EARLY PSEUDOMONAS AERUGINOSA TREATMENT AND LONG TERM LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS

Isabel Gascón Casaredi

EARLY *PSEUDOMONAS AERUGINOSA* TREATMENT AND LONG TERM LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS

Universitat Autònoma
de Barcelona

EARLY *PSEUDOMONAS AERUGINOSA* TREATMENT AND LONG TERM LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS

Doctoral Thesis carried out by:
Isabel Gascón Casaredi

Supervisor:
Montserrat Bosque García

Tutor:
Eduard Monsó Molas

Doctoral Program in Medicine
Departament of Medicine

Barcelona, 2022

Papá y mamá esta tesis es para vosotros, que me disteis la oportunidad de vivir, que disteis lo mejor de vosotros mismos para que creciera y me formara como Pediatra, que me habéis dado todo vuestro amor y que siembre habéis estado a mi lado, a pesar de las dificultades y de los baches en el camino.

A todos mis pacientes, sin ellos hoy yo no estaría aquí.

AGRADECIMIENTOS

A la Dra. Montse Bosque García, para mí ha sido siempre un ejemplo tanto a nivel personal, como a nivel profesional, ella siempre ha estado a mi lado, transmitiéndome su fuerza, su apoyo y su cariño, tanto en los momentos bonitos, como en los difíciles, sin ella todo este proyecto no se habría hecho realidad.

A todos los profesionales con los que he coincidido a lo largo de mi carrera profesional. Desde mis inicios en el Hospital Clínico de Valencia, mis compañeras de residencia, la Dra. Amparo Escribano, y todos los adjuntos que creyeron en mí y me enseñaron todo su conocimiento, para que yo pudiera forjar los cimientos de mi práctica Pediátrica y Neumológica.

Posteriormente a mis compañeros de Llefià y de Can Ruti, en especial a la Dra. Rosa Clofent, que para mí ha sido un gran referente y una muy buena amiga, que siempre me ha ofrecido su cariño y sus mejores consejos.

A mis compañeros de la Marina, tanto médicos como enfermeras, en especial al Dr. Martí, que también siempre estuvo a mi lado y me transmitió su espíritu de eterno aprendizaje y curiosidad.

A mis compañeros del Máster de Neumología y Alergia, a todos mis compañeros del Hospital Parc Taulí, porque todos ellos me apoyaron y han contribuido a que hoy pueda escribir estas palabras.

A mis compañeras y residentes del Hospital del Mar de Barcelona. Gracias Asun y gracias Txell por creer en mí hacer que las cosas fueran fáciles, agradables y divertidas.

A mi Fellowship en el Hospital for Sick Children of Toronto. Allí coincidí con un grupo maravilloso de compañeros que a día de hoy llevo todavía en mi corazón.

A mis compañeros del Hospital Sant Joan de Déu, por darme la gran oportunidad de trabajar con ellos, de compartir nuestros pacientes y de seguir creciendo día a día a su lado.

Especial mención de agradecimiento al Dr. Félix Rajen y a Michelle Shaw, por haberme dado la oportunidad de trabajar con ellos y haber hecho posible que este proyecto se haya hecho realidad.

A mis amigas; Marta, Ana, Conchis, Leire, Anna, Magda, Carol, Nana, a mis amigas del Ruti, a Cristina, mi profesora de piano y todas y tantas otras amigas que he ido conociendo a lo largo de este camino. Os doy las gracias, por teneros en mi vida, por darme la oportunidad de ser vuestra amiga, por todos los momentos vividos juntas, y por todos aquellos que aún están por llegar. A David, por ayudarme con la maquetación del trabajo. A Antonio Lozano por acompañarme en el camino.

A mi familia, a mi hermana por traer al mundo a mis dos soles favoritos: Luca y Yann. A mi prima M. Ángeles, que como su nombre indica, ha sido siempre un ángel para mí, a todos mis primos y tíos, tanto españoles, como italianos y finalmente a mis abuelos y ancestros, sin su esfuerzo esto no habría sido posible.

ABREVIATIONS

AB: Ana Blanchard
ABPA: Allergic bronchopulmonary aspergillosis
AET: Antibiotic eradication therapy
ATP: Adenosine triphosphate
BAL: Bronchoalveolar lavage
BC: *Burkholderia cepacia*
BMI: Body mass index
CFTR: Cystic fibrosis transmembrane conductance regulator
CFTR: Gene of the cystic fibrosis transmembrane conductance regulator
CFSPID: Cystic fibrosis screen positive, with inconclusive diagnosis
CFTR2: CFTR webside
CI: Confidence interval
DNAse: Dornase alfa
ENaC: Amiloride-sensitive sodium channel
FEV1: Forced expiratory volume in 1 second
HRCT: High-resolutions computed tomography
ICU: Intensive care unit
IGC: Isabel Gascón Casaredi
IgE: Immunoglobulin E
iRT: Immunoreactive trypsin
IQR: Interquartile range
IV: Intravenous
LCI: Lung clearance index
MSSA: *Methicillin-sensible S. aureus*
MRSA: *Methicillin-resistant S. aureus*
MDR-PA= Drug multiresistant Pa
NBD1 and NBD2: Nuclear binding domain NBD1 and NBD2
NETs: Neutrophil extracellular traps
NBS: Newborn screening
R-domain: Regulatory domain
REB: Research ethics board
ON: Ontario
OP: Oropharyngeal
Pa: *Pseudomonas aeruginosa*
PAP: pancreatitis associated protein
PFT: Pulmonary function tests
TIS: Tobramycin inhaled solution
TMEM16a: Transmembrane member 16a
NBDs: Two nucleotide-binding domains
US: United States
UK: United Kingdom

INDEX

ABSTRACT

Introduction: While antibiotic eradication therapy (AET) of early *Pseudomonas aeruginosa* infection is considered standard of care, its long-term effect on the subsequent course of cystic fibrosis (CF) lung disease remains unclear.

Methods: CF patients who were *Pseudomonas aeruginosa*-free for at least a year and had a minimum of 10 years of pulmonary function measurements were included. Subjects were categorized as **Never** if they never had *Pseudomonas aeruginosa* isolated from a respiratory tract sample. Subjects changed to the **Eradicated** group if they had a *Pseudomonas aeruginosa* infection, were treated with AET, and subsequently cleared their infection. Subjects changed to the **Chronic** group if AET did not clear their *Pseudomonas aeruginosa*. The primary outcome was absolute FEV1 decline over time, with age as the time variable. Mixed-effects linear regression models were used to account for the repeated lung function measurements over time within each patient.

Results: 205 CF subjects (48% were female) were included; the median (IQR) age at first infection was 9.6 (5.6, 14.6) years. The median (IQR) follow-up was 10.2 (5.7, 14.7) years for the Eradicated group, 8.8 (4.5, 14.9) years for the Chronic group and 2.8 (1.0, 5.7) years for the Never group was among those patients that had at least one *Pseudomonas aeruginosa* infection over the study period, annual lung function decline of FEV1 was significantly less (-1.11% predicted/year; 95% CI: -1.18, -1.04) in the Eradication group compared to the Chronic group (-1.57%; -1.64, -1.50) ($p<0.001$).

Conclusions: AET against *Pseudomonas aeruginosa* improves lung function trajectory in CF patients.

RESUMEN

Introducción: Si bien la terapia de erradicación para la infección precoz por *Pseudomonas aeruginosa* se considera el tratamiento habitual de los pacientes afectos de fibrosis quística (FQ), su efecto a largo plazo en el curso de la función pulmonar, sigue sin estar claro. Nuestro objetivo es valorar el efecto de la erradicación precoz de la infección por *Pseudomonas aeruginosa* en la caída largo plazo de la función pulmonar.

Métodos: pacientes afectos de FQ, libres de infección por *Pseudomonas aeruginosa* al menos desde el último año, con un seguimiento mínimo de función pulmonar de 10 años, fueron incluidos en este análisis. Los sujetos fueron categorizados como **Nunca**, si no habían tenido un aislamiento previo de *Pseudomonas aeruginosa*. Los sujetos cambiaban al grupo **Erradicado** si habían tenido una infección por *Pseudomonas aeruginosa*, que una vez tratada con la terapia de erradicación precoz, habían eliminado la infección. Los sujetos cambiaban al grupo **Crónico** si con la terapia de erradicación precoz no conseguía eliminar la infección por *Pseudomonas aeruginosa*. El resultado principal fue la caída del FEV1 absoluto en el tiempo, con la edad siendo utilizada como la variable de tiempo. Se utilizaron modelos de regresión lineal de efectos mixtos para tener en cuenta las mediciones repetidas de la función pulmonar a lo largo del tiempo en cada paciente.

Resultados: 205 pacientes afectos de FQ (48% mujeres), fueron incluidos. La edad mediana [IQR] de la primera infección fue de 9.6 (5.6, 14.6) años. La edad media de entrada en el estudio [IQR] fue de 5.6 (5.1, 10.5) años. La media de seguimiento [IQR] para el grupo nunca fue de 2.8 (1.0, 5.7) años, 10.2 (5.7, 14.7) años para el grupo Erradicados y 8.8 (4.5, 14.9) años para el grupo Crónicos. En los pacientes que tuvieron como mínimo una infección por *Pseudomonas aeruginosa* durante el periodo de estudio, la caída de función pulmonar anual fue de forma significativa más baja -1.11% predicho/año (95% IC -1.18,-1.04) en el grupo de pacientes erradicados, comparado con el grupo de pacientes crónicos -1.57%
(-1.64,-1.50) en un 0.46% ($p<0.001$).

Conclusiones: La terapia de erradicación precoz de la infección por *Pseudomonas aeruginosa* mejora la trayectoria de la función pulmonar en los pacientes afectos de FQ.

1 INTRODUCTION

1.1 Definition

Cystic fibrosis (CF) is a classic Mendelian autosomal recessive disorder. It is most common in populations with northern European ancestry where the predominant mutation is Phe508del (also known as F508del). People with cystic fibrosis from other regions have a wider range of mutations with the Phe508del mutation being much less prevalent. More than 2000 gene variants have been identified, many of which have been associated with disease causation. Mutations have different effects on the manufacture of the cystic fibrosis transmembrane conductance regulator protein (CFTR), its processing function and its stability at the cell membrane (1).

1.2 Cystic Fibrosis History

Nearly 80 years ago, Dorothy Andersen defined CF as a disease (Figure 1). In 1938 she described cystic fibrosis of the pancreas in 49 patients and the disorder was subsequently associated with lung infection and salt loss during a heat wave in New York. (1) The recognition of the increased salt content of sweat in people with cystic fibrosis by di Sant´Agnese and colleagues in the early 1950s led to the development of the stimulated sweat test, using pilocarpine iontophoresis as a diagnostic method.
In the 1980s, Quinton showed chloride impermeability in sweat glands to be the basis of the raised sweat electrolytes in patients with cystic fibrosis. The gene responsible for cystic fibrosis, an autosomal recessive disorder, was discovered in 1989 by teams led by Tsui, Riordan and Collins, with the subsequent identifications of its protein product, termed the cystic fibrosis transmembrane conductance regulator (CFTR)(2)(Figure 2). In the 1950s, the median life expectancy for patients with cystic fibrosis was a few months; the leading cause of death was meconium ileus and malnutrition subsequent to pancreatic malabsorption. During the past six decades, the median age of survival has increased progressively and is now more than 40 years in developed countries. Respiratory failure secondary to progressive lung disease is now the most common cause of death in individuals who do not receive a lung transplant. This improvement in life expectancy has been achieved by understanding the importance of improving airway clearance, aggressively treating infection and correcting nutrition deficits. Recently, with the understanding of how abnormal ion transport

in airways epithelial cells results in impaired mucus clearance and infection has lead the development of effective mucolytic agents and antipseudomonal antibiotics and the delivery of dedicated multidisciplinary cystic fibrosis care. Since the sequencing of the causative cystic fibrosis transmembrane conductance regulator (CFTR) gene, laboratory research has focused on developing therapies that correct the underlying basic defect in CFTR function. This approach has started to deliver transformational therapies for patients (1).

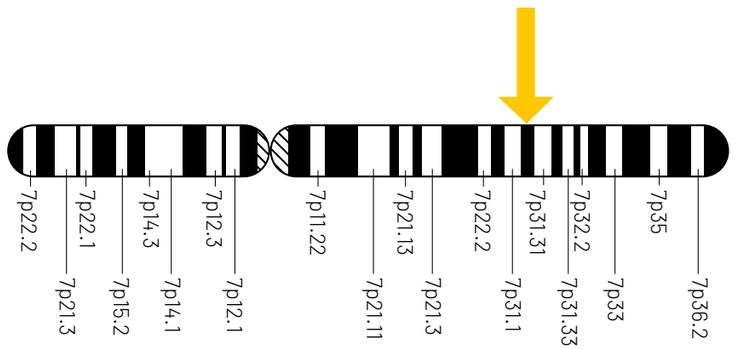


Figure 2: Chromosome 7. Region 7q31

1.3 Epidemiology

1.3.1. Prevalence

Estimates of disease incidence are around one in 3000 live births in persons of northern European descent. (3)

1.3.2. Sex and ethnicity distribution

CF can affect all ethnicities; however, Caucasians are the most common affected by this disease. Other ethnicities such as African American, Latin American or Asian have a lower incidence. Only one in 15.000 to 20.000 African Americans have CF and only one in 4.000 to 10.000 of Latin Americans, with even lower incidence rates in people of Asian background (4).
Regarding sex distribution, the disease affects both males and females, but for unknown reasons, males have a better expectancy of live. (5)

1.3.3. Prognosis/Survival

With the implementation of newborn screening (NBS), specific units of care and new therapies, the survival of CF patient's has improved in the last years. In 1959, the median of survival was 6 months and in 2008 was of 37.4 years (33.7-40) (Figure 3) (Figure4).

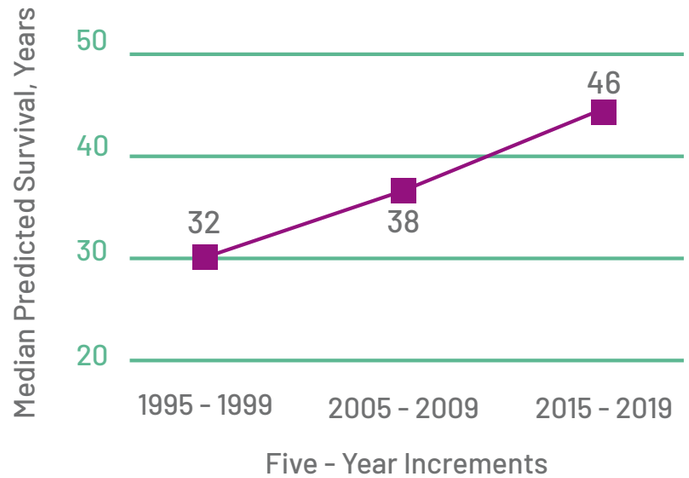


Figure 3: Median predicted survival in the United States (US). 2019 register.

