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Assessing the long-term clinical effectiveness of inhaled and anti-inflammatory therapies for lung disease in cystic fibrosis

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ASSESSING THE LONG-TERM CLINICAL EFFECTIVENESS OF INHALED AND ANTI-INFLAMMATORY THERAPIES FOR LUNG DISEASE IN CYSTIC FIBROSIS

by

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PH.D. THESIS

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To Ma, Dad, Bhaiya, Anju and Tia

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ABSTRACT

Cystic fibrosis (CF) is the most common life-restricting, genetically inherited disease among Caucasians affecting approximately 30,000 people in the United States. Lung disease is the major cause of morbidity and mortality in CF. A number of oral, inhaled, and intravenous therapies are available to combat CF lung disease. Of these, this research project focused on inhaled dornase alfa, oral azithromycin, inhaled tobramycin, and inhaled aztreonam. Data to address three research aims were requested and obtained from the Cystic Fibrosis Foundation Patient Registry (CFFPR).

The first aim examined the use of inhaled dornase alfa in younger children with CF. With no clinical efficacy data of dornase alfa in children ≤ 6 years of age, the study utilized subsequent forced expiratory volume in 1 second (FEV_1) measured between 6 – 7 years of age, to assess the effectiveness of long-term dornase alfa use ≤ 6 years of age. Propensity score methods were used to reduce the likelihood of treatment indication bias. The results suggested that receiving treatment with dornase alfa before 6 years of age did not improve FEV_1 between 6 – 7 years. Unmeasured covariates leading to treatment indication bias were likely one of the key explanations for these results. Additionally, lack of a more sensitive outcome than FEV_1 to assess lung function in young patients with early lung damage was thought to be another reason for the failure to reject the null hypothesis.

The second aim assessed the long-term clinical effectiveness of chronic azithromycin use on the rate of FEV_1 decline in CF patients between 6 – 20 years of age. This study was novel in that the rate of FEV_1 decline, rather than change in FEV_1 from baseline, was the primary outcome, which was characterized using propensity score matching followed by a linear mixed model analysis. The results of the analysis suggested that the rate of FEV_1 decline was slower in patients who did not receive chronic treatment with azithromycin. Treatment indication bias was thought to play an important role in the direction of the association between treatment and outcome.

Associations between FEV_1 % predicted and many of the other study variables included in the analysis were consistent with previous studies.

The final aim compared the clinical effectiveness of a combination of inhaled tobramycin and aztreonam with inhaled tobramycin alone on the rate of FEV_1 decline in CF patients between 6 – 20 years of age. This aim was novel in that the effect of this combination treatment on rate of decline in FEV_1 has never been assessed. A linear mixed model analysis was used after matching patients in the two treatment groups on their propensity scores. Once again, the results were contrary to the alternative hypothesis with the combination group having a steeper rate of FEV_1 decline than the group that was treated with tobramycin alone. An important reason for this result was thought to be unresolved treatment indication bias that could not be eliminated even with the use of the propensity score methods used to test the associated hypothesis.

The use of validated methods of analysis, i.e., propensity scores, to counter treatment indication bias using the largest available observational dataset for CF, was one of the key strengths of this study. Moreover, this study highlighted important weaknesses in the CFFPR with regards to lack of data on patient and physician-level variables – an area of active interest for the Cystic Fibrosis Foundation.

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CHAPTER I - INTRODUCTION

Cystic fibrosis (CF) is a genetically inherited disease, which affects a number of organs in the human body. There is currently no cure for CF; however a number of treatment modalities are used in the clinical care of patients with CF. The focus of this dissertation was to assess the long-term clinical effectiveness of therapies used to treat lung disease in CF. This introductory chapter presents the history, epidemiology, pathogenesis, and clinical features of CF; it then describes the background of the specific therapies studied in this research project – dornase alfa, azithromycin, inhaled tobramycin and inhaled aztreonam.

History of Cystic Fibrosis

CF is the most common life-restricting, genetically inherited disease among Caucasians. It affects mucus glands and leads to accumulation of thick, viscous mucus, affecting the lungs, and organs in the digestive and genitourinary systems (1, 2). Based on the history of CF provided by Littlewood (3), CF was first recognized as a specific disease entity in 1938 by Dorothy Andersen (4) and was termed as "cystic fibrosis of the pancreas." However, it was soon recognized that the thick viscous mucus was responsible for plugging the ducts of the mucus glands not only in the pancreas but also other organs in the body, which lead to the disease being labeled as "mucoviscidosis" by Sidney Farber in 1943 (5). In 1946, CF was identified to be familial in origin with an autosomal recessive inheritance pattern (6).

Paul di Sant'Agnese, a pediatrician in New York, demonstrated that patients with CF have markedly higher sweat chlorides and sodium compared to those who did not have the disease (7). This led to the development of the pilocarpine iontophoresis technique of sweat stimulation for sweat chloride testing by Gibson and Cooke in 1959 (8). Paul Quinton, in 1983, identified chloride transport as the basic defect in CF, and noted that the mucus abnormalities in CF were due to the anomalous fluid environment in which it was secreted (9). However, it was not until 1989 that the CF gene was finally

identified by three scientific groups and called the 'cystic fibrosis transmembrane conductance regulator' (*CFTR*) (10-12). Over the last 25 years, nearly 2,000 mutations of the *CFTR* gene have been identified (13). With the identification of the *CFTR* gene, rapid progress has been made in understanding the disease process, which, in turn, has influenced how CF is clinically managed (1).

Epidemiology of Cystic Fibrosis

Study of the epidemiology of CF has been greatly enhanced by the maintenance of large-scale patient registries in North America and Europe. The Cystic Fibrosis Foundation (CFF) is an organization that maintains a registry for patients treated across more than 110 CF care centers across the United States (US), with almost 28,000 patients enrolled in the registry. It is estimated that in the US, about 30,000 people have CF, yielding a prevalence of 9.6 per 100,000 (2), based on 2012 US population estimates (14). Furthermore about 10 million Americans are carriers of an abnormal *CFTR* gene (15). About one in every 3,500 newborns in the US is estimated to have CF (2).

All fifty states and the District of Columbia have had newborn screening programs in place for CF since 2010. As a result, in 2012, more than 61% of new CF cases were diagnosed due to an abnormal newborn screen. Moreover, most of the cases of CF are diagnosed before 2 years of age (Figure 1-1). The most common *CFTR* gene mutation in the US is *delta*F508 with about 87% of the people with CF having at least one and about 47% having two *delta*F508 mutations, followed by *G542X* and *G551D* at 4.6% and 4.3%, respectively (2).

The CFF patient registry has a slightly higher proportion of males at 51.7% as compared to females. The racial distribution of CF patients in the US in 2012 was primarily composed of Caucasians at 94.1% followed by Hispanics and African Americans at 7.7% and 4.5%, respectively. Almost 35% of the adults with CF had a college degree or higher, with about 45% of all adult patients working at least part-time.

Approximately 40% of adults with CF were either married or living together in 2012 (2) (Figure 1-2).

The proportion of CF patients who are 18 years of age and older has increased over the years. In 1986, only 29% of people with CF were adults; this increased to 49% in 2012 (Figure 1-3). The median predicted age of survival in the US for people with CF has increased from 31.3 years in 2002, to 41.1 years in 2012 – an increase of almost 10 years (2). In 2012, based on US population estimates, the crude mortality rate for people with CF in the US was 1.4 per million (14).

Pathogenesis of Cystic Fibrosis

CF is an autosomal recessive disease caused by mutations in the *CFTR* gene (10-12). Mutations in the *CFTR* gene can be classified into five major classes based on how they affect *CFTR* function (16). Class I mutations lead to defective *CFTR* protein synthesis and include nonsense and frameshift mutations. Class II mutations lead to defective *CFTR* protein processing, by interfering with glycosylation and folding of the protein post-translation. This leads to the degradation of the abnormally processed protein. The most common mutation observed in CF patients in the US, *delta*F508, belongs to this class. In class III mutations, the *CFTR* protein is transported to the plasma membrane but it has a defective regulation as it cannot be activated by adenosine triphosphate (ATP) or cyclic adenosine monophosphate (cAMP). Class IV mutations lead to an altered conductance with a reduction in the chloride transport rate. Finally, in class V mutations, normal *CFTR* protein is produced by the cell; however, the amount of the protein is reduced due to a promoter or splicing abnormality (17, 18). Class I – III mutations generally lead to more severe disease with regards to pancreatic insufficiency and high sweat chloride levels, whereas Class IV and V mutations lead to comparatively milder disease generally associated with pancreatic sufficiency and normal to intermediate sweat chloride levels (16).

The *CFTR* protein is expressed in many cells and primarily functions as an ion channel that controls liquid volume on the epithelial surfaces through the secretion of chloride ions and by inhibiting the absorption of sodium (1). Additionally, *CFTR* has been shown to regulate the calcium-activated chloride and potassium channels serving important roles in exocytosis and forming molecular complexes in the plasma membrane (17). The epithelial surfaces affected by CF have different functions in their un-affected state – some of these epithelia are volume-absorbing, such as the ones in the airways and distal intestines, some are volume-secretory, such as those in the pancreas and proximal intestines, whereas others are salt-but not volume-absorbing such as those in the sweat ducts (16).

Organ-Specific Pathophysiology in Cystic Fibrosis

Lung

Lung disease is the major cause of morbidity and mortality in CF. In people with CF, an absent or abnormal *CFTR* protein leads to aberrations of salt and fluid transport in the lung. This is reflected in the increased transepithelial potential difference across the airway epithelia, which was demonstrated by Knowles et al. by measuring the potential difference across the nasal and lower airways using mucosal super-perfusion (19). It was observed that the transepithelial potential difference was two to three times greater in people with CF compared with those who did not have the disease. This can be explained by the low-volume model (17). This model hypothesizes that *CFTR* absence is responsible for an increased absorption of sodium ions from the airway lumen with no *CFTR*-driven chloride secretion. At the same time, chloride absorption also increases through non-*CFTR* chloride uptake channels. However, the overall chloride absorption is less than that of sodium leading to a more negative airway epithelial surface. Additionally, an increase in sodium and chloride absorption is accompanied by an increase in fluid absorption. This leads to the dehydration of epithelial surfaces with a decrease in the airway surface liquid volume and a decrease in mucociliary clearance –

one of the lungs' primary innate defense mechanisms. Furthermore, dehydration of airway mucus leads to adhesion of this mucus to the epithelial surface making it even more difficult to clear it by cough-dependent mechanisms (16). As a result, CF patients have impaired clearance of inhaled bacteria.

Airway pathogens such as *Hemophilus influenzae*, *Staphylococcus aureus,* *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Burkholderia cepacia* become chronically colonized within the firmly adhered airway secretions and cannot be eradicated easily (17). The reasons for this high prevalence of bacteria in early lung disease in CF remain unclear. Various hypotheses have been proposed to explain this phenomenon, most of which suggest either a problem with decreased bacterial clearance, or issues with intrinsic hyper-inflammation or decreased bacterial killing (20). Chronic infection with these bacteria and the associated inflammation impair innate immunity in the lungs by a number of proposed mechanisms. The mucociliary clearance in the lungs is affected by factors involved in inflammation (21, 22) and infection in CF (23, 24), with advancing lung disease leading to a progressive impairment in mucociliary clearance (25).

In addition, a disproportionate inflammatory response is responsible for a decline in respiratory function in people with CF. According to Davies et al. (2007), for a given bacterial load in the lower airway, the inflammatory response is about 10 times greater in people with CF compared to those that do not have the disease (1). The exact mechanisms for this disproportionate airway inflammation are not clearly understood. An important characteristic of CF lung disease is the prevalence of high numbers of neutrophils in the airway. These neutrophils are a primary factor leading to the exaggerated inflammatory response resulting in the release of toxic cellular compounds that damage the airways and the lung parenchyma leading to bronchiectasis. Moreover, release of intracellular contents from dying neutrophils is responsible for deposition of high amounts of deoxyribonucleic acid (DNA) in the sputum, which in turn further increases the viscosity of the mucus (25). A viscous mucous leads to plugging of the

airways, which leads to increased bacterial persistence and further airway damage (25). Progressive airway damage increasingly impairs mucociliary clearance. This vicious cycle of infection, inflammation and airway damage leads to the development of progressive lung disease and respiratory failure in CF (26).

P. aeruginosa, an important pathogen in CF lung disease, specifically adapts to this microenvironment in the airways in CF through the formation of biofilms and the production of a capsular polysaccharide that prevents antibiotic penetration and aids in chronic colonization in the lungs (17, 27, 28).

Gastro-intestinal Tract

Absent or abnormal *CFTR* protein in the gastrointestinal tract has varied effects on organs such as the pancreas, intestines and the liver. However, the end result is obstruction and irreversible damage in all these organs. In the exocrine pancreas, a deficient *CFTR* protein leads to the restriction of chloride-bicarbonate exchange across the ductal epithelia. This limits the secretion of bicarbonate into the ducts along with restriction of the passive flow of sodium and water along with bicarbonate. This impairs the flushing of mucus from the exocrine glands, which in turn, leads to accumulation of mucus, obstruction and retention of pancreatic enzymes within the organ leading to a destruction of pancreatic tissue. (16, 17). Similarly, in the intestines, a deficient *CFTR* protein leads to the inhibition of chloride and water secretion into the intestinal lumen. Moreover, an increased sodium and water absorption from the lumen, results in the accumulation of dehydrated mucus and other intraluminal contents ensuing into intestinal obstruction (16). Almost 20% of people with CF present within the first 24 hours of life with intestinal obstruction called meconium ileus. Meconium ileus can be life-threatening if it is not treated (16).

Similar thickening of glandular secretions is also observed in the hepatobiliary system with deficient chloride and water transport. This can cause biliary cirrhosis along

with bile duct proliferation in the liver or chronic cholecystitis and cholelithiasis in the gallbladder (16).

Sweat Glands

In people without CF, sodium and chloride are absorbed from the sweat gland ducts mainly through apical sodium channels and *CFTR* protein (17). However, in CF, due to a deficient *CFTR* protein, chloride cannot be absorbed across the ductal epithelium. This limits the absorption of sodium from the ducts thereby making the secreted sweat highly salty, a dysfunction that can be measured by the pilocarpine iontophoresis technique (8, 16).

Male Reproductive Tract

In men with CF, glandular obstruction of the vas deferens can occur *in utero* leading to involution of the vas deferens and associated structures, a frequent cause of infertility in these men (17).

Clinical features of Cystic Fibrosis

A majority of CF patients present with signs and symptoms early in childhood. Almost 20% of patients present within the first 24 hours of life with intestinal obstruction called meconium ileus (16). If left untreated, this can lead to bowel perforation and peritonitis. In infants and young children, the disease usually manifests as recurrent respiratory symptoms such as cough and wheeze along with pneumonia. Failure to thrive due to exocrine pancreatic insufficiency is also observed in 85 – 90% of children in this age group, as pancreatic insufficiency can lead to steatorrhea (fat in stools), diarrhea, and abdominal distension (1).

Older children and adults usually present with recurrent respiratory symptoms. Chronic sinusitis and nasal polyps are also common presentations in this group of people with CF (1). Lung disease in this age group can present as pulmonary complications such as pneumothorax, which may be present in > 10% of people with CF (16). It may also manifest as blood-streaked sputum or frank hemoptysis (coughing of fresh blood). CF

patients can also present with late pulmonary events such as respiratory failure and cor pulmonale (16).

Distal intestinal obstruction syndrome (DIOS) can manifest in children and adults with symptoms such as loss of appetite, lower abdominal pain, and vomiting. Pancreatic beta cells, responsible for insulin production are usually affected later in life leading to a decreased insulin production. This coupled with insulin resistance caused by inflammation, can cause high blood sugar levels, which may require treatment with insulin (16). Pubertal development is usually delayed in CF and is likely to be related to nutritional defects and an increase in the number of infections (29). More than 95% of men with CF are azoospermic due to the obliteration of the vas deferens *in utero*. Also, about 20% of women with CF present with infertility due to thick cervical mucus that may hinder sperm migration. Furthermore, effects of chronic lung disease on the menstrual cycle may also lead to infertility in these women (16). Adults can also present with liver dysfunction in the form of focal biliary cirrhosis, portal hypertension and cholestasis with or without gallstones (30).

Lung Disease in Cystic Fibrosis

A majority of CF patients succumb to respiratory failure – a result of lung disease progression due to a cycle of chronic infection, inflammation and lung damage. Lung disease in CF is insidious, with patients being relatively asymptomatic before permanent lung damage ensues. The chief symptom in early CF is cough with about 50% of CF patients having a history of frequent cough as early as 10.5 months of age (31). However, the earliest evidence of lung disease in CF is infection with or without associated inflammation in bronchoalveolar lavage fluid as evidenced by the existence of microorganisms and raised levels of inflammatory markers like interleukin-8 and neutrophilia (32-35). *Staphylococcus aureus*, *Hemophilus influenza*, and *P. aeruginosa* are the most predominant early microorganisms isolated from bronchoalveolar lavage fluids (36). This early infection is highlighted in microbiological data from the CFF

patient registry (2) (Figure 1-4). Of these bacteria, chronic colonization with *P. aeruginosa* is associated with a rapid decline in lung function (37, 38).

Progression of lung disease in CF is also reflected in spirometry (Appendix I). It is the most common type of pulmonary function test and can assess both reversible and irreversible changes in functional vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1). The reversible component is due to accumulation of secretions in the airways, with or without a reaction of the airways to those secretions and occurs in 40 – 60% of patients with CF. The irreversible component is due to the chronic destruction of the conductive airway wall and bronchiolitis (16).

Diagnosis of Cystic Fibrosis

In 1998, a CFF Consensus Panel formulated criteria for the diagnosis of CF (39). The diagnostic criteria are as follows: a) one or more clinical features consistent with CF or a history of CF in a sibling or a positive newborn screening test; and b) a sweat chloride concentration > 60 mmol/L by the quantitative pilocarpine iontophoresis on two or more occasions or identification of two CF mutations or an abnormal nasal transepithelial potential difference.

Sweat Test

Measurement for sweat electrolyte levels remains the optimal diagnostic test for CF (1). In the majority of people with CF who have typical clinical features and mutations consistent with CF, the sweat test is diagnostic (30). However, intermediate levels of sweat chlorides have been observed in people with an atypical CF phenotype. Moreover, there have been rare reports of a normal sweat test in people who were found to have mutations consistent with CF on genetic testing.

A sweat chloride concentration > 60 mmol/L is considered diagnostic of CF. However, sweat chloride levels of > 40 mmol/L have been shown to be highly suggestive of a CF diagnosis in infants younger than 3 months of age (39). Furthermore, there are a number of rare clinical conditions where the sweat test has been found to be positive but

these can be ruled out based on their distinguishable clinical features. As suggested by the CFF consensus panel, the only acceptable method to conduct sweat testing is quantitative pilocarpine iontophoresis. This technique requires skill and should be administered by someone who is trained and experienced (1). A single positive sweat test alone is not diagnostic of CF. In the absence of a positive CF mutation analysis or in people where the initial sweat test is borderline, a repeat sweat test may be needed to establish the diagnosis (30).

Mutation Analysis

The identification of the CF gene brought along with it the hope that problems associated with the diagnosis of CF were over. Although the cure for CF from this discovery has been elusive, it has greatly enhanced the diagnosis of CF. The presence of mutations consistent with CF was found to predict with a certain degree of confidence that a person has CF (39). Thus far almost 2,000 mutations of the *CFTR* gene have been identified (13). However, given this large number of CF alleles, the confirmation of a CF diagnosis on the basis of two CF-producing mutations, while not very sensitive, is highly specific (39). As a result, most clinical laboratories will search for the commonest mutations within their geographic region. Customization of mutation panels to reflect the ethnic background and clinical characteristics of a person with CF, can help increase the sensitivity of mutation analysis but is not a routine clinical practice (39). Failure to find two mutations consistent with CF does not preclude the diagnosis of CF. In most cases, the diagnosis of CF will be confirmed by a positive sweat chloride test along with clinical characteristics consistent with CF and not by the presence of two CF mutations. Nonetheless, in these cases mutation analysis is still appropriate as it can be utilized for confirmation of the CF diagnosis, prediction of certain phenotypic characteristics such as pancreatic insufficiency, and provision of information for interested family members. Moreover, mutation analysis may also help with categorization of people with CF for research purposes. The results of the mutation analysis should be offered to people with

CF or their family members after providing appropriate genetic counselling (39). Additionally, there are now specific gene-based therapies to correct the CFTR transportation defects and to potentiate defective CFTR at the cell surface.

Nasal Potential Difference Measurements

Ion transport abnormalities in nasal epithelia of people with CF are associated with a different pattern of nasal potential difference compared with the normal nasal epithelia. In particular, three features are characteristic of nasal potential difference testing in CF: a) a raised nasal potential difference reflecting enhanced sodium ion transport across an epithelial surface that is relatively impermeable to chloride ions; b) nasal perfusion with amiloride (a sodium channel inhibitor), leads to greater inhibition of nasal potential difference; and c) nasal perfusion with a chloride-free solution along with isoproterenol (a beta-adrenergic agonist) causes little or no change in nasal potential difference reflecting absence of *CFTR*-mediated chloride secretion (39).

A raised basal nasal potential difference may suggest a strong likelihood of CF. However, its absence does not rule out CF as a false-negative test may be caused by an inflamed nasal epithelium. The absence of a large upswing in the nasal potential difference after perfusion with a chloride-free solution and a beta-adrenergic agonist is not confirmatory of CF. However, a large response is strong grounds for dismissing a diagnosis of CF. The nasal potential difference test must be duplicated in order for it be accepted in the diagnosis of CF (39).

Additional tests are required to clinically evaluate people with an atypical CF phenotype. Persistent airway colonization with *P. aeruginosa* – especially of the mucoid type – detected through the culture of throat swabs, sputum or bronchoalveolar fluid is highly suggestive of CF. Moreover, chronic airway colonization with other bacteria such as *Staphylococcus aureus*, *Hemophilus influenzae*, and *Burkholderia cepacia* may lend credence to a diagnosis of CF (39). Assessment of exocrine pancreatic function is important in CF as a vast majority of people with CF have abnormal pancreatic function

due to a deficient *CFTR* protein. A number of direct and indirect pancreatic function tests are available for testing the exocrine pancreatic function but all currently available tests have some drawbacks (39). Another consistent feature of CF is obstructive azoospermia in 98 – 99% of post-pubertal males with CF (40, 41). In this majority of CF males, the azoospermia is a result of absent or rudimentary vas deferentia. As a result while evaluating a post-pubertal male with an atypical presentation, it is important to conduct a thorough urogenital evaluation (39).

Treatment of Cystic Fibrosis

In 1964, Matthews et al. recognized the three pillars of treatment in CF – nutritional repletion, airway obstruction relief, and treatment of lung infections with antibiotics (42). In recent years, based on results of scientific studies, a fourth pillar has been established – suppression of inflammation (43).

Nutritional Repletion

As more than 85% of people with CF are pancreatic insufficient at birth, treatment with pancreatic enzyme supplementation is an important tool in the clinicians' armamentarium. Some clinicians administer gastric acid blockers to minimize stomach acidity and try to increase the bioavailability of pancreatic enzyme supplements (43, 44). Fat malabsorption due to pancreatic insufficiency leads to impaired absorption of fat-soluble vitamins like vitamins A, D, E, and K. These need to be supplemented to avoid problems of vitamin deficiency. Nutritional supplementation in the form of calorie-dense oral supplements, along with regular enteral feeding is also needed in people with CF because of issues with increased caloric demand due to severe lung disease, impaired enterohepatic circulation of bile, and anorexia associated with any chronic disease. Furthermore, relative underweight has been observed to be a negative prognostic indicator in CF (43, 45).

Airway Obstruction Relief

A key pathognomonic feature in CF is the plugging of airways with thick, tenacious mucus, which leads to a vicious cycle of infection, inflammation and airway damage. Hence, clearance of airway secretions has an important role in CF clinical management. Postural drainage and thoracic percussion by "clapping" on the chest wall seems to be a method of choice in younger people with CF and in people too sick to cooperate with active clearance techniques (43). Modern devices such as high frequency chest wall oscillatory mechanical vests help by vibrating the chest to release impacted secretions by rapid inflation and deflation. This method does not require assistance from a partner. Autogenic drainage is another type of airway clearance where breathing is controlled to expel mucus. Finally, people with CF who are healthier can enhance clearance with aerobic exercises that stimulate deep breathing and cough (43).

Over the years, drugs have been developed to help with the clearance of mucus from airways. An important drug which helps in the breakdown of mucus is dornase alfa – a highly purified solution of recombinant human DNase (rhDNase). By breaking down the DNA in the thick mucus from dead cells and neutrophils, dornase alfa reduces the viscosity of mucus, thereby making it easier for a person with CF to expel it. More recently, use of hypertonic saline aerosols has been shown to help with airway clearance by improving mucus hydration (46). Many CF clinicians also use bronchodilators to relax airway smooth muscles thereby relieving bronchoconstriction and increasing airway lumen size (43).

Treatment of Lung Infections with Antibiotics

Lung infections accompanied by increased pulmonary symptoms or a decline in lung function require treatment with antibiotics (43). Generally, for mild exacerbations of pulmonary symptoms, use of oral antibiotics based on bacterial culture results is satisfactory. However, severe exacerbations warrant treatment with intravenous antibiotics. More recently, aerosols of antibiotics such as tobramycin and aztreonam have

been formulated and used successfully in the treatment of lung infections. These aerosols have the advantage of reaching high concentrations in the lungs thereby reducing systemic adverse effects. Moreover, the use of aerosol treatments as suppressive therapy in the absence of pulmonary symptoms of a decline in lung function have also led to a decrease in the number of hospitalizations for exacerbations and an improvement in lung function (43, 47).

