSpO_2$, overnight RR, t ≤ 90%, and spirometric parameters were maintained over time, with no further significant changes detected. The results are shown in Table 2. While the comparison between parameters previously mentioned are presented in Figure 1.

Table 2. Comparison between T1 and T2.

	T1	T2	z	*p*	r
AHI	2.20 (1.80–3.80)	2.20 (0.80–3.40)	−1.400	0.161	0.24
ODI	3.20 (1.60–4.20)	3.00 (2.27–4.30)	−0.081	0.936	0.01
$mSpO_2$	97.0 (96.0–98.0)	96.8 (96.0–97.0)	−1.234	0.217	0.21
t ≤ 90%	0.00 (0.00–0.00)	0.00 (0.00–0.00)	−1.572	0.116	0.27
overnight RR	22.0 (22.0–23.0)	22.5 (20.7–24.0)	−0.467	0.641	0.08
FEV1	87.8 (68.8–107.0)	91.1 (65.5–107.9)	−0.483	0.629	0.08
FVC	93.7 (82.1–110.4)	96.0 (74.6–104.8)	−1.549	0.121	0.26
FEF-25	78.6 (46.3–104.5)	89.5 (36.4–106.0)	−0.089	0.929	0.02
FEF-50	60.5 (35.6–93.8)	71.1 (20.9–94.6)	−0.597	0.551	0.10
FEF-75	36.4 (24.1–67.1)	56.0 (23.0–87.4)	−1.511	0.131	0.26
BMI	22.4 (20.1–24.7)	22.4 (20.1–24.7)	−0.704	0.481	0.12

Legend: $mSpO_2$: mean nocturnal oxygen saturation; t ≤ 90%: time spent with SpO_2 at or below 90%; RR: mean overnight respiratory rate; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; BMI: body mass index; Data are expressed as median and interquartile range. Effect sizes are reported as r, calculated from the z-value ($r = z/\sqrt{N}$). Significance level was set at $p < 0.05$.