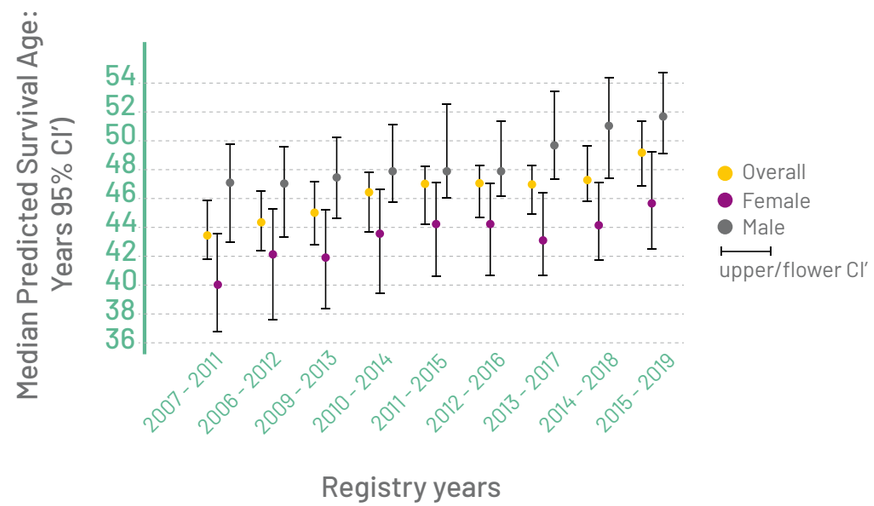


Figure 4: Median predicted survival in the United Kingdom (UK). 2019 register.

1.4 Mechanisms/pathophysiology

1.4.1. CFTR protein and genetic mutations

CF is caused by gene mutations of the CFTR gene on the long arm of the chromosome 7. This gene is a unique member of the adenosine triphosphate (ATP) binding cassete (ABC) or traffic ATPase family of genes, which carries a regulatory domain that is actively phosphorylated. CFTR functions primarily as an apical anion channel of chloride and bicarbonate, rather than an active pump. Like other members of the ABC protein family, it houses two nucleotide-binding domains (NBDs) encodings sites capable of binding and hydrolyzing ATP (Walker and B motifs) and membrane spanning domains that serve as the ion channel pore through the plasma membrane (figure 5)(6).

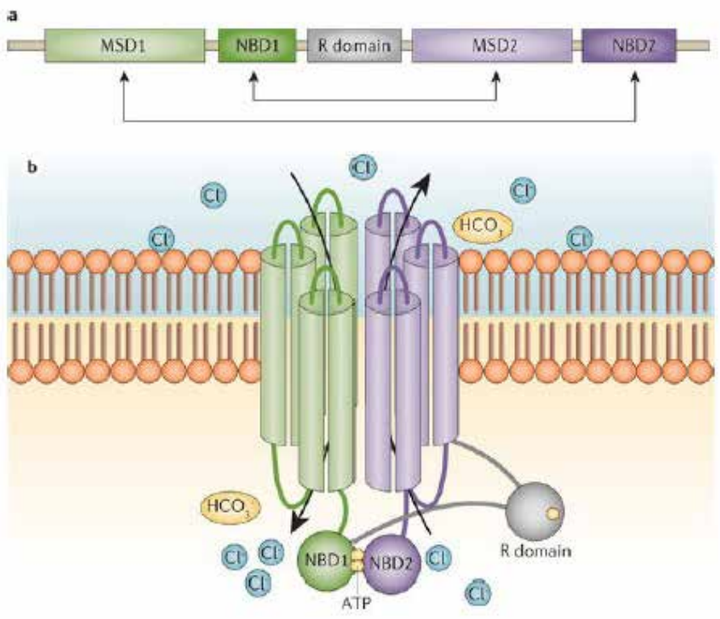


Figure 5: Structure of CFTR. A/ linear structure. B/ The cystic fibrosis transmembrane conductance regulator (CFTR) protein is comprised of two, six span membrane-bound regions each connected to a nuclear binding domain (NBD1 and NBD2), which bind ATP, as well as regulatory (R) domain that is comprised of many charged amino acids. The channel opens when its R-domain is phosphorylated by protein kinase A and ATP is bound at the NBDs. (6)

Most mutations of the *CFTR* gene are missense alterations, but frameshifts, splicing, nonsense mutations, and inframe deletions and insertions have been described. About 15% of identified genetic variants are not associated with disease. *CFTR* mutations can be divided into six classes according to their effects on protein function. Class I, II, and III mutations are associated with no residual *CFTR* function and patients with these mutations have a severe phenotype, whereas individuals with class IV, V and VI mutations have some residual function of *CFTR* protein and have mild lung phenotype and pancreatic sufficiency (figure 6). The potential role of other genes, which might affect other important pathways in the pathophysiology of CF, is of interest. Several extensive studies using a genome-wide association approach have identified genes that are particular associated with non-pulmonary complications (1).

To help the CF community to understand some of the complexities and clinical implications of the wide range of identified *CFTR* mutations, a website has been developed to provide information about specific cystic fibrosis mutations (http://cftr2.org). The CFTR2 website includes information about the most common 160 mutations worldwide from 39696 people with CF. The researchers have provided helpful information about genotype-phenotype relations by analyzing the registry and available data from cell studies (1)(6).

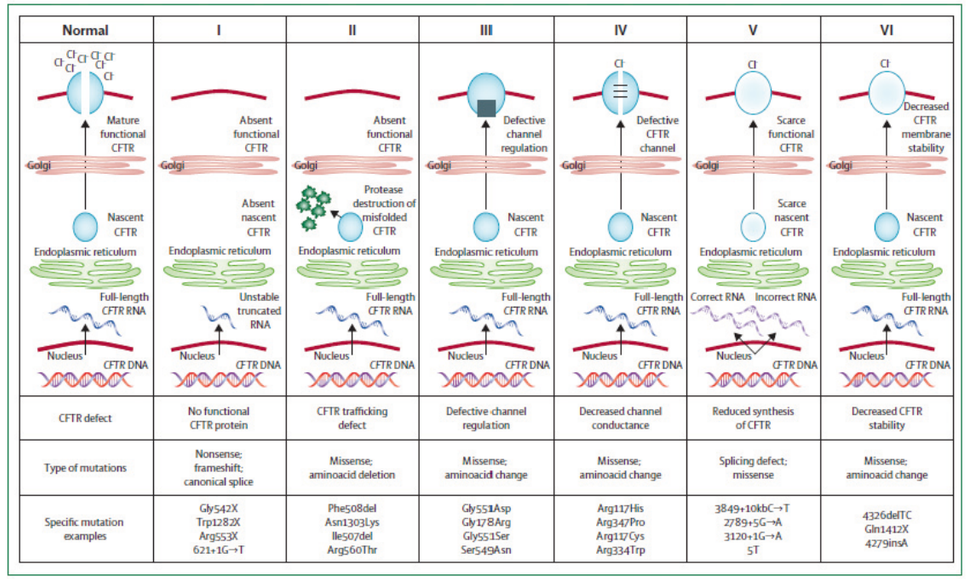


Figure 6: Classes of CFTR mutation (1)

1.4.2. Airway pathophysiology

1.4.2.1. Airway pathophysiology summary

CF affects the function of epithelial tissues in which CFTR is highly expressed, mainly glandular epithelia. The disease primarily manifests in the lung, pancreas, gastrointestinal tract, vas deferens, and sweat glands, although airway disease is the leading cause of morbidity and mortality. Airway disease is thought to begin in the small airways. In the lungs, cystic fibrosis results in mucus accumulation that compromises the airway lumen and contributes to obstructive pulmonary disease (figure 7). Submucosal gland hyperplasia and thickened mucus secretions are also prominent. The development of bronchiectasis leads to irreversible changes that encourage continued infection and accelerate disease pathogenesis (6).

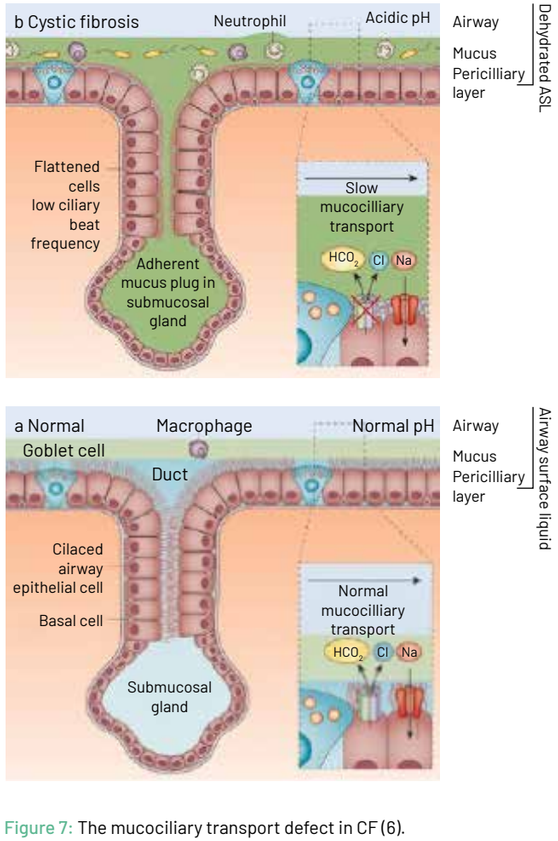


Figure 7: The mucociliary transport defect in CF (6).

Despite decades of research, understanding the origins of airway pathogenesis remains incomplete and continues to evolve. Several key manifestations include delayed mucociliary clearance through the airway surface, liquid depletion, abnormalities of the physical properties and adhesion of mucus, and a predisposition to infection owing to abnormal mucosal defences. Dysregulated inflammation intrinsic to the CFTR defect is also apparent. These processes initiate and perpetuate a cycle of destruction that ultimately results in irreversible lung injury, bronchiectasis and respiratory failure (6).

Loss of apical CFTR leads to reduced chloride and bicarbonate secretion. Given that the release of water and electrolytes onto the airway surface is largely driven by CFTR-dependent fluid secretion through both the glands and the surface epithelia, CFTR deficiency leads to diminished airway surface hydration, which can impair mucociliary transport itself. CFTR has also been shown to regulate the activity of the epithelial sodium channel (ENaC; also known as the amiloride-sensitive sodium channel), which is also activated by cleavage events conferred by free proteases such as prostasin and neutrophil elastase, which are enriched in the inflamed airway. CFTR defect also confers unopposed ENaC-dependent sodium and water absorption, which exacerbates airway surface liquid depletion. Moreover, the periciliary layer, a mucin gel layer between the cell surface and the mucus layer (Figure 3), is sensitive to the osmolar forces of the overlying mucus; as the overlying mucins become. The researchers have provided helpful information about genotype-phenotype relations by analyzing the registry and available data from cell studies concentrate in the absence of adequate fluid transport, the periciliary layer collapses and failure of mucociliary transport ensues. (6).

1.4.3. Mucus and glandular epithelium abnormalities.

Hyper viscous respiratory secretions obstruct small and medium airways, leading to a profound failure of mucociliary clearance that can be verified macroscopically by radioligand imaging. Mucus includes a complex array of extracellular proteins found in high concentrations in the airway lumen (6).

In extrapulmonary organs (for example, the pancreas and the liver), profound ductular obstruction is observed without polymicrobial infection. An emerging notion implicates defective bicarbonate transport as a mediator of hyperviscosity and mucosal adhesion in cystic fibrosis. In this model, exocrine mucus-which is highly damaging in charge-is produced by acinar and other epithelial cells. This mucus binds calcium ions, condenses the mucus and shields the opposing repulsive force between sulfates and other anionic groups on constituent mucins. Bicarbonate secretion via CFTR chelates calcium and permits mucinous expansion and viscoelastic state compatible with physiologic clearance. Failure of bicarbonate release is hypothesized to result in defective mucin expansion, leading to hiperviscous secretion with abnormally adherent properties; that is, the mucus is tightly bound to the epithelial surface and is difficult to mobilize (6).

1.4.4. Defects in airway clearance.

Although CFTR mainly functions as an anion transporter, it regulates numerous processes, including fundamental aspects of airway defence and inflammatory cell function. CFTR is situated within membrane complexes close to several integral membrane proteins, including other ion channels. In addition to ENaC, CFTR can directly or indirectly regulate anion secretion through other chloride channels, such as transmembrane member 16a (TMEM16a; also known as anoctamin-1), or contribute to airway pH regulation through chloride exchangers, including anion exchanger type 2. The absence of bicarbonate secretion leads to an acidic pH airway surface liquid in cystic fibrosis, which has been reported as a possible cause of defective bacterial killing by the highly pH-sensitive innate defensins.

CFTR also directly affects neutrophil killing, as it affects degranulation by interfering with granule trafficking. Dysfunctional macrophages might also be biased towards a pro-inflammatory response. Proteomic and transcriptome analyses demonstrate hundreds of cellular gene products that directly bind to or are regulated by CFTR (6).

1.4.4.1. Inflammation in lung disease

Whether the infection is required to cause airway inflammation in CF remains under debate. Despite this controversy, what is clear is that infection exaggerates the inflammatory milieu.
Factors released in chronic neutrophilic inflammation in CF can profoundly reduce airway surface liquid height. As well as aggravating airway dehydration, the imbalance between neutrophil elastase, other proteolytic enzymes (derived from inflammatory cells or bacteria) and anti-proteases results in excessive tissue damage. Oxidative stress and persisting airway inflammation might be associated with local airway deficiency in glutathione (which usually protects from reactive oxygen species). Over time, a vicious circle of reduced mucus clearance, neutrophil-dominated inflammation and bacterial infection damages the airway (6).

1.4.4.2. Lung infection

Airway pathogens most commonly detected include *Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus*, and *Aspergillus species*. Fungi, including *Aspergillus* species, are also increasingly recognized as pathogens in cystic fibrosis and are associated with an increased rate of pulmonary exacerbations (6) (figure 8). Viral infection is also a common cause of exacerbations in people with cystic fibrosis; although these patients are not more susceptible to viral infection, the impact is more significant.