Suppression of Inflammation

Compared with infants who do not have CF, infants with CF whose airways get infected with bacteria have been found to have higher levels of interleukin-8 and neutrophil counts in bronchoalveolar lavage fluid. This is true even after adjustment for the bacterial burden and suggests an exaggerated inflammatory response (43, 48, 49). This knowledge has led to the use of alternate-day steroids and high dose ibuprofen to suppress the inflammation, which has helped with reducing the rate of decline in lung function in young people with CF (50, 51). However, with increasing rates of adverse effects of alternate-day steroids such as growth-impairment, diabetes, and cataracts, the use of alternate-day steroids has declined over the years. Recent clinical studies have shown that continuous oral alternate-day azithromycin therapy helps people with CF colonized with *P. aeruginosa* by improving their lung function and also reducing the number of pulmonary exacerbations (52). How azithromycin aids in CF is not clearly known but it is hypothesized that it has both antimicrobial and anti-inflammatory properties (52-54).

Management of Complications

The disease process in CF affects other organs like the liver, intestines and pancreas. Liver disease in CF manifests mainly in the form of hepatic steatosis, obstructive biliary cirrhosis, and in some cases gallstones. Ursodeoxycholic acid is very frequently used to reduce the progression of liver disease. Similarly, CF can cause intestinal obstruction in infants, as well as older children and adults. Oral use of osmotic

laxatives and Gastrograffin enemas in CF helps prevent or relieve intestinal obstruction. Progressive disease in the pancreas may lead to destruction of the islet cells responsible for insulin production. As a result, CF-related diabetes is quite frequently noted in older people with CF and requires treatment with insulin (43).

Even with the available clinical therapies, the decline in lung function in a majority of people with CF continues and eventually leads to respiratory failure. To extend survival in these people, lung transplantation is an option. According to the CFF, about 200 people in the US with CF received a lung transplant in 2012. However, due to a limitation in the supply of donor lungs, many people with CF on the transplant wait list do not survive. Moreover, there are additional risks associated with a lung transplant, and long-term medical care is needed for it to be successful. The 1-year survival rate after a lung transplant is about 80% with a 5-year survival rate of almost 50% (55).

Cystic Fibrosis Foundation Patient Registry

The CFF Patient Registry (CFFPR) is a large observational database that has collected encounter-level data for almost 28,000 CF patients (almost 95% of all CF patients in the US) receiving care at more than 110 CFF-accredited CF care centers across the US. These data include height, weight, gender, state of residence, CF mutations, pulmonary function test results, medication use and complications related to CF. These data are collected from all centers using online forms developed by the CFF for each clinic encounter. The CFFPR serves as a valuable tool for health care providers and scientific researchers by providing information that might help to: 1) improve the delivery of clinical care; 2) study the treatment effects of various CF therapies; 3) formulate clinical care guidelines; and 4) design clinical trials for testing new therapies (2). Moreover, the CFFPR also provides CF patients and their families an outlook on the overall health of those with the disease through an annual registry report that is publically available on their website (56).

The CFF develops CF care guidelines by reviewing medical literature and data from the CFFPR (57). These care guidelines recommend the number of clinic visits, lab tests, pulmonary function testing (PFT), etc., to be carried out each year. The outcomes and measures are defined by the CFF on an annual basis. Each center enters site-specific data in the CFFPR, which are then compared with other CF centers in the US. The CF centers are provided with such an annual report by the CFF with analysis of CFFPR data summarizing how their center compares for specific measures at the national level (58, 59).

Another large CF database, the Epidemiologic Study of Cystic Fibrosis (ESCF), has been used by CF researchers to conduct analysis of observational data. This is a large industry-sponsored prospective database with data for more than 24,000 CF patients collected from 1994 to 2005. The ESCF was designed to collect detailed, longitudinal data on CF clinical encounters for CF patients at many centers in the US and Canada. The main objectives of this database were to characterize the decline in lung function, pulmonary exacerbation rates requiring antibiotic treatment, and safety and effectiveness of long-term dornase alfa use in CF patients > 6 years of age (60).

The focus of this introductory chapter will now be on the specific CF therapies that were studied in this research project – dornase alfa, azithromycin, inhaled tobramycin, and inhaled aztreonam.

Dornase alfa

Mechanism of action

Dornase alfa is a purified solution of rhDNase. The inflammatory response in CF leads to an abundance of long strands of neutrophilic DNA in the airway. This DNA is a major contributor to the higher viscosity of CF sputum. Dornase alfa slices extracellular DNA present in the airways thereby reducing the viscosity of the mucus and promoting improved clearance of airway secretions. This, in turn, aids in improving lung function in CF patients treated with dornase alfa, as shown by the results of randomized clinical trials

(61-63). This is the primary mechanism by which dornase alfa is believed to bring about its action. However, clinical trials also noted a significant reduction in the risk of pulmonary exacerbations in patients being treated with dornase alfa (61-63). This effect may be due to a reduction in the rate of bacterial infections as a result of the improved mucociliary clearance and/or direct effects on the host defenses against infection (64).

Studies evaluating the efficacy of dornase alfa

A number of clinical trials have been carried out to evaluate the efficacy of dornase alfa in CF patients with lung disease. These clinical trials noted an improvement in forced expiratory volume in 1 second (FEV_1) in CF patients ≥ 5 years of age (61-63). Among the pulmonary function test measurements, FEV_1 is the single best predictor of mortality in CF (65). FEV_1 values in children depend on factors such as age, sex, race, and height. The CFF recommends the use of two different prediction equations to standardize FEV_1 measured in liters to % predicted. Equations from Wang et al., (66) are used for FEV_1 values obtained for males through age 17 years and for females through age 15 years, and the equations of Hankinson et al.,(67) are used for FEV_1 values obtained beyond these ages.

Fuchs et al. (61) conducted a randomized, double-blind, placebo-controlled clinical trial with three treatment arms – once daily 2.5 mg nebulized dornase alfa, twice daily 2.5 mg nebulized dornase alfa, and placebo, over a period of 24 weeks. This multicenter trial included 968 CF patients ≥ 5 years of age with a forced vital capacity (FVC) > 40% predicted recruited from 51 institutions. The primary outcomes in this study were the relative risk of pulmonary exacerbations requiring treatment with intravenous (IV) antibiotics as analyzed by a Cox proportional-hazards model, and mean % change in FEV_1 as analyzed by analysis of variance (ANOVA). The study detected a lower age-adjusted relative risk of exacerbations in the once daily ($p = 0.04$) and the twice daily dornase alfa groups ($p < 0.01$), compared with the placebo group. Furthermore, use of once daily and twice daily dornase alfa was associated with a mean

improvement of 5.8 ± 0.7% (mean ± SE) and 5.6 ± 0.7% in FEV_1, respectively ($p < 0.01$ as compared with the placebo).

Another study by McCoy et al. (62) evaluated the 12-week efficacy of once daily 2.5 mg dornase alfa in CF patients with advanced lung disease by conducting a multicenter, double-blind, placebo-controlled trial. This trial recruited 320 clinically stable CF patients with FVC < 40% predicted from 65 centers across the US. The two primary outcomes were time from randomization to first pulmonary exacerbation as analyzed by relative risk estimation using a Cox proportional-hazards model, and mean % change in FEV_1 from baseline, as analyzed by a Student's *t*-test. The relative risk of developing pulmonary exacerbations for the two groups was not significantly different from the null value of 1.0 ($p = 0.52$). However, treatment with dornase alfa was associated with a significant mean improvement in FEV_1 of 9.4% (95% CI = 6.81 – 11.96) compared with a 2.1% (95% CI = 0.07 – 4.18) mean improvement in the placebo group ($p < 0.001$).

More recently, Quan et al. (63) carried out a randomized, double-blind, placebo-controlled trial over 96 weeks. 474 CF children between 6 – 10 years of age with FVC ≥ 85% predicted were randomized to receive once daily 2.5mg nebulized dornase alfa or placebo. The main primary outcomes in this study were mean change in FEV_1 % predicted from baseline as analyzed by a repeated measures model, and the relative risk for first pulmonary exacerbation for dornase alfa compared with placebo as analyzed by a Cox proportional-hazards model. At 96 weeks, the mean difference in FEV_1 % predicted in the dornase alfa group compared with the placebo group was 3.2 ± 1.2% (mean ± SE) ($p = 0.006$). Moreover, the risk of pulmonary exacerbations was reduced by 34% in the dornase alfa group compared with the placebo group (relative risk – 0.66, 95% CI = 0.44 – 1.00, $p = 0.048$).

These results were reflected in a recent Cochrane review of 15 clinical trials with 2,469 patients, which observed an improvement in lung function in the treated groups at

one, three, and six months, and then at the end of two years. Voice alteration and rash were the only related adverse events; they were reported more frequently in the treated group only in one trial (68).

Based on the results of these clinical trials and the existing Cochrane review, an expert committee convened by the CFF **strongly recommended** treatment with once daily 2.5 mg dornase alfa in CF patients ≥ 6 years of age with moderate (FEV_1 % predicted 40 to < 70) to severe lung disease (FEV_1 % predicted < 40) and **recommended** use of dornase alfa in CF patients ≥ 6 years of age with mild lung disease (FEV_1 % predicted 70 to < 100) (57).

A recent observational study conducted by Konstan et al. (69) evaluated the effectiveness of dornase alfa use in CF using a large database – the ESCF. This study was unique in that it assessed the rate of decline in FEV_1 % predicted rather than mean change in lung function from baseline. The study utilized data from CF patients aged 8 – 38 years and divided them into two groups – one group with patients who were enrolled in the ESCF for at least two years before being treated with dornase alfa for a period of ≥ 2 years, and the comparator group consisting of patients who were not treated with dornase alfa. The analysis was carried out using a linear mixed-effects model with both random slopes and random intercepts and was adjusted for age, gender, pulmonary exacerbations, respiratory therapies, and nutritional supplements. The study noted a reduction in the mean rate of decline in FEV_1 % predicted after starting treatment with dornase alfa in children (8 – 17 years) ($p < 0.001$) and adults (≥ 18 years); however, the reduction was not statistically significant in adults ($p = 0.068$). This study outlines the importance of the use of large observational CF databases in assessing the effectiveness of CF therapies on change in the rate of decline in FEV_1.

Azithromycin

Mechanism of action

Azithromycin belongs to a class of antimicrobials called macrolides. It is an orally available drug that is used as a broad spectrum antibiotic to treat community-acquired pneumonia and skin infections. For its antimicrobial action, it is known that azithromycin works by inhibiting protein synthesis in bacteria (52). Azithromycin has direct killing properties against bacteria prevalent in CF such as *Staphylococcus aureus*, and *Hemophilus influenza*. However, it has no direct killing activity against *P. aeruginosa* and acts on it indirectly through mechanisms not well understood. It is hypothesized that azithromycin may have anti-inflammatory properties (53, 54). It has also been suggested that azithromycin acts by reducing the virulence factors associated with *P. aeruginosa*, thereby reducing its activity. These virulence factors involved in the production of mucoid biofilm may be important for the pathogenicity of *P. aeruginosa* in CF (52). Azithromycin can reach high tissue concentrations and has a long half-life; both these properties make it an attractive treatment option in CF (70).

Studies evaluating the efficacy of azithromycin

A number of clinical trials have been conducted to evaluate the efficacy of chronic use of azithromycin in CF, both in patients with chronic colonization with *P. aeruginosa* (71-73) and those without (74-76).

Efficacy of azithromycin in CF patients with chronic colonization with P. aeruginosa

Equi et al. (71) conducted a randomized double-blind, placebo-controlled crossover trial to evaluate the efficacy of azithromycin in CF. This trial was conducted in 41 CF children, 8 – 18 years of age over a period of six months. Previous chronic infection with *P. aeruginosa* was not an entry criterion in this study. The treatment group received daily treatment with azithromycin (≤ 40 kg – 250 mg daily ; > 40 kg – 500 mg daily), whereas the control group received a placebo. The treatments were crossed over

after two months of washout. The main outcome in this study was the median relative difference in FEV_1 % predicted between the two groups as analyzed by the sign test. The median relative difference in FEV_1 % predicted between the two groups was 5.4% (95% CI = 0.8 – 10.5%). The study did not observe any noticeable side-effects.

Another study conducted in Australia by Wolter et al. (73), enrolled 60 adult CF patients in a randomized placebo-controlled trial where enrolled patients were randomized to receive either 250 mg of azithromycin or placebo daily for a period of three months. The primary outcome in this study was the change in FEV_1 % predicted from baseline as analyzed by repeated measures ANOVA models. FEV_1 % predicted was maintained in the treatment group while in the placebo group there was a decline of -3.62 ± 1.78% (p = 0.047). Moreover, the study also noted significant improvements in quality of life scores, C-reactive protein levels, and the number of pulmonary exacerbations.

Saiman et al. (72) conducted a multicenter, randomized, double-blind, placebo-controlled trial at 23 CF centers in the US, which enrolled 185 patients ≥ 6 years of age and chronically colonized with *P. aeruginosa*. The enrolled patients were randomized to receive either azithromycin (< 40 kg – 250 mg; ≥ 40 kg – 500 mg) or placebo, three days a week for 168 days. The primary outcome was mean change in FEV_1 from baseline as analyzed by piecewise linear regression analysis followed by a 2-sided *t*-test. The study also analyzed time to first pulmonary exacerbation using a Cox proportional-hazards model. The mean relative change from baseline in FEV_1 % predicted was found to be significantly different between the two groups (mean difference = 6.2%; 95% CI = 2.6 – 9.8%; p = 0.001). Moreover, the patients in the azithromycin group were less likely to have an exacerbation compared to those in the placebo group (hazard ratio = 0.665; 95% CI = 0.44 – 0.95; p = 0.03).

Efficacy of azithromycin in CF patients uninfected with P. aeruginosa

A French study conducted by Clement et al. (74) enrolled 82 CF patients ≥ 6 years of age with a FEV_1 % predicted ≥ 40% in a multicenter, randomized, double-blind, placebo-controlled trial. The enrolled patients were randomized to receive azithromycin (< 40 kg – 250 mg; ≥ 40 kg – 500 mg) or placebo, three days a week for 12 months. The primary end point in the study was the mean relative change in FEV_1 as analyzed by using a linear mixed model approach. The study also analyzed the mean number of pulmonary exacerbations in the two groups using a Poisson regression model, as well as the time to first exacerbation using a Cox proportional-hazards model. The study did not observe a significant mean relative change in FEV_1 between the two groups. However, the number of pulmonary exacerbations (count ratio = 0.5; 95% CI = 0.32 – 0.79; $p < 0.005$), and the time to first exacerbation (hazard ratio = 0.37; 95% CI = 0.22 – 0.63; $p < 0.0001$) were significantly reduced in the treatment group irrespective of the infection status with *P. aeruginosa*.

Saiman et al. (75) conducted another multicenter, randomized, double-blind, placebo-controlled trial at 40 CF centers in the US and Canada to assess the efficacy of azithromycin in CF patients uninfected with *P. aeruginosa*. Two hundred sixty *P. aeruginosa*-negative patients between 6 – 18 years of age and having a FEV_1 % predicted ≥ 50% were randomized to receive either azithromycin (18-35.9 kg – 250 mg; ≥ 36 kg – 500 mg) or placebo, three days per week for 168 days. The primary outcome in this study was the mean change in FEV_1 as analyzed by a piecewise linear repeated measures regression model followed by a 2-sided *t*-test. Difference in time to pulmonary exacerbation was assessed as a secondary outcome using a Cox proportional-hazards model. The mean improvement in FEV_1 % predicted was 5.4 ± 13.3% in the azithromycin group and 3.4 ± 12.4% in the placebo group, yielding a non-significant mean relative difference in FEV_1 % predicted of 2% (95% CI= -1.2 – 5.2%; $p = 0.22$).

Patients in the treatment group had a 50% reduction in exacerbations (95% CI = 31 – 79%) compared with the placebo group. Finally, no increased risk of adverse events was noted in the azithromycin group.

As a follow-up to the Saiman et al. (2010) study, the authors conducted an open-label 24-week study of azithromycin in 146 CF patients aged 6 – 18 years, wherein patients originally in the azithromycin group in the 2010 study continued (azithromycin-azithromycin group), whereas patients initially on placebo received azithromycin (placebo-azithromycin group) (76). The dose of azithromycin was the same as in the 2010 study. The main outcome once again was mean change in FEV_1; however, in this case in the azithromycin–azithromycin group the outcome was compared in those participants during the 2010 trial versus during this open-label study. Similarly, the outcome was compared between the two study phases in patients in the placebo–azithromycin group. Similar analysis was carried out to assess the rate of pulmonary exacerbations. There were no significant improvements in lung function in either of the two groups. In the placebo-azithromycin group, no significant differences were noted in change in FEV_1 % predicted between the 2010 trial (-2.42 ± 11.73%) and this open-label study (1.78 ± 12.62%), however there was a trend towards improvement (relative change = 4.48%; 95% CI = 1.04 – 9.99%). Moreover, in the placebo-azithromycin group, the odds of a pulmonary exacerbation were comparable between the 2010 study and the open-label study results (odds ratio = 0.7; 95% CI = 0.4 – 1.5). In the azithromycin-azithromycin group, continued stability of treatment response was noted with a mean FEV_1 % predicted change of -0.06% (95% CI = -3.92 – 3.8%) and an odds ratio of experiencing a pulmonary exacerbation of 1.6 (95% CI = 0.8 – 3.0).

The results from these clinical trials were reflected in a Cochrane review that included 10 studies with 959 patients (52). The authors noted a mean improvement in FEV_1 % predicted of 3.97% (95% CI = 1.74– 6.19%) at six months. Furthermore, they observed that patients on azithromycin were about twice as likely to be free of pulmonary

exacerbations at six months compared to those in the placebo group (odds ratio = 1.96; 95% CI = 1.15 – 3.33).

Based on the cumulative clinical trial results, the expert committee convened by the CFF **recommended** the use of azithromycin three times per week in children ≥ 6 years of age with CF and with chronic colonization with *P. aeruginosa*, to improve lung function and reduce pulmonary exacerbations (57).

Inhaled Tobramycin and Aztreonam

Mechanism of Action

Tobramycin is an aminoglycoside antibiotic that acts primarily on bacteria by disrupting protein synthesis and leading to altered cell membrane permeability and eventually cell death (77). Tobramycin has activity against a wide range of gram-negative bacteria, more importantly *P. aeruginosa*. Aztreonam is a beta-lactam antibiotic and brings about its action by binding to penicillin-binding proteins in the bacteria leading to inhibition of cell wall synthesis and death of bacteria (78). Similar to tobramycin, aztreonam has activity against *P. aeruginosa* and other gram-negative bacteria commonly found in the airways of patients with CF.

Studies evaluating the efficacy of inhaled tobramycin and aztreonam

Chronic therapy with inhaled anti-pseudomonal antibiotics is the standard of care in patients with CF, who have chronic P. aeruginosa airway infection (57). Currently, two inhaled antibiotics are FDA approved for treatment of chronic P. aeruginosa infection in CF – inhaled tobramycin and inhaled aztreonam.

Inhaled tobramycin

Many clinical trials have been carried out to assess the efficacy of inhaled tobramycin in CF patients. In 1999, Ramsey et al. conducted a pivotal Phase III clinical trial to evaluate the efficacy of intermittent use of inhaled tobramycin in CF (47). A total of 520 CF patients were randomized to receive either 300 mg of inhaled tobramycin or

placebo twice daily for 28 days, with no study drug for the following 28 days. Enrolled patients received treatment or placebo in three such 28 days on/28 days off cycles for a total of 24 weeks. The rationale for the 28 days on/28 days off cycle was to lessen the likelihood of antimicrobial resistance. The authors reported a 10.08% increase in FEV_1 % predicted in the inhaled tobramycin group compared to a 1.79% decrease in the placebo group ($p < 0.001$). Additionally, the sputum *P. aeruginosa* density showed a significant reduction in the treatment group compared with the placebo group ($p < 0.001$). The patients in the treatment group were significantly less likely to be hospitalized and receive intravenous anti-pseudomonal antibiotics.

In 2004, Murphy et al. conducted a randomized open-label clinical trial in young CF patients with mild lung disease (79). One hundred eighty-four CF patients between 6 – 16 years with FEV_1 % predicted values between 70 and 110% were enrolled in this study. The treatment group received inhaled tobramycin twice daily for seven cycles (28 days on/28 days off) along with routine clinical care for a total of 56 weeks. The placebo group received routine clinical care with no inhaled tobramycin for 56 weeks. The study could not evaluate an effect on lung function decline rate due to inadequate enrollment and early study termination. However, a significantly higher hospitalization rate was noted in the placebo group compared with the treatment group ($p = 0.011$). Moreover, significantly fewer patients in the tobramycin group received treatment with oral antibiotics ($p = 0.009$).

Randomized clinical trials carried out by Lenoir et al. (80) and Chuchalin et al. (81) showed improvements in FEV_1 in the inhaled tobramycin group compared with the placebo group. Moreover, both studies noted a significant weight gain in the tobramycin group with Chuchalin et al. also reporting a significant decrease in hospitalizations and use of intravenous tobramycin and other anti-pseudomonal antibiotics. Konstan et al. conducted a randomized clinical trial in 2010 to assess the efficacy of a powdered form of inhaled tobramycin in CF patients (82). In this study 95 patients between 6 – 21 years

of age were randomized to receive either tobramycin inhalation powder (112 mg tobramycin) or placebo twice daily for one cycle (28 days on/28 days off). This was followed by two open-label cycles for all patients. The authors reported a significant improvement in FEV_1 % predicted in the treatment group compared with the placebo ($p = 0.002$). The treatment group also had reduced sputum *P .aeruginosa* density, anti-pseudomonal antibiotic use and hospitalization due to respiratory symptoms.

As a comparison study, Konstan et al. in 2011 conducted a randomized open-label trial, where 553 CF patients were randomized to either receive the inhaled tobramycin powder or the inhaled tobramycin solution twice daily for three treatment cycles. The authors observed that the rate of cough and the overall discontinuation rate were higher in the tobramycin powder group compared to the solution group. However, the increase in FEV_1 % predicted and the reduction in sputum *P. aeruginosa* density was comparable between the two groups.

Based on the results of these clinical trials, the CFF expert committee **strongly recommended** the chronic use of inhaled tobramycin in CF patients ≥ 6 years of age, with moderate to severe lung disease and chronic airway colonization with *P. aeruginosa* to reduce exacerbations and improve lung function and overall quality of life. Moreover, the committee also **recommended** the chronic use of inhaled tobramycin in CF patients ≥ 6 years of age, with mild lung disease and chronic airway colonization with *P. aeruginosa* to reduce pulmonary exacerbations (57).

Inhaled aztreonam

A number of clinical trials have been conducted to assess the clinical efficacy of inhaled aztreonam in people with CF. In a Phase II clinical trial conducted in 2008 by Retsch-Bogart et al., 31 CF patients ≥ 6 years of age were randomized to each of the three groups – inhaled aztreonam (225 mg twice a day), inhaled aztreonam (75 mg twice a day), and placebo (83). The study observed an increase in FEV_1 in both the 225 mg and the 75 mg groups on day 7 compared with the baseline. However, by day 14, no

significant differences were noted between the two treatment groups and the placebo group. Furthermore, the authors observed a significant reduction in *P. aeruginosa* bacterial density in both the 75 and 225 mg groups at days 7 and 14 ($p < 0.001$). McCoy et al. carried out another clinical trial to assess the efficacy of inhaled aztreonam, where 211 CF patients ≥ 6 years of age were randomized to receive 75 mg of inhaled aztreonam three times daily, or 75 mg inhaled aztreonam twice daily or placebo, and were monitored for 56 days (84). Inhaled aztreonam was found to significantly increase the median time to use of additional antipseudomonal antibiotics ($p = 0.007$). The inhaled aztreonam groups also had significantly higher FEV_1 values compared with the placebo group ($p = 0.001$).

In another clinical trial conducted by Retsch-Bogart et al. in 2009, 164 CF patients ≥ 6 years of age, were randomized to the inhaled aztreonam group (75 mg three times daily) or the placebo group (85). The aztreonam group had significantly higher mean Cystic Fibrosis Questionnaire-Revised Respiratory Symptom (CFQ-R) score ($p < 0.001$), mean predicted FEV_1 values ($p < 0.001$) as well as lower sputum *P. aeruginosa* density ($p< 0.001$). Omermann et al. in 2010, carried out an 18-month open-label study to assess the efficacy and safety of alternate-month repeated courses (28 days on/28 days off) of inhaled aztreonam in CF patients ≥ 6 years of age (86). 274 patients were enrolled in this study and were randomized to receive either 75 mg of inhaled aztreonam, three times daily, or 75 mg twice daily. The study noted that both groups had improved mean CFQ-R scores and FEV_1 values with a reduction in the sputum *P. aeruginosa* density. However, these improvements disappeared in the off-months when the patients were not receiving treatment with inhaled aztreonam. On the other hand, gain in weight was sustained over the 18 months. Moreover, a dose-response was noted, with patients receiving the drug three times daily demonstrating greater improvements in FEV_1 and CFQ-R over 18 months.