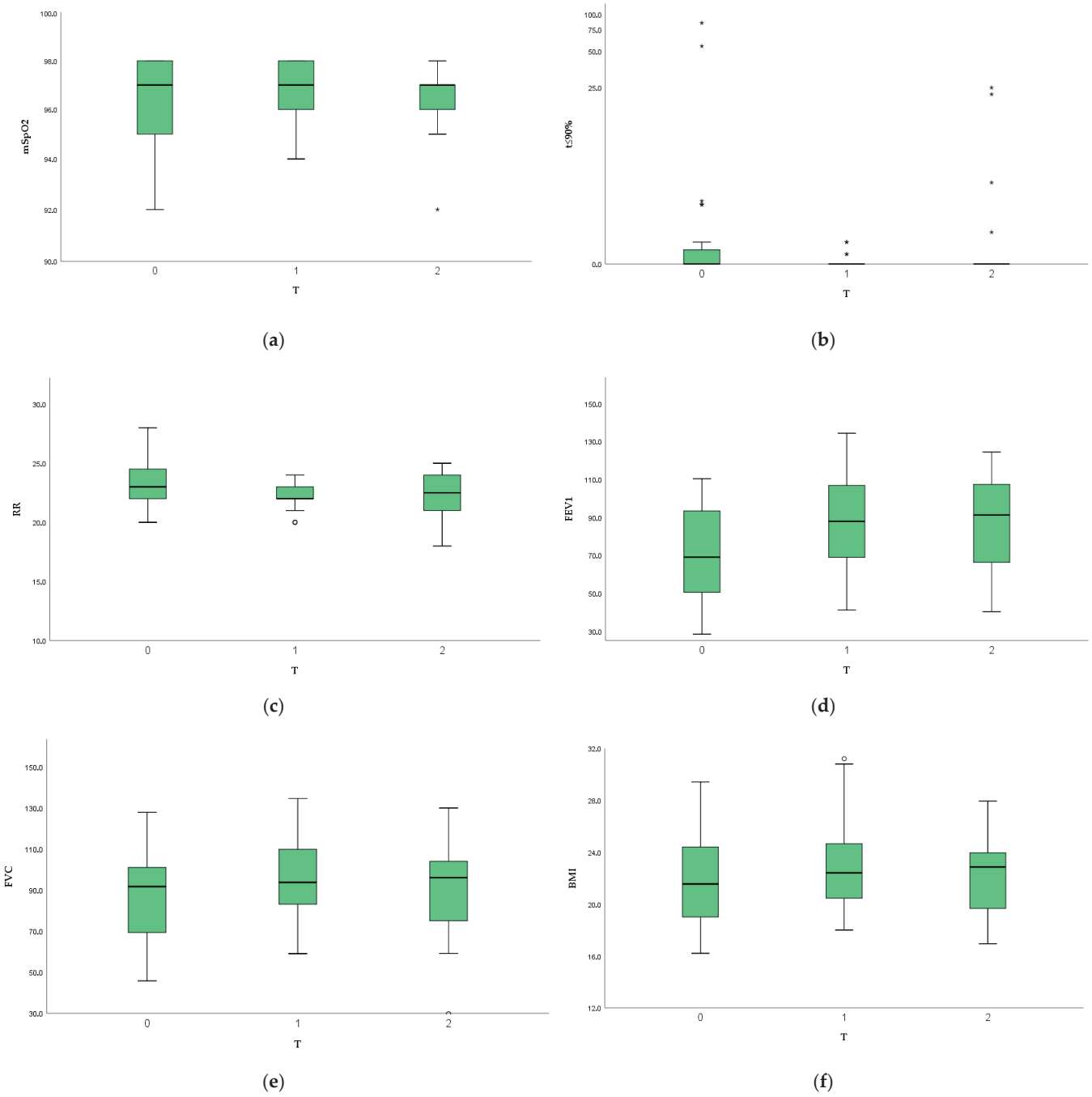


Figure 1. Comparison of $mSpO_2$ (**a**), t ≤ 90% (**b**), RR (**c**), FEV1 (**d**), FVC (**e**), BMI (**f**) recorded at T0, T1, and T2. **Legend:** $mSpO_2$: mean nocturnal oxygen saturation; t ≤ 90%: time spent with SpO_2 at or below 90%; RR: mean overnight respiratory rate; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; BMI: body mass index.

We calculated Spearman correlation coefficient for the variables that showed a significant change from baseline to T1. Notably, we found a positive correlation between $mSpO_2$ and FEV1 ($\rho = 0.515$, $p = 0.002$), and between FEV1 and FVC ($\rho = 0.894$, $p < 0.001$). In contrast to these results, we noted a negative correlation between t ≤ 90% and FEV1 ($\rho = 0.404$, $p = 0.016$). No significant correlation was found between BMI and any of the other variables. The results are shown in Figure 2, and the full correlation values are provided in Table S2.