With increasing age, infections with other bacteria, including *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* (BC) complex, become increasingly common. *Methicillin-resistant Staphylococcus* aureus (MRSA) is an increasing threat in up to 30% of patients presenting at some US centres, though the rates are lower in Europe. In the late

1980s, epidemic strains of BC were associated with clear evidence of cross-infection, rapid clinical deterioration in many and poor outcomes following lung transplantation. Both MRSA and BC infection in patients with cystic fibrosis have been reported to have adverse effects on prognosis.

Non-tuberculous mycobacteria cause infection in 3-30% of patients with CF, and prevalence rates have increased over the past ten years (6).

Over the past several years, the airway has been revealed as not sterile in health and the airway in cystic fibrosis is now considered to harbours a polymicrobial milieu. Both non-cultured-based and cultured-based methods have demonstrated a wide range of atypical bacterial species, including *Prevotella*, *Fusobacterium*, and *Veillonella spp*. amongst others (6).

The lung in CF is a microaerophilic environment and Pa survives and in fact thrives within low oxygen tension biofilms. Chronic Pa infection is also associated with ongoing microevolution, which alters virulence, regulatory networks and acquisition of antimicrobial-resistance mechanisms. These factors likely contribute to the persistence of Pa. The interaction of the host with pathogens is crucial for the development and progression of pulmonary disease in CF, leading to perpetual neutrophil recruitment to the lung. A key component of this dysregulation is the development of neutrophil extracellular traps (NETs), which are stimulated by bacterial pathogens (such as Pa and MARS NETs have detrimental effects in the CF s airway by enhancing the viscosity of airway secretions, dampening pathogen clearance and potentially enhancing biofilm development and persistence (6).

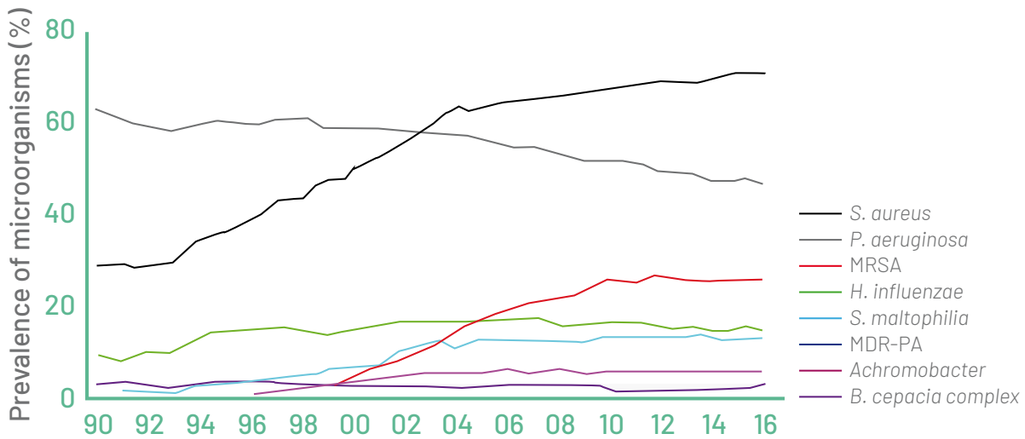


Figure 8: Prevalence of microorganism in 2016. MARS= Methicillin-resistant S. aureus, MDR-PA= Drug multiresistant Pa. American Cystic Fibrosis Foundation.

1.5 Diagnosis screening and prevention

1.5.1. Principles of diagnosis

In 1998 Rosenstein's criteria for the diagnosis of CF were defined to more precisely define the criteria for a diagnosis of CF. Rosenstein's diagnostic criteria for cystic fibrosis are the following:
Individuals must have:

- at least two clinical features previously described
- or a history of a CF sibling
- or a positive newborn screening test result

AND an increased sweat chloride concentration by pilocarpine iontophoresis on two or More occasions

- Or identification of two CF mutations
- Or demonstration of abnormal nasal epithelial ion transport(7).

The conventional diagnosis of CF is given with the criteria presented below, and increasingly diagnosis is by NBS. However, physicians should be aware of symptoms and signs of disease in older children and adults, in particular, bronchiectasis, recurrent respiratory tract infections, nasal polyps, male infertility and portal hypertension; late presenting patients are usually (but not invariably) pancreatic sufficient. Screening can miss some mild cases if the child is born in a region without routine screening, the child missed screening despite being born in a screening region, or laboratory error occurred. More than 95% of patients presenting symptomatically can be diagnosed with a sweat test (figure 9). The test measures chloride concentration after pilocarpine application, which stimulates sweat production. The upper limit of average sweat chloride concentration remains under contention; in Europe, the upper limit of normal for an indeterminate result is 30 mmol/L regardless of age. Given that sweat chloride concentration can increase with age, 40 mmol/L is considered the upper limit after six months of age only in the US. Above 60 mmol/L is abnormal and diagnostic, provided the clinical presentation is compatible (6).

Genetic testing is essential to diagnosis because mutation-specific therapy is an increasing reality and can help resolve unclear cases. Given the marked variation in the prevalence of the CFTR mutations between different ethnic groups, test panels that account for this variation should be used unless whole-exome sequencing is performed (6)(Figure 11).

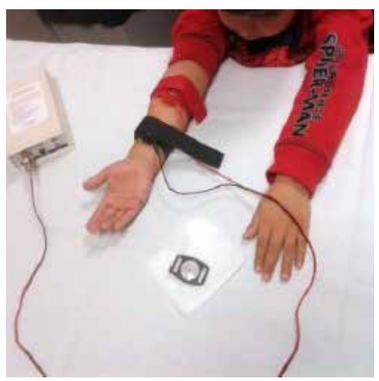


Figure 9: Sweat test performance.

Measurement of transepithelial potential difference is an adjunct diagnostic test but is only available in only a few specialist centres. The in vivo technique measures the potential difference across the nose or respiratory epithelium via a catheter referenced to a peripheral electrode (Figure 8). Rectal potential difference can also be measured in vivo; alternatively, in vitro measurement of CFTR activity on excised rectal biopsy tissue can be performed via open-circuit or closed-circuit currents. Other in vitro methods of assessing CFTR function, such as intestinal organoid swelling or the function of nasal epithelial cells grown in culture are also of emerging interest.

Other supportive tests can be considered to clarify the diagnosis, including stool human faecal elastase measurement for pancreatic insufficiency and, in postpuberal men, semen analysis to test for azoospermia (6).

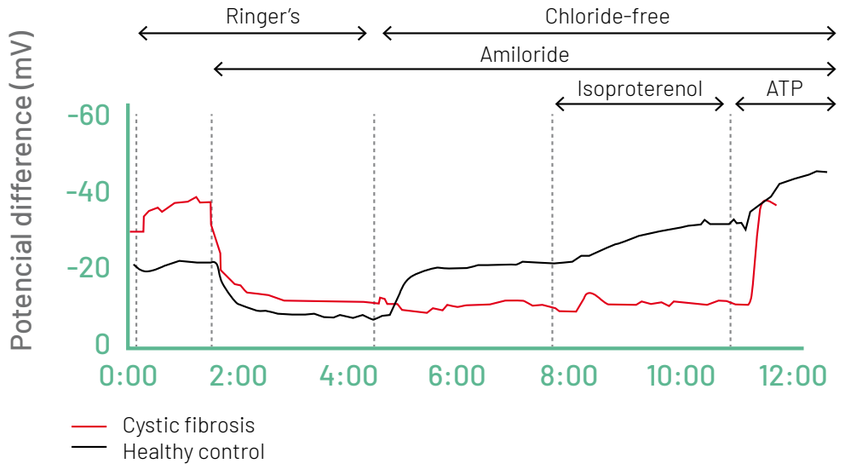


Figure 10: Representative nasal potential difference tracings from a healthy control (blue) and a patient with cystic fibrosis (red) (6).

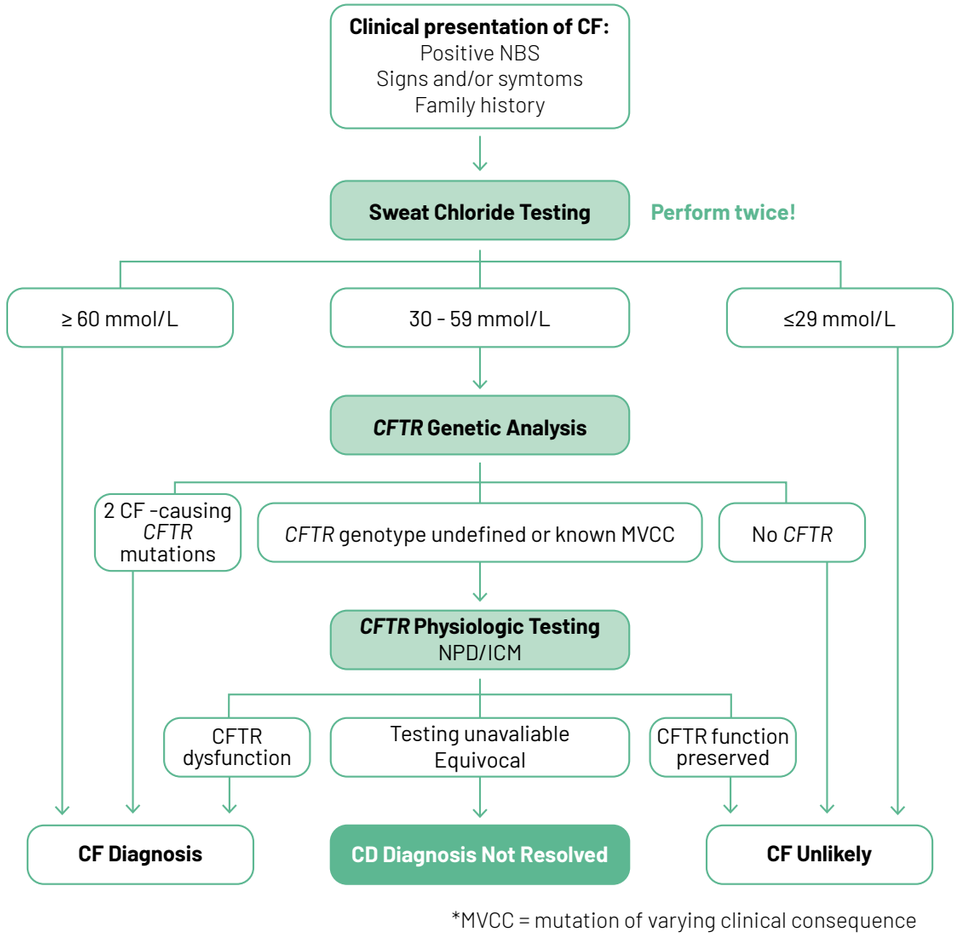


Figure 11: CF diagnosis (8).

1.5.2. Newborn screening

A large number of NBS protocols are available, including measurements of serum immunoreactive trypsin (iRT), pancreatitis associated protein (PAP) and CFTR mutation analysis. Screening diagnosis must always be confirmed with a sweat test, not least to ensure that there has been no error in the screening laboratory.

The possible outcomes are:

- Definite diagnosis of classic CF depending on the protocol,
- CF definitely excluded or an indeterminate outcome.
- Indeterminate outcomes are the most challenging situations; these children fall into two groups, namely those harboring CFTR mutations who will develop late-onset, mild-variant disease and those with genuine diagnostic uncertainty.

Thus, screening can lead to the child being given a diagnosis of CF that might not have been possible until middle age. Such situation raises the question of whether an early diagnosis impacts the quality of life of the individual and their family. If accurate information is carefully given, and the intensity of treatment and monitoring is appropriately applied according to the severity of the clinical state, quality of life need not be negatively affected (6).

True diagnostic uncertainty is another outcome of screening. The US CF Foundation has coined the phrase ´CFTR-related metabolic syndrome `to describe infants with sweat chloride values < 60 mmol/L (which could be too high an upper limit of normal in infancy, given that virtually all healthy babies have a sweat chloride < 30 mmol/L) and two CFTR mutations, one of which is has not been shown to be disease causing. An alternative terms; cystic fibrosis screen positive with inconclusive diagnosis (CFSPID) has been proposed by the European Cystic Fibrosis Working Group. However, some of these infants develop cystic fibrosis-like symptoms, and current recommendations are for careful follow-up monitoring (6).

1.5.3. Respiratory manifestations

The onset and progression of clinical manifestations of CF lung disease are highly variable, and detecting the presence of disease in infants and children can be challenging.

Most children will present with cough as their primary symptom, which becomes increasingly productive, pulmonary exacerbations leading to bronchiectasis and chronic lung disease. As the lung disease progresses, CF patients can experience exercise intolerance, dyspnea, and shortness of breath. *Pulmonary Hypertension* is a complication of hypoxemia that is most commonly seen in older patients with advanced disease. With more advanced diseases, the presence of pulmonary hypertension significantly reduces life expectancy.

On the other hand, CF patients have chronic upper respiratory tract involvement, clinically manifested as nasal congestion and rhinorrhea. Pansinusitis is almost universally present in affected individuals. The percentage of CF patients who have features consistent with clinical sinusitis varies widely. Occurring in 7%-56% of CF patients, nasal polyposis is a common complication leading to nasal obstruction and congestion.

Besides the clinical manifestations pointed out before, the most important pulmonary complications of CF are:

Bronchiectasis and Pulmonary exacerbations

Typically, bronchiectasis begins in the upper lobes in CF patients and the progresses to involve the whole lung.

Atelectasis often coexists with bronchiectasis because of the accumulation of purulent secretions and airway obstruction.

The altered balance between airway pathogens and local host defences leads to acute changes in respiratory signs and symptoms; this phenomenon is known as pulmonary exacerbation. Clinically a pulmonary exacerbation is manifested as increased respiratory symptoms such as cough, dyspnea, and sputum production, often accompanied by systemic symptoms such as fatigue, anorexia, and weight loss. Pulmonary function values usually decrease during exacerbations, and a therapeutic goal is to restore the best baseline of pulmonary functions, regardless of the patient's symptoms.

Hemoptysis and Pneumothorax

As airway disease progresses, there is an increased likelihood of respiratory complications, including hemoptysis and pneumothorax. Approximately 3% of CF patients will experience pneumothorax during their lifetime, and the average annual incidence of pneumothorax is 0. 6%. Hemoptysis is common in older CF patients, particularly during pulmonary exacerbations.

As CF lung disease progresses, bronchial arteries or collateral vessels enlarge and may rupture into an inflamed airway, producing massive hemoptysis. This is a medical emergency, and roughly, 4% of all patients with CF will suffer massive hemoptysis in their life.