More recently, Wainwright et al., in 2011, performed a double-blind, multicenter, randomized, placebo-controlled trial in CF patients ≥ 6 years of age with mild lung function impairment (FEV_1 > 75 % predicted) (87). 157 patients were randomized to receive either 75 mg of inhaled aztreonam three times daily or placebo for 28 days with a follow-up of 14 days. The authors noted significant improvements in the inhaled aztreonam group for mean FEV_1 % predicted values (p = 0.021), and sputum *P. aeruginosa* density (p = 0.016). Additionally, a non-significant increase in the mean CFQ-R score of 1.8 points (p = 0.443) was observed in the inhaled aztreonam group.

Based on the results of these clinical trials, the CFF expert committee **strongly recommended** the use of inhaled aztreonam in CF patients ≥ 6 years of age, with moderate to severe lung disease and chronic colonization with *P. aeruginosa* to improve lung function and quality of life (88). Moreover, the expert committee also **recommended** its use in CF patients ≥ 6 years of age with mild lung disease and chronic colonization with *P. aeruginosa*.

Comparison of clinical efficacy between inhaled tobramycin and aztreonam

There are few comparative effectiveness studies of inhaled tobramycin and aztreonam. A recently published study by Assael et al. comparing inhaled aztreonam and tobramycin over three 28-day-treatment courses, concluded that aztreonam was superior in lung function improvement and reduction in acute pulmonary exacerbations (89). However, some have felt the study design in this industry-sponsored trial may have been biased toward aztreonam because 85% of patients enrolled in this study reported inhaled tobramycin use for ≥ 84 days in the previous year (i.e., they were on inhaled tobramycin prior to the study) (89). This is important because in the initial clinical trial of inhaled tobramycin by Ramsey et al. (47), they observed that after an initial improvement in the predicted FEV_1 from baseline values in the first month on therapy, the FEV_1 improvements did not return to baseline values. On subsequent months of treatment,

FEV_1 improvement ranged from about 10% on treatment months to about 7 – 8% on off months. Therefore, it would be expected that patients continuing on inhaled tobramycin would only have a modest improvement (0.55% in the Assael et al., study) compared to those that were inhaled antibiotic-naive (12% in the Ramsey et al., study with patients that were inhaled antibiotic-naive). Another study conducted to observe the long-term safety and efficacy of inhaled aztreonam found that about 52% of the enrolled patients had ≥ 1 course of inhaled tobramycin and about 23% had > 3 courses of inhaled tobramycin during aztreonam-off treatment intervals (86). Given the need for additional courses of tobramycin while on aztreonam, it appears that treatment with inhaled aztreonam in conjunction with inhaled tobramycin could have a more pronounced effect on slowing FEV_1 decline in CF patients.

Specific Aims

The primary goal of this project was to assess the clinical effectiveness of long-term therapies in CF and help refine existing guidelines for the clinical management of this disease. Using the CFFPR, the following specific aims were investigated:

1. Specific Aim 1: To compare the highest FEV_1 % predicted value measured between 6 – 7 years of age in CF patients who received treatment with dornase alfa when they were ≤ 6 years of age, and those who did not receive treatment with dornase alfa.
2. Specific Aim 2: To compare the rate of decline in FEV_1 % predicted in CF patients, 6 – 20 years of age, who received chronic treatment with oral azithromycin, with the rate of FEV_1 decline in CF patients who did not receive chronic treatment with oral azithromycin.
3. Specific Aim 3: To compare the rate of decline in FEV_1 % predicted in CF patients, 6 – 20 years of age, who received combined alternate-month treatment with inhaled tobramycin and aztreonam, with the rate of FEV_1

decline in CF patients who received treatment with inhaled tobramycin alone.

Significance

The three aims investigated the clinical effectiveness of commonly prescribed chronic medications in CF – inhaled dornase alfa, oral azithromycin, and inhaled tobramycin and aztreonam. No long-term effectiveness studies to date have focused on use of dornase alfa in children ≤ 6 years of age and its effects on FEV_1 % predicted measured between 6 – 7 years of age, or on treatment with alternate-day oral azithromycin or concurrent therapy with inhaled tobramycin and aztreonam and their effects on rate of decline in FEV_1. These aims were designed to address important clinical questions in the management of CF. Given that long-term multicenter clinical trials to evaluate efficacy of these treatments are expensive and difficult to conduct, analysis of data from such longitudinal observational disease-specific databases provides a more practical approach in CF clinical research. If, as we hypothesized, these treatments were noted to have long-term clinical effectiveness leading to higher FEV_1 % predicted or slower rate of FEV_1 decline, it would make the case for initiation of these treatments early in the disease process to improve patient survival. However, after taking into account the study limitations, if no significant changes in outcomes were observed it would make the case for reducing the burden of these chronic treatments on patients with CF. This, in turn, would help increase patient adherence and improve their quality of life.

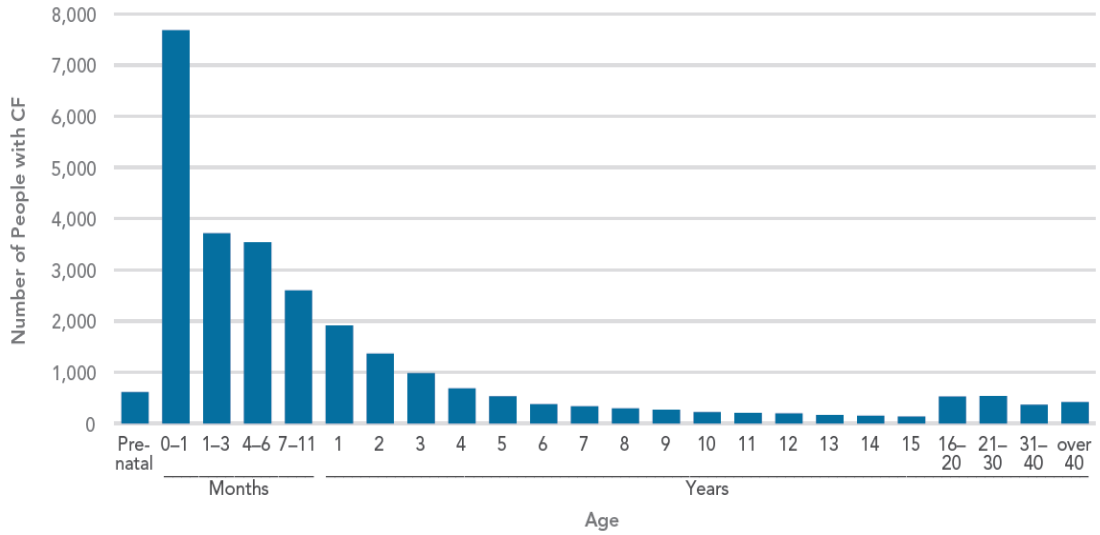

Figure 1-1. Age at Diagnosis of All People with Cystic Fibrosis in the United States Enrolled in the CFFPR, 2012. Reproduced with permission from the Cystic Fibrosis Foundation.

Source: Cystic Fibrosis Foundation Patient Registry. 2012 Annual Data Report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2013.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

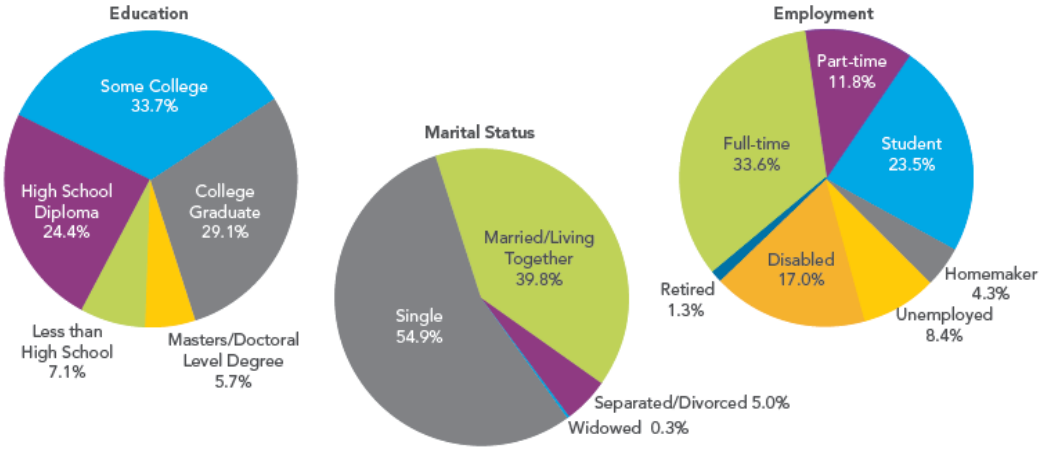

Figure 1-2. Characteristics of Adults with Cystic Fibrosis, 18 years of Age and Older in the United States enrolled in the CFFPR, 2012. Reproduced with permission from the Cystic Fibrosis Foundation.

Source: Cystic Fibrosis Foundation Patient Registry. 2012 Annual Data Report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2013.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

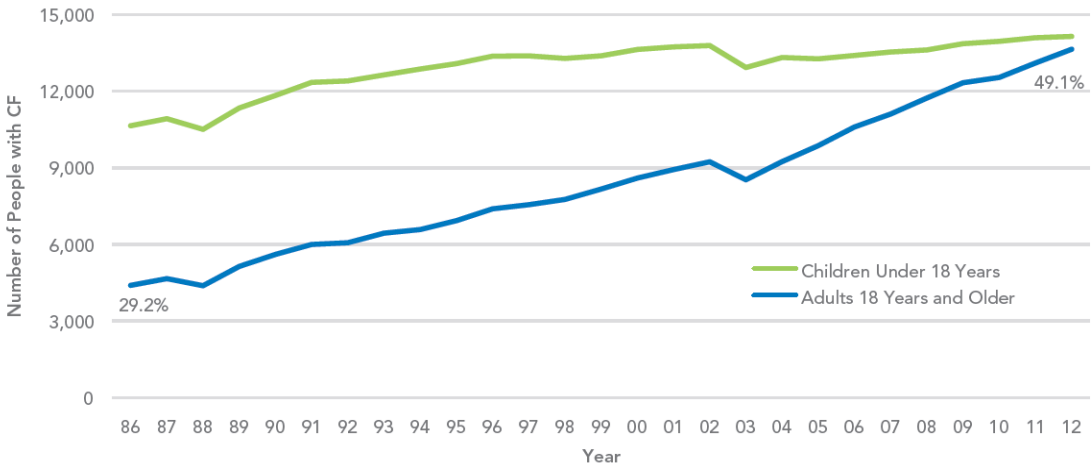

Figure 1-3. Number of Children and Adults with Cystic Fibrosis, 1986 – 2012, in the United States enrolled in the CFFPR. Reproduced with permission from the Cystic Fibrosis Foundation.

Source: Cystic Fibrosis Foundation Patient Registry. 2012 Annual Data Report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2013.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

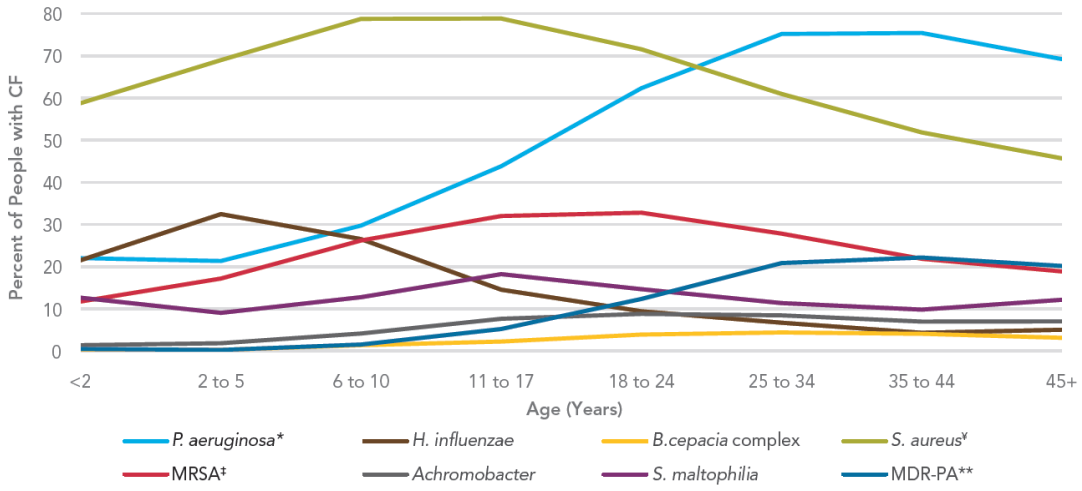

Figure 1-4. Age-specific prevalence of airway infections in Cystic Fibrosis Patients in the United States as reported in the CFFPR, 2012. Reproduced with permission from the Cystic Fibrosis Foundation.

Source: Cystic Fibrosis Foundation Patient Registry. 2012 Annual Data Report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2013.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

**P. aeruginosa* includes *MDR-PA*; ***MDR-PA* is multi-drug resistant *Pseudomonas aeruginosa*; ¥*S. aureus* includes people with *MRSA*; ‡*MRSA* – methicillin-resistant *Staphylococcus aureus*

CHAPTER II - METHODS USED IN THE ANALYSIS OF DATA TO ADDRESS STUDY AIMS

This chapter describes methods that were used to create and analyze datasets generated to address the three study aims. The focus of the research project was on assessing the long-term effects of therapies used in cystic fibrosis (CF) on forced expiratory volume in 1 second (FEV_1) in CF patients between 3 – 20 years of age.

Creating Analysis Dataset

Data for the study aims were requested and obtained from the Cystic Fibrosis Foundation Patient Registry (CFFPR). This request for data access involved submission of an application along with a detailed study proposal and approval from the University of Iowa Institutional Review Board. Once the application was approved by the CFF review committee, a list of variables available in the CFFPR that were necessary to assess the study aims was sent to the Cystic Fibrosis Foundation (CFF). The data were compiled by the CFF and mailed to the study author for analysis to address the aims of this research project.

This study utilized two different datasets. The first dataset included data for patients who were ≤ 6 years of age at some time between 2000 and 2011. This dataset was used to evaluate study Aim 1. The second dataset, which included data from 2004 to 2011, included data for patients ≥ 6 years of age at some time during the study period and was used to evaluate study Aims 2 and 3. These time periods of 2000-11 for Aim 1 and 2004-11 for Aims 2 and 3 were finalized and approved by the CFF review committee since it was felt that these study periods should have all the relevant data for analysis of the respective study aims. The basic demographic factors for the two groups are summarized in Table 2-1.

Both datasets included data on the "treatment" variable as well as FEV_1 data along with other variables necessary to assess baseline characteristics and potential confounders based on results from previous studies in CF patients. For Aim 1, the highest

FEV_1 % predicted value between 6 – 7 years of age was defined as the outcome; whereas for Aims 2 and 3 the rate of decline in FEV_1 % predicted was the outcome of interest. Since these datasets included data for individual clinic visits, RETAIN statements were used in multiple DATA steps in SAS 9.3 (SAS Institute, Cary, NC) to create the final datasets that included patients who fulfilled study criteria for the individual study aims.

The analysis for Aim 1 was focused on outcome assessment at a single time point for an individual patient. Hence, each patient in the final dataset for Aim 1 had only one row of observations in the final dataset. The analysis for Aims 2 and 3 was focused on the longitudinal assessment of outcomes, and the final dataset was created in the "long form." Each patient had multiple rows of observations depending on the number of clinic visits they had during the study period.

Analysis of Baseline Variables

For all study aims, the baseline variables were summarized using means and standard deviations (SD) for continuous variables and using percentages for the categorical variables. The different study groups in each aim were then compared using the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables.

Propensity Score Methods and Final Analysis

All three study aims used propensity score methods to assess the association between the primary dependent and independent variables. This analytic approach was used to reduce treatment indication bias, which is often encountered in observational studies evaluating the effectiveness of a treatment. The propensity score is the probability or the likelihood of receiving treatment given a set of baseline variables and was calculated by fitting a logistic regression model. This model included all available baseline covariates that could have affected the likelihood of receiving treatment with the study drug. The logistic model was fitted in SAS using the PROC LOGISTIC procedure. The predicted probability of receiving treatment with the study drug obtained from this

model for each patient was their propensity score. This propensity score was then utilized in different ways for the three study aims.

For Aim 1, the propensity score was used as a covariate in a multivariable linear regression model that was fitted in SAS to predict FEV_1 % predicted values between 6 – 7 years of age using PROC GLM. The model included the propensity score, along with variables that were shown to be potential confounders of the association between treatment and the outcome. The FEV_1 % predicted measures for patients in the dornase alfa-treated and untreated groups were compared using the difference in group-specific least squares means.

Previous studies have suggested that using the propensity score as a covariate in a regression model could lead to biased estimates in both non-linear (90) and linear (91) regression models. However, Hade et al. also noted that model-based adjustment using the propensity score may have an advantage if the two study groups (treatment and control) do not have too much overlap of their propensity scores (91). This was the case when the propensity score distribution was compared between the dornase alfa and the untreated groups for study Aim 1. Nonetheless, as an additional analysis, the two groups were matched on their propensity score. Matching was carried out using the local optimal (greedy) algorithm with a 1:1 caliper matching. This was carried out in SAS using the "%PSMatching" macro (92). The two treatment groups were randomly sorted and then the first patient from the treated group was selected to find its closest "control" match in terms of the propensity score if the control's propensity score was within a certain distance (caliper) (92).

Following the matching, the two groups were again compared on their baseline characteristics to assess how well the matching had worked. A multivariable linear regression model was then fitted using data from this matched set of patients. Furthermore, in order to better characterize the unmatched patients, the dornase alfa-treated and untreated groups consisting of unmatched patients were compared on their

baseline characteristics. Data from this set of unmatched patients were also used to fit a multivariable linear regression model similar to the one specified in the primary analysis to assess the magnitude and direction of association between the treatment and the outcome.

Additionally for Aim 1, a multivariable linear regression model was fitted that included all baseline covariates and potential study confounders, as part of the final analysis. This was carried out in SAS using PROC GLM. Once again the two groups were compared using the difference in their least squares means.

For Aims 2 and 3, the patients in the two study groups were matched on their propensity scores. The matching methods were similar to those described for Aim 1. After matching, the two groups were once again compared on the baseline characteristics. A linear mixed model with a random intercept and a random slope for the time variable was then fitted using the restricted maximum likelihood method of estimation. This was done using PROC MIXED in SAS. Patient identification number was the random variable in this linear mixed model. The "no diagonal" factor analytic covariance structure was used in PROC MIXED to produce an unstructured covariance matrix that is nonnegative definite – FA0(q). Data from the propensity score-matched set of patients that included some baseline covariates that were found to be different between the two groups after propensity score matching, along with potential study confounders were used in this analysis. Since the rate of decline in FEV_1 % predicted was the primary focus of these two study aims, an interaction term with time was included for the primary treatment group variable.

Similar to Aim 1, the unmatched patients from the two groups were characterized by comparing their baseline characteristics. As an additional step, data from this group of unmatched patients were used to fit a linear mixed model to assess the magnitude and the direction of the association between the rate of FEV_1 decline and the treatments. The

model specifications were similar to those used in the linear mixed model for the primary analysis.

Model diagnostics were assessed for all three study aims. For Aim 1, the PLOTS = ALL option was used in the PROC GLM procedure to obtain plots of residuals and evaluate the influential observations. For Aims 2 and 3, PLOTS (MAXPOINTS = NONE) = ALL option was used in the PROC MIXED procedure to obtain marginal as well as conditional residual plots for FEV_1 % predicted.

Table 2-1. Demographic and Clinical Characteristics of Patients with Cystic Fibrosis in the CFFPR Datasets before Applying Study Criteria

Variables[a]	Aim 1	Aims 2 & 3
Age, years, mean (SD)	1.6 (1.8)	11.2 (4.7)
Females (%)	49.5	48.5
Race (%)		
• Caucasians	92.9	93.9
• African Americans	5.4	4.8
Genotype (%)		
• Homozygous *delta*F508	46.3	45.9
• Heterozygous *delta*F508	36.7	34.4
• No *delta*F508	17.0	19.7
Pancreatic insufficiency[b] (%)	24.3	32.4
CF-related diabetes (%)	0.04	4.8
FEV_1 % predicted[c], mean (SD)	100.1 (18.7)	83.2 (22.8)

Note: SD = standard deviation; CF = cystic fibrosis; FEV_1 = forced expiratory volume in 1 second; CFFPR = Cystic Fibrosis Foundation Patient Registry

[a]Values for all the variables included in the table except FEV_1 % predicted are for the first visit in the dataset

[b]Pancreatic insufficiency was defined as receiving supplementation with pancreatic enzymes.

[c]For Aim 1, FEV_1 % predicted was defined as the highest FEV_1 % predicted between 6 – 7 years of age. For Aims 2 & 3, FEV_1 % predicted was defined as FEV_1 % predicted value at the first visit in the dataset.

CHAPTER III - CLINICAL EFFECTIVENESS OF DORNASE ALFA USE IN CHILDREN SIX YEARS OF AGE AND YOUNGER WITH CYSTIC FIBROSIS

Background

Lung disease in cystic fibrosis (CF) is characterized by airway obstruction along with chronic infection and a neutrophil predominated inflammatory response leading to permanent lung damage. DNA from neutrophils is hypothesized to be the primary cause of an increase in the viscosity of the mucus secretion leading to plugging of the airways, which in turn leads to a vicious cycle of infection, inflammation, and lung damage ultimately leading to progressive lung disease (25). To counter this viscous mucus, many mucolytic therapies have been developed and tested. Of these, Dornase alfa – a highly purified solution of recombinant human deoxyribonuclease (rhDNase), has been found to be effective. Dornase alfa reduces the viscosity of sputum in CF patients by hydrolyzing the excessive DNA (93).

Clinical trials carried out in CF patients, ≥ 5 years of age, have shown that dornase alfa is effective in improving forced expiratory volume in 1 second (FEV_1), an important predictor of mortality in CF (61-63). The Cystic Fibrosis Foundation (CFF) not only recommends the use of dornase alfa in patients ≥ 6 years of age with moderate to severe disease but also in patients with mild lung disease as it reduces pulmonary exacerbations and improves lung function (57). Moreover, high lung function at an early age increases the risk of rapid decline in FEV_1 % predicted (60). Results from a recent study by Konstan et al. that evaluated the association between dornase alfa use and rate of decline in FEV_1, provide further evidence that initiating treatment with dornase alfa early in the disease process slows the decline in lung function and may hypothetically prolong survival (69). Given these data in children ≥ 6 years of age, clinicians have also prescribed dornase alfa in children < 6 years of age to slow the progression of lung disease. However, clinical outcomes data for evaluation of early use of dornase alfa in

this age group are lacking. An important reason for this is a general lack of good clinical tools to assess lung function in early childhood, before these patients learn to carry out pulmonary function tests. Moreover, the financial costs associated with treatment with dornase alfa are substantial, with a year's supply costing about $34,800 (94). With no efficacy studies on dornase alfa use in children < 6 years of age and the costs associated with its use, it's important to evaluate its effectiveness in clinical care in CF.

As is evident from the 2012 CFF Patient Registry (CFFPR) data for different birth cohorts, one of the key improvements in CF lung disease has been the increase in median FEV_1 % predicted values, first measured around 6 years of age (Figure 3-1) (2). The differences in the median FEV_1 values at 6 years of age across the three birth cohorts are maintained over time, suggesting that interventions before this age have the greatest potential for further improving lung function. Hence, this study used a unique outcome in the form of lung function defined as the highest FEV_1 value between 6 – 7 years of age. The specific aim of this study was to compare the FEV_1 % predicted values between CF patients who received treatment with dornase alfa when they were ≤ 6 years of age, and those who did not receive treatment with dornase alfa. We hypothesized that the use of dornase alfa in children with CF ≤ 6 years of age is associated with improved subsequent FEV_1 measurements between 6 – 7 years of age compared to CF children of the same age group who did not receive treatment with dornase alfa.

This study was the first to evaluate the effectiveness of dornase alfa use in children ≤ 6 years of age, utilizing subsequent initial lung function as an outcome in these children. An investigation of the role of dornase alfa use on clinical outcomes in young CF patients holds great potential for developing appropriate treatment strategies to control progression of lung disease and to improve the quality of life for young patients with CF.

Methods

Study Population

Data for this study were obtained from the CFFPR. The CFFPR is a large database that includes data for nearly 95% of all CF patients treated at more than 110 accredited CF centers in the United States (US). For this study, eligible CF patients were those enrolled in the CFFPR from 2000-11, who were ≤ 6 years of age at some time between 2000-11 and had subsequent pulmonary function measurements between 6 – 7 years of age. CF patients who were treated with dornase alfa (treated group) when they were ≤ 6 years of age were compared with those who were not (untreated group). These age criteria were chosen as the primary aim was to assess the effectiveness of dornase alfa in young children ≤ 6 years of age.

For the treated group, a patient was classified as being on dornase alfa if they had more than two clinic visits in a year and were on the drug for > 50% of those visits. To be included in the study, they had to be on the drug for three such consecutive years before subsequent FEV_1 measurements between 6 – 7 years of age. As a result, the age of treatment initiation for a majority of the patients in this group fell between 3 − 3.5 years. To make the two groups comparable on age, the untreated patients who had follow-up data between 3 − 6 years of age with subsequent FEV_1 measurement between 6 − 7 years were included in the untreated group. Both the treated and the untreated groups could continue to receive treatments with other CF therapies such as nutritional supplements, antibiotics, and anti-inflammatory medications.