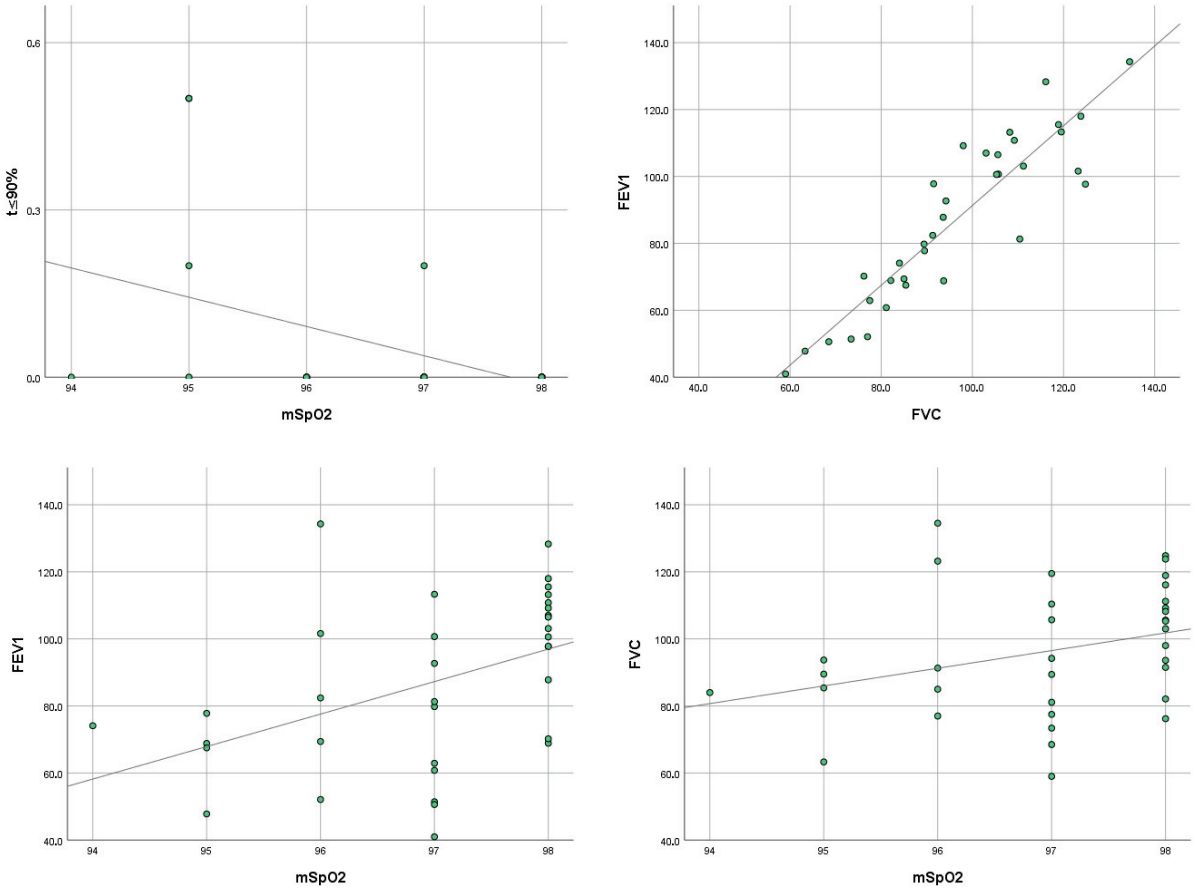


Figure 2. Correlation between $mSpO_2$, $t \leq 90\%$, FEV1 and FVC. **Legend:** $mSpO_2$: mean nocturnal oxygen saturation; $t \leq 90\%$: time spent with SpO_2 at or below 90%; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity.

4. Discussion

Sleep-related respiratory disorders are a significant yet often overlooked comorbidity in patients with CF, even among those who are clinically stable [15]. These disorders may be related to chronic lung disease, nocturnal cough, chronic sinusitis, increasing rates of obesity, and gastrointestinal symptoms [16–18,32]. They often arise early in the disease course and negatively affect physical, psychological, and cognitive health [17] underscoring the need for effective screening and treatment strategies to improve patients' quality of life.

Triple combination therapy has marked a turning point in the natural history of CF. While the efficacy and safety of ETI are well established [33], its effectiveness in managing sleep-related respiratory disorders is still poorly characterized. Our study is the first to evaluate the impact of ETI on nocturnal cardiorespiratory polygraphy parameters with a follow-up period of 24 months. Although polysomnography is considered the gold standard for assessing sleep architecture and arousals, it was not included in this study because our outcomes focused specifically on respiratory sleep parameters, which can be reliably captured using cardiorespiratory polygraphy. Mean oxygen saturation, time with $SpO_2 \leq 90\%$, and mean respiratory rate, together with spirometric measurements, showed significant improvement after 12 months, with these gains remaining stable at 24 months of therapy.

An increasing number of studies advocate for the routine use of polysomnography in CF management [19–21]. Our results support the integration of sleep studies as a regular component of CF patient evaluations from the early stages of disease.

We observed an increase in nocturnal $mSpO_2$ in CF patients after 12 months of ETI therapy. During sleep, especially in the REM phase, episodes of hypoxemia may occur, which can be attributed to hypoventilation or an alteration of the ventilation-perfusion ratio [34].