Airway hiperrecativity

Airway hyperreactivity is often diagnosed in patients with CF. Roughly half of CF children and adults have a bronchodilator response, even if they do not have asthma-like symptoms, and the degree of airway hyperresponsiveness may change over time.

Allergic bronchopulmonary Aspergillosis (ABPA)

The defect in airway clearance associated with CF may also allow for endobronchial fungal growth and the development of ABPA. ABPA is an inflammatory complication, clinically manifested by wheezing and cough refractory to standard therapies. It is an intense immunologic response to surface colonization with the fungus Aspergillus fumigatus, which is characterized by clinical deterioration that is not explained by other etiologies, elevates serum quantitative immunoglobulin (Ig)E concentrations (> 500 UI/ml), positive skin prick test to Aspergillus fumigatus or elevated in vitro Aspergillus specific immunoglobulin E (IgE) levels, and Aspergillus-specific IgG levels or precipitins. Features that should raise concerns for the diagnosis include high-resolution computed tomography (HRCT) findings of mucus impaction, central bronchiectasis, tree-in-bud opacities, and centrilobular nodules (9).

1.5.4. Extrapulmonary manifestations

CF is a multi-system disease that affects many organs in which CFTR is expressed. The gastrointestinal tract is affected in numerous ways, including increased nutrient loss secondary to pancreatic insufficiency, reduced fat-soluble vitamin absorption, fat-soluble vitamin deficiency states, frequent gastro-oesophagal reflux, and impaired bowel transit (complicated by distal intestinal obstruction syndrome, constipation, and small intestinal bacterial overgrowth). Hepatic involvement is also common, with up to one in three patients affected, including those with evidence of hepatic steatosis, ductal stenosis and focal biliary cirrhosis. Biliary cirrhosis usually becomes clinically evident in late childhood or early adolescence and leads to portal (6).

Cystic-fibrosis-related diabetes is increasingly common with advancing age and represents a clinically distinct form from type 1 and type 2 diabetes mellitus in the general population. Other endocrinological complications of CF include delayed menarche in malnourished adolescent females and reduced bone mineral density, which can increase the risk of bone fractures and is multifactorial. Congenital bilateral absence of the vas deferens occurs in 98% of males with cystic fibrosis and results in azoospermia. Renal complications can occur and include Nephrocalcinosis and salt and water depletion owing to excess fluid losses, which contribute to acute kidney injury and proteinuria. Chronic kidney disease is more common in adult patients, and risk factors for its development include age, diabetes, prior episodes of acute kidney injury and prior organ transplantation. As survival for patients with CF has increased, a number of complications have been recognized. These include an increased risk of colorectal and other gastrointestinal malignancies, the potential for macrovascular disease to complicate longstanding inflammatory disease and venous insufficiency (6).

1.6 Monitoring and management of CF disease

1.6.1. Monitoring lung disease

Several Clinical Practice Guidelines that outline standards for routine monitoring and intervention to slow progressing of lung disease have been published (10).

Patients should be seen in a multidisciplinary clinic; evaluations include clinical evaluation, regular cultures of airway secretions, pulmonary function testing; such as forced expiratory volume in 1 second (FEV1), blood testing and radiological testing such as HRCT.

Clinical evaluations are essential and include monitoring weight loss, anorexia, exercise tolerance, airway clearance technique and school attendance, which is indirect, measures of pulmonary morbidity. Indeed, the child´s nutritional health is relevant to pulmonary outcomes (10).

Moreover, both physiological and social supports are an essential key point to be considered in the follow up of CF patients and their family (10).

Following the suggestions of several guidelines published about monitoring CF disease, CF patients should be seen in clinics every 2 or every 3 months, and if needed more often depending on the patient´s situation (10).

Infants diagnosed by NBS should be seen monthly in the first years and sometimes more frequently in the first months following diagnosis (10).

Therefore, the team should see patients at least four times each year and patients with complex clinical issues should be seen as much as necessary, and one of this visits should include the annual follow up. In order to avoid cross infections, clinics in CF patients are ruled in a segregation format (10).

1.6.2. Pulmonary disease treatment

Treatment strategies for CF lung disease include antibiotics, mucolytic therapy, anti-inflammatory therapies and monitoring of airway clearance.

1.6.2.1. Mucolytic and hydrator therapies

Inhaled therapy offers targeted drug delivery and is relatively simple and quick to take. There have been significant improvements in both delivery devices and the formulation of drugs, with "intelligent nebulizers" and dry-powder inhalers becoming commonplace. The advantages of inhaled medications are that they generate high drug levels within the airways with limited systemic toxicity.
Regarding mucolytic and hydrator therapies, the most common mucolytic and hydrator therapies used in CF patients are hypertonic saline and Dornase alfa (DNAse).
Hypertonic saline acts as a hydrating agent that increases mucociliary clearance and has been demonstrated to improve lung function and reduce exacerbations in both short-term and long-term trials (11).
DNAse is a recombinant human DNA that breaks down DNA derived from degrading neutrophils that accumulate in the airways of patients with CF therefore reducing the viscosity of airway secretions and leading to improved lung function and reduced exacerbations (12). This therapy is considered a standard of care for patients≥ 5 years (6).

Inhaled dry-powder mannitol is another osmotic agent that has showed lung function improvements with more consistent effects in the adult population (13).

1.6.2.2. Antibiotic therapy

Pulmonary infection in patients with CF has been associated with poorer lung function, lower weight percentiles and increased morbidity and mortality. Therefore, antibiotic therapy in CF is used to prevent, eradicate and control respiratory infection to delay pulmonary infection and improve clinical and functional outcomes.
In CF patients, antibiotic therapy is usually different and more aggressive than other populations. Usually, the antibiotic dose is doubled and prescribed for 15 days, and in particular cases with poor response, they can be prolonged up to 21 days. Depending on the severity of the pulmonary exacerbation and the disponibility of the antibiotic, the therapy can be oral, inhaled, or intravenous (IV)(14).

1.6.2.3. Airway clearance and exercise

Modes of airway clearance include device assistance (positive expiratory pressure and vest and handheld vibratory devices), breathing modalities (autogenic drainage) and percussion.
In terms of exercise, aerobic exercise results in improved exercise tolerance, and increased physical activity has been shown to reduce lung function decline (15).

1.6.2.4. Macrolides

The mechanism of action of macrolide antibiotics is unknown; however, in vitro studies suggest that macrolide agents may decrease the production of bacterial virulence factors, modulate the inflammatory response, or both (16).
When used in Pa infected patients, several randomized controlled trials have demonstrated to reduce the time to next exacerbation, improve lung function, quality of life and weight (6).

1.6.2.5. Anti-inflammatory therapies

Several anti-inflammatory therapies have been studied in CF patients including non-steroidal anti-inflammatory drugs (such as ibuprofen), both oral an inhaled corticosteroids and leukotriene inhibitors.

1.6.2.6. Lung Transplantation

End- stage respiratory insufficiency remains the cause of death in the vast majority of CF patients, therefore lung transplantation is the treatment stablished for these patients (6). An FEV1 < 30% of predicted values and a rapid decline in FEV1 despite optimal conservative treatment, malnutrition, diabetes, female gender, frequent exacerbations and/or increasing need for intravenous antibiotic therapy, recurrent massive hemoptysis, which cannot be controlled by bronchial artery embolization, relapsing or complicated pneumothorax or the need for intensive care unit (ICU) admission are all indicators that a pretransplant assessment is needed (17).
For patients with predominant hypoxic respiratory failure, non-invasive ventilations can be a useful symptomatic treatment and can provide a bridge to transplantation (1).

1.6.3. Extrapulmonary disease treatment

The rapidly increasing size of the adult population with CF and the associated complications in these patients creates challenges for the delivery of care. Nowadays, there are several treatments for extrapulmonary disease treatments like as pancreatic enzymes, vitamins and nutritional support.
Due to the rapidly increasing size of the adult population, metabolic and endocrine complications, hyperlipidemia, and gastrointestinal malignancy have emerged in the adult CF population (18).

Therefore the involvement of an interdisciplinary and the adequate transition from de pediatric age to adulthood is increasingly essential for the adult with CF (19).

1.6.4. Treatment of the basic defect

Several therapies intend to treat the primary defect from gene therapy, improving the expression of CFTR on the cell surface, or increasing the "open probability of existing channels to targeting other ion channels to compensate for its dysfunction (6).

1.7 Pa infection and lung function

Pa is one of the common pathogens of CF lung disease. Pa infection begins as intermittent isolation of typically non-mucoid Pa strains from the airways, which, unless treated with targeted antibiotic therapy, will establish chronic infection in most individuals (20). Early infection with Pa is a major predictor of mortality and morbidity in young children with CF (21). Given the potential negative impact of Pa infection, antibiotic treatment protocols have been developed to aim for eradication early on before chronic infection has been established. Treatment protocols include inhaled therapy alone or combined with systemic antibiotics; the success rate of different protocols is high with negative cultures being accomplished in more than 80 percent of patients (22)(23)(24)(25).

While antibiotic eradication therapy (AET) of Pa infection is now considered the standard of care (20) (21) (22) (23) (26) and clearance of Pa can be achieved in most patients, the longer term impact of eradication therapy is less clear. The EPIC trial showed that a failure to eradicate was associated with a higher risk of exacerbation (27) and studies have demonstrated that treatment lengthens the time to chronic infection, but a positive impact on lung function measures has not been established. Since most patients are asymptomatic when a new infection with Pa is detected in respiratory cultures, early infection is not usually associated with a deterioration in lung function and treatment has no immediate effects on the most commonly used lung function measure, forced expiratory volume in 1 second (FEV1)(28). Recent data suggest that this is also the case for sensitive outcome measures such as LCI (29). It has been previously demonstrated that CF patients in whom Pa infection was cleared had similar FEV1 trajectories over subsequent years compared to children who were never infected (30). However, this previous study had limited follow-up data, so long term effects of AET on the subsequent course of lung disease therefore remains unclear (31).

2 RATIONALE OF THE DOCTORAL PROJECT

The CF Unit at the Hospital for Sick Children in Toronto, directed by Dr Felix Ratjen and comprising a multidisciplinary team, has many years of experience managing Pediatric CF. The doctoral candidate has had the chance to be a part of this medical team as a fellow and has been involved in the everyday clinical work of the Unit, providing clinical attention to CF patients.

During that time, the doctoral candidate could work with CF patients in wards and clinics. Therefore, she had the opportunity to deeply learn about the disease and ask questions about the treatment, evolution and prognosis of CF patients.

As a part of her clinical Fellowship, she realized she had an excellent opportunity to develop research skills with Dr Rajten's team and asked to develop research work regarding CF. Her research objective during the Fellowship was to run/conduct a project regarding early Pa eradication therapy (AET) and its long-term effect on lung function. Moreover, why discuss early AET and long-term lung function in CF?

Over the last two decades, with the implementation of NBS, the use of effective therapies, and the emergence of new therapies, the survival of CF patients has increased, and incident cases of Pa have decreased significantly.

However, as noted above, Pa is one of the most common pathogens in CF patients. Chronic Pa infection leads to accelerated lung function decline, increased mortality and morbidity in CF patients. Many studies have been designed to address the efficacy and safety of strategies used for Pa. eradication therapy, and now a day, AET is a standard gold of care in patients with early Pa infection.

Nevertheless, regarding Pa infection, one particular question has not been addressed before the evidence of the effect of AET on initial Pa infection and the effect on the long-term course of lung function.

While several authors have demonstrated the short-term effect of Pa AET on lung function, its long-term effect on the subsequent course of lung disease remains unclear. Reweaving the literature, several studies have tried to answer this question. However, no one has shown a clear relationship between AET for Pa infection and its impact on long-term lung function in CF patients.

The lack of evidence regarding this specific question cannot be stressed enough. It is probably due to the difficulties in collecting a large sample and the need for more extended follow-up periods of the same cohort of patients. In particular, few previous studies have addressed the impact of AET for Pa infection on long-term lung function, and their results have been inconclusive. Therefore, this particular question remains unanswered. A fuller answer to this question will provide more scientific evidence proving that AET can result in improved microbiological outcomes and clinical outcomes.

The justification of the present thesis is to address this particular question from the perspective of a pediatric respirologist. Providing the answer to this clinical question requires the participation of an experienced clinical team and expert in the management of pediatric CF.

A well-designed database and a specific CF research team are essential to conducting a retrospective study with a large, homogeneous sample. The CF research team, also directed by Dr Felix Rajten, has developed many relevant studies in CF that have been capital for both the understanding of the disease and the development of new therapies that now a day are considered standard of care in CF. Therefore, the candidate has had the opportunity to coordinate with the research team, participating in the study's design, the collection of data and the drafting of the manuscript.

If the objectives of this research work are achieved, the clinical applicability of this project will consist of two crucial/main points. On one side, to improve Pa infection AET protocols and consequently tries to implement different research lines, trying to find new antibiotic treatment for Pa infection with a safe and compelling profile that can be used in pediatric cystic fibrosis patients. On the other side, to implement new research lines regarding monitoring techniques of early disease/lung function, with higher sensitivity and effectivity than the techniques/ones that are currently available.

3 HYPOTHESIS

Successful Pa eradication therapy in patients with CF is associated with significantly reducing progressive deterioration of long-term lung function.

3 HYPOTHESIS


4 AIMS

4 AIMS

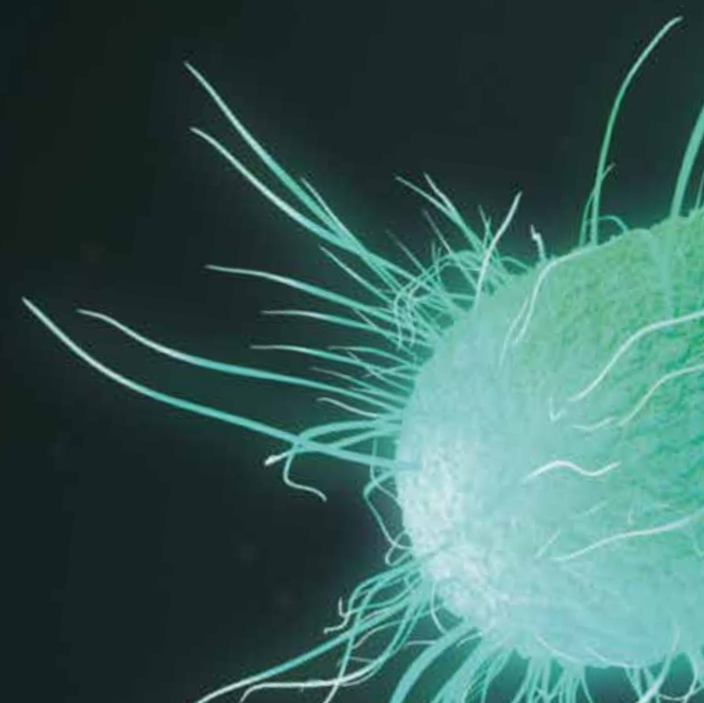
Primary aim:

- To assess the long-term differences in lung function decline between the Eradicated and Chronic groups.