FEV_1 measurement requires active participation from the patient as it is the volume of air expired in the first second during forceful expiration, and children require training to be able to do it correctly. This can lead to highly variable FEV_1 measurements early on. Therefore it has become the norm in clinical studies that use FEV_1 as an outcome, to use measurements only after 6 years of age. In this study the outcome was defined as the highest FEV_1 value measured between 6 − 7 years of age. The highest

value in a year was chosen, as low values are possible due to bad technique which may falsely reflect lowered lung function, as would the mean of all available measurements during the age 6 – 7 timeframe. Additionally, it is highly unlikely to have falsely raised FEV_1 values due to an improper technique. Although newer devices have made it possible to perform this test in infants, they require sedation and extensive training of the person performing the test. Additionally, infant pulmonary function tests are only available up to 2 years of age. Hence, results from early pulmonary function tests cannot be used beyond this age range.

Study Design

The study used a retrospective cohort design. The primary outcome in this study of young CF patients was defined as the highest FEV_1 measured between 6 − 7 years of age, and was compared between those who received treatment with dornase alfa and those who did not. The CFFPR standardizes the FEV_1 measurements in liters to percent predicted values for a given age, gender, race and height using reference equations. As these are percent predicted values, it is possible to have a FEV_1 measurement that is > 100% of predicted. The main predictor variable was use of dornase alfa (Figure 3-2). Data on baseline patient characteristics were requested from the CFFPR. These included:

- Age – This was defined as the age at which a patient in the treated group was started on dornase alfa and continued to receive treatment for at least 3 years. For the untreated group, this variable was defined as the age at which follow-up began for a patient and lasted for at least 3 years before the measurement of the primary outcome.
- Gender - Gender was categorized as female or male.
- Genotype – Genotype was categorized into homozygous *deltaF508* – the most common mutation in CF patients in the US, heterozygous *deltaF508*, or no *deltaF508*.

- Body mass index (BMI) percentile – This was defined as the highest BMI percentile in the year prior to the start of treatment in the treated group and the start of follow-up in the untreated group.
- Chronic *Pseudomonas aeruginosa* (*Pa*) status – This was defined using the modified Leeds criteria (95), i.e., patients who had more than two sputum or throat cultures in the year prior to the start of treatment or follow-up and grew *Pa* in more than 50% of those cultures.
- Total number of pulmonary exacerbations in the year prior to the start of treatment in the treated group and start of follow-up in the untreated group.
- Total number of hospitalizations in the year prior to the start of treatment in the treated group and start of follow-up in the untreated group.
- CF-related diabetes (CFRD) (with or without fasting hyperglycemia) – CFRD status was defined based on presence or absence of a diagnosis in the CFFPR in the year prior to the start of treatment in the treated group and start of follow-up in the untreated group.
- Pancreatic insufficiency – This was defined as having received treatment with pancreatic enzymes in the year prior to the start of treatment in the treated group and start of follow-up in the untreated group.
- Center size – This was defined based on the number of patients who received care at a CF center between 2000 and 2011 and was categorized into small (≤ 60 patients) or large (> 60 patients). This categorization was based on the distribution of patients receiving care at various CF centers.
- Center-specific prescription rate of dornase alfa in infants – This was defined as the proportion of children ≤ 1 year of age who received treatment with dornase alfa at a CF center between 2000 and 2011.

Data on other potential confounders during the visit when the subsequent FEV_1 was recorded between 6 – 7 years of age were also extracted from the CFFPR database. These included use of inhaled antibiotics such as tobramycin, aztreonam, and colistin, presence of bacteria on sputum cultures that have been previously noted to be associated with lower lung functions such as *Pa,* mucoid *Pa, Burkholderia cepacia, methicillin-resistant Staphylococcus aureus*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia* (96), CFRD status, pancreatic insufficiency status, BMI percentile, use of hypertonic saline, chronic oral antibiotic use, and chronic macrolide use. The total follow-up time and total number of exacerbations and hospitalizations during follow-up were calculated and used as covariates in the analyses.

Statistical Analyses

The study data were summarized using means with standard deviations (SD) for continuous measures and percentages for categorical variables. The two groups were compared on these baseline characteristics using the Wilcoxon rank-sum test for continuous data and the chi-square test for categorical data.

Comparison of the FEV_1 between 6 – 7 years of age between the two treatment groups was accomplished using two complementary modeling approaches, both of which incorporated the information on baseline characteristics and potential confounders: standard multivariable linear regression analysis, and propensity score analysis. The multivariable linear regression model was fitted to compare the dornase alfa-treated group with the untreated group on their highest FEV_1 measurement between 6 – 7 years of age. This model adjusted for baseline characteristics as well as potential confounders measured at the time of the outcome assessment. The two treatment groups were compared using the difference between their least squares means. Regression diagnostic statistics were obtained to assess the residuals and identify any influential observations.

The propensity score analysis estimated the likelihood of receiving treatment with dornase alfa for each of the patients included in the linear regression analysis by

fitting a logistic regression model that included the baseline covariates listed above. The propensity score, thus calculated, was used as a covariate in a multivariable linear regression model along with adjustment for other confounders measured at the time of outcome assessment. As in the previous multivariable linear regression model, regression diagnostics were obtained to evaluate the residuals and the influential observations.

All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). *P* values < 0.05 were considered significant. This study was approved by the University of Iowa Institutional Review Board.

Results

Characteristics of the Study Cohort

A total of 13,802 patients enrolled in the CFFPR were ≤ 6 years of age at some time between 2000 and 2011 (Figure 3-3). After excluding patients with less than three years of data in the CFFPR (N =3,472), 9,058 patients were noted to have been treated with dornase alfa at some point during the study period. The remaining 1,272 were never treated with dornase alfa. In the treated group, 340 patients fulfilled the study criteria of being on dornase alfa for at least three years and having a subsequent FEV_1 value between 6 − 7 years of age. Of these, 323 patients had complete data for baseline characteristics and potential study confounders. In the untreated group, 357 patients met the criteria for highest FEV_1 between 6 − 7 years of age and complete data for baseline characteristics and other study variables. As a result, data from 680 patients were available for the analysis. The median number of CF patients ≤ 6 years of age at each center between 2000 and 2011 was 62 (interquartile range = 29 − 106).

The baseline characteristics for patients in both groups are summarized in Table 3-1. Patients who were treated with dornase alfa were more likely to be females, and be homozygous for the *deltaF508* mutation. Additionally, the treated group had a significantly higher number of exacerbations and hospitalizations in the previous year, a higher proportion of patients who were pancreatic insufficient, and a significantly higher

proportion of patients who were chronically colonized with *Pa*. The center-specific prescription rate for dornase alfa in infants in the treated group was significantly higher than that in the untreated group. However, the two groups did not differ in their baseline BMI percentile, the proportion of patients who received treatment at a small CF center, or the prevalence of CFRD.

Multivariable Linear Regression Analysis

Standard linear regression models were used to analyze the relationship between dornase alfa use and FEV_1. The results of both the unadjusted as well as the multivariable regression models are summarized in Table 3-2. The unadjusted model showed that patients who received treatment with dornase alfa had a significantly lower FEV_1 measurement between 6 – 7 years of age compared with those who did not (101.87% predicted in the treated group vs. 106.03% predicted in the untreated group, $p = 0.002$). However, after adjusting for the baseline characteristics and potential confounders in a multivariable model, the direction of the difference between the two group means switched albeit with a much smaller magnitude and this difference was not significant (104.31% predicted in the treated group vs. 103.44% predicted in the untreated group, $p = 0.71$). Most of the potential confounders were not significantly associated with the outcome, with the exception of center size ($p < 0.0001$), BMI percentile at the time of FEV_1 measurement ($p < 0.0001$), total number of exacerbations during follow-up ($p = 0.0006$), total number of hospitalizations during follow-up ($p = 0.01$), and duration of follow-up ($p = 0.01$).

Propensity Score Analysis

Propensity scores were estimated for each patient in the two groups. Patients in the treated group had a mean propensity score of 0.68 (SD = 0.27), whereas those in the untreated group had a mean propensity score of 0.29 (SD = 0.22) (Figure 3-4). Propensity score was included as a covariate in the multivariable regression model in lieu of the baseline characteristics. The same covariates treated as potential confounders in the

standard multivariable linear regression model were included in this analysis. Similar to the results of the multivariable regression analysis without the propensity score covariate, this analysis did not find a significant difference in FEV_1 % predicted between the two groups although the treated group had a slightly higher FEV_1 (104.30% predicted in the treated group vs. 103.85% predicted in the untreated group, $p = 0.85$) (Table 3-2). The covariates that were found to have a significant association were BMI percentile at the time of FEV_1 measurement ($p < 0.0001$), total number of exacerbations during follow-up ($p = 0.0007$), total number of hospitalizations during follow-up ($p = 0.01$), and duration of follow-up ($p = 0.01$). Chronic use of oral antibiotics was also found to be significantly associated with FEV_1 % predicted ($p = 0.04$).

To assess a dose-response trend, similar propensity score analyses were also carried out for patients who received treatment with dornase alfa for one and two years, respectively. These analyses did not find a significant difference in FEV_1 % predicted between the two groups. In the group of patients treated with dornase alfa for one year, the untreated group had a higher FEV_1 % predicted compared with the treated group (103.07% predicted in the treated group vs. 104.20% predicted in the untreated group, $p = 0.48$). Similarly, in the group of patients treated with dornase alfa for two years, the untreated group had a higher FEV_1 compared with the treated group (101.80% predicted in the treated group vs. 104.72% predicted in the untreated group, $p = 0.12$).

Additionally, since exacerbations and hospitalizations after start of treatment with dornase alfa could be in the causal pathway for the association between treatment and lung function, these two variables were excluded from the analysis. This analysis also did not find a significant difference in FEV_1 % predicted between the two groups (103.79% predicted in the treated group vs. 104.30% predicted in the untreated group, $p = 0.84$).

Discussion

This study indicated that the use of dornase alfa in children ≤ 6 years of age is not associated with a significantly higher FEV_1 measurement between $6 - 7$ years of age. It is

the first study to assess the clinical effectiveness of dornase alfa in this age group using subsequent FEV_1 as an outcome. The study not only used standard multivariable regression methods but also propensity score analysis using measured covariates to reduce the likelihood of treatment indication bias. As is evident from the baseline characteristics of the two groups, the group that received treatment with dornase alfa had factors that have been shown to be associated with worse lung disease prognosis, such as female gender, homozygous *deltaF508* status, chronic *Pa* infection, and pancreatic insufficiency (65, 97-100). Additionally, the treated group had a higher mean number of exacerbations and hospitalizations at baseline. Hence, adjusting for these baseline characteristics in the analysis either as individual covariates or as a combined propensity score variable appears to have helped in reducing some of the issues associated with treatment indication bias.

Even after adjustment for the baseline covariates and potential confounders, the study failed to reject the null hypothesis suggesting that dornase alfa use in CF patients ≤ 6 years of age did not have a positive effect on FEV_1 between 6 – 7 years of age. These results are different from the results of dornase alfa studies in CF patients ≥ 6 years of age. One possible explanation for these differences is that FEV_1 may yield poor sensitivity for detecting early lung disease in the preschool age range. Another possible explanation for this may be that despite the fact that lung disease in CF begins early in life the variables available in the database may have poor sensitivity to detect inflammation and airway damage. In fact, none of the novel clinical tools available to evaluate lung damage in this younger age group have been assessed for evidence of clinical utility (101). Hence, the CFFPR does not collect data on these novel methods of assessing lung function in preschool children. As a result, this study utilized FEV_1 measured between 6 – 7 years as an outcome. A major issue with using FEV_1 as an outcome is the absence of baseline measurements of lung function to assess and control for severity of lung disease. Additionally, as seen in this study, the patients who were

treated with dornase alfa had the presence of other factors indicating more severe lung disease as compared with the untreated group. Hence, by the time these patients had completed three years of treatment with dornase alfa and were able to perform valid lung function measurements, they likely would have been suffering with more intense lung damage as compared with the untreated group.

A propensity score matching was also carried out (Appendix II) as part of the propensity score analysis of these data. This analysis also did not find a significant difference between the mean FEV_1 % predicted between the two groups. However, this analysis had a post-hoc power of only 64% to detect a mean difference of 4.74% in FEV_1 % predicted as compared to 87% power to detect a mean difference of 4.16% in FEV_1 % predicted in the regression analysis with and without propensity score as a covariate. This decrease in power may have been due to the reduced sample size as a result of the propensity score matching. Additionally, the patients who were left without a match in the two groups were characterized. The mean propensity scores in the two groups were significantly different from each other (0.87 in the treated group vs. 0.16 in the untreated group, $p < 0.0001$) (Figure B-1).

This study had several limitations. As an observational study that utilized data from the CFFPR, patient adherence to dornase alfa could not be assessed. If dornase alfa was listed under treatments for a clinic visit, it was assumed that the patient was receiving it. Additionally, given the observational design of this study, it is subject to treatment indication bias. Use of baseline covariates in the model and propensity score methods may have helped reduce some of that bias but it was carried out using only the measured covariates. The analyses included data for all available covariates that could possibly affect the likelihood of receiving treatment with dornase alfa. However, it could not account for unmeasured covariates. Additionally, this model also adjusted for potential confounders by including data on these variables at the time of FEV_1 measurement. This

should have helped to reduce confounding due to the fact that the treated group received more therapies compared to the untreated group.

Another important limitation of this study is the relatively small sample size. Given the study criteria for eligibility, the number of patients available for the final analysis was reduced by a substantial amount (10,330 to 683). This number dropped down to 300 after propensity score matching. These study criteria were important in assessing the long-term effectiveness of dornase alfa – as a result three years of consecutive use of dornase alfa in the treated group seemed appropriate. Additionally, the untreated group only included patients who had never received treatment with dornase alfa but could continue to receive other CF therapies. Post-hoc power analysis suggested that the multivariable regression analysis both with and without the propensity score as a covariate had a power of 87% (two-tailed alpha = 0.05) based on the distribution of FEV_1 measured between 6 – 7 years of age in the 680 patients that were included in the final analysis, suggesting that the non-significance of the study results may not be due to a lack of power.

In conclusion, children with CF treated with dornase alfa before 6 years of age did not show a significantly higher FEV_1 % predicted values measured between 6 – 7 years of age. Multicenter randomized clinical trials are needed to assess the efficacy of dornase alfa use in preschool children. In addition, this study also highlights the problems associated with using FEV_1 as an outcome to assess effectiveness of dornase alfa in young children. More sensitive clinical tools such as multiple breath washout with measurement of lung clearance index may be needed to evaluate effectiveness of therapies in preschool CF patients.

Table 3-1. Baseline characteristics of study cohort

Characteristics	No Dornase Alfa (N = 357)	Dornase Alfa (N = 323)	*P* value*
Age (years), mean (SD)	3.1 ± 0.12	3.4 ± 0.1	< 0.0001
Females - N (%)	184 (51.5)	184 (57.0)	0.16
Genotype - N (%) • Homozygous *delta*508 • Heterozygous *delta*508 • No *delta*508	 147 (41.2) 152 (42.6) 58 (16.2)	 169 (52.3) 111 (34.4) 43 (13.3)	0.02
Body mass index percentile[a], mean (SD)	63.78 (26.75)	65.67 (24.76)	0.52
Exacerbations (per year)[b], mean (SD)	0.18 (0.56)	0.69 (1.11)	< 0.0001
Hospitalizations (per year)[c], mean (SD)	0.13 (0.45)	0.47 (0.81)	< 0.0001
Center size[d], Small, N (%)	71 (19.9)	48 (14.9)	0.09
Prescription Rate in infants[e] (%), mean (SD)	13 (11)	30 (18)	< 0.0001
Chronic *Pseudomonas aeruginosa* - N (%)	10 (2.8)	23 (7.1)	0.01
CF-related diabetes - N (%)	1 (0.3)	0 (0)	0.34
Pancreatic insufficiency - N (%)	274 (76.8)	299 (92.6)	< 0.0001

Note: SD = standard deviation; CF = cystic fibrosis.

[a]Highest body mass index percentile in the year prior to age of start of treatment.

[b]Number of pulmonary exacerbations in the year prior to age of start of treatment.

[c]Number of hospitalizations in the year prior to age of start of treatment.

[d]Center was classified into small and large centers based on a cut-off value of 60 patients between 2000 – 2011.

[e]Center-specific prescription rate of dornase alfa in CF patients ≤ 1 years of age

**P* values from Wilcoxon rank-sum test or chi-square test.

Table 3-2. Parameter Estimates (SE) from fitting Linear Regression Models to Predict FEV_1 % Predicted

Variables	Type of Model					
	Unadjusted		Multivariable Linear Regression[a]		Multivariable Linear Regression[b]	
	Estimate (SE)	*P* value	Estimate (SE)	*P* value	Estimate (SE)	*P* value
Dornase alfa use (No vs. yes)	4.07 (1.35)	0.002	-0.87 (2.34)	0.71	-0.45 (2.4)	0.85
Chronic use of oral antibiotics	-	-	-6.01 (3.61)	0.1	-7.25 (3.59)	0.04
BMI percentile	-	-	0.14 (0.03)	<0.0001	0.14 (0.02)	<0.0001
Exacerbations during follow-up	-	-	-0.94 (0.27)	0.0006	-0.91 (0.27)	0.0007
Hospitalizations during follow-up	-	-	-1.43 (0.57)	0.01	-1.38 (0.54)	0.01
Follow-up duration	-	-	5.8 (2.23)	0.009	5.5 (2.2)	0.01

Note: SE = standard error; BMI = body mass index; *Pa* = *Pseudomonas aeruginosa*

[a]Multivariable regression model including baseline characteristics and potential confounders

[b]Multivariable regression model including propensity score as a covariate and potential confounders

Potential confounders included in models a and b were age, inhaled tobramycin, aztreonam, colistin, presence of bacteria on sputum cultures – *Pa*, mucoid *Pa*, *methicillin-resistant Staphylococcus aureus*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia*, CF-related diabetes status, pancreatic insufficiency status, use of hypertonic saline, chronic macrolide use, high-dose ibuprofen use, body mass index percentile, total exacerbations during follow-up, during hospitalizations during follow-up, follow-up duration.

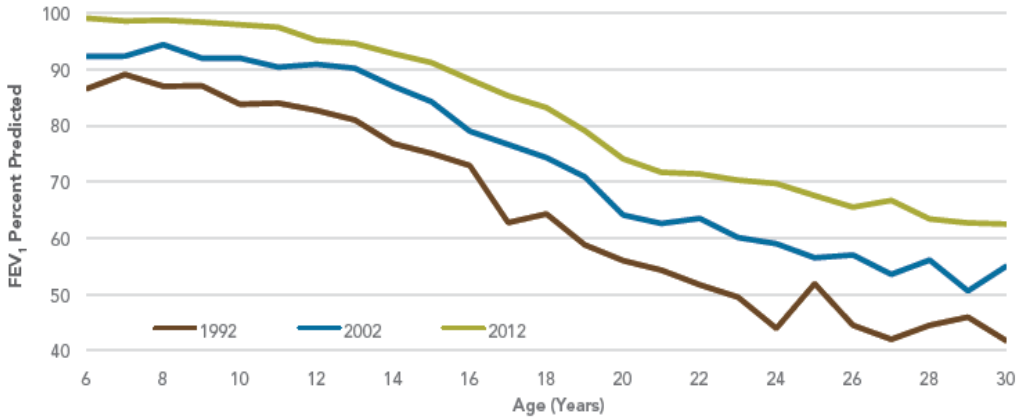

Figure 3-1. Median FEV_1% Predicted by Age – 1992, 2002 and 2012 in Patients with Cystic Fibrosis in the United States as Reported in the CFFPR. Reproduced with permission from the Cystic Fibrosis Foundation.

Source: Cystic Fibrosis Foundation Patient Registry. 2012 Annual Data Report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2013.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

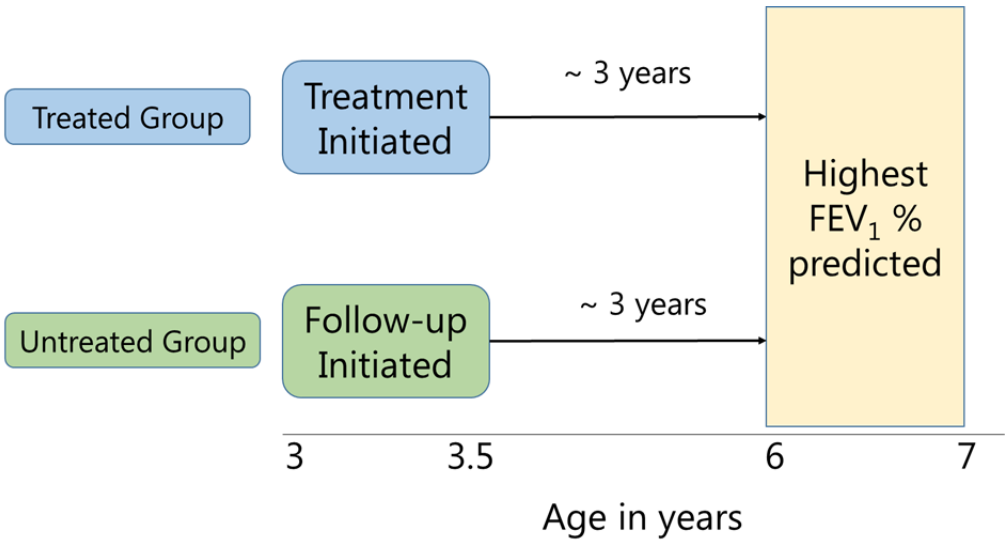

Figure 3-2. Study Timeline for Evaluating the Clinical Effectiveness of Dornase Alfa in Children Six Years of Age and Younger.

Note: FEV_1 = forced expiratory volume in 1 second

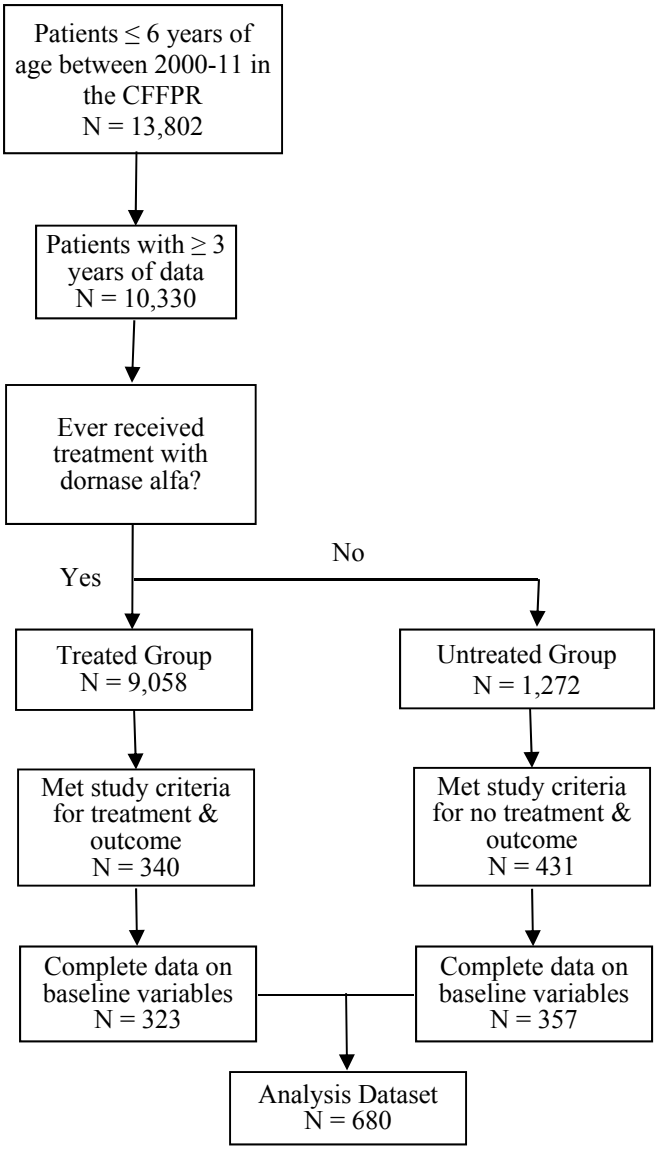

Figure 3-3. Identification of Patients for the Treated and Untreated Groups.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

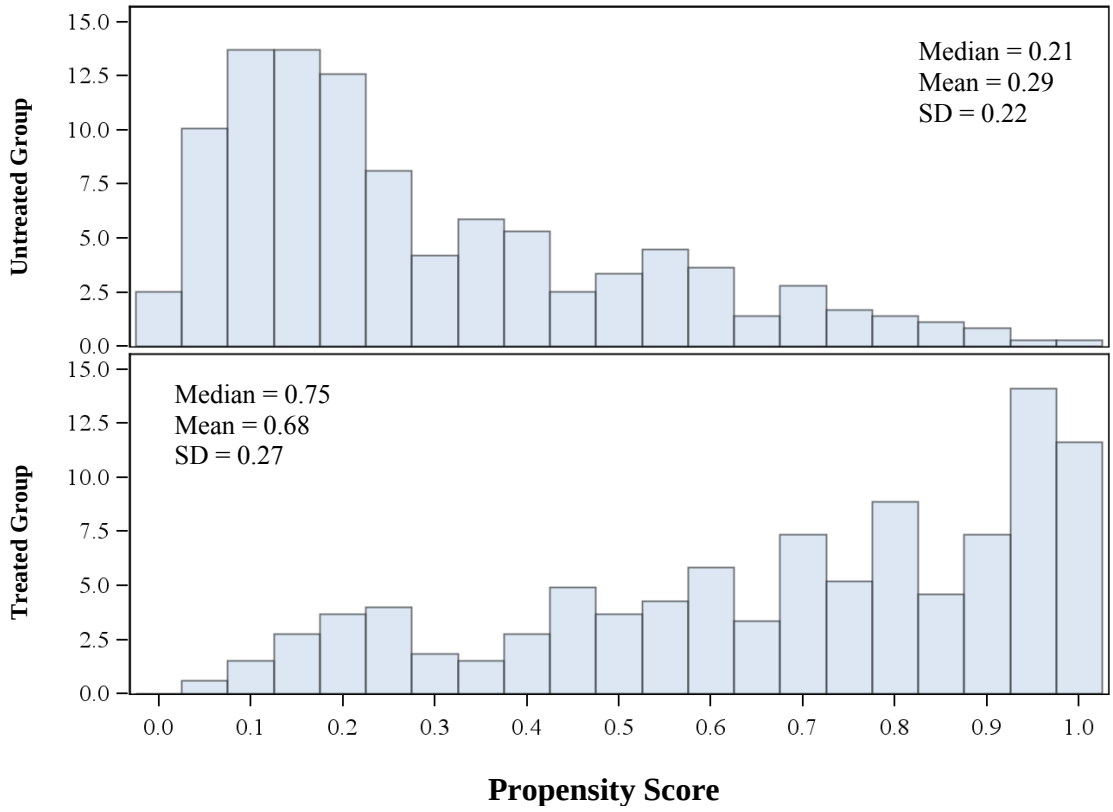

Figure 3-4. Distribution of Propensity Scores in the Dornase Alfa-Treated (N = 323) and Untreated (N = 357) Groups of Young Children with Cystic Fibrosis.