In the review by Reiter et al. [17], both adults and children with CF experience lower nadir saturation levels and overall SpO_2 values compared to healthy individuals. Similarly, Silva et al. [35] found decreased nocturnal SpO_2 levels in a group of children and adolescents with normal or mildly impaired lung function, while Naqvi et al. reported reduced minimal SpO_2 in the absence of significant respiratory events, even among patients with moderate to severe lung disease [36]. In our cohort, aside from one patient with an average saturation below 92%, no pathological values were identified at baseline. Nevertheless, we showed that ETI therapy significantly improves mean nocturnal oxygen saturation, even when baseline values are within the low-normal range.

Consistent with this improvement, $t \leq 90\%$ also showed a statistically significant increase after 12 months of therapy. However, it is important to note that only two of our patients had a pathological value at T0. We anticipate similar therapeutic success even with a larger sample that includes patients with pathological baseline values. These results emphasize the potential of the therapy to enhance respiratory function during sleep.

In line with our results, a previous study conducted at our center documented the effectiveness of ETI therapy on nocturnal cardiorespiratory polygraphy parameters in nine patients with advanced lung disease and nocturnal ventilation. We concluded that ETI therapy improved nocturnal saturation, both in terms of $mSpO_2$ and $t \leq 90\%$, with benefits evident even three months after initiating therapy, and this improvement was sustained at 12 months [23].

In the present study, however, we found a significant long-term improvement in nocturnal oxygenation, despite involving patients with less severe lung disease. These findings further underscore the importance of incorporating sleep assessment into the routine clinical management of clinically stable CF patients, as they demonstrate that ETI-related benefits extend to earlier stages of the disease.

Furthermore, nocturnal monitoring also revealed a reduction in overnight RR, which had been slightly elevated at baseline. This statistically significant improvement has been documented in the literature [22]. It may be hypothesized that the reduction in respiratory effort following ETI therapy is the result of an overall enhancement in respiratory function and the subsequent increase in average SpO_2. Conversely, a lower RR decreases oxygen demand and may help maintain more stable nocturnal oxygen saturation. In a previous study, we attributed this improvement to enhanced respiratory muscle strength, as indicated by increases in Maximum Inspiratory Pressure (MIP) and Maximum Expiratory Pressure (MEP) [23].

These findings are consistent with those reported by Dietz-Terjung et al. [24], who also observed improvements in nocturnal respiratory parameters—including AHI, ODI, and respiratory rate—after ETI therapy, although sleep quality, particularly among women, remained suboptimal despite objective gains.

Additionally, our study evaluated another clinically relevant outcome of ETI therapy. At T0, five patients were using nocturnal non-invasive ventilation, and after 12 months of therapy, all were able to discontinue ventilatory support due to overall clinical improvement. None required ventilatory support at T1 or T2. Previous studies have shown similar cases where ETI-induced improvements allowed patients with advanced lung disease to stop ventilation or oxygen therapy, even delaying lung transplantation [23,37].

Although most baseline parameters were within normal limits, we still observed statistically significant improvements following ETI initiation. While the clinical relevance

of small changes in already normal parameters may be limited, these findings suggest a favorable trend toward greater respiratory stability that could have implications for long-term health outcomes.

Results regarding the efficacy of ETI on the ODI are inconsistent. While Welsner et al. [22] reported a significant increase in ODI, our previous study [23] in advanced lung disease showed an improvement that was not statistically significant. In our current sample, only one patient presented a pathological ODI value at baseline, which improved after 12 months of ETI therapy. However, overall, we did not observe any statistically significant changes in ODI values in the sample.

In our cohort, AHI and ODI displayed small numerical variations over time, although these did not reach statistical significance. Given the low prevalence of abnormal baseline values, it is unclear whether these trends reflect early treatment-related effects or natural variability. Larger studies including individuals with a greater baseline SRD burden will be needed to determine whether ETI may influence these parameters in a clinically meaningful way.