Secondary aims:

- To assess the age of first Pa infection in our cohort of patients.
- To assess the percentage of patients that were in each group of Pa status infection, the frame of time that patients were in each group of Pa status infection and the number of patients over time in each group of Pa status infection at selected age snapshots.
- To assess the baseline pulmonary function measured as forced expiratory volume in one second (FEV1) of the patients at the study entry.

5 METHODS



Study population and data collection

This study was a retrospective analysis of individuals with CF followed at the Hospital for Sick Children and St. Michael's Hospital (Toronto, ON, Canada), captured in the Toronto CF Database from 1998 to 2018. Data were extracted from the database housed at the Hospital for Sick Children which contains information on patients' pulmonary function tests (PFT), microbiology, and medications. The data was supplemented with specifics on AET Treatments and outcomes through systematic review of health records by the doctoral candidate and other study investigators (figure one consort diagram) (I.G.C., A.B.). The Research Ethics Board at the Hospital approved this study for Sick Children (REB#1000027683).

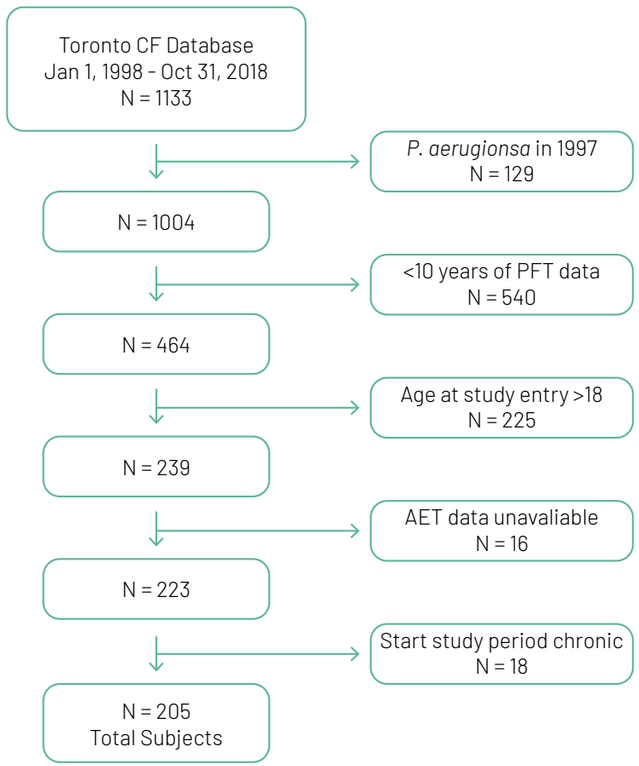


Figure 1: CONSORT Diagram.

Inclusion criteria:

Patients were included according the following inclusion criteria:

- Patients younger than 18 years of age at the cohort entry
- Patients with a confirmed diagnosis of CF. The diagnosis of CF was defined by:
- The presence of clinical features consistent with CF or
- A positive family history of CF plus
- Either two documented sweat chlorides of > 60 mEq/L by quantitative pilocarpine iontophoresis test, a genotype showing two well-characterized disease-causing mutations, or a nasal potential difference consistent with CF.

- For patients who entered the study cohort in 1998, a full year of negative Pa cultures of respiratory tract specimens prior to cohort entry.
- At least 10 years of spirometry measurements in order to qualify for inclusion and could not enter the cohort if chronic Pa infection was already present at that time point.
- Data was included until the last patient encounter in the time frame, until lung transplantation or death.

Definitions: *Pseudomonas aeruginosa* eradication protocol and outcomes

Early infection for Pa as defined as either the first positive respiratory culture for Pa within the study period or a positive after at least one year of negative cultures whilst not on antibiotics with activity against Pa. The details of the AET protocol used at our center have been published by Stanojevic et al. (14). Initial treatment of Pa in asymptomatic patients consisted of tobramycin inhalation solution (TIS) (either 1 year of tobramycin 80mg/2ml or 28 days of inhaled tobramycin 300 mg/5ml). Symptomatic patients with Pa infection were given 14 days of intravenous antibiotics and a subsequent course of inhaled tobramycin (14).

Patients who cleared Pa infection after the end of AET were classified as eradicated patients who did not clear their Pa infection after AET was completed were classified as chronically infected.

Work plan

An excel database was designed with all the study variables and a q/3 months record of each of them. The doctoral candidate with other investigators of the study did and extended chart review of all the patients who were eligible for the study and collected all the data necessary for the development of the project.

Study variables recorded during the 20 years of the data review were:

- Patients characteristic:
  - age at study entry
  - gender
  - age at diagnosis
  - CFTR functional class
  - ethnicity
  - pancreatic status
- Age at first Pa infection.
- FEV1% predicted at study
- FEV1% every 3 months
- Follow up time in the study.
- Categorization of Pa infection status according to the criteria describe above.
- Microbiological data q/3 months
- Follow up time in each group of categorization
- Pa eradication therapy as described above.

Once the excel database was designed and completed with all the data, the statistic study described in the following paragraphs was applied, obtaining the results that we are presenting in the following sections.

Statistical analyses

Due to the long-term nature of the follow-up of this study, the majority of the covariates of interest were non-time-varying, defined based on data from their first encounter in the cohort. These included gender, CFTR functional class (classes I-III vs classes IV-V), age at diagnosis (<= 2 years versus > 2 years), pancreatic status, and starting FEV1 % predicted measurement.

The exposure of interest in this study was the time-varying categorization of Pa status. Subjects are categorized in the Never group if, since cohort entry, or in their lifetime microbiology samples they never had Pa isolated from a respiratory tract sample. Subjects changed to the Eradicated group if they had a P.a infection, treated with the AET protocol, and subsequently cleared their infection at first post-AET regimen clinic visit. Finally, subjects changed to the Chronic group upon having a full AET regimen, which did not clear their Pa infection at first post-AET regimen culture, resulting in a clinical categorization of chronic Pa infection. This could have occurred on their first AET regimen, representing a shift from the Never to Chronic group, or after a previously successful AET attempt, representing a change from the Eradicated to the Chronic group.

The primary outcome for this study was the absolute FEV1 decline over time, with age being used as the time variable. This accounts for both the amount of time in the study cohort and simultaneously adjusts for age. FEV1 was reported as percent predicted using the GLI reference equations (18). Mixed-effects linear regression models were used to account for the repeated lung function measurements over time within each patient. Subjects could change exposure group from Never to Eradicated, Never to Chronic, or Eradicated to Chronic. Outcome slopes represent the absolute change in FEV1 % predicted per year, adjusted for age or any other specified covariate.

Qualitative variables are expressed in the form of absolute frequencies and percentages. Quantitative variables in the form of median (minimum and maximum values, and IQR). The comparison of the quantitative variables is done using The Student's test with a value of statistical significance being p less than 0.05

5 METHODS

6 RESULTS

A total of 205 patients were included in the analysis (Figure 1), of which 48% were female, 88% had a Class I-III mutation, 74% were diagnosed before the age of two years, and 90% were pancreatic insufficient (Table 1). The median (IQR) age at entry into the study was 5.6 years [5.1, 10.5 years] with a mean (SD) first reported FEV1 % predicted within the study period of 83.4% predicted (19.4%).

Of the 205 patients, 23 (11%) were censored at transplant, and 18 (9%) were censored at death. Mean (SD) follow-up time within the study was 17.3 years (2.7 years) (Table 1). During the study period, patients had a median (IQR) of 68 [51, 93] reportable PFTs performed, and 74 (47, 99) microbiology samples assessed. This represents a mean (range) of 5.9 (1, 35) PFTs per year and 5.2 (1, 27) microbiology samples per year.

Of the total 205 patients, 165 (80.5%) entered the study period as having Never had Pa and 40 (19.5%) entered as Eradicated. These 165 patients contributed a median (IQR) 2.8 years (1.0, 5.7 years) to the Never group. Among patients that had at least one Pa infection over the study period (n=203), the median (IQR) age at first infection was 9.6 years (5.6, 14.6 years). One year of 80 mg, inhaled tobramycin was the treatment course for 448 Pa infections among 186 patients in our cohort whereas 28 days of 300 mg tobramycin was used for 280 eradication attempts among 96 participants. The median (IQR) duration of time contributing to the Eradicated group was 10.2 years (5.7, 14.7 years) (n=172), and 8.8 years (4.5, 14.9 years) for the Chronic group (n=119).

At the age of 10 years of a total of 158 patients, 44 patients were in the Never group, 92 patients were in the Eradicated group and 22 patients were in the Chronic group. At the age of 15 years, of a total of 195 patients, 31 were in the Never group, 107 were in the Eradicated group, and 57 were in the Chronic group. At the age of 20 years of a total of 129 patients, 10 were in the Never group, 60 were in the Eradicated group, and 59 were in the Chronic group. Finally, at the age of 25 years of a total of 71 patients, two patients were in the Never group, 21 were in the Eradicated group, and 48 were in the Chronic group.

Table 1: Patient characteristics at study entry

Patient Characteristic	Total N=205
Age at study entry (years), median (IQR)	5.6 [5.1, 10.5]
Female, n (%)	98 (48%)
Age at diagnosis, n (%) <=2 years >2 years	 152 (74%) 53 (26%)

CFTR Functional Class, n (%)
  I-III
  IV-V
  Missing
	
	181 (88%)
	17 (8%)
	7 (3%)

Ethnicity, n (%)
  Caucasian
  Black
  Asian
  Other
	
	184 (90%)
	10 (5%)
	2 (1%)
	9 (4%)

Pancreatic Insufficient, n (%)	185 (90%)
Age at first *P. aeruginosa* infection (years), median (IQR)	9.6 [5.6, 14.6]
FEV_1 % predicted at study entry, median (IQR)	83.6 [68.7, 98.3]
Follow-up time in study (years), median (IQR)	18.3 [15.9, 19.3]

Follow-up time in each group (years), median (IQR)
  Never (n=165)
  Eradicated (n=172)
  Chronic (n=119)
	
	2.8 [1.0, 5.7]
	10.2 [5.7, 14.7]
	8.8 [4.5, 14.9]

Number of patients in each group at selected age snap-shots
  Age: 10 years
  Never
  Eradicated
  Chronic
  Age: 15 years
  Never
  Eradicated
  Chronic
  Age: 20 years
  Never
  Eradicated
  Chronic
  Age: 25 years
  Never
  Eradicated
  Chronic
	
	44
	92
	22
	
	31
	107
	57
	
	10
	60
	59
	
	2
	21
	48

IQR: interquartile range; CFTR: CF transmembrane conductance regulator; FEV1 % predicted: forced expiratory volume in one second, as measured by spirometry and standardized by GLI (32).

Lung function trajectories

Due to the long-term nature of this analysis, we considered non-time-varying covariates when developing the explanatory multivariable model. Covariates were attempted forward-stepwise based on univariable association with FEV1 decline over time, and kept in the model if they were statistically significant. While FEV1 % predicted at study entry and pancreatic status were kept in the final model, neither covariate substantially changed the effect estimate of the overall decline in FEV1 by Pa infection group.

The decline for the Never group was -0.77% per year (95% CI -0.94, -0.60%). In the final model (Table 2), the Eradicated group had an overall annual decline in FEV1 of -1.11% predicted/year (95% CI -1.18, -1.04) and the Chronic group had a decline of -1.57% predicted/year (95% CI -1.64, -1.50). This represented a 0.46% predicted per year less decline for subjects who successfully eradicated compared to those where AET failed to eradicate Pa and they developed chronic infection (95% CI 0.37, 0.55; p<0.001)(Figure 2).

Table 2: Patient characteristics at study entry

Covariate	Slope (95% CI)	p-value
Age (years)	-1.11%/year (95% CI -1.18, -1.04)	<0.001
*Eradication Status**		
Eradicated	0.46%/year (95% CI 0.37, 0.55)	<0.001
Chronic	Reference	
FEV_1 % predicted at study start**	0.50 (95% CI 0.38, 0.61)	<0.001
Pancreatic status		
Insufficient	Reference	0.003
Sufficient	11.38 (95% CI 3.86, 18.89)	

*Eradication status was entered in the model as an interaction with age; the slope refers to the additional decline in FEV1 per year on top of that of eradicated individuals, which would be the decline per year in the age row.

**Represents the difference in FEV1 for each unit increase in FEV1 % predicted at the study start.

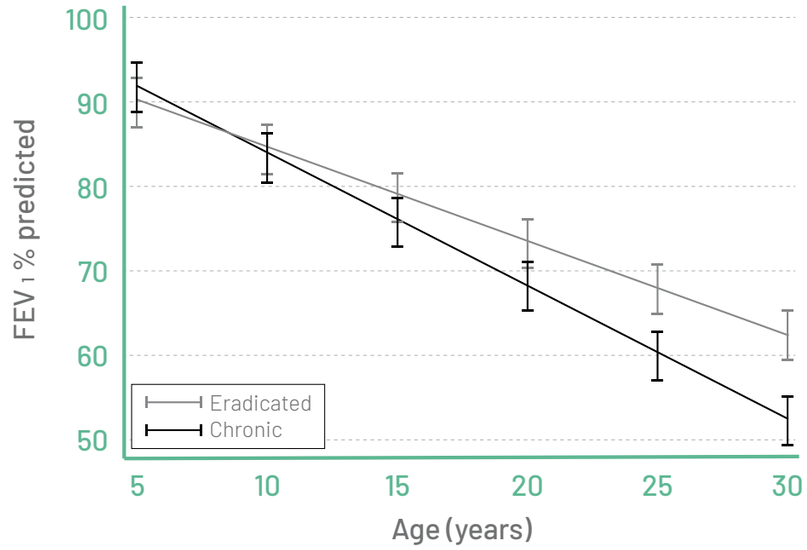


Figure 1: Progression of FEV1 % predicted among those who successfully eradicate Pa after AET initiation, compared to those who develop chronic Pa.