Note: SD = standard deviation

CHAPTER IV - LONG-TERM CLINICAL EFFECTIVENESS OF AZITHROMYCIN IN CYSTIC FIBROSIS

Background

Cystic Fibrosis (CF) is characterized by a vicious cycle of pulmonary infection, inflammation, and airway damage, eventually leading to progressive lung disease and a drop in lung function. Hence, a major focus in CF lung disease management has been to break this cycle with the use of antibiotics and anti-inflammatory agents (25). Azithromycin, a macrolide antibiotic, is one such drug in the armamentarium of clinicians treating CF. The exact mechanism by which azithromycin is effective in treating CF is not known; however it has been suggested that azithromycin may have anti-inflammatory properties (24, 25). Randomized clinical trials carried out in recent years have noted its efficacy in reducing pulmonary exacerbations and antibiotic use with improved weight gain in patients uninfected with *Pseudomonas aeruginosa* (*Pa*) (74-76) as well as in improving lung function in CF patients chronically infected with *Pa* (71-73).

Most of these trials followed up patients for a short duration (6 to 12 months), with no long-term data on the effectiveness of azithromycin in improving lung outcomes in CF. Moreover, the primary outcome of interest for lung function in all these trials was a change in lung volume [forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC)] at the end of treatment from the baseline. CF therapies that have a higher probability of improving survival should not only cause a prompt improvement in FEV_1, but should also reduce the rate of decline of FEV_1 over time (26).

This retrospective study was carried out to investigate the effects of azithromycin on rate of decline in lung function in CF patients, who were treated for a period of at least two years. The specific aim of this study was **to compare the rate of decline in lung function between CF patients who received chronic treatment with azithromycin and those who did not receive treatment with it.** We hypothesized that the rate of decline in FEV_1 would be significantly lower in CF patients receiving treatment with

azithromycin than in those not receiving treatment. Longitudinal FEV_1 data for patients enrolled in the Cystic Fibrosis Foundation Patient Registry (CFFPR), receiving azithromycin, were compared with that for patients not receiving azithromycin. Patients in both the groups continued to receive treatment with routine CF therapies. This comparison was adjusted for important potential confounders such as age, gender, concomitant use of routine therapies, chronic airway infection with *Pa* and pulmonary exacerbations as defined in the CFFPR. Based on the published literature, this was the first study to evaluate the long-term effectiveness of azithromycin on the rate of decline in lung function.

Methods

Study Population

All CF patients enrolled in the CFFPR aged 6 to 20 years, between 2004 and 2011, were eligible for this study. These age restrictions for eligibility were enforced since lung function tests are usually available after 6 years of age and body mass index (BMI) percentile based on the Centers for Disease Control and Prevention (CDC) growth charts are available only until 20 years of age. BMI percentile has been noted to be an important confounder affecting treatment assignment as well as lung function (2). Hence, availability of BMI percentile data was important to assess the likelihood of treatment assignment. Additionally, this age criterion enabled a concentration on areas of increased risk for rapid decline in lung function (102, 103). In patients who received lung transplantation, all post-transplant data were excluded from the analysis.

Study Design

The study used a retrospective cohort design wherein the rate of decline in FEV_1 was compared between patients who did and did not receive treatment with azithromycin (Figure 4-2). Chronic use of azithromycin was defined as having received oral azithromycin for at least two years during which azithromycin use was recorded for at least 50% of the clinic visits. Patients in both the groups could continue to receive other

CF therapies such as inhaled antibiotics, dornase alfa, hypertonic saline, pancreatic enzyme supplements, high-dose ibuprofen and chronic oral antibiotics.

The primary outcome in this study was rate of decline in FEV_1 % predicted. The FEV_1 % predicted values were calculated using the Global Lung Initiative (GLI) reference equations, which were developed using data from people between 3 and 95 years of age with healthy lungs (104). Using the patients' age, gender, height, and race in these reference equations, the absolute FEV_1 measurements in liters were converted to % predicted values. As a result it was possible for a CF patient to have a FEV_1 % predicted value of over 100% if she/he had a FEV_1 measurement that was above that predicted for their combination of age, gender, height, and race. To be able to calculate the change in slope of FEV_1, eligible patients had to have at least three FEV_1 measurements spanning at least six months. Data were also collected on a number of baseline factors that could affect treatment assignment or could be potential confounders of the association between azithromycin use and rate of FEV_1 decline (99). These variables were:

- Baseline age – This was defined as the age at the start of azithromycin treatment in the treated group. In the untreated group it was defined as the age before which the patient had three FEV_1 measurements spanning at least six months.
- Gender – Gender was defined as male or female.
- Baseline FEV_1 – This was defined as the FEV_1 measurement at the baseline age.
- Genotype – This was defined based on number of *delta*F508 alleles and was categorized into: a) homozygous *delta*F508; b) heterozygous *delta*F508; and c) no *delta*F508.
- Pre-baseline FEV_1 slope – This was defined as the slope of FEV_1 change before the start of the azithromycin treatment in the treated group and before the start of follow-up in the untreated group. For the pre-baseline

FEV_1 slope calculation the eligible patients had to have at least three FEV_1 measurements spanning at least six months.

- Baseline Body Mass Index (BMI) percentile – This was defined as the highest BMI percentile in the year prior to the baseline age.
- Chronic *Pseudomonas aeruginosa* (*Pa*) infection – This was defined using the modified Leeds criteria (95). Patients who had at least two respiratory cultures in the year prior to the baseline age, with at least 50% of the cultures positive for *Pa* were assumed to have chronic *Pa* infection.
- Baseline CF-related diabetes mellitus status – This was defined as presence of CF-related diabetes mellitus in the year prior to baseline age.
- Baseline Pancreatic Insufficiency – A patient was assumed to be pancreatic insufficient if she/he was receiving pancreatic enzyme supplements at any time during the year prior to the baseline age.
- Baseline Dornase Alfa use – This was defined as dornase alfa use at baseline age.
- Center-specific prescription rate of azithromycin in children < 6 years of age – This variable was defined as the proportion of patients < 6 years of age receiving care at a center, who ever received treatment with azithromycin irrespective of their *Pa* infection status. This variable was created based on the assumption that if a center was more likely to prescribe azithromycin to a patient < 6 years of age, it was more likely to prescribe it to a patient ≥ 6 years of age.
- Center size – Based on the distribution of patients across the CF centers, a CF center was classified as a small center if it provided care for ≤ 90 patients between 2004 and 2011, and a large center if > 90 patients received care.

- Total number of exacerbations – This was defined as the total number of pulmonary exacerbations as identified in the CFFPR in the year prior to the baseline age.
- Total number of hospitalizations – This was defined as the total number of hospitalizations as identified in the CFFPR in the year prior to the baseline age.

Data on other variables for each clinic visit were also collected for the follow-up period. These included binary variables such as exacerbation, hospitalization, use of other CF therapies such as inhaled antibiotics like tobramycin, aztreonam, and colistin, dornase alfa, hypertonic saline, high dose ibuprofen, and chronic oral antibiotics, presence of bacteria such as *Pa*, *Burkholderia cepacia*, *methicillin-resistant Staphylococcal aureus* (MRSA), mucoid *Pa*, *Alcaligenes xylosoxidans*, and *Stenotrophomonas maltophilia*. Visit-specific data on CF-related diabetes mellitus status was also collected and treated as a categorical variable with three categories: normal; impaired glucose tolerance; and CF-related diabetes mellitus with or without fasting hyperglycemia.

Statistical Analyses

Baseline patient characteristics were summarized using means and standard deviations (SD) for continuous variables, and percentages for categorical variables. The two groups were compared on these baseline characteristics using the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables.

To account for treatment indication bias, propensity scores were calculated for patients in the study to estimate the likelihood of receiving treatment with azithromycin, given a set of baseline factors. The propensity scores were calculated by fitting a logistic regression model that included the baseline covariates listed above. Patients in the two treatment groups were then matched on their propensity scores using the local optimal (greedy) algorithm. The analysis used caliper, 1:1, matching – a method in which the treated and the untreated groups were randomly sorted and then the first treated patient

was selected to find its closest untreated match in terms of the propensity score if the control's propensity score was within a certain distance (caliper) (92). A caliper width of 0.05 was used in this study and matching was carried out without replacement.

This matched group of treated and untreated patients was used in the final analysis, where a linear mixed model was fitted to compare the rate of decline of FEV_1 % predicted between the two groups using the restricted maximum likelihood method of estimation. The time interval in years between the FEV_1 measurement and baseline age was included in both the fixed and random parts of the model. The treatment group by time interaction term was used to estimate the difference in the rate of decline in FEV_1 % predicted between the two groups. This model adjusted for the potential confounders listed above.

All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). *P* values < 0.05 were considered significant. This study was approved by the University of Iowa Institutional Review Board.

Results

Characteristics of the Study Cohort

A total of 18,653 patients enrolled in the CFFPR were 6 – 20 years of age at some time between 2004 and 2011 (Figure 4-3). Of these patients, 9,240 were noted to have been chronically treated with azithromycin at some point during the study period. The remaining 9,413 were never treated with azithromycin. In the treated group, 2,461 patients fulfilled the study criteria of being treated chronically with azithromycin for a period of at least two years with at least three FEV_1 % predicted measurements spanning at least six months. These 2,461 patients also had complete data for baseline characteristics and potential study confounders. In the untreated group, 5,023 patients met the study criteria and had complete data for baseline characteristics and other study variables. Propensity score matching was carried out on this set of 7,484 patients which

resulted in a matched set of 1,514 patients in each of the two groups. This set of 3,028 patients was used for the linear mixed model analysis.

The baseline characteristics for patients in both groups are summarized in Table 4-1. The azithromycin treatment group included patients who were older and were more likely to be females, and be homozygous for the *delta*F508 mutation. Additionally, the azithromycin treated group had a significantly higher proportion of patients who were chronically infected with *Pa*, and also had a higher proportion of CF-related diabetes and pancreatic insufficiency. The use of dornase alfa in the treated group was significantly higher than in the untreated group. The treated group also had significantly lower baseline BMI, higher total number of exacerbations and hospitalizations, higher center-specific prescription rates of azithromycin in children < 6 years of age, and lower baseline FEV_1. The two groups did not differ in the proportion of patients receiving care at a small CF center or the pre-baseline FEV_1 slope.

Propensity Score Calculation and Matching

Propensity scores were calculated for patients in both groups to assess the likelihood of receiving chronic treatment with azithromycin given the set of baseline characteristics. The mean propensity score in the treated group was 0.56 ± 0.26, whereas the mean propensity score in the untreated group was 0.22 ± 0.20 (Figure 4-4). The two groups were then matched on their propensity scores. After matching, the mean propensity scores for the treated and the untreated groups were 0.42 ± 0.22 and 0.41 ± 0.22, respectively (Figure 4-5). The baseline characteristics of the patients after propensity score matching are summarized in Table 4-2. After matching on the propensity score, the two treatment groups were similar with respect to most baseline characteristics except dornase alfa use ($p = 0.04$), and chronic infection with *Pa* ($p = 0.06$).

Linear Mixed Model Analysis

After the propensity score matching, data for this set of patients were analyzed using a linear mixed model analysis. The results of this analysis, summarized in Table 4-3, suggested that the average annual rate of FEV_1 decline in patients not treated with azithromycin was 0.44% per year slower than that in patients chronically treated with azithromycin (-2.73% per year in the treated group vs. -2.29% per year in the untreated group, $p = 0.007$). Additionally, on average, the untreated group had a FEV_1 % predicted value that was 0.46% higher than that in the treated group, although this result was not significant ($p = 0.29$). Other variables that were found to be significant predictors of FEV_1 decline were: baseline FEV_1 (estimate = 0.81% (0.01), $p < 0.0001$); chronic *Pa* infection (no vs. yes, estimate = 2.24% (0.44), $p < 0.0001$); center-specific prescription rate for azithromycin in children < 6 years of age (estimate = 3.52% (1.73), $p = 0.04$); presence of exacerbations during follow-up (no vs. yes, estimate = 5.84% (0.08), $p < 0.0001$); hospitalizations during follow-up (no vs. yes, estimate = 9.59% (0.22), $p < 0.0001$); hypertonic saline use during follow-up (no vs. yes, estimate = -1.45% (0.20); $p < 0.0001$); colistin use during follow-up (no vs. yes, estimate = 0.53% (0.20), $p = 0.008$); and presence of *Pa* (no vs. yes, estimate = 0.69% (0.24), $p = 0.005$), MRSA (no vs. yes, estimate = 1.07% (0.23), $p < 0.0001$), and *Alcaligenes xylosoxidans* (no vs. yes, estimate = 0.81% (0.24), $p = 0.0009$) in respiratory cultures during follow-up. Interaction with time for some variables that could affect the slope of FEV_1 decline based on results from previous studies (99), was also included in the model to assess their effect on the slope of FEV_1 decline. Of these, only hypertonic saline was noted to have a significant effect on the slope of FEV_1 decline (no vs. yes, estimate = 0.43% per year (0.07), $p < 0.0001$). However, the direction of this association was in favor of the untreated group, which had a slower rate of decline compared to the hypertonic saline treated group.

Additionally, since exacerbations and hospitalizations during follow-up could be in the causal pathway for the association between treatment with azithromycin and lung

function, these two variables were excluded from the linear mixed model. The results of this analysis, suggested that the average annual rate of FEV_1 decline in patients not treated with azithromycin was 0.33% per year slower than that in patients chronically treated with azithromycin (-2.44% per year in the treated group vs. -2.11% per year in the untreated group, $p = 0.05$). Additionally, on average, the untreated group had a FEV_1 % predicted value that was 0.49% higher than that in the treated group, although this result was not significant ($p = 0.27$).

To preserve matching between patients in the two groups on their propensity scores, a matched analysis using PROC MIXED was also carried out, where the propensity score-matched pair identification number was treated as a random effect along with patient identification number. The results of this analysis were similar to the primary analysis. The average annual rate of FEV_1 decline in patients not treated with azithromycin was 0.44% per year slower than that in patients chronically treated with azithromycin (-2.73% per year in the treated group vs. -2.29% per year in the untreated group, $p = 0.007$). Additionally, on average, the untreated group had a FEV_1 % predicted value that was 0.46% higher than that in the treated group ($p = 0.29$).

Discussion

The results of this study indicated that the chronic use of azithromycin was not associated with a slower decline in FEV_1. On the contrary, the study found a significantly slower rate of FEV_1 decline in the untreated group compared to the treated group. This was the first study to evaluate the long-term effectiveness of chronic azithromycin use on the rate of FEV_1 decline. This study was also the first to use the GLI reference equations for standardizing FEV_1 % predicted and to assess the rate of decline. The CFF recommends using the Wang et al. (66) prediction equation for females less than 15 years old and for males less than 17 years old (105). Beyond these ages, the recommendation is to switch over to the Hankinson et al. prediction equation (67). However, a difference of up to 8% in predicted FEV_1 values obtained from these two prediction equations has been

observed in individuals with CF at the time of this switch (106). Hence, this study used the all-age GLI equation. Additionally, the study used propensity score matching methods to account for the treatment indication bias that usually is present when assessing long-term effects of CF treatments. The treatment indication bias was reflected in the comparison of the baseline characteristics between the two groups. The treated group had a significantly higher proportion of factors that have been shown to be associated with FEV_1 decline such as: a higher proportion of females and homozygous *delta*F508 genotype; lower BMI percentile; a higher number of exacerbations and hospitalizations; a higher proportion of patients with chronic *Pa* and CF-related diabetes; and a lower baseline FEV_1. Hence by matching the two groups based on the propensity score calculated using baseline characteristics, the study was able to reduce some of the bias associated with treatment assignment.

Even after adjustment utilizing baseline characteristics and potential confounders, the study failed to reject the null hypothesis and found results that were contrary to the expected outcome. Since the rate of decline over the long-term use of azithromycin has never been assessed before, it is difficult to ascertain if these results are consistent with reality. However, given that the untreated group on average had higher FEV_1 values on follow-up compared with the treated group, although not significant, these results are different from what has been noted in short-term clinical trials testing the efficacy of azithromycin. A possible explanation for this may be the presence of treatment indication bias that wasn't controlled by propensity score matching. One of the limitations of propensity score matching is that it can only account for measured differences between the two groups. If unmeasured covariates have an influence on the likelihood of a patient receiving a therapy, then propensity score matching cannot control for their effects. This seems to be a likely scenario in this study. The CFFPR does not collect any information on patient symptom scores or physician prescription preferences. Moreover, no data on patient quality of life measures are collected. These variables may be the missing link in

combating treatment indication bias using propensity score methods. The inability of propensity score methods to eliminate treatment indication bias has been previously highlighted by VanDyke et al. (103). The authors of that study evaluated the effectiveness of inhaled tobramycin on reducing FEV_1 decline using CFFPR data and found that the treated group had significantly lower values of FEV_1 compared to the untreated group (estimate = -1.74% (0.31), $p < 0.0001$).

Additionally, as part of the propensity score matched analysis in this study, the unmatched patients were characterized. The unmatched treated and untreated patients differed significantly on all baseline variables except center size and pre-baseline FEV_1 slope. The unmatched patients in the treated group (n = 947) had a mean propensity score of 0.77 (SD = 0.11) while those in the untreated group (n = 3,509) had a mean score of 0.13 (SD = 0.11). When a linear mixed model was fitted to analyze the effectiveness of azithromycin on rate of FEV_1 decline, the untreated group was noted to have a slower average rate of FEV_1 decline (untreated vs. treated, estimate = 0.98%, $p < 0.0001$).

This study also assessed the rate of FEV_1 decline where the duration of follow-up was limited to two years. This was done to mimic study eligibility criteria recently used by Konstan et al. to assess the effectiveness of dornase alfa and inhaled tobramycin on the rate of FEV_1 decline (69, 107). Once, again the results did not show a significant difference in the rate of FEV_1 decline between the azithromycin-treated and untreated groups, although the direction of the association changed (untreated vs. treated, estimate = -0.03% (0.42), $p = 0.95$). Additionally, this study evaluated the effectiveness of azithromycin on the rate of FEV_1 decline in the entire population of eligible patients without propensity score matching. A linear mixed model was fitted to include all baseline covariates as well as potential confounders. This analysis yielded similar results to those obtained from the propensity score matched method using all follow-up data. The rate of FEV_1 decline per year was 0.78% slower in the untreated group compared with the treated group ($p < 0.0001$).

Since physician's prescription preferences may be guided by an unexpected decline in FEV_1 greater than what was anticipated based on the pre-baseline FEV_1 slope, an additional variable was created to account for this unexpected decline in FEV_1. It was defined as the difference between the estimated FEV_1 % predicted at baseline calculated from the pre-baseline FEV_1 slope and the observed baseline FEV_1 % predicted value. However, adding this variable to the logistic model for propensity score calculation and adjusting for it in the final linear mixed model analysis did not affect final results comparing the rate of FEV_1 decline in the two groups (untreated vs. treated, estimate = 0.46% (0.17), p = 0.005).

An important limitation of this study was lack of medication adherence monitoring. Given the retrospective nature of the data obtained from the CFFPR, there was no way to monitor patient adherence to medication use. If the medication was listed for a clinic visit, it was assumed that the patient was taking the medication.

The results from the primary propensity score matched analysis for some variables are consistent with results from previous studies (47, 99, 108). Patients with normal blood sugars had higher FEV_1 values, on average, compared with patients with a diagnosis of CF-related diabetes mellitus, although this association was not significant. Also, patients, who did not have exacerbations or hospitalizations during follow-up, had mean FEV_1 values that were significantly higher than patients who had exacerbations. Additionally, presence of *Pa* and MRSA on respiratory cultures during follow-up was associated with significantly lower mean FEV_1 values. Presence of mucoid *Pa* was associated with lower FEV_1 values; however this association was not significant. Finally, patients treated with inhaled hypertonic saline and inhaled tobramycin had FEV_1 values higher than the untreated group though this association was only significant for hypertonic saline.

In conclusion, this study did not find patients who were chronically treated with azithromycin to have a slower rate of FEV_1 decline. On the contrary, a significantly

slower rate of FEV_1 decline was observed in the untreated group. The inability of propensity score methods to eliminate treatment indication bias seems to have had an effect on the study results. Additional variables, unavailable in the CFFPR, need to be investigated to assess their role in treatment assignment and included in the propensity score calculation. As an alternative to propensity score analysis, an instrumental variable analysis could also be carried out; although several challenges still need to be addressed when working with data assessed at multiple time points before that method can be applied to the current study design.

Table 4-1. Baseline Characteristics of Study Cohort by Treatment Status

Characteristics	Treated with Azithromycin (N = 2,461)	Not treated with Azithromycin (N = 5,023)	*P* value*
Age, mean (SD), years	12.84 (3.5)	11.42 (4.3)	<0.0001
Female sex, N (%)	1,227 (50.0)	2,366 (47.1)	0.03
Genotype, N (%) • Homozygous *delta*F508 • Heterozygous *delta*F508 • No *delta*F508	 1,287 (52.3) 863 (35.1) 311 (12.6)	 2,246 (44.7) 1,796 (35.8) 981 (19.5)	<0.0001
Body Mass Index percentile[a], mean (SD)	51.5 (26.3)	54.4 (27.2)	<0.0001
Exacerbations[b], mean (SD)	0.48(1.08)	0.29 (0.74)	<0.0001
Hospitalizations[c], mean (SD)	0.71 (1.11)	0.47 (0.95)	<0.0001
Center size[d], Small, N (%)	598 (24.3)	1,204 (24.0)	0.75
Center-Prescription Rate[e], mean (SD)	0.09 (0.11)	0.06 (0.08)	<0.0001
Chronic *Pseudomonas aeruginosa* infection, N (%)	900 (36.6)	535 (10.7)	<0.0001
CF-related diabetes, N (%)	193 (7.8)	246 (4.9)	<0.0001
Pancreatic Insufficiency, N (%)	2,379 (96.7)	3,994 (79.5)	<0.0001
Baseline FEV_1 % predicted , mean (SD)	80.7 (21.2)	87.7 (20.9)	<0.0001
Pre-baseline FEV_1 % predicted slope, mean (SD)	-0.33 (9.77)	-0.41 (22.28)	0.31
Dornase alfa use, N (%)	1,890 (76.8)	1,909 (38.0)	<0.0001

Note: SD = standard deviation; CF = cystic fibrosis; FEV_1 = forced expiratory volume in 1 second.

[a]Highest body mass index percentile in the year prior to start of treatment or follow-up.

[b]Number of pulmonary exacerbations in the year prior to start of treatment or follow-up.

[c]Number of hospitalizations in the year prior to start of treatment or follow-up.

[d]Center was classified into small and large centers based on a cut-off value of 90 patients between 2004 – 2011.

[e]Center-specific prescription rate of azithromycin in CF patients < 6 years of age irrespective of *Pseudomonas aeruginosa* infection status.

**P* values from Wilcoxon rank-sum test or chi-square test.

Table 4-2. Baseline Characteristics of Study Cohort after Propensity Score Matching by Treatment Status

Characteristics	Treated with Azithromycin (N = 1,514)	Not treated with Azithromycin (N = 1,514)	*P* value*
Age, mean (SD), years	12.1 (3.4)	12.5 (4.7)	0.75
Female sex, N (%)	728 (48.1)	738 (48.8)	0.72
Genotype, N (%) • Homozygous *delta*F508 • Heterozygous *delta*F508 • No *delta*F508	 772 (51.0) 517 (34.1) 225 (14.9)	 770 (50.9) 501 (33.0) 243 (16.1)	0.62
Body Mass Index percentile[a], mean (SD)	53.2 (26.2)	52.4 (27.9)	0.59
Exacerbations[b], mean (SD)	0.48 (1.01)	0.48 (1.0)	0.75
Hospitalizations[c], mean (SD)	0.61 (1.01)	0.61(1.06)	0.53
Center size[d], Small, N (%)	375 (24.8)	376 (24.8)	0.97
Center-Prescription Rate[e], mean (SD)	0.08 (0.09)	0.08 (0.09)	0.1
Chronic *Pseudomonas aeruginosa* infection, n (%)	295 (19.5)	337 (22.3)	0.06
CF-related diabetes, N (%)	94 (6.2)	103 (6.8)	0.51
Pancreatic Insufficiency, N (%)	1,432 (94.6)	1,414 (93.4)	0.17
Baseline FEV_1 % predicted , mean (SD)	83.74 (20.7)	82.19 (22.0)	0.21
Pre-baseline FEV_1 % predicted slope, mean (SD)	-0.16 (10.45)	-0.46 (21.91)	0.63
Dornase alfa use, N (%)	986 (65.1)	931 (61.5)	0.04

Note: SD = standard deviation; CF = cystic fibrosis; FEV_1 = forced expiratory volume in 1 second.