Our analysis demonstrates an improvement in AHI after ETI therapy, although this change did not reach statistical significance. However, aside from two patients at T0 who were diagnosed with mild obstructive sleep apnea (OSA) (AHI $\geq$ 5), the nocturnal polygraphy performed prior to therapy did not reveal a pathological AHI in the remainder of the sample. Literature reports a low incidence of nocturnal apneas in adult CF patients [17,22], while OSA is much more prevalent among pediatric CF patients [14] due to chronic sinonasal disease and adeno-tonsillar hypertrophy [38]. In their prospective, observational sleep study, Welsner et al. [22] observed a reduction in AHI and ODI after the initiation of ETI in CF adults, despite normal pre-treatment AHI levels. This was one of the few studies conducted prior to ours that specifically examined the effects of triple combination therapy on nocturnal cardiorespiratory polygraphy parameters; however, the average duration of treatment was only 194 days, which is considerably shorter than that of our participants.

Conversely, Shakkottai et al. [39] were the first to report the effects of CFTR modulator therapy on OSA in children with CF. In contrast to previous findings, this retrospective study observed that children on modulator therapy who underwent overnight monitoring were approximately four times more likely to have OSA compared with those not receiving modulators, despite comparable nutritional status, lung function, and upper airway pathology. The authors therefore highlight the importance of ongoing OSA surveillance in children with CF. However, the study's limitations include the referral-based population—comprising children with a high clinical suspicion of OSA—and the inclusion of earlier modulator therapies predating the introduction of ETI. Further studies are needed to clarify the true prevalence of OSA in children with CF and to better characterize the modulators' impact on upper-airway physiology.

In our study, we assessed FEV1, FVC, and FEF-25, FEF-50, FEF-75, all of which demonstrated significant and statistically meaningful improvements following ETI therapy.

Spirometric improvements are well documented in the literature for both adults and children [33,40]. For instance, Sutharsan et al. [40] confirmed an 11.2% increase in FEV1 at 6 months following ETI therapy, which was notably higher than the 1.0% increase observed in the tezacaftor plus ivacaftor group. Similarly, Olivier et al. [41] conducted a retrospective study involving children aged 6 to 17, showing an 11.4% improvement in FEV1 after 3 months of therapy. Savi et al. [42] also reported FEV1 increases of +12.5% at 12 months and +13% at 24 months in 36 patients with advanced lung disease. In contrast, our cohort showed greater FEV1 gains (+27.4% at T1, +32.4% at T2), likely reflecting the inclusion of

clinically stable patients with mild to moderate lung disease, consistent with improvements reported in comparable ETI-treated cohorts.

We also assessed BMI, given that adequate nutritional support is essential for optimizing pulmonary function. Before treatment, five patients were underweight (i.e., using the WHO adult criteria, BMI lower than 18.5 kg/m^2), while the remaining patients had normal or normal-to-low BMI. Consistent with previous reports [43–45], all patients gained weight after starting ETI and median BMI increased significantly. Several mechanisms may explain this improvement. This new CFTR modulator increases caloric intake by improving appetite [46] and requiring administration with dietary fats. Additionally, the improvement in respiratory function and the reduction in pulmonary exacerbations may decrease energy expenditure associated with respiratory muscle work and appetite loss, although this has not yet been definitively demonstrated [47,48]. To date, it remains unclear whether ETI is capable of restoring exocrine pancreatic function [49]. However, in our study, fecal elastase was not routinely assessed in patients undergoing pancreatic enzyme therapy. The significant increase in BMI is an encouraging finding; however, as nutritional biomarkers were not collected and nutrition was not a predefined study endpoint, further studies are needed to determine whether this change reflects true nutritional improvement or other ETI-related mechanisms.

Spearman correlation showed a positive correlation at T1 between $mSpO_2$ and FEV1 as well as between FEV1 and FVC. The latter correlation was expected, given that FVC is strongly related to FEV1 values. The moderate correlation between $mSpO_2$ and FEV1 suggests that improved $mSpO_2$ after ETI therapy may depend on FEV1 gains. A negative correlation was also found between $t \leq 90\%$ and FEV1. Previous studies have already investigated the correlation between nocturnal pulse oximetry parameters and respiratory function. Many studies have examined respiratory function parameters (primarily expressed as FEV1, LCI, or daytime saturation) as predictive factors for nocturnal hypoxemia [42,43]. The interplay between these parameters suggests that improvements in spirometric measurements not only directly affect daytime respiratory function but may also contribute to better nocturnal oxygen saturation. This strengthens the idea that the benefits of ETI therapy extend beyond daytime pulmonary function, contributing to improved nocturnal respiratory parameters and better management of sleep-related breathing disorders. These functional correlations are consistent with imaging evidence showing that ETI leads to structural lung improvements, including reductions in mucus plugging, airway wall thickening, air trapping, and inflammatory changes. By enhancing airway patency and ventilation homogeneity, these anatomical modifications provide a common mechanistic basis for the parallel improvements observed in both spirometric indices and nocturnal oxygenation. Thus, pulmonary function tests and SRD changes should be interpreted as concomitant manifestations of the same ETI-induced structural recovery, rather than as a direct causal relationship between daytime and nocturnal respiratory function.