7 DISCUSSION

To the best of our knowledge, this is the first retrospective study with a large cohort of CF patients, demonstrating the effect of early eradication of Pa infection on long-term lung function decline in CF patients. We have observed in this 20-year retrospective study that there is a significantly less deterioration in FEV1 in those for whom Pa eradication was successful compared to those without and who developed a chronic infection. These findings confirm our initial hypothesis that successful Pa eradication therapy in patients with CF is associated with improved long-term lung function decline. According to the literature, as described by many authors like Ratjen et al. (20), Pa is one of the most common pathogens of CF lung disease. Pa infection begins as intermittent isolation of typically non-mucoid Pa strains from the airways. Unless treated with targeted antibiotic therapy, it will establish chronic infection in most individuals, with subsequent deterioration of pulmonary function.

We will begin analyzing the secondary aims of this work. In this line, the first secondary aim was to assess the age of the first Pa infection in our cohort of patients. In our study of 205 patients, 165 (80.5%) entered the study period as having Never had Pa and 40 (19.5%) entered as Eradicated. The median age in years (IQR) at the study entry was 5.6 years [5.1, 10.5]. The median (IQR) age of first Pa infection in this cohort of patients was of 9.6 years of age [5.6, 14.6]. This study's result contrasts those obtained by other authors in which the age of early Pa infection was lower that the age of initial Pa infection of our cohort of patients. In a recent original article published by Jackson and Waters (33), they reported that the initial age of Pa acquisition could be as early as within the first year of life (33); in this paper, they reviewed the incidence of Pa infection in different countries including Canada, the US and Australia. They reported that the initial age of Pa acquisition could be as early as within the first year of life reflect that in 2017, the Australian CF Registry reported that 5% of patients between 0-1 years old tested positive for Pa from respiratory tract cultures. In-between 2016-2018, they reported number of CF patients between the ages 0-2 that cultured positive for Pa ranged from 10-20% in Canadian and US CF patients' registries, with an increase to around 30% by age 18 years. However, in very young children, Pa is usually cultured from an oropharyngeal (OP) swab, and many do not reflect lower airway infection. Other authors like Douglas et al. (34), Liczak et al. (35) and Burns et al. (36) used bronchoalveolar lavage (BAL) specimens to detect Pa in young children and have reported acquisition rates of as low as 10% up to 33% in children under five or under six years of age, respectively. From these studies, Pa was detected in the lower airways of CF patients as early as three months of age, and the average age of acquisition was over two years old. In our study, we did not analyze early Pa infection, as most of the authors were studying the first Pa infection as early infection; otherwise, we analyzed the age of the first Pa infection at cohort entry. As explained above, the age of the first Pa infection in our cohort of patients was higher than the age reported in the literature. A primary factor explains the difference found within the age of initial Pa infection of our cohort of patients and the results of the literature, which is the inclusion criterion that were used in the study.

The second secondary aim of our work was to assess the percentage of patients in each group of categorization over time, the time that patients were in each group of status infection and the number of patients over infection group and at selected age snapshots time in each status. Of the 205 patients followed for 20 years, 165 (80.5%) entered the study period as the Never group; these 165 patients contributed a median (IQR) of 2.8 years [1.0, 5.7 years] to this group; 40 patients (19.5%) entered as the Eradicated group. Over the study period time, 172 patients (83%) contributed to this group with a median (IQR) duration of contributing to the Eradicated group of 10.2 years [5.7, 14.7 years]. Finally, 119 patients (58%) were in the Chronic group at some point in the study and contributed a median (IQR) of 8.8 years [4.5, 14.9 years] for this group. The exposure of interest of this study was the time-varying categorization of Pa status infections. Therefore, categorizing groups of Pa status in each moment were not static but dynamic, meaning that one patient could move from one categorization group to another, depending on its status infection. No published studies analyze the status of Pa infection over 20 years and allow patients included to change from different categorization groups over time.

Regarding the number of patients over infection group and at selected age snapshots time in each status. At the age of 10 years, of a total of 158 patients, 44 were in the Never group, 92 were in the Eradicated group, and 22 were in the Chronic group. At the age of 15 years, of a total of 195 patients, 31 were in the Never group, 107 were in the Eradicated group, and 57 were in the Chronic group. At the age of 20 years of a total of 129 patients, ten were in the Never group, 60 were in the Eradicated group, and 59 were in the Chronic group. Finally, at the age of 25 years of a total of 71 patients, two patients were in the Never group, 21 were in the Eradicated group, and 48 were in the chronic group (table 1).

Analyzing the Never group, 165 (80.5%) entered the study period as the Never group; these 165 patients contributed a median (IQR) of 2.8 years [1.0, 5.7 years] to the Never group and the age of first Pa infection was of 9.6 years [5.6, 14.6] as previously mentioned. If we analyze the results of the age snapshot, we can see that in the Never group, at the age of 10 years, 44 (28%) patients were free of Pa infection; at the age of 15 years, 31 (15%) patients, still were free of a Pa infection, at the age of 20 years 10 patients (7.7%) were in the Never group. Finally, at the age of 25 years, two patients (3%) remained free from Pa infection. As we read through these results, age is a significant cause of Pa acquisition; therefore, patients with CF will have an infection by Pa at some point in their natural history. However, if the first Pa infection occurs later the morbidity associated with Pa infection in CF patients will be reduced. In this line, authors such as Emerson et al. (21) and Ronsefeld et al. (37) refer that the impact of Pa infection is established by 8 to 13 years of age. Among subjects whom Pa was isolated during the first five years of life, the risk of death during 8 years of following/up was 2.6 times higher than among subjects whom Pa was not isolated early in life. Our promising results regarding the time patients were free from Pa infection during the study period which was a media (IQR) of 2.8 years [1.0, 5.7], are probably related to the implementation of the NBS and the specialized multidisciplinary unit of CF patients that has been running for a long time in the Hospital of Sick Children of Toronto. The results presented in the this paragraph reassure the importance of the presence of multidisciplinary units of CF patients to achieve an early detection and early monitoring of status infection in patients with CF to prevent lung damage.

Analyzing the Eradicated group, 40 (19.5%) entered as this group. During the 20 years of follow-up, 172 (83%) patients contributed to the Eradicated group with a median time (IQR) of 10.2 years [5.7, 14.7]. The number of patients over time in the Eradicated group and at selected age snapshots was: at the age of 10 years, 92 (58%) patients were in this group; at the age of 15 years, 107 (54%) of patients; at the age of 20 years, 60 (46.5%) patients. Finally, at the age of 25 years, 21 (29.5%) patients were in this group. First of all, from this data, we can re-assert that with the implementation of NBS, with the specialized multidisciplinary unit of CF patients that has been running for a long time in the Hospital of Sick Children of Toronto, with the early detection and eradication of Pa infection and the use of effective AET therapies to clear Pa infection, the rate of successful eradication was as high as 172 patients (83%). This eradication success rate was due to the AET therapy mentioned in the methods section, used in our population according to the protocol used at the Sick Children's Hospital during the study period. Regarding AET therapy and its effectiveness in clearing Pa infections and leading patients free from Pa infection, many studies have been conducted (38); most of these studies report an 80 to 100% eradication success rate with a median time to Pa recurrence of 7.5 to 26 months (38) These data are consistent with the rate of Eradication success previously reported in our study. Regarding the period that patients contributed to the Eradicated group, our cohort of patients contributed to this group with a median (IQR) of 10.2 years [5.7, 14.7]. These results differ from the studies published in the literature, reporting a median time to Pa recurrence of 7.5 to 26 months (38). This difference is again related to one main factor, which is the study design. Patients may have had more than one infection and subsequent clear their infection after a complete AET regimen, described in the methods section (page 47). As long as they eradicate that subsequent infection, they would remain in the Eradicated group, the only move to the chronic group upon failing a complete AET regimen. One of the most representative trials analyzing AET therapy and its effectiveness is the

one performed by Ratjen et al. (24). The ELITE trial was an open-label randomized multicenter study, which tried to determine the optimal length of inhaled tobramycin treatment for Pa eradication and the median time to recurrence of Pa infection. The effect of 28 versus 56 days of treatment with inhaled tobramycin (300 mg twice daily) was compared. Both open-label regimens were effective in clearing the organism. The study suggested that treatment with 56 days of inhaled tobramycin does not have an additional benefit over 28 days of therapy (24) . In this study, over 90% of the randomized patients in the 28-day and 56-day tobramycin inhaled solution (TIS) groups had negative cultures for Pa 1 month after the treatment. Most of these patients remained free of infection for up to 27 months. Other studies have tried to assess the optimal AET therapy for Pa infection as the one performed by Blanchard et al. (39) and the randomized studies performed by Treggiari et al. (40). Nevertheless, questions remain on the optimal way of administration of antibiotics (intravenous/oral/inhalation or combinations) and the optimal treatment duration for long-lasting eradication. Finally, analyzing the number of patients over time in the Eradicated group and at selected age snapshots, our results again show that AET therapy is highly effective. However, at an older age, patients will become chronically infected by Pa; therefore, two main points concerning Pa infection must be pointed out in this discussion. On the one hand, the importance of early detection and eradication of Pa infection, and on the other hand, the importance of keeping patients without Pa infection or in an eradicated status to postpone chronic Pa infection and prevent the detriment of morbidity and mortality associated with Pa infection.

Regarding the chronic group, our results related with this group chronic show that 119 patients (58% of patients) at some point in the study period become chronically infected by Pa according to our categorization of Chronic Pa infection. During the 20 years of follow-up, these patients contributed a median (IQR) of 8.8 years [4.5, 14.9 years] for the chronic Pa infection group. The number of patients over time in the Chronic group and at selected age snapshots was: at the age of 10 years, 22 (14%) patients were in this group at the age of 15 years, 57 (29%) patients; at the age of 20 years of a total of 59 (45.7%) patients. Finally, at the age of 25 years, 48 (67.6%) of patients were in the Chronic group. These results are consistent or even slightly better than the ones reported in the literature, probably due to the early implementation of the AET therapy protocol described in the methods section (page 47) for Pa infection in the SickKids Hospital. Authors like Rosenfeld et al. (37) refer to an increasing prevalence of chronic Pa infection with age. The data published by the annual CF report of 2001; as many as 20-30% of infants, 30 to 40% of children to 2 to 10 years of age, approximately 60% of adolescents and approximately 80% of adults with CF were infected by Pa. Regarding the percentage of Chronic infection; our results are consistent with the data presented by the 2013 annual reports of the UK reporting a 60% of patients chronically infected by Pa and by the American Cystic Fibrosis Foundation reporting a 60% of chronic infections. More recent reports of chronic Pa infection, such as the 2018 American Annual Cystic Fibrosis Foundation report, refer that Pa status using Leed´s criteria showed that 45,3% had a chronic pattern of infection(41). Other reports like as Garcia Clemente el al. (42) published in 2017 refer that CF units in Spain have reported chronic Pa infection in 46% of people with CF (42). Despite in our cohort of patients, we did not categorized Chronic status as per Leed´s criteria, patients categorized as Chronic infection had more than 50% positive Pa cultures in one year; therefore, our results are comparable with the results of the studies using Leed´s criteria to define Chronic Pa infection. Therefore, the difference between our results and the results of these studies in the percentage of chronic Pa infection is that our study was a retrospective study of 20 years of follow up from 1997 to 2018. During these 20 years, several new therapies have been introduced in treatment for CF patients; therefore, Pa infection has decreased over the last few years. In their review paper, Jackson et al. (33) refer that the decreasing prevalence of Pa may be due to several factors, either host, environmental or pathogen-related. One of the possible explanations is the widespread adoption and success of the early Pa antimicrobial eradication program, as previously discussed in the Eradication section. In their retrospective cohort study, Crull et al. (43) observed a significant decrease in the risk rate of developing chronic Pa infection between 2003 and 2012. The decreased incidence of Pa may be attributable to several

changes, like introducing eradication therapies for Pa infection and introducing CFTR modulator agents. In this line, Heltshe et al. (44) demonstrated significantly reduced odds of isolating Pa from airways and a 23% reduction in mucoid Pa following ivacaftor therapy compared to culture data prior to initiation. Despite the differences in the design and length of our study compared with other studies published in the literature, there are few differences observed between our results and those published in the literature.

In the following paragraphs, we are going to discuss both the third secondary aim and the primary aim of the work, which were, respectively; to assess the baseline FEV1% predicted within the study entry and to assess the long-term differences in lung function decline between the Eradicated and Chronic groups.

Regarding baseline lung function, in our cohort of patients, the mean first reported FEV1% predicted within the study period was 83.6% [68.7, 98.3]. As previously explained in our study, 165 (80.5%) patients entered the study period as having Never had Pa, and 40 (19.5%) patients entered as Eradicated. Therefore the 83.6% predicted FEV1 value accounted for both the Eradicated and the Never groups. These results are comparable with the results of baseline FEV1% published by Konstan et al. (45). They analyzed the rate of decline of FEV1 in children and adolescents with CF. They identified and compared risk factors associated with FEV1 decline. They reported that baseline FEV1 of patients at ages 6-8 years was 88.4% +/- 20.5%, at ages 9-12 years, FEV1 was 85.3% +/- 20.8 and at ages between 13-17 years, FEV1 was 78.4%+/-22.

Finally, we will analyze the primary aim which was assessing long-term differences in lung function decline between the Eradicated and Chronic groups and try to confirm our initial hypothesis, defending that successful Pa eradication therapy in patients with Cystic Fibrosis is associated with improved long-term lung function. In our cohort of patients, the Eradicated group had an overall annual decline in FEV1 of -1.11% predicted/year (95% CI -1.18, -1.04). The Chronic group had a decline of -1.57% predicted/year (95% CI -1.64, -1.50). This represented a 0.46% predicted per year less decline for subjects who successfully eradicated compared to those where AET failed to eradicate Pa, and they developed a chronic infection (95% CI 0.37, 0.55; p<0001) (Figure 2). Assessing the primary aim regarding long-term differences in lung function decline between the Eradicated and Chronic groups and therefore looking in general at the effect of AET on long-term lung function, we see that there are few previous studies examining the effect of AET on Pa infection on measures other than microbiology in CF. Zemanick et al. (46) evaluated clinical outcomes associated with initial isolation of Pa in a large CF cohort. Pa acquisition was associated with an increased rate of pulmonary exacerbations, more frequent detection of crackles or wheeze on physical exam and increased risk for emergence of MRSA , Stenotrophomonas maltophilia and Achromobacter xylosoxidans on respiratory cultures. However, there was no association between initial acquisition of Pa and more rapid decline in lung function or change in growth parameters. Similarly, Mayer-Hamblett et al. (47) in their five year follow-up study of over 200 children with CF who underwent Pa eradication treatment, observed increased antimicrobial usage in individuals who failed AET versus those who successfully cleared Pa, but there was no association between eradication status and clinical outcomes including lung function decline over 5 five years following treatment. Both of the aforementioned studies did not find an association between AET and FEV1 decline, consistent with prior studies performed in that line (46) (47), but had shorter follow up periods than the current study. In a study with a follow up period of several decades, Frederiksen et al. (48) compared CF patients treated with an intensive protocol consisting of colistin inhalations and oral ciprofloxacin at the time of initial Pa colonization with historic controls who had never participated in any early-treatment trials. They found that aggressive treatment maintained or increased pulmonary function during the year after inclusion, compared with the control group. While these data are consistent with our current findings, the use of historic controls limits a direct comparison of a treatment effect. In this study we have chosen to show, in Figure 2, the long-term differences in lung function decline between the Eradicated and

Chronic groups, we did look into the differences between the Never group and the Eradicated group. The challenge is that our question focuses on the long-term effects on lung function, and there are very few patients who stay in the Never group for a long time. As is shown in Table 1, the median (IQR) total time contributed to the Never group was 2.8 [1.0, 5.7] years, which is significantly less than the time contributed to either the Eradicated or Chronic groups. While we presented the annual decline in the Never group in the second paragraph of the Results section: lung function trajectories section, we felt decided that presenting the progression of FEV1 of the Never group in our final model and comparing to the other groups was not appropriate. In previously study performed by Amin et al. (30) they demonstrated that CF patients in whom Pa infection was cleared had similar FEV1 trajectories over subsequent years compared to children who were never infected (30) .