[a]Highest body mass index percentile in the year prior to start of treatment or follow-up.

[b]Number of pulmonary exacerbations in the year prior to start of treatment or follow-up.

[c]Number of hospitalizations in the year prior to start of treatment or follow-up.

[d]Center was classified into small and large centers based on a cut-off value of 90 patients between 2004 – 2011.

[e]Center-specific prescription rate of azithromycin in CF patients < 6 years of age irrespective of *Pseudomonas aeruginosa* infection status.

**P* values from Wilcoxon rank-sum test or chi-square test.

Table 4-3. Parameter Estimates (SE) from fitting a Linear Mixed Model to Predict the Rate of Decline in FEV_1 % Predicted

Variables	Coefficient (SE)	*P* Value
Azithromycin (untreated vs. treated)	0.46 (0.43)	0.29
Azithromycin×Time (untreated vs. treated)	0.44 (0.16)	0.007
Time	-2.73 (0.20)	<0.0001
Baseline FEV_1	0.81 (0.01)	<0.0001
Chronic *Pseudomonas aeruginosa* infection (no vs. yes)	2.24 (0.44)	<0.0001
Center-specific prescription rate	3.52 (1.73)	0.04
Pre-Baseline age FEV_1 slope	-0.003 (0.01)	0.75
CF-related diabetes mellitus (Normal blood sugar vs. CF-related diabetes mellitus) (Impaired glucose tolerance vs. CF-related diabetes mellitus)	 0.29 (0.17) 0.08 (0.31)	 0.09 0.79
Exacerbations (no vs. yes)	5.84 (0.08)	<0.0001
Hospitalizations (no vs. yes)	9.59 (0.22)	<0.0001
Dornase alfa use (no vs. yes)	-0.03 (0.22)	0.88
Inhaled tobramycin use (no vs. yes)	-0.25 (0.25)	0.30
Azithromycin×Inhaled tobramycin (not on both vs. on both)	-0.54 (0.37)	0.15
Inhaled aztreonam use (no vs. yes)	0.49 (0.70)	0.48
Hypertonic saline use (no vs. yes)	-1.45 (0.20)	<0.0001
High dose ibuprofen use(no vs. yes)	-0.45 (0.33)	0.17
Chronic oral antibiotic use (no vs. yes)	0.07 (0.16)	0.64
Colistin use (no vs. yes)	0.53 (0.20)	0.008
Pseudomonas aeruginosa[a] (no vs. yes)	0.69 (0.24)	0.005
Burkholderia cepacia[a] (no vs. yes)	0.11 (0.34)	0.75
MRSA[a] (no vs. yes)	1.07 (0.23)	<0.0001
Mucoid *Pseudomonas aeruginosa*[a] (no vs. yes)	0.38 (0.31)	0.21
Alcaligenes xylosoxidans[a] (no vs. yes)	0.81 (0.24)	0.0009
Stenotrophomonas maltophilia[a] (no vs. yes)	0.24 (0.16)	0.14
Dornase alfa×Time (no vs. yes)	-0.08 (0.07)	0.28
Inhaled tobramycin×Time (no vs. yes)	0.13 (0.08)	0.08
Azithromycin×Inhaled tobramycin×Time (not on both vs. on both)	0.14(0.12)	0.26
Inhaled aztreonam×Time (no vs. yes)	0.01 (0.16)	0.93
Hypertonic saline×Time (no vs. yes)	0.43 (0.07)	<0.0001
Pseudomonas aeruginosa×Time[a] (no vs. yes)	-0.05 (0.08)	0.59
MRSA×Time[a] (no vs. yes)	0.08 (0.08)	0.27
Mucoid *Pseudomonas aeruginosa*×Time[a] (no vs. yes)	0.16 (0.10)	0.11

Note: FEV_1 = forced expiratory volume in 1 second; MRSA = Methicillin resistant *Staphylococcus aureus*

[a]Respiratory cultures for these bacteria were found to be positive during follow-up after baseline.

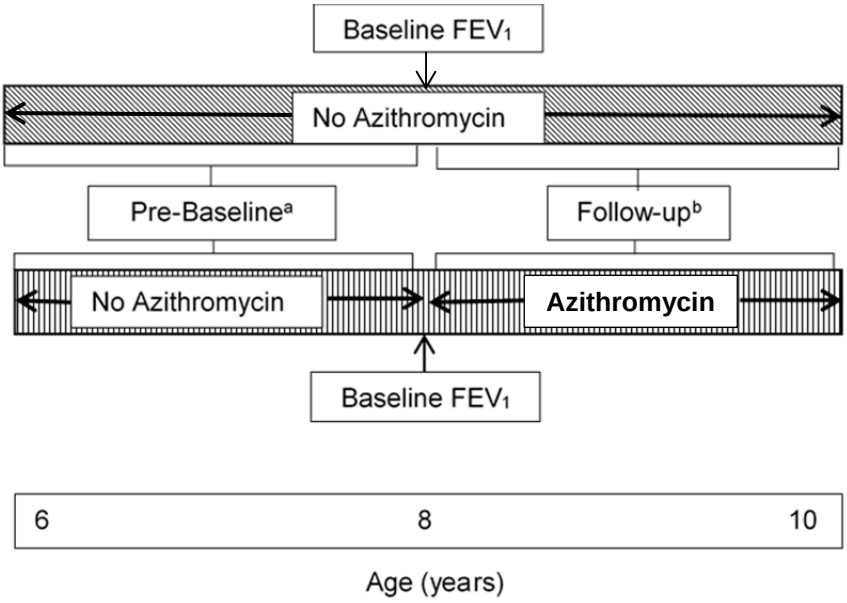

Figure 4-1. Study Timeline for a Scenario where Treatment with Azithromycin (Treated Group) or Follow-up (Untreated Group) Began at 8 Years of Age.

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[a]In the treated group the pre-baseline period was defined as the period before the start of treatment with azithromycin. In the untreated group, the pre-baseline period was defined as the period before start of follow-up, in which, the patient had at least three FEV_1 measurements spanning at least six months. This period could be less than two years as long as there were three FEV_1 measurements spanning at least six months.

[b]Follow-up period lasted for at least two years in both groups. This period had to include at least three FEV_1 measurements spanning at least six months in both groups.

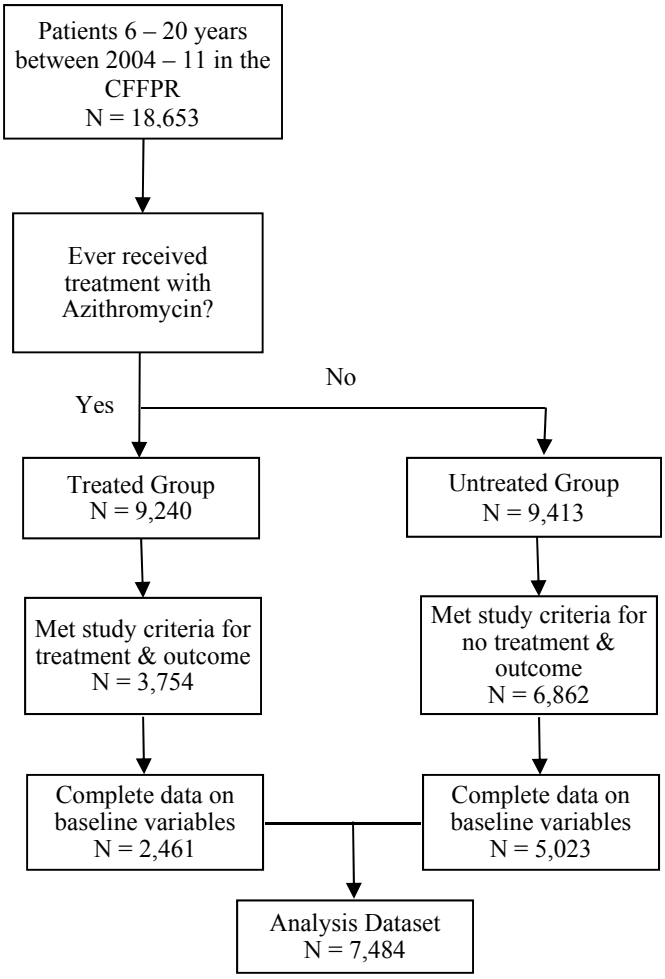

Figure 4-2. Identification of Patients for the Treated and Untreated Groups.

Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

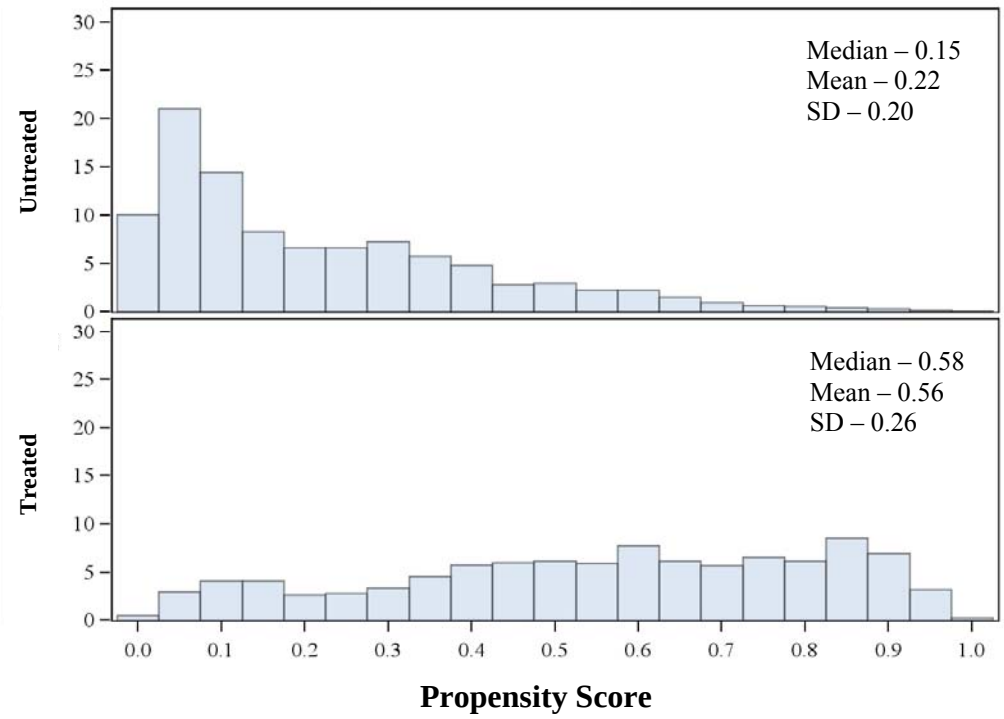

Figure 4-3. Distribution of Propensity Scores in the Azithromycin-Treated (N = 2,461) and Untreated (N = 5,023) Groups of Patients with Cystic Fibrosis.

Note: SD = standard deviation

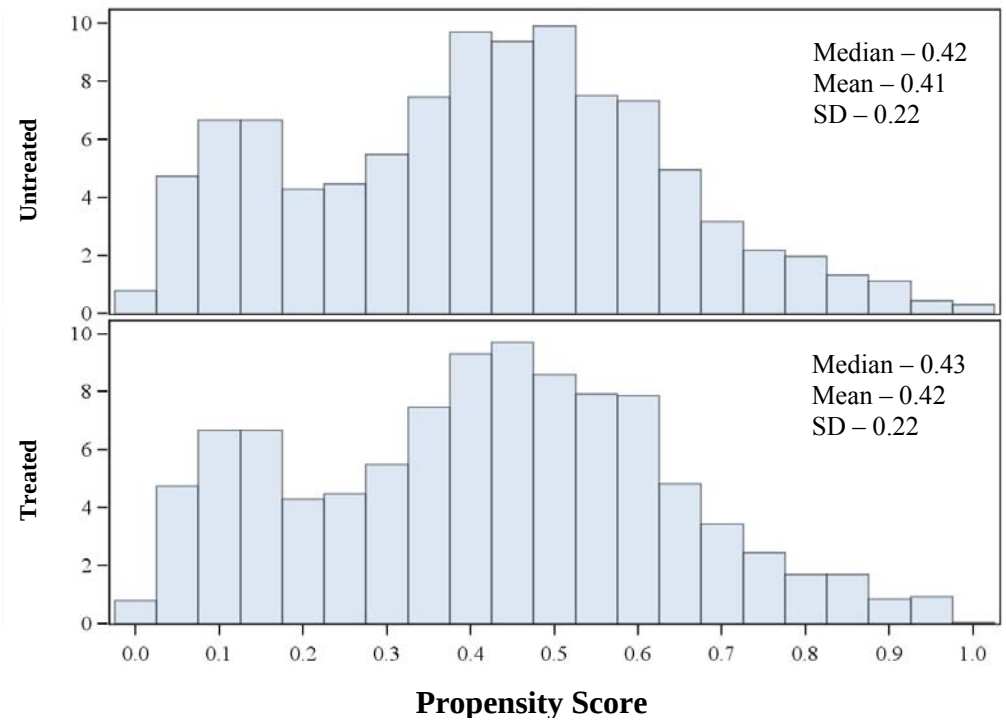

Figure 4-4. Distribution of Propensity Scores in the Azithromycin-Treated and Untreated Groups of Patients with Cystic Fibrosis after Propensity Score Matching (N = 1,514 pairs).

Note: SD = standard deviation

CHAPTER V - LONG-TERM CLINICAL EFFECTIVENESS OF COMBINED INHALED TOBRAMYCIN AND AZTREONAM ON LUNG FUNCTION IN CYSTIC FIBROSIS

Background

Chronic therapy with inhaled anti-pseudomonal antibiotics is the standard of care in patients with cystic fibrosis (CF), 6 years of age and older, who have chronic *Pseudomonas aeruginosa* (*Pa*) airway infection (109). Currently, two inhaled antibiotics are FDA approved for treatment of chronic *Pa* infections in CF patients: tobramycin, available as either tobramycin inhalation powder (TIP) or nebulized tobramycin inhaled solution (TIS) (Novartis Pharmaceuticals Corporation); and aztreonam for inhalation solution (Cayston, Gilead Sciences, Inc.). Compared to a placebo control, both of these inhaled treatments have been found to improve clinical outcomes in CF patients in the form of lung function improvements, reduced courses of IV antibiotics, and/or reduced number of pulmonary exacerbations (47, 84-86). These inhaled antibiotics are generally used in one of two patterns: every other month monotherapy; and continuous alternate month therapy. Different inhaled antibiotics are used together for the continuous alternating month pattern. For example, 28 days on inhaled tobramycin followed by 28 days on inhaled aztreonam. The every other month pattern for a specific antibiotic in both of these patterns is utilized to help decrease the likelihood of selecting for bacterial antibiotic resistance. Continuous alternate month therapy is generally reserved for patients who are sicker, have more rapidly progressing disease, and/or are having more frequent exacerbations or hospitalizations.

There are few comparative effectiveness studies of inhaled tobramycin and aztreonam. A recently published study by Assael et al. comparing inhaled aztreonam and tobramycin over three treatment courses, each of 28 days, concluded that aztreonam was superior in lung function improvement and reduction in acute pulmonary exacerbations (89). However, some have felt the study design in this industry-sponsored trial may have

been biased toward aztreonam because 85% of patients enrolled in this study reported inhaled tobramycin use for ≥ 84 days in the previous year (i.e., were on inhaled tobramycin prior to the study) (89). This is important because in the initial clinical trial of inhaled tobramycin by Ramsey et al. (47), they observed that after an initial improvement in the FEV_1 % predicted from baseline values in the first month on therapy, the FEV_1 did not return to baseline values. On subsequent months of treatment, FEV_1 improvement ranged from about 10% on treatment months to about 7 to 8% during off months. Therefore, it would be expected that patients continuing on inhaled tobramycin would only have a modest improvement (0.55% in the Assael et al. study) compared to those that were inhaled antibiotic-naive (12% in the Ramsey et al. study with patients that were inhaled antibiotic naive). Another study conducted to observe the long-term safety and efficacy of inhaled aztreonam found that about 52% of the enrolled patients had at least one course of inhaled tobramycin and about 23% had > three courses of inhaled tobramycin during aztreonam-off treatment intervals (86). Given the need for additional courses of tobramycin while on aztreonam, it appears that treatment with inhaled aztreonam in conjunction with inhaled tobramycin could have a more pronounced effect on slowing FEV_1 decline in CF patients. In clinical practice, many patients are being treated with a combination of alternating months of inhaled tobramycin and aztreonam to achieve the desired improvement in lung function.

The use of continuous alternate month inhaled tobramycin and aztreonam may be reflected in the CF Foundation Patient Registry (CFFPR) data from 2008-2011 that reflect little to no change in use of inhaled tobramycin but a significant increase in inhaled aztreonam use (Figure 5-1) (110-112). With no efficacy data on the combined use of inhaled tobramycin and aztreonam, and the high costs (currently about $7,000 for a month's supply of either therapy (94)), and increased time and treatment burdens associated with their use, this study was designed to address a critical need to evaluate the clinical effectiveness of the combination in CF patients with chronic *Pa* airway infection.

This retrospective study was conducted to assess the effects of combined use of inhaled tobramycin and inhaled aztreonam on the rate of decline in lung function, in patients who received treatment with this combination for a period of at least one year. The specific aim of this study was **to compare the rate of decline in lung function between CF patients who received a combination of inhaled tobramycin and aztreonam and those who received inhaled tobramycin alone**. We hypothesized that the rate of decline in FEV_1 would be significantly lower in CF patients who received the combination than in those who received inhaled tobramycin alone. Longitudinal FEV_1 data for patients enrolled in the CFFPR, receiving inhaled tobramycin and aztreonam, were compared with that for patients receiving inhaled tobramycin alone. Patients in both groups continued to receive treatment with routine CF therapies. This comparison was adjusted for important potential confounders such as age, gender, concomitant use of routine therapies, chronic airway infection with *Pa* and pulmonary exacerbations as defined in the CFFPR. Based on the published literature, this was the first study to evaluate the long-term effectiveness of inhaled tobramycin and aztreonam on the rate of decline in lung function.

Methods

Study Population

All CF patients enrolled in the CFFPR aged 6 – 20 years, between 2004 and 2011, were eligible for this study. These age restrictions for eligibility were enforced since lung function tests are usually available after 6 years of age and body mass index (BMI) percentile based on the Centers for Disease Control and Prevention (CDC) growth charts are available only until 20 years of age. BMI percentile has been noted to be an important confounder affecting both treatment assignment as well as lung function (9). Hence, availability of data on BMI percentile was important to assess likelihood of treatment assignment. Additionally, this age criterion enabled the study to concentrate on areas of

increased risk for rapid decline in lung function (10, 11). In patients who received lung transplantation, all data post-transplant, were excluded from the analysis.

Study Design

The study used a retrospective cohort design wherein the rate of decline in FEV_1 was compared between patients who received combined treatment with inhaled tobramycin and aztreonam and those who received treatment with tobramycin alone (Figure 5-2). Chronic use in both these groups was defined as having received the treatment for at least one year and encompassing three or more clinic visits while on the respective treatments. Patients in both the groups continued to receive treatment with other CF therapies such as inhaled antibiotics, dornase alfa, hypertonic saline, pancreatic enzyme supplements, high-dose ibuprofen and chronic oral antibiotics.

The primary outcome was the rate of decline in FEV_1 % predicted. The Global Lung Initiative (GLI) reference equations were used to calculate the FEV_1 % predicted values. These reference equations were developed using data from people between 3 and 95 years of age with healthy lungs (12). Using the patients' age, gender, race, and height in these reference equations, the absolute FEV_1 measurements in liters were converted to % predicted values. Eligible patients had to have at least three FEV_1 measurements spanning at least six months to allow for the calculation of the slope of FEV_1 decline. Data were also collected on a number of baseline factors that could affect treatment assignment and/or could be potential confounders of the association between inhaled antibiotic use and rate of FEV_1 decline (13). These variables were:

- Baseline age – This was defined as the age at start of the combined inhaled tobramycin and aztreonam treatment in the combined treatment group. In the "tobramycin only" group it was defined as the age at which inhaled tobramycin was started.
- Gender – Gender was defined as male or female.

- Genotype – Genotype was defined based on number of *delta*F508 alleles and was categorized into: a) homozygous *delta*F508; b) heterozygous *delta*F508; and c) no *delta*F508.
- Baseline FEV_1 – This was defined as the FEV_1 measurement at the baseline age.
- Baseline Body Mass Index (BMI) percentile – BMI percentile was defined as the highest BMI percentile in the year prior to the baseline age.
- Pre-baseline FEV_1 slope – This was defined as the slope of FEV_1 change before baseline age. For the pre-baseline FEV_1 slope calculation the eligible patients had to have at least three FEV_1 measurements spanning at least six months prior to the baseline age.
- Chronic *Pseudomonas aeruginosa* (*Pa*) infection – This was defined using the modified Leeds criteria (95). Patients who had at least two respiratory cultures in the year prior to the baseline age, with at least 50% of the cultures positive for *Pa* were defined to have chronic *Pa* infection.
- Baseline Pancreatic Insufficiency – A patient was assumed to be pancreatic insufficient if she/he was receiving pancreatic enzyme supplements at any time during the year prior to the baseline age.
- Baseline CF-related diabetes mellitus status – This variable was defined as presence of CF-related diabetes mellitus in the year prior to baseline age.
- Baseline Dornase Alfa use – This was defined as dornase alfa use at baseline age.
- Center-specific prescription rate of inhaled tobramycin or inhaled aztreonam in children < 6 years of age – This variable was defined as the proportion of patients < 6 years of age receiving care at a center, who ever received treatment with either inhaled tobramycin or inhaled aztreonam. This variable was created based on the assumption that if a center was

more likely to prescribe inhaled antibiotics to a patient < 6 years of age, it was more likely to prescribe these antibiotics or their combination to a patient ≥ 6 years of age.

- Total number of exacerbations – This was defined as the total number of pulmonary exacerbations as identified in the CFFPR in the year prior to the baseline age.
- Total number of hospitalizations – This was defined as the total number of hospitalizations as identified in the CFFPR in the year prior to the baseline age.
- Center size – Based on the distribution of patients across the CF centers, a CF center was classified as a large center if > 90 patients received care at a center between 2004 and 2011, and a small center if it provided care for ≤ 90 patients.

Data on other potential confounders for each clinic visit were also collected for the follow-up period. These included binary variables such as exacerbation, hospitalization, use of other CF therapies such as chronic azithromycin, inhaled colistin, dornase alfa, hypertonic saline, high dose ibuprofen, and chronic oral antibiotics. Also, presence of bacteria such as *Pa*, *Burkholderia cepacia*, *methicillin-resistant Staphylococcal aureus* (MRSA), mucoid *Pa*, *Alcaligenes xylosoxidans*, and *Stenotrophomonas maltophilia* was recorded as a binary variable. Visit-specific data on CF-related diabetes mellitus status were also collected and treated as a categorical variable with three categories: normal; impaired glucose tolerance; and CF-related diabetes mellitus with or without fasting hyperglycemia.

Statistical Analyses

Baseline patient characteristics were summarized using means and standard deviations (SD) for continuous variables, and percentages for categorical variables. The two treatment groups were also compared on these baseline characteristics using the

Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables.

To combat treatment indication bias, propensity scores were calculated for patients in the study to estimate the likelihood of receiving treatment with the combination therapy, given a set of baseline characteristics listed above. A logistic regression model including the baseline variables was fitted to calculate the propensity scores. Patients in the two groups were then matched on their propensity scores using the local optimal (greedy) algorithm. The analysis used 1:1, caliper, matching. This is a method in which the two treatment groups were randomly sorted and then the first combined treated patient was selected to find its closest "tobramycin only" match in terms of the propensity score if the control's propensity score was within a certain distance (caliper) (92). This study used a caliper width of 0.05 and matching was carried out without replacement.

Propensity score matching was followed by fitting a linear mixed model to compare the rate of decline of FEV_1 % predicted between the two groups with the restricted maximum likelihood method of estimation, using data from the matched group of patients. The time interval in years between baseline age and the FEV_1 measurement was included in both the fixed and random components of the model. The treatment group by time interaction term was used to estimate the difference in the rate of decline in FEV_1 % predicted between the two groups. This model adjusted for potential confounders listed above.

All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). *P* values < 0.05 were considered significant. This study was approved by the University of Iowa Institutional Review Board.

Results

Characteristics of the Study Cohort

Between 2004 and 2011, 18,653 patients enrolled in the CFFPR were between 6 – 20 years of age (Figure 5-3). Of these, 12,389 patients had ever received treatment with inhaled tobramycin. A total of 2,257 patients were noted to have ever received treatment with a combination of inhaled tobramycin and aztreonam, while the remaining 10,132 patients had at some time during the study period received treatment with inhaled tobramycin alone. In the combination group, 1,270 patients fulfilled the study eligibility criteria. Of these, 1,013 patients were found to have complete data for all baseline characteristics. In the "tobramycin only" group, 7,629 patients fulfilled the study eligibility criteria. Of these, 4,077 patients had complete data for all baseline characteristics. Propensity score matching was carried out on this set of 5,090 patients which resulted in a matched set of 711 patients in each of the two treatment groups. This set of 1,422 patients was used for the final linear mixed model analysis.