Ultimately, BMI improvements did not correlate with the other variables. Thus, the weight gain observed in CF patients after treatment appeared to be independently associated with improvements in both spirometric and polygraphic parameters, which may be interpreted as a general improvement in health among these individuals.

In our study, we compared outcomes after 12 and 24 months of ETI therapy. The significant clinical improvements observed from baseline to 12 months, including both spirometric parameters and nocturnal cardiorespiratory polygraphy measures, suggesting a broader overall enhancement in health status in these individuals.

To our knowledge, all studies on the efficacy of ETI therapy have reported improvements in the measured parameters within the first year of treatment [23,41]. In particular, our group and Olivier et al. [23,41] previously demonstrated a positive impact on pul-

monary function tests after 3 months of ETI therapy, which was sustained at the 12 and 6-month follow-up, respectively. Our study is among the few to demonstrate sustained clinical benefits of ETI therapy over a 24-month period, confirming that the improvements observed at one year persist for another 12 months. This supports the conclusion that the clinical improvement achieved after one year of ETI therapy are stable and enduring throughout an additional 12 months of treatment. Overall, our study adds valuable data to the existing evidence, showing that the therapeutic benefits of ETI therapy are not transient but rather sustained over a longer period.

This study has some limitations. The absence of a predefined power calculation and the modest sample size, particularly given the low prevalence of abnormal baseline sleep parameters, may have limited the ability to detect significant changes in AHI and ODI. Although we did not use validated questionnaires to assess subjective sleep quality—which could have added insight into patients' perceptions—the objective improvements in nocturnal respiratory parameters support the relevance of instrumental sleep studies in monitoring ETI therapy. Despite the limited sample size, the consistency of our findings highlights the need for validation in larger, preferably multicenter, studies. The use of race-based spirometric equations is another limitation to be addressed in future analyses. Further research should include subjective assessments, broader demographic representation, control groups, and extended follow-up beyond 24 months to better define the clinical significance and long-term impact of ETI on nocturnal respiratory health.

5. Conclusions

Current evidence on sleep disturbances in individuals with cystic fibrosis—particularly those receiving ETI therapy—remains limited. This study provides additional insight into the effects of ETI therapy on nocturnal cardiorespiratory polygraphy parameters in people with CF over a 24-month period. Our findings indicate that ETI therapy significantly enhances mean oxygen saturation levels, reduces the time spent with oxygen saturation below 90%, and lowers mean respiratory rate after 12 months. These improvements likely reflect the same ETI-induced structural and physiological benefits that also drive the spirometric gains. Importantly, the persistence of these improvements over 24 months indicates that the benefits of ETI therapy are sustained in the long term.

These findings emphasize the importance of incorporating the evaluation of cardiorespiratory parameters through sleep studies into the clinical management of people with CF, beginning in the early stages of the disease. For this reason, nocturnal respiratory monitoring should be considered an integral component of routine clinical follow-up in people with CF, including those who appear clinically stable, to ensure early detection of sleep-related disturbances and a more comprehensive assessment of ETI's long-term benefits.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/life15121942/s1. Table S1: Characteristics of patients at T0. Table S2: Correlation between mSpO2, t $\leq$ 90%, FEV1, FVC, and BMI.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethical committee of Azienda Ospedaliero-Universitaria Policlinico "G. Rodolico—San Marco" di Catania (Ethical Committee no. 278/2021, date of approval 20 December 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. For patients under 18 years of age, consent was obtained both from the patients and from their legal guardians. Written informed consent was also obtained to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy.

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