The baseline characteristics for patients in both treatment groups are summarized in Table 5-1. Patients in the combined treatment group were more likely to be older and belong to the female gender as compared with the "tobramycin only" group. Also, the combined treatment group had a significantly lower baseline BMI percentile, and pre-baseline FEV_1 % predicted slope. It had a significantly higher number of exacerbations and hospitalizations, and a lower baseline FEV_1 % predicted. Additionally, it also had a higher proportion of patients with chronic *Pa* infection, CF-related diabetes, and pancreatic insufficiency. Dornase alfa use at baseline was also significantly higher in this group. The two groups did not differ in the proportion of patients receiving treatment at a small center or the proportion of patients with the homozygous *delta*F508 genotype.

Propensity Score Calculation and Matching

Propensity scores were calculated for patients in both groups to assess the likelihood of receiving combined treatment with inhaled tobramycin and aztreonam,

given the set of baseline variables. The mean propensity score in the combined treatment group was 0.49 ± 0.3, whereas the mean propensity score in the "tobramycin only" group was 0.13 ± 0.16 (Figure 5-4). The two groups were then matched on their propensity scores. The post-match mean propensity scores for the combined treatment and the "tobramycin only" groups were 0.35 ± 0.23 and 0.35 ± 0.23, respectively (Figure 5-5). The baseline characteristics of the patients after propensity score matching are summarized in Table 5-2. After matching on the propensity score, the two treatment groups were similar with respect to most baseline characteristics except total number of exacerbations ($p = 0.0001$), and pre-baseline FEV_1 % predicted slope ($p = 0.009$).

Linear Mixed Model Analysis

After the propensity score matching, data on the matched set of patients from the two treatment groups were analyzed using a linear mixed model analysis. The results of this analysis are summarized in Table 5-3. This model suggested that rate of FEV_1 decline in patients in the "tobramycin only" group was 0.8% per year slower than that in the combined treatment group (-3.53% per year in the combined treatment group vs. -2.73% per year in the "tobramycin only" group, $p = 0.04$). Also, on average the "tobramycin only" group had a FEV_1 % predicted value that was 2.08% higher than that in the combined treatment group ($p < 0.0001$). Other variables that were found to be significant predictors of FEV_1 decline were: baseline FEV_1 (estimate = 0.88% (0.01), $p < 0.0001$); presence of exacerbations during follow-up visits (no vs. yes, estimate = 5.27% (0.14), $p < 0.0001$); hospitalizations during follow-up visits (no vs. yes, estimate = 8.73% (0.24), $p < 0.0001$); hypertonic saline use during follow-up (no vs. yes, estimate = -1.2% (0.28), $p < 0.0001$); high dose ibuprofen use during follow-up (no vs. yes, estimate = -1.3% (0.50), $p = 0.01$); inhaled colistin use during follow-up (no vs. yes, estimate = 0.75% (0.30), $p = 0.01$) ; and presence of *Pa* (no vs. yes, estimate = 0.91% (0.27), $p = 0.0009$), *Burkholderia cepacia* (no vs. yes, estimate = 1.26% (0.54), $p = 0.02$), MRSA (no vs. yes, estimate = 1.34% (0.20), $p < 0.0001$), mucoid *Pa* (no vs. yes, estimate =

1.07% (0.24), $p < 0.0001$) and *Alcaligenes xylosoxidans* (no vs. yes, estimate = 0.78% (0.38), $p = 0.04$) in respiratory cultures during follow-up. Some variables that have been previously shown to affect the slope of FEV_1 decline (99) were also included as "interaction with time" variables in the model. Of these, only hypertonic saline had a significant association with the rate of FEV_1 decline, wherein patients not treated with hypertonic saline had slower decline in the rate of FEV_1 compared with those who were treated with it (no vs. yes, estimate = 0.39% per year (0.12), $p = 0.001$).

Additionally, since exacerbations and hospitalizations during follow-up could be in the causal pathway for the association between treatment with inhaled antibiotics and lung function, these two variables were excluded from the linear mixed model. The results of this analysis, suggested that the rate of FEV_1 decline in patients in the "tobramycin only" group was 0.72% per year slower than that in the combined treatment group (-3.50% per year in the combined treatment group vs. -2.78% per year in the "tobramycin only" group, $p = 0.06$). Also, on average the "tobramycin only" group had a FEV_1 % predicted value that was 1.52% higher than that in the combined treatment group ($p = 0.004$).

To preserve matching between patients in the two groups on their propensity scores, a matched analysis using PROC MIXED was also carried out, where the propensity score-matched pair identification number was treated as a random effect along with patient identification number. The results of this analysis were similar to the primary analysis. The rate of FEV_1 decline in patients in the "tobramycin only" group was 0.79% per year slower than that in the combined treatment group (-3.53% per year in the combined treatment group vs. -2.74% per year in the "tobramycin only" group, $p = 0.04$). Also, on average the "tobramycin only" group had a FEV_1 % predicted value that was 2.08% higher than that in the combined treatment group ($p < 0.0001$).

Discussion

This was the first study to assess and compare the rate of FEV_1 decline in patients who received combination treatment with inhaled tobramycin and aztreonam, and those who received inhaled tobramycin alone. Additionally, this study was the first to use GLI reference equations to standardize FEV_1 absolute values to % predicted values that were used to assess the rate of FEV_1 decline. The CFF recommends using the Wang et al. (66) prediction equation for males less than 17 years old and for females less than 15 years old (105). Beyond these ages, the recommendation is to switch over to the Hankinson et al. prediction equation (67). However, Stanojevic et al. observed a difference of up to 8% in predicted FEV_1 values obtained from these two prediction equations in individuals with CF at the time of this switch (106). Hence, the all-age GLI equation was used in this study. The results of this study suggest that patients on inhaled tobramycin alone have higher FEV_1 % predicted values on average as well as slower rate of FEV_1 decline compared to patients who received treatment with both inhaled tobramycin and aztreonam. Propensity score matched analysis was conducted in this study to combat treatment indication bias. Significant indication bias was reflected in the comparison of baseline characteristics between the two treatment groups. The combination treatment group had a higher proportion of patients with factors that have been previously shown to be associated with worse lung function outcomes and as a result more likely to receive treatment with a combination of drugs rather than one drug alone. Hence, propensity score matching was carried out, which reduced this treatment indication bias. This was revealed in the comparison of baseline characteristics between the two groups after matching.

However, even with the adjustment to reduce indication bias, this study failed to reject the null hypothesis and yielded results that were contrary to the hypothesis. Since, a combination therapy with inhaled tobramycin and aztreonam has never been compared with inhaled tobramycin treatment alone, it is difficult to evaluate if these results are

consistent with the truth. Moreover, the rate of FEV_1 decline has never been used as an outcome to assess this comparison between the two treatment groups. The only study that compared inhaled aztreonam with inhaled tobramycin found that inhaled aztreonam was superior to inhaled tobramycin in improving lung function (89). Antagonism between the two study drugs has not been documented in the scientific literature to date, hence the study hypothesis. However, given the results of this study in terms of higher average FEV_1 values in the "tobramycin only" group, it appears that the propensity score matching was unable to effectively reduce the treatment indication bias. A major limitation of propensity score methods is that they can only account for the measured differences between the two treatment groups. If unmeasured covariates play a part in treatment assignment, then propensity score methods cannot control for them. The CFFPR does not collect any information on physician prescription preferences or patient symptom scores. Additionally, no data on health-related quality of life measures are collected. These variables may further aid in reducing the treatment indication bias using propensity score methods. This inability of propensity score methods to eliminate treatment indication bias has been previously documented by VanDyke et al. (103). That study, while evaluating the effectiveness of inhaled tobramycin on reducing FEV_1 decline using CFFPR data, found that the treated group had significantly lower values of FEV_1 compared to the untreated group (estimate = -1.74% (0.31), $p < 0.0001$).

As part of the propensity score matched analysis, the unmatched patients were characterized to assess the distribution of baseline variables. There were significant differences between the two groups on all baseline variables except genotype and center size. The combined treatment group with unmatched patients (n = 302) had a mean propensity score of 0.82 ± 0.15 and "tobramycin only" group (n = 3,366) had a mean propensity score of 0.08 ± 0.08. Results of the linear mixed model analysis to compare the rate of FEV_1 decline between the two groups in this set of unmatched patients yielded

similar results to the primary propensity score matched analysis ("tobramycin only" vs. combined treatment, estimate = 1.6%, $p < 0.0001$).

This study also evaluated the rate of FEV_1 decline where the duration of follow-up was limited to two years. This was done to follow study eligibility criteria recently used by Konstan et al. to assess the effectiveness of inhaled tobramycin and dornase alfa on rate of FEV_1 decline (69, 107). The results were similar to those from the primary propensity score matched analysis ("tobramycin only" vs. combined treatment, estimate = 1.02% (0.49), $p = 0.04$). Additionally, this study evaluated the effectiveness of combined treatment on the rate of FEV_1 decline in the entire population of eligible patients without propensity score matching. A linear mixed model was fitted to include all baseline covariates as well as potential confounders. This analysis yielded similar results to those obtained from the primary propensity score matched analysis using all follow-up data. The rate of FEV_1 decline per year was 1.34% slower in the "tobramycin only" group compared with the combined treatment group ($p < 0.0001$).

As physician's prescription preferences may be guided by an unexpected decline in FEV_1 greater than what was anticipated based on the pre-baseline FEV_1 slope, an additional variable was created to account for this unexpected decline in FEV_1. This variable was defined as the difference between the estimated FEV_1 % predicted at baseline calculated from the pre-baseline FEV_1 slope and the observed baseline FEV_1 % predicted value. Adding this variable to the logistic regression model for propensity score calculation and adjusting for it in the final linear mixed model analysis did not affect the direction of the result comparing the rate of FEV_1 decline in the two treatment groups but made the difference non-significant. ("tobramycin only" vs. combined treatment, estimate = 0.56% (0.39), $p = 0.15$).

A significant limitation of this study was the inability to monitor treatment adherence. Since this was a retrospective study using data from a patient registry, there was no way to monitor patient adherence to these treatments. If the inhaled treatments

and other medications were listed in the CFFPR, it was assumed that the patient was taking the drug.

This study found results for some important confounders that were consistent with findings from previous studies (17, 20). Presence of exacerbations or hospitalizations during follow-up was significantly associated with FEV_1 decline. Hypertonic saline use during follow-up was associated with higher mean FEV_1 values. Presence of *Pa*, mucoid *Pa*, *Burkholderia cepacia*, MRSA, and *Alcaligenes xylosoxidans* on respiratory cultures was associated with lower mean FEV_1 values.

In conclusion, this study did not find patients treated with a combination of inhaled tobramycin and aztreonam to have a slower rate of decline. In contrast, the group that received treatment with inhaled tobramycin alone had a significantly slower rate of FEV_1 decline. This may be explained by the inability of propensity score methods to combat treatment indication bias effectively in the presence of unmeasured covariates. There is a need to record more symptom and physician-centric variables in the CFFPR and use these to assess the propensity of receiving CF therapies. As an alternative to propensity score analysis, an instrumental variable analysis could help with eliminating treatment indication bias if the appropriate instrument is identified. However, several challenges that exist when using instrumental variable analysis with, data assessed at multiple time points need to be addressed before long-term effectiveness of CF therapies can be assessed using this method.

Table 5-1. Baseline Characteristics of Study Cohort by Treatment Status

Characteristics	Combination Group (N = 1,013)	Tobramycin Only Group (N = 4,077)	*P* value*
Age, mean (SD), years	15.0 (3.5)	13.6 (3.8)	<0.0001
Female sex, N (%)	592 (58.4)	1,898 (46.6)	<0.0001
Genotype, N (%) • Homozygous *delta*F508 • Heterozygous *delta*F508 • No *delta*F508	 520 (51.3) 350 (34.6) 143 (14.1)	 2,139 (52.5) 1,327 (32.5) 611 (15.0)	0.45
Body Mass Index percentile[a], mean (SD)	47.8 (26.9)	50.0 (26.6)	0.02
Exacerbations[b], mean (SD)	2.54(2.89)	0.47 (1.14)	<0.0001
Hospitalizations[c], mean (SD)	1.84 (1.77)	0.97 (1.35)	<0.0001
Center size[d], Small, N (%),	227 (22.4)	939 (23.0)	0.67
Center-Prescription Rate[e], mean (SD)	0.19 (0.16)	0.15 (0.15)	<0.0001
Chronic *Pseudomonas aeruginosa* infection, N (%)	600 (59.2)	1324 (32.5)	<0.0001
CF-related diabetes, N (%)	214 (21.1)	486 (11.9)	<0.0001
Pancreatic Insufficiency, N (%)	988 (97.5)	3,802 (93.3)	<0.0001
Baseline FEV_1 % predicted , mean (SD)	66.7 (23.2)	79.6 (21.6)	<0.0001
Pre-baseline FEV_1 % predicted slope, mean (SD)	-1.98 (5.15)	-0.18 (13.67)	<0.0001
Dornase alfa use, N (%)	750 (74.0)	1,661 (40.7)	<0.0001

Note: SD = standard deviation; CF = cystic fibrosis; FEV_1 = forced expiratory volume in 1 second.

[a]Highest body mass index percentile in the year prior to start of treatment.

[b]Number of pulmonary exacerbations in the year prior to start of treatment.

[c]Number of hospitalizations in the year prior to start of treatment.

[d]Center was classified into small and large centers based on a cut-off value of 90 patients between 2004 – 2011.

[e]Center-specific prescription rate of inhaled tobramycin or aztreonam in CF patients < 6 years of age irrespective of *Pseudomonas aeruginosa* infection status.

**P* values from Wilcoxon rank-sum test or chi-square test.

Table 5-2. Baseline Characteristics of Study Cohort after Propensity Score Matching by Treatment Status

Characteristics	Combination Group (N = 711)	Tobramycin Only Group (N = 711)	*P* value*
Age, mean (SD), years	14.5 (3.5)	14.6 (3.8)	0.35
Female sex, N (%)	391 (55.0)	389 (54.7)	0.92
Genotype, N (%) • Homozygous *delta*F508 • Heterozygous *delta*F508 • No *delta*F508	 376 (52.9) 237 (33.3) 98 (13.8)	 387 (54.4) 238 (33.5) 86 (12.1)	0.62
Body Mass Index percentile[a], mean (SD)	48.3 (26.9)	49.0 (27.2)	0.66
Exacerbations[b], mean (SD)	1.55 (1.77)	1.43 (2.04)	0.0001
Hospitalizations[c], mean (SD)	1.45 (1.51)	1.47(1.58)	0.67
Center size[d], Small, N (%)	152 (21.4)	144 (20.3)	0.60
Center-Prescription Rate[e], mean (SD)	0.18 (0.14)	0.19 (0.2)	0.15
Chronic *Pseudomonas aeruginosa* infection, N (%)	367 (51.6)	375 (52.7)	0.67
CF-related diabetes, N (%)	117 (16.5)	125 (17.6)	0.57
Pancreatic Insufficiency, N (%)	689 (96.9)	685 (96.3)	0.56
Baseline FEV_1 % predicted , mean (SD)	70.63 (23.2)	70.75 (22.4)	0.91
Pre-baseline FEV_1 % predicted slope, mean (SD)	-1.68 (5.58)	-0.9 (11.9)	0.0093
Dornase alfa use, N (%)	497 (69.9)	495 (69.6)	0.91

Note: SD = standard deviation; CF = cystic fibrosis; FEV_1 = forced expiratory volume in 1 second.

[a]Highest body mass index percentile in the year prior to start of treatment.

[b]Number of pulmonary exacerbations in the year prior to start of treatment.

[c]Number of hospitalizations in the year prior to start of treatment.

[d]Center was classified into small and large centers based on a cut-off value of 90 patients between 2004 – 2011.

[e]Center-specific prescription rate of inhaled tobramycin or aztreonam in CF patients < 6 years of age irrespective of *Pseudomonas aeruginosa* infection status.

**P* values from Wilcoxon rank-sum test or chi-square test.

Table 5-3. Parameter Estimates (SE) from fitting a Linear Mixed Model to Predict the Rate of Decline in FEV_1 % Predicted

Variables	Coefficient (SE)	*P* Value
Inhaled antibiotic (tobramycin only vs. combination)	2.08 (0.52)	<0.0001
Inhaled antibiotic×Time (tobramycin only vs. combination)	0.80 (0.38)	0.04
Time	-3.53 (0.34)	<0.0001
Baseline FEV_1	0.88 (0.01)	<0.0001
Total exacerbations before baseline	0.20 (0.12)	0.10
Center-specific prescription rate[a]	-0.51 (1.41)	0.72
Pre-Baseline age FEV_1 slope	-0.05 (0.03)	0.09
Exacerbations (no vs. yes)	5.27 (0.14)	<0.0001
Hospitalizations (no vs. yes)	8.73 (0.24)	<0.0001
Dornase alfa use (no vs. yes)	-0.35 (0.30)	0.24
Chronic Azithromycin use (no vs. yes)	0.16 (0.29)	0.57
Hypertonic saline use (no vs. yes)	-1.20 (0.28)	<0.0001
High dose ibuprofen use(no vs. yes)	-1.26 (0.50)	0.01
Chronic oral antibiotic use (no vs. yes)	0.08 (0.24)	0.73
Colistin use (no vs. yes)	0.75 (0.30)	0.01
Pseudomonas aeruginosa[b] (no vs. yes)	0.91 (0.27)	0.0009
Burkholderia cepacia[b] (no vs. yes)	1.26 (0.54)	0.02
MRSA[b] (no vs. yes)	1.34 (0.20)	<0.0001
Mucoid *Pseudomonas aeruginosa*[b] (no vs. yes)	1.07 (0.24)	<0.0001
Alcaligenes xylosoxidans[b] (no vs. yes)	0.78 (0.38)	0.04
Stenotrophomonas maltophilia[b] (no vs. yes)	-0.08(0.25)	0.77
Dornase alfa×Time (no vs. yes)	0.19 (0.12)	0.13
Chronic Azithromycin×Time (no vs. yes)	-0.07(0.13)	0.59
Hypertonic saline×Time (no vs. yes)	0.39 (0.12)	0.001
Pseudomonas aeruginosa×Time[b] (no vs. yes)	-0.02 (0.10)	0.81

Note: FEV_1 = forced expiratory volume in 1 second; MRSA = Methicillin resistant *Staphylococcus aureus*

[a]Center-specific prescription rates for inhaled tobramycin or aztreonam in children < 6 years of age.

[b]Respiratory cultures for these bacteria were found to be positive during follow-up after baseline.

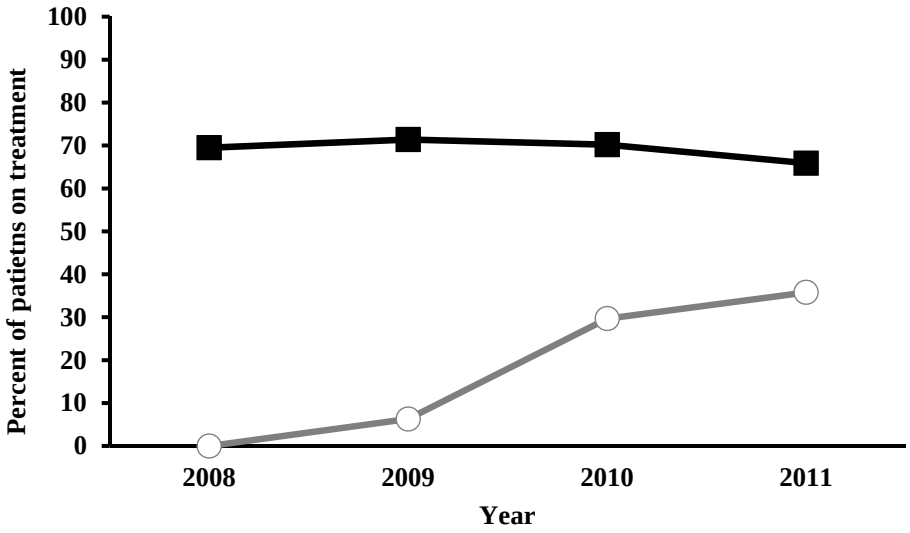

Figure 5-1. Percent of Patients who met the Criteria for Treatment with Inhaled Antibiotics and were prescribed the Treatment.

Source: Cystic Fibrosis Foundation. Patient Registry 2011 Annual Data Report. Bethesda, Maryland: Cystic Fibrosis Foundation; 2012.

Note: The black line indicates inhaled tobramycin use and the grey line indicates inhaled aztreonam use.

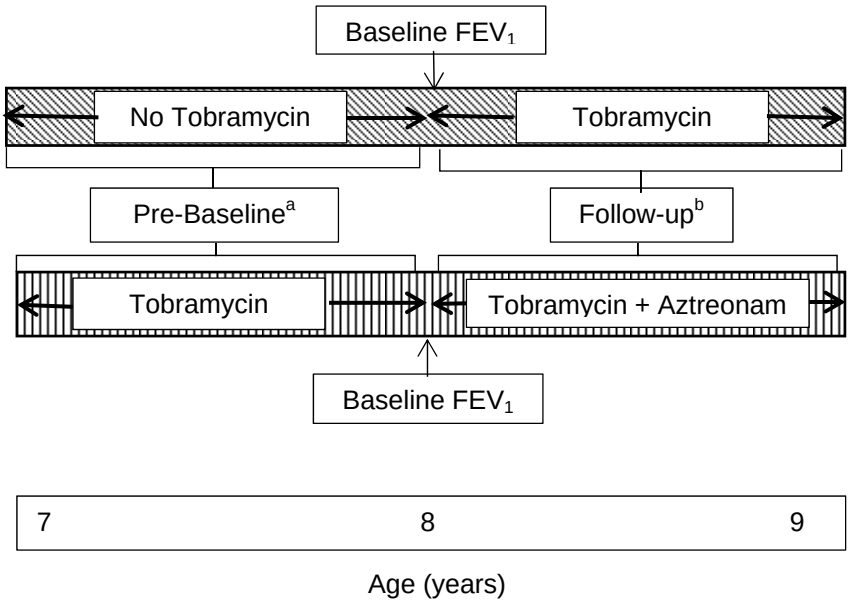

Figure 5-2. Study Timeline for a Scenario where Treatment with Combined Inhaled Tobramycin and Aztreonam Treatment or Tobramycin Alone Began at 8 Years of Age.

Note: [a]In both groups the pre-baseline period was defined as the period before the start of treatment with combination of inhaled tobramycin and aztreonam or inhaled tobramycin alone. This period could be less than one year as long as there were three FEV_1 measurements spanning at least six months.

[b]Follow-up period lasted for at least one year in both groups. This period had to include at least three FEV_1 measurements spanning at least six months in both groups.

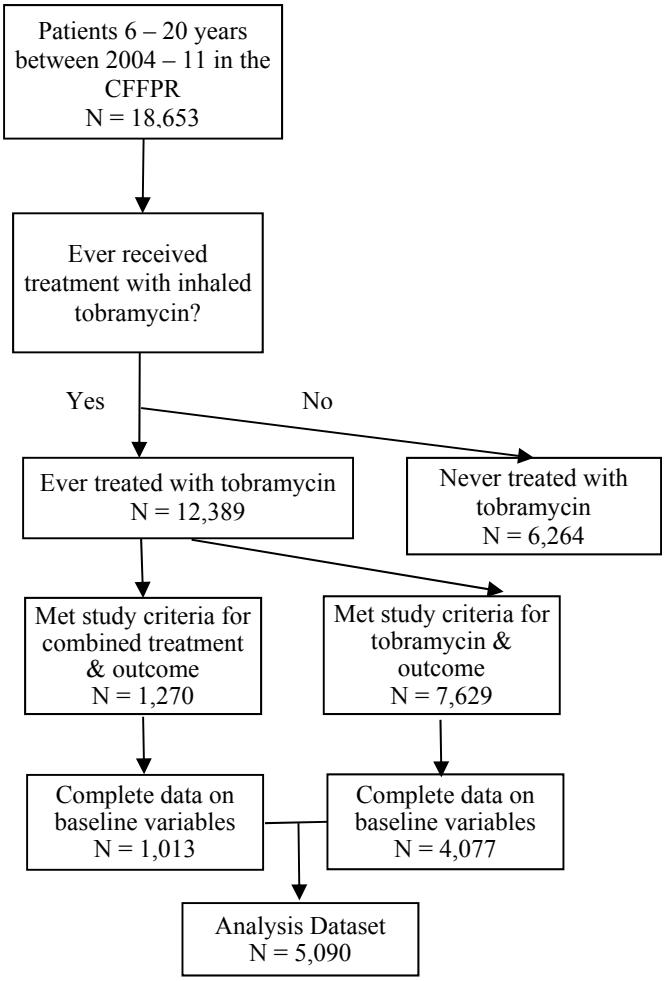

Figure 5-3. Identification of Patients for the Combined Inhaled Tobramycin and Aztreonam Treatment and Tobramycin Alone Treatment Groups

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Note: CFFPR = Cystic Fibrosis Foundation Patient Registry

Combined treatment was defined as treatment with both inhaled tobramycin and aztreonam.

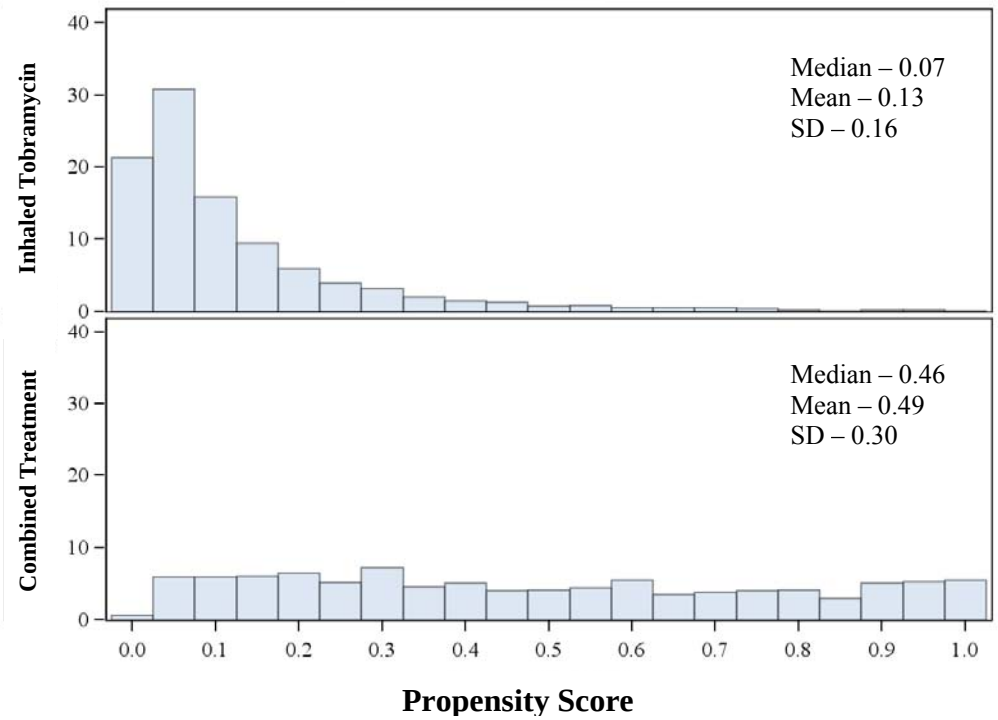

Figure 5-4. Distribution of Propensity Scores in the Combined Inhaled Tobramycin and Aztreonam Treatment (N = 1,013) and Tobramycin Alone Treatment (N = 4,077) Groups of Patients with Cystic Fibrosis.

Note: SD = standard deviation

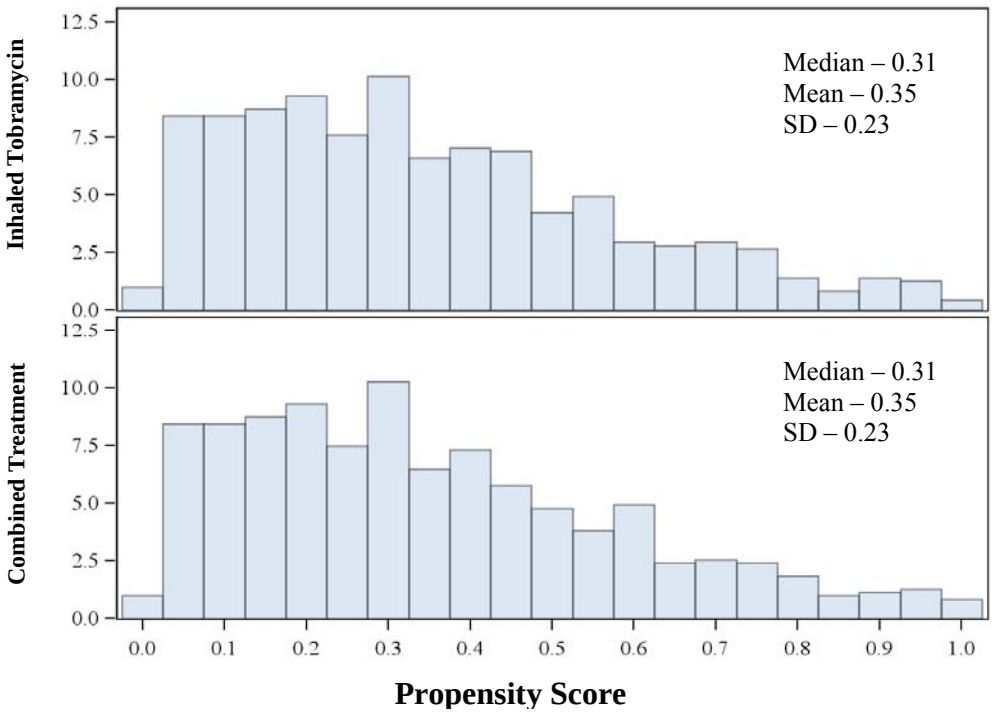

Figure 5-5. Distribution of Propensity Scores in the Combined Inhaled Tobramycin and Aztreonam Treatment and Tobramycin Alone Treatment Groups of Patients with Cystic Fibrosis after Propensity Score Matching (N = 711 pairs).

Note: SD = standard deviation

CHAPTER VI - CONCLUSIONS AND FUTURE DIRECTIONS

This research project was designed to address gaps in the knowledge of the long-term clinical effectiveness of therapies frequently used in cystic fibrosis (CF). Large-scale multicenter randomized clinical trials should ideally be conducted to answer the research questions addressed in this study. However, use of an existing observational database such as the Cystic Fibrosis Foundation Patient Registry (CFFPR) was the most pragmatic approach for this initial investigation.

The first research aim focused on use of dornase alfa in younger children with CF. With no clinical efficacy data of dornase alfa in children ≤ 6 years of age, the study utilized subsequent forced expiratory volume in 1 second (FEV_1) between 6 – 7 years of age, to assess the effectiveness of early long-term dornase alfa use. The analytical findings for this aim suggested that receiving treatment with dornase alfa before 6 years of age did not improve FEV_1 between 6 – 7 years of age. Unmeasured covariates leading to treatment indication bias are likely one of the key explanations for these results. Additionally, lack of a more sensitive outcome than FEV_1 to assess lung function in young patients with early lung damage is another likely reason for the failure to reject the null hypothesis. Selection bias may have also played a role since the analysis was restricted to 323 patients out of the 9,058 patients, who ever received treatment with dornase alfa in the CFFPR dataset constructed to address Aim 1. The study eligibility criterion of treatment with dornase alfa for at least three years was specified since the focus of this study was on assessing long-term clinical effectiveness of dornase alfa. Nonetheless, exploratory analyses that relaxed the study criterion for use of dornase alfa to both one and two years provided similar results.

The second research aim was to assess the long-term clinical effectiveness of chronic azithromycin use on the rate of FEV_1 decline. This study was novel in that the rate of FEV_1 decline, rather than change in FEV_1 from baseline, was the primary outcome. The results of the analysis suggested that the rate of FEV_1 decline was slower in

patients who did not receive chronic treatment with azithromycin. Once again, treatment indication bias is likely to have played an important role in the direction of the association between treatment and outcome. Associations between FEV_1 % predicted and many of the other study variables included in the analysis were consistent with those of previous studies.

The final research aim was focused on assessing the clinical effectiveness of a combination of inhaled tobramycin and aztreonam on the rate of FEV_1 decline. This aim was novel in that the effect of this combination treatment on rate of decline in FEV_1 had never been assessed. Once again, the results were contrary to the alternative hypothesis with the combination group having a steeper rate of FEV_1 decline than the group that was treated with tobramycin alone. An important reason for this result was thought to be unresolved treatment indication bias that could not be eliminated with the use of the propensity score methods used to test the associated hypothesis.

Comparison with Findings from Clinical trials

This observational study assessed efficacy of inhaled dornase alfa, oral azithromycin, and inhaled tobramycin and aztreonam in CF patients enrolled in the CFFPR who met study eligibility criteria. The exposure and outcome assessment was based on data obtained during routine clinical visits and not as a part of a controlled trial, which usually does not reflect real world clinical use of a treatment.

Clinical trials have not been conducted in CF patients ≤ 6 years of age to assess the efficacy of dornase alfa making this the first study to do so. Measurement of lung function in this age group is not carried out routinely as part of clinic visits. Hence, the study utilized a unique outcome – highest FEV_1 % predicted measured between 6 – 7 years of age - an outcome that has never been used before in CF clinical trials to assess efficacy of drugs.

Clinical trials to assess the efficacy of oral azithromycin, and inhaled tobramycin and aztreonam have been conducted in CF patients ≥ 6 years of age. Most of these trials

had selection criteria for FEV_1 % predicted that limited the inclusion of patients either with low or high FEV_1 values indicating severe or mild lung disease, respectively. In this observational study there were no restrictions on subject eligibility based on FEV_1 % predicted. CF clinical trials usually limit the use of concurrent medications when assessing the efficacy of the primary drug; that was not the case in this study. Also, patients were not excluded if they exacerbations or were hospitalized at baseline – a practice routinely followed in clinical trials at the screening or baseline visit. Moreover, this study used a unique outcome – rate of decline in FEV_1 % predicted – for assessing efficacy of azithromycin and inhaled antibiotics. This outcome has not been previously used to test their efficacy in clinical trials, which commonly use change in FEV_1 from baseline as a primary or secondary outcome.

Thus, this observational study utilized routine clinical data from a broad spectrum of CF patients and used unique outcomes to assess the long-term effectiveness of important CF therapies as opposed to conducting a controlled clinical trial in a small subset of CF patients with the exclusion of a substantial proportion of the CF population.

Strengths and Limitations

This was the first study to evaluate the effectiveness of dornase alfa use on lung function in children ≤ 6 years of age. Also, this was the first study to assess the long-term clinical effectiveness of chronic azithromycin and inhaled antibiotics on the rate of FEV_1 decline. Data for all three study aims were obtained from the CFFPR, a patient registry that includes data on almost 95% of all CF patients in the United States. Clinic visit-level data are available for all patients enrolled in the registry. As a result, eligible groups of patients could be created based on specified eligibility criteria. Moreover, the presence of longitudinal data enabled the assessment of long-term clinical effectiveness of treatments on the rate of FEV_1 decline for Aims 2 and 3. This study was the first to use the all-age Global Lung Initiative (GLI) reference equations for standardizing FEV_1 % predicted and to assess the rate of decline in Aims 2 and 3 (104). The CFF recommends using the Wang

et al. (66) prediction equation for females less than 15 years old and for males less than 17 years old (105), beyond which, the recommendation is to switch over to the Hankinson et al. prediction equation (67). However, a difference of up to 8% in predicted FEV_1 values obtained from these two prediction equations has been observed in individuals with CF at the time of this switch (106). Hence, this study used the all-age GLI equation. Propensity score methods were used as the primary analytic approach to address all three study aims. These methods have been previously used successfully in research studies to combat treatment indication bias.

This study had several limitations. Firstly, this was an observational study with no monitoring of patient adherence to medication. Secondly, the direction of the results and some of the inconsistencies with existing data from previous studies lead to the conclusion that treatment indication bias was not completely eradicated. This may be due to the lack of patient- and physician-level variables such as symptom scores, prescribing preferences and quality of life measures in the CFFPR. Additionally, more sensitive measures of early lung damage such as the lung clearance index (LCI) are currently being evaluated in clinical trials in CF (113). Availability of LCI data in observational datasets will help in assessing the effectiveness of treatments used to prevent early lung damage. However, it may be a while before these data are used routinely for clinical management in CF and incorporated into the CFFPR.

Importance of Study Results

Given the strengths of this research project, it is important to highlight the current limitations of the CFFPR in evaluating the long-term clinical effectiveness of treatments used in CF. The use of validated methods of analysis, i.e., propensity scores, to counter treatment indication bias using the largest available observational dataset for CF, make this a strong research project. However, this study could not control for covariates for which no data were available in the CFFPR. These mainly include patient symptom scores and quality of life assessment along with physician prescription preferences.

Additionally, the lack of more sensitive lung function measures such as LCI makes the CFFPR a less valuable data source for assessing effectiveness of CF treatments in infants and young children. In the face of these limitations in the CFFPR data, CF epidemiologists may move towards alternative advanced epidemiologic methods such as instrumental variable analysis to account for the unmeasured covariates. Recent evidence of this was seen in a study evaluating the effectiveness of inhaled tobramycin on FEV_1 decline (103). The authors found results consistent with those in this research project using propensity score methods. However, they also analyzed the data using center-specific prescription rates of inhaled tobramycin as an instrument and reported results similar to those found in clinical trials of inhaled tobramycin.

Nonetheless, instrumental variable analysis is not without its own pitfalls. A major impediment to the use of instrumental variable analysis is the requirement of an observable instrument that should satisfy unverifiable and highly restrictive assumptions (114). Moreover, current instrumental variable approaches suffer from their inability to assess long-term treatment effectiveness utilizing datasets with multiple time points and time-varying covariates (103, 115). Advancements in the instrumental variable methods are needed before they can be used in evaluating the long-term effectiveness of CF treatments.

Future Directions

Factors at the level of both the patient and the physician play a role in whether a patient receives a particular treatment. This seems to be an active area of interest for the CFF. It is important to collect data on measures such as patient symptom scores, physician prescription preferences along with quality of life measures, which are currently missing in the CFFPR. In a recent publication by the CFF Therapeutics group, a call for focus on the age-appropriate tools to assess patient- and observer-reported outcomes along with health-related quality of life outcome measures was made (116). Inclusion of these measures in the CFFPR should aid in the further refinement of

propensity score methods in CF research. Furthermore, addressing the challenges in the use of advanced epidemiologic methods such as instrumental variable methods is important before they can be applied to assess long-term effectiveness of CF therapies.

An interesting area of development in CF in the last couple of years has been the testing of novel drugs that modulate the cystic fibrosis transmembrane conductance regulator (*CFTR*) protein. These drugs act at the level of the gene or the *CFTR* protein and hence are mutation specific. Ivacaftor, one such drug that acts as a potentiator of the *CFTR* protein, was approved by the FDA in 2012 for use in CF patients ≥ 6 years of age, with at least one copy of the *G551D* mutation (58). About 1,200 CF patients in the US have this mutation. Recently, in February 2014, it was approved for use in patients with eight additional rare CF mutations (117). Also, there are ongoing Phase III clinical trials focusing on drugs or a combination of drugs that can be used in patients who are either heterozygous or homozygous for the *delta*F508 mutation, the most common CF mutation in the United States (2).

Approval of these novel drugs is a boon for CF patients and physicians caring for them. However, it complicates the use of CFFPR as a data source for assessing the effectiveness of other commonly used treatments in CF. Given that Ivacaftor, currently the only approved *CFTR* modulator, can be used only in 5% of the patients enrolled in the CFFPR, it would still be possible to exclude these patients for an observational study of the effectiveness of other therapies. However, once drugs that can also be used to treat patients with common mutations become available, it will be important to account for these drugs in the design and analysis of observational studies focused on assessing the effectiveness of other CF treatments using data from the CFFPR.

In summary, the use of the CFFPR as a data source for observational studies in CF will pose interesting challenges for the CF epidemiologist. On one hand, the availability of more sensitive measures of lung function along with patient- and observer-based outcomes will help in honing existing advanced epidemiologic tools in their

application in CF research. On the other hand, the availability and use of novel CF modulators will make it challenging to assess the effectiveness of other CF treatments in observational studies.

APPENDIX A - PULMONARY FUNCTION TESTING USING SPIROMETRY

Lung function measurement is a key component of the clinical assessment of lung disease in cystic fibrosis (CF). However, measuring lung function in infants is technically difficult, laborious, expensive, and requires sedation. Additionally, preschool children are too old to undergo sedation and too young to follow instructions for lung function measurement (118). As a result, lung function measurement is used for clinical decision making and as an outcome in clinical trials in CF patients ≥ 6 years of age. Spirometry – a type of pulmonary function testing – is the most frequently used method to assess lung function. In spirometry, maximal inhalation is followed by a forceful and rapid exhalation into a spirometer. It is used to measure forced expiratory volume in the first second of expiration (FEV_1), forced vital capacity (FVC) – the volume of air that can be blown out after maximal inhalation, and other measurements such as forced expiratory flow at 25, 50 and 75% of FVC (119). The results of spirometry are typically represented as a flow-volume loop and volume-time graph (Figure A-1).

To be able to compare the measurements generated by spirometry with normative data, they are converted to appropriate % predicted values using the patient's height, age, gender, and ethnicity (119). Different prediction equations are used in CF to calculate the % predicted values for lung function depending on age and gender. The CFF recommends using the Wang et al. (66) prediction equation for females less than 15 years of age and for males less than 17 years of age (105). Beyond these ages, the recommendation is to switch over to the Hankinson et al. prediction equation (67). However, a difference of up to 8% in predicted FEV_1 values obtained from these two prediction equations has been observed in individuals with CF at the time of this switch (106). Recently, there has been a push to move towards new all-age reference equations. The Quanjer et al. Global Lung Function Initiative (GLI)-2012 equations are multi-ethnic all-age reference equations that have been endorsed by all major international respiratory

societies (104). These equations offer a unified approach to the interpretation of FEV_1 and other spirometry outcomes (104).

In CF, FEV_1 is frequently used to make clinical decisions on treatment strategies. Moreover, it has been shown to be the single best predictor of mortality in CF (65). Based on the FEV_1 % predicted, lung disease in CF can be categorized into the following categories: a) normal lung function – FEV_1 of $\geq$ 90 % predicted; b) mild lung disease – FEV_1 between 70 and 89 % predicted; c) moderate lung disease – FEV_1 between 40 and 69 % predicted; and d) severe lung disease – FEV_1 < 40 % predicted (2).

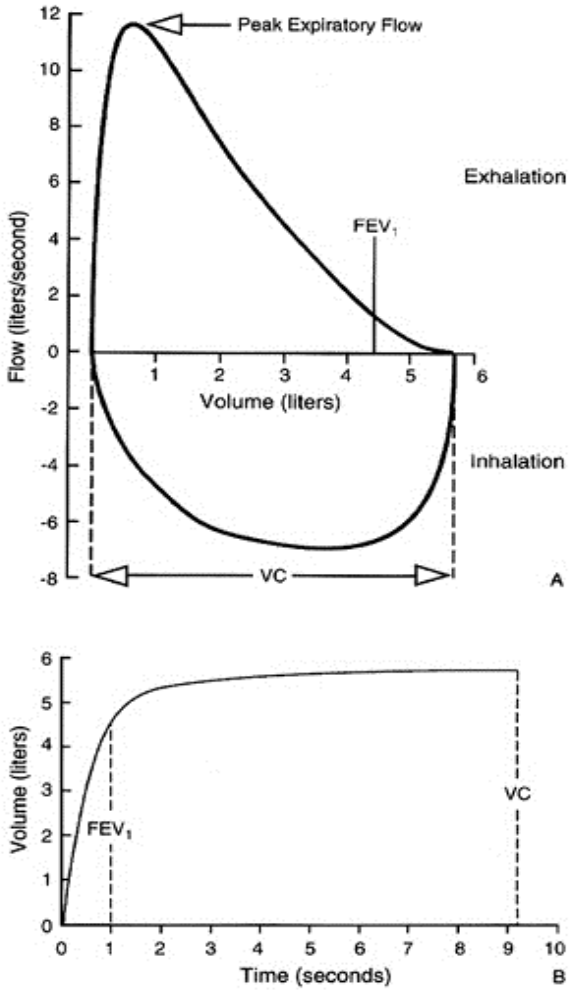

Figure A-1. Normal Flow-Volume (A) and Volume-Time (B) Curves.

Source: Reproduced with permission from Crapo RO. Pulmonary-function testing. N Engl J Med. 1994;331(1):25-30., Copyright Massachusetts Medical Society.

Note: FEV_1 = forced expiratory volume in 1 second; VC = vital capacity

APPENDIX B - PROPENSITY SCORE MATCHED ANALYSIS FOR AIM 1

As part of the propensity score analysis, a subset analysis of the cohort based on the matched propensity scores was also conducted. The matching was carried out using the local optimal (greedy) algorithm and the SAS macro "%PSMatching" (92). The analysis used caliper matching – a method in which the treated and the untreated groups were randomly sorted and then the first treated patient was selected to find its closest untreated match in terms of the propensity score if the control's propensity score was within a certain distance (caliper) (92). A caliper width of 0.05 was used in this study and matching was carried out without replacement. The propensity score matching resulted in a dataset of 150 patients who received dornase alfa and 150 patients who did not. After the match, the two study groups were comparable on the baseline covariates with mean propensity scores of 0.46 (SD = 0.22) in the untreated group and 0.47 (SD = 0.22) in the treated group. This matched set of patients was analyzed using a multivariable linear regression model with the same potential confounders as in the standard regression model. It was observed that the treated group had a mean FEV_1 % predicted value that was 2.25% lower than the untreated group (104.53% predicted in the treated group vs. 106.79% predicted in the untreated group, $p = 0.49$). However, a post-hoc power analysis showed that the matched analysis had a power of only 64% compared to 87% in the standard analysis.

To preserve matching between subjects in the two groups on their propensity scores, a matched analysis using PROC MIXED was also carried out, where the propensity score-matched pair identification number was treated as a random effect along with patient identification number. In this analysis, the treated group had a mean FEV_1 % predicted value that was 2.64% lower than untreated group ($p = 0.42$).

Additionally, as part of the propensity score matched analysis, the unmatched patients were characterized. The unmatched treated and untreated patients differed

significantly on the total number of hospitalizations in the prior year (0.67 vs. 0.05), total number of exacerbations in the prior year (0.98 vs. 0.06), center-specific prescription rate in infants (0.39 vs. 0.09), chronic *Pa* colonization (9.8 vs. 1.0%), proportion of females (62.43 vs. 48.31%), homozygous *deltaF508* status (57.23 vs. 38.16%), and pancreatic insufficiency (94.22 vs. 69.08%). The unmatched patients in the treated group (N = 173) had a mean propensity score of 0.87 (SD = 0.12) while those in the untreated group (N = 207) had a mean score of 0.16 (SD = 0.1) (Figure B-1). An unadjusted linear regression model was fitted to evaluate the association between dornase alfa use and FEV_1 % predicted in this unmatched set of patients. In that analysis, the untreated group had a mean FEV_1 % predicted value that was 3.94% higher than that in the treated group (100.7% predicted in the treated group vs. 104.6% predicted in the untreated group, p = 0.03). When a multivariable regression analysis was carried out on these unmatched patients using the same potential confounders as covariates, the treated group was noted to have a higher FEV_1 % predicted compared to the untreated group (106.75% predicted vs. 98.80% predicted, p = 0.06). A probable reason for this result could be that propensity score matching lead to the exacerbation of bias in the dornase alfa treatment estimates by creating imbalance in the unmeasured covariates. This may have a greater impact if the unmeasured covariates are confounders of the association between dornase alfa and subsequent FEV_1 measurement (120).

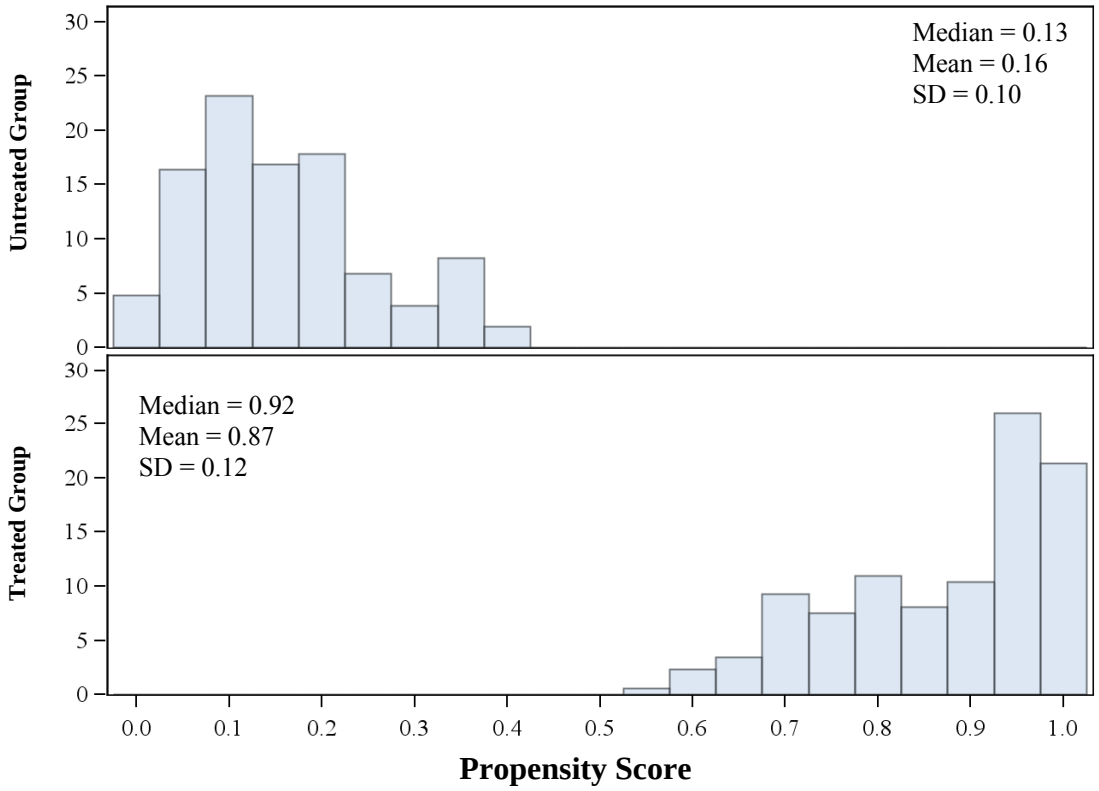

Figure B-1. Propensity Score Distribution in Unmatched Patients in Dornase Alfa-Treated (N = 173) and Untreated (N = 207) Groups of Young Children with Cystic Fibrosis.

Note: SD = standard deviation

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