There were several strengths as well as limitations to our study. The main strength is the 20 years of follow-up data (prospectively collected database). Moreover, strengths include the length of time, the number of encounters that the study has, and the thoroughness of the dataset. The dataset has every hospital encounter over the time frame at either site, including the following patients transitioning from a pediatric to an adult site. The data set also had every culture, whether it was associated with admission, a hospital visit, or just dropped off. Finally, the dataset also has every technically acceptable spirometry measurement during the time frame.

The statistical analysis allowed us to detect the change in long-term lung function decline. There were considered as many common confounders in the final model as there were available, which include age, gender, CFTR functional class, ethnicity, and pancreatic status. While there could be confounders we did not consider, those would be confounders that are difficult to measure or are not standard in the literature.

The limitations were the retrospective nature of the study, not allowing direct comparison of treatment versus no treatment for early Pa infection, which would not be possible given that AET is the standard of care for CF patients.

There were some changes in the AET protocol over the 20-year study period, namely the switch from 1 one year of TIS (80 mg/2ml) to 28 days of TIS (300 mg/5 ml), but we have previously shown these two regimens to be comparable in terms of AET efficacy. However, the change in care over time was accounted for by using age as a covariate in the model. All the studies collecting data through decades could suffer from implementing changes in the protocols that affect the measured variable. Given the study's retrospective nature, we cannot prove causality between AET and improved lung function trajectory, and other factors could be responsible for the observed group differences.

Nevertheless, the statistical analysis adjusted by some of these factors suggests that eradication is probably an important protective factor in avoiding a faster decline in respiratory function.

FEV1 decline could have been assessed FEV1 as non-linear, but by assessing long-term decline as linear, we have presented results that are directly comparable to what has been presented in the past, allowing for an expansion of the literature.

Pulmonary exacerbation was not assessed as an outcome that has previously been reported to differ between individuals with successful Pa eradication and those who fail AET. Different time points rather than the initial culture end of AET could be chosen as an endpoint for successful eradication. Finally, the success of an eradication protocol can be influenced by multiple factors, including Pa phenotypes as described before and host factors affecting bacterial clearance and adherence to treatment that we cannot control for in this analysis.

7 DISCUSSION

8 CONCLUSIONS

After analyzing our data, we have obtained the follow conclusions:

1 During the 20 years of follow up of the study the Eradicated group showed a 0.46% predicted FEV1 per year less decline compared to the Chronic group ($p<0.001$).

2 The median age (IQR) of acquisition Pa infection in our cohort was 9.6 years [5.6, 14.6].

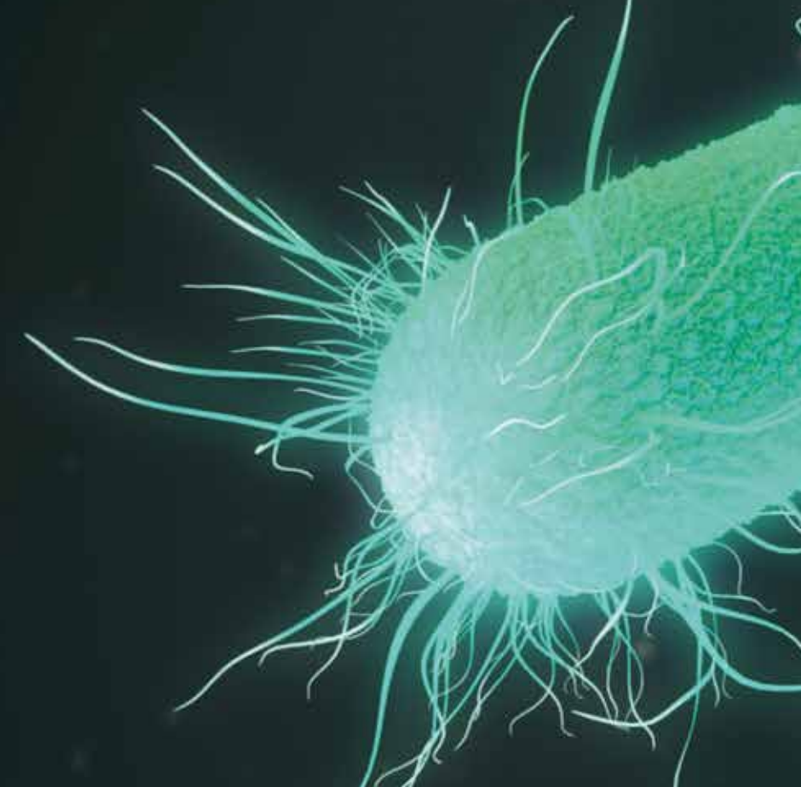
3 The percentage of patients that contributed at some point of the study for the Never group was of 80.5% of patients; 83% patients was for the Eradicated group and 58% of patients became Chronic at some point in the study.
The categorization group with more years of follow up was the Eradicated group with a median (IQR) of 10.2 years, followed by the Chronic group with a media (IQR) of 8.8 years. Finally the Never group had a median (IQR) of 2.8 years.
The number of patients overtime in each group of Pa status infection at selected age snapshots showed a huge reduction in both the Never and the Eradicated group. By contrast the Chronic group had a great increase in the number of patients; going from 22 patients at 10 years to 48 patients at 25 years of age. Expected results according to the natural history of CF disease.

4 Patients at the entry of the study, representing both the Never and the Eradicated group, with a median (IQR) age of 5.6 years [5.1, 10.5] had a good pulmonary function test with a value of FEV1% predicted of 83.4% [68.7, 98.3].

5 We have demonstrated for the first time that successful Pa eradication therapy is associated with improved long-term lung function.

8 CONCLUSIONS

9 FUTURE LINES OF RESEARCH



9 FUTURE LINES OF RESEARCH

In this study, we have demonstrated that early eradication of Pa infection impacts long-term lung function decline in CF patients. Therefore the future lines of research and potential questions that could be addressed are:

First, a potential line of future research is to implement new monitoring techniques to early detect the detrimental effect of Pa infection on lung function and, therefore, to improve clinical outcomes in CF patients. Nowadays, the universal techniques used to monitor lung disease in CF patients are the FEV1% predicted the decline and the HRCT images. In this line, since most patients are asymptomatic when a new infection with Pa is detected in respiratory cultures, early infection is not usually associated with deterioration in lung function, and treatment has no immediate effects on the most commonly used lung function measure, the FEV1. There are other newer techniques as lung clearance index (LCI), to monitor early disease in CF, but recent data suggest that this is also the case for this sensitive outcome measure. Therefore, finding a new technique to monitor lung disease that could be sensitive enough to detect early effects of Pa infection on lung function, feasible and economically sustained, could be another potential line of research.

Secondly, another line of potential research would be to find other diagnostic tools for Pa infection which were more sensible that the sputum culture or the Nasopharyngeal swab, and less invasive than BAL samples.

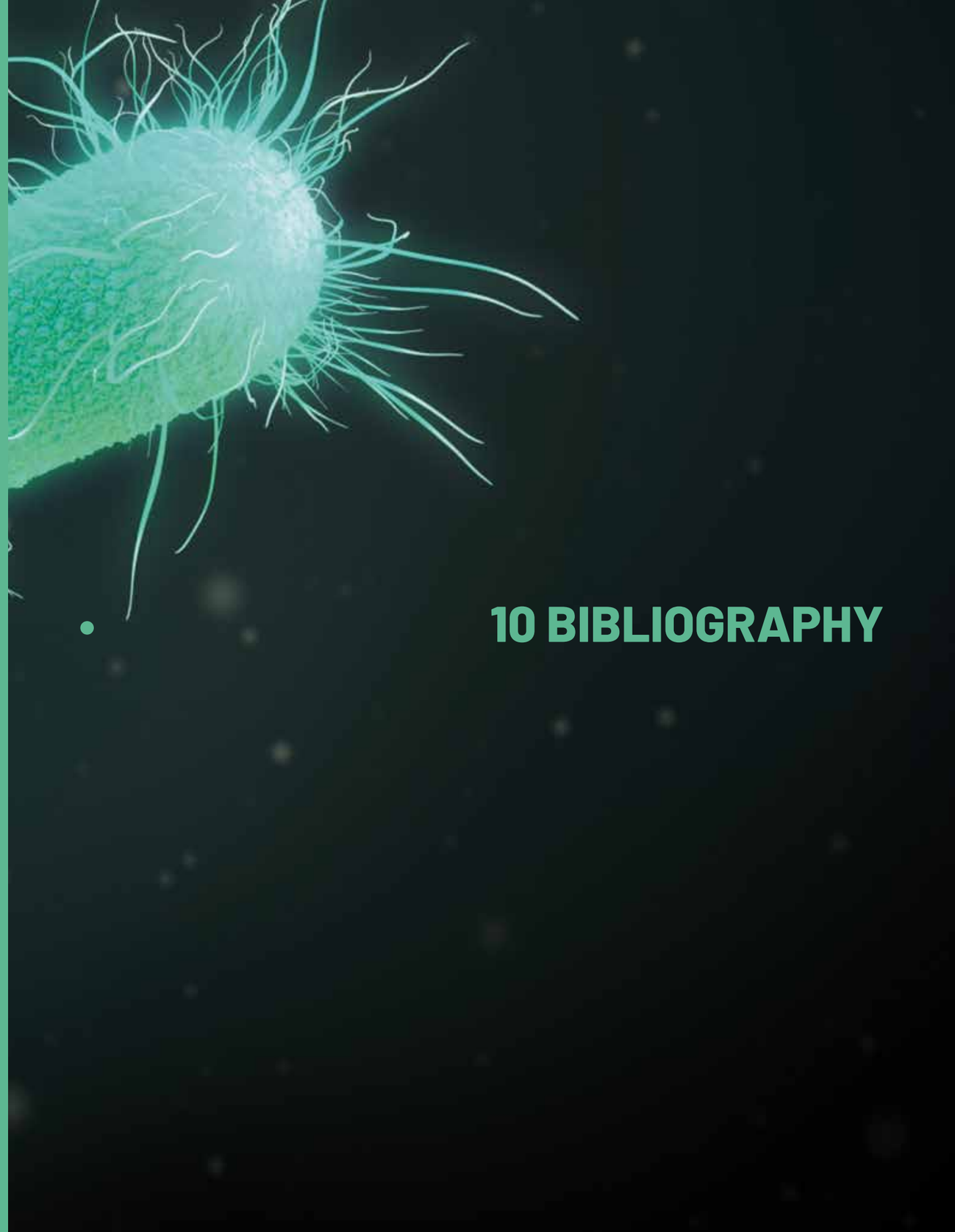
Thirdly, it would be exciting to study potential mechanisms to clear Pa infection more effectively as a key to protecting lung health and improving clinical outcomes in CF. In this line, several therapies could be studied in the future:

- Therapies that restore CFTR function can enhance mucociliary clearance and either delay or prevent Pa infection. Identifying highly effective modulator therapy for individuals with all CF genotypes could be a research line.
- On the other hand, immune-modulating agents could help the host eradicate Pa from the airways. Several studies are currently conducted; to define whether this therapy, used in conjunction with antimicrobial therapy, can improve the eradication of Pa from CF airways.
- Finally, as the effectiveness of antibiotics is often limited in the CF lung environment, adjuvant compounds may have a role that can target bacterial biofilms. In this line, several studies are currently conducted

Fourthly, study the potential factors contributing to a failure to eradicate Pa within the CF lung. These factors include:

- Host factors include CFTR dysfunction leading to impairment in the function of monocytes, alveolar macrophages and neutrophils.
- Bacterial factors such as: Chronic phenotype leads to decreased motility, mucoid, wrinkly phenotype, biofilm formation and persister cells.
- Polymicrobial infections such as lack of protective effect of anaerobes; interaction of Pa with MSSA leading to tobramycin tolerance.
- Conditions limiting antimicrobial effectiveness such as: Anaerobic environment, low pH, slows bacterial growth and bacterial biofilm formation.

Finally to study new antibiotic compounds with a higher effectivity to treat
Pa infection and with an easily delivery in order to facilitate treatment to the Cystic Fibrosis patients, especially children, and to improve their quality of life.



10 BIBLIOGRAPHY

1. Elborn JS. Cystic fibrosis. Lancet. 2016;388(10059):2519–31.

2. Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, et al. The future of cystic fibrosis care: a global perspective. Lancet Respir Med. 2020;8(1):65–124.

3. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet [Internet]. 2009;373(9678):1891–904. Available from: http://dx.doi.org/10.1016/S0140-6736(09)60327-5

4. Walters S MA in H and G. Cystic Fibrosis 3rd Ed. Cystic Fib. ed. Hodson ME, Geddes DM BA, editor. London: Hodder Arnold; 3rd New edition (25 May 2007); 2007. 21–45 p.

5. Verma N, Bush A, Buchdahl R. Is There Still a Gender Gap in Cystic Fibrosis? Chest [Internet]. 2005;128(4):2824–34. Available from: http://dx.doi.org/10.1016/S0012-3692(15)52709-8

6. Alexandra L. Quittner AML-R. 20 Facts about Cystic Fibrosis. Adherence Self-Management Pediatr Popul [Internet]. 2020;196:107–32. Available from: www.informahealthcare.com

7. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: A consensus statement. J Pediatr. 1998;132(4):589–95.

8. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, et al. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. J Pediatr [Internet]. 2017;181:S4-S15.e1. Available from: http://dx.doi.org/10.1016/j.jpeds.2016.09.064

9. Keown K, Abbott S, Kuzeljevic B, Rayment JH, Chilvers MA, Yang CL. An investigation into biomarkers for the diagnosis of ABPA and aspergillus disease in cystic fibrosis. Pediatr Pulmonol [Internet]. 2019;54(11):1787–93. Available from: http://dx.doi.org/10.1002/ppul.24465

10. Hospital RB, Alexander S, Alshafi K, Al-yaghchi C, Anderson A, Balfour- I, et al. Clinical Guidelines : Care of Children with Cystic Fibrosis. 2020;

11. Sacks FM, Harris A, Sc M, Johnson DW, Ph D, Kesselhut J, et al. New England Journal CREST. Science (80-). 2009;360:609–19.

12. Robert G, Stevens A, Colin-Jones D. Dornase alfa for cystic fibrosis. Bmj. 1995;311(7008):813.

13. Bilton D, Robinson P, Cooper P, Gallagher CG, Kolbe J, Fox H, et al. Inhaled dry powder mannitol in cystic fibrosis: An efficacy and safety study. Eur Respir J. 2011;38(5):1071–80.

14. Stanojevic S, Waters V, Mathew JL, Taylor L, Ratjen F. Effectiveness of inhaled tobramycin in eradicating *pseudomonas aeruginosa* in children with cystic fibrosis. J Cyst Fibros [Internet]. 2014;13(2):172–8. Available from: http://dx.doi.org/10.1016/j.jcf.2013.09.002

15. Schneiderman JE, Wilkes DL, Atenafu EG, Nguyen T, Wells GD, Alarie N, et al. Longitudinal relationship between physical activity and lung health in patients with cystic fibrosis. Eur Respir J. 2014;43(3):817–23.

16. Saiman L, Marshall BC, Mayer-hamblett N, Burns JL, Quittner AL, Cibene D a, et al. Azithromycin in Patients With. J Am Med Assoc. 2003;290(13):1749–56.

17. Hirche TO, Knoop C, Hebestreit H, Shimmin D, Solé A, Elborn JS, et al. Practical guidelines: Lung transplantation in patients with cystic fibrosis. Pulm Med. 2014;2014.

18. Plant BJ, Goss CH, Plant WD, Bell SC. Management of comorbidities in older patients with cystic fibrosis. Lancet Respir Med [Internet]. 2013;1(2):164–74. Available from: http://dx.doi.org/10.1016/S2213-2600(13)70025-0

19. Smyth AR, Bell SC, Bojcin S, Bryon M, Duff A, Flume P, et al. European cystic fibrosis society standards of care: Best practice guidelines. J Cyst Fibros [Internet]. 2014;13(S1):S23–42. Available from: http://dx.doi.org/10.1016/j.jcf.2014.03.010

20. Bendiak G, Ratjen F. CFTR, Mucins, and Mucus in Cystic Fibrosis. Semin Respir Crit Care Med. 2009;30(05):587–95.

21. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol. 2002;34(2):91–100.

22. Blanchard AC, Rooney AM, Yau Y, Zhang Y, Stapleton PJ, Horton E, et al. Early detection using qPCR of *Pseudomonas aeruginosa* infection in children with cystic fibrosis undergoing eradication treatment. J Cyst Fibros [Internet]. 2018;17(6):723–8. Available from: https://doi.org/10.1016/j.jcf.2018.02.008

23. Ratjen F, Moeller A, McKinney ML, Asherova I, Alon N, Maykut R, et al. Eradication of early *P. aeruginosa* infection in children <7 years of age with cystic fibrosis: The early study. J Cyst Fibros [Internet]. 2019;18(1):78–85. Available from: https://doi.org/10.1016/j.jcf.2018.04.002

24. Ratjen F, Munck A, Kho P, Angyalosi G. Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: The ELITE trial. Thorax. 2010;65(4):286–91.

25. Early T. Comparative Efficacy and Safety of 4 Randomized Regimens to Treat Early. Arch Pediatr Adolesc Med. 2011;165(9):847–56.

26. Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, Khan U, Kulich M, Kronmal R, et al. Comparative efficacy and safety of 4 randomized regimens to treat early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. Arch Pediatr Adolesc Med. 2011;165(9):847–56.

27. Mayer-Hamblett N, Kronmal RA, Gibson RL, Rosenfeld M, Retsch-Bogart G, Treggiari MM, et al. Initial *Pseudomonas aeruginosa* treatment failure is associated with exacerbations in cystic fibrosis. Pediatr Pulmonol. 2012;47(2):125–34.

28. Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF, et al. Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. J Pediatr. 2001;138(5):699–704.

29. Stanojevic S, Davis SD, Retsch-Bogart G, Webster H, Davis M, Johnson RC, et al. Progression of lung disease in preschool patients with cystic fibrosis. Am J Respir Crit Care Med. 2017;195(9):1216–25.

30. Amin R, Lam M, Dupuis A, Ratjen F. The effect of early *Pseudomonas aeruginosa* treatment on lung function in pediatric cystic fibrosis. Pediatr Pulmonol. 2011 Jun;46(6):554–8.

31. Santos S, Perrem L, Shaw M, Hewko S, Sanders DB, Davis S, Stanojevic S, Solomon M, Grasemann H, Sweezey N, Waters V, Ratjen FSantos S, Perrem L, Shaw M, Hewko S, Sanders DB, Davis S, Stanojevic S, Solomon M, Grasemann H, Sweezey N, Waters V RF. LCI response to acquisition and eradication of *Pseudomonas aeruginosa* in children with CF. North American Cystic Fibrosis Conference, October 2020, Phoenix, USA (virtual conference). Pediatr Pulmonol., 48(S36): S471. Pediatr Pulmonol. 2020;55:S38–361.

32. Quanjer PH, Cole TJ, Hall GL, Culver BH. Report of the Global Lung Function Initiative (GLI), ERS Task Force to establish improved Lung Function Reference Values, includng supplement. Eur Respir J. 2013;40(6):1324–43.

33. Jackson L, Waters V. Factors influencing the acquisition and eradication of early *Pseudomonas aeruginosa* infection in cystic fibrosis. J Cyst Fibros [Internet]. 2021;20(1):8–16. Available from: https://doi.org/10.1016/j.jcf.2020.10.008

34. Douglas TA, Brennan S, Gard S, Berry L, Gangell C, Stick SM, et al. Acquisition and eradication of *P. aeruginosa* in young children with cystic fibrosis. Eur Respir J. 2009;33(2):305–11.

35. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clin Microbiol Rev. 2002;15(2):194–222.

36. Burns JL, Gibson RL, McNamara S, Yim D, Emerson J, Rosenfeld M, et al. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. J Infect Dis. 2001;183(3):444–52.

37. Rosenfeld M, Ramsey BW, Gibson RL. Pseudomonas acquisition in young patients with cystic fibrosis: Pathophysiology, diagnosis, and management. Curr Opin Pulm Med. 2003;9(6):492–7.

38. Schelstraete P, Haerynck F, Van daele S, Deseyne S, De Baets F. Eradication therapy for *Pseudomonas aeruginosa* colonization episodes in cystic fibrosis patients not chronically colonized by *P. aeruginosa*. J Cyst Fibros [Internet]. 2013;12(1):1–8. Available from: http://dx.doi.org/10.1016/j.jcf.2012.07.008

39. Blanchard AC, Horton E, Stanojevic S, Taylor L, Waters V, Ratjen F. Effectiveness of a stepwise *Pseudomonas aeruginosa* eradication protocol in children with cystic fibrosis. J Cyst Fibros [Internet]. 2017;16(3):395–400. Available from: http://dx.doi.org/10.1016/j.jcf.2017.01.007

40. Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, Khan U, Kulich M, Kronmal R, et al. Comparative efficacy and safety of 4 randomized regimens to treat early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. Arch Pediatr Adolesc Med. 2011 Sep;165(9):847–56.

41. Cystic Fibrosis Foundation. 2018 Patient Registry Annual Data Report. Cyst Fibros Found Patient Regist 2018 Annu Data Rep [Internet]. 2018;92. Available from: https://www.cff.org/Research/Researcher-Resources/Patient-Registry/2018-Patient-Registry-Annual-Data-Report.pdf%0Ahttp://www.cff.org/UploadedFiles/research/ClinicalResearch/Patient-Registry-Report-2009.pdf

42. Garcia-Clemente M, de la Rosa D, Máiz L, Girón R, Blanco M, Olveira C, et al. Impact of *Pseudomonas aeruginosa* Infection on Patients with Chronic Inflammatory Airway Diseases. J Clin Med. 2020;9(12):3800.

43. Crull MR, Somayaji R, Ramos KJ, Caldwell E, Mayer-Hamblett N, Aitken ML, et al. Changing Rates of Chronic *Pseudomonas aeruginosa* Infections in Cystic Fibrosis: A Population-Based Cohort Study. Clin Infect Dis. 2018;67(7):1089–95.

44. Heltshe SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW, et al. *Pseudomonas aeruginosa* in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. Clin Infect Dis. 2015;60(5):703–12.

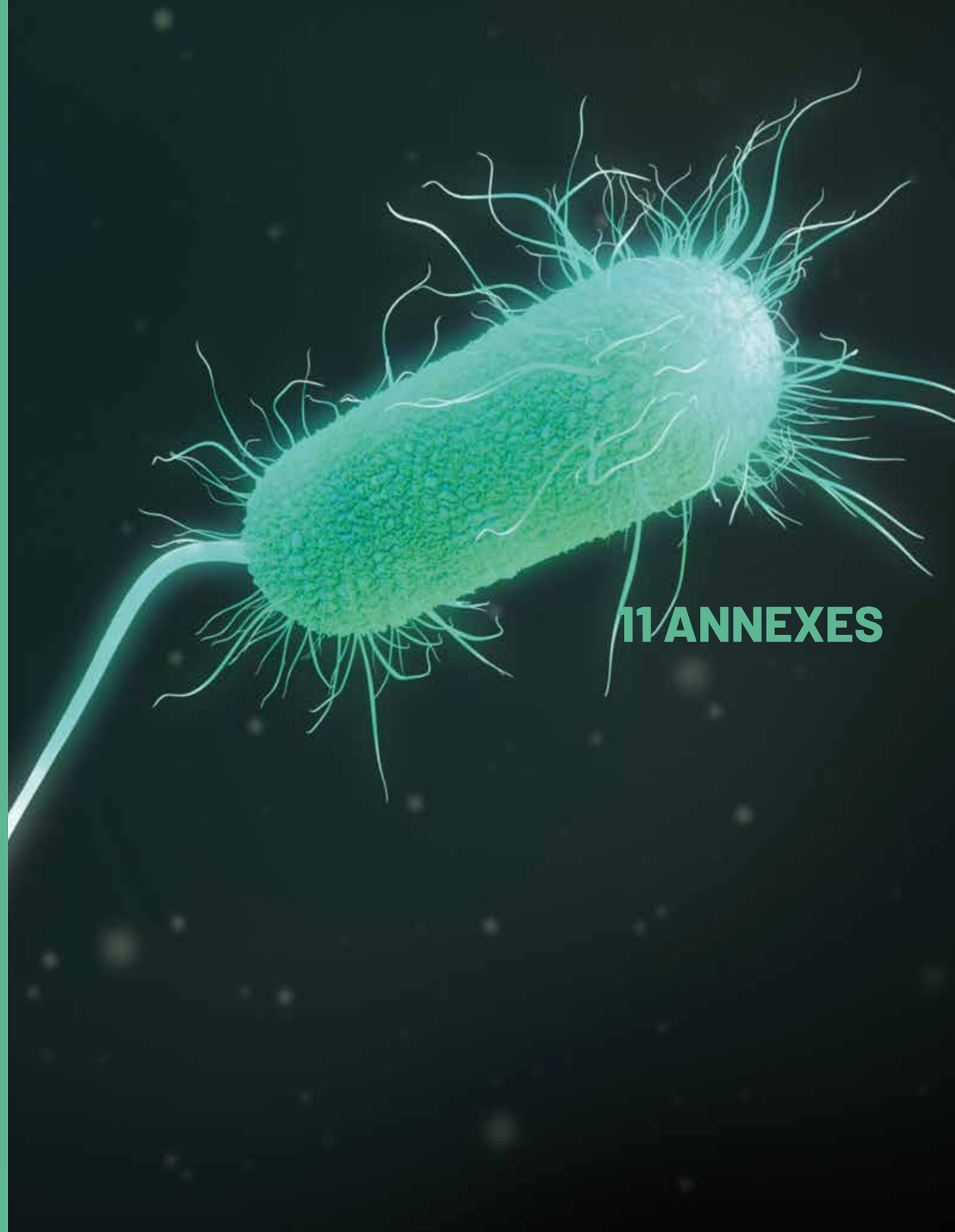
45. Konstan MW, Morgan WJ, Butler SM, Pasta DJ, Craib ML, Silva SJ, et al. Risk Factors For Rate of Decline in Forced Expiratory Volume in One Second in Children and Adolescents with Cystic Fibrosis. J Pediatr. 2007;151(2).

46. Zemanick ET, Emerson J, Thompson V, McNamara S, Morgan W, Gibson RL, et al. Clinical outcomes after initial pseudomonas acquisition in cystic fibrosis. In: Pediatric Pulmonology. John Wiley and Sons Inc.; 2015. p. 42–8.

47. Mayer-Hamblett N, Kloster M, Rosenfeld M, Gibson RL, Retsch-Bogart GZ, Emerson J, et al. Impact of sustained eradication of new *pseudomonas aeruginosa* infection on long-term outcomes in cystic fibrosis. Clin Infect Dis. 2015;61(5):707–15.

48. Frederiksen B, Koch C, Høiby N. Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Pediatr Pulmonol. 1997;23(5):330–5.

10 BIBLIOGRAPHY



11 ANNEXES

ANNEX 1

Isabel Gascon Casaredi, Michelle Shaw, Valerie Waters, Ryan Seeto, Ana C. Blanchard, Felix Ratjen. Impact of antibiotic eradication therapy of *Pseudomonas aeruginosa* on long term lung function in cystic fibrosis, Journal of Cystic Fibrosis, 2022, ISSN 1569-1993, https://doi.org/10.1016/j.jcf.2022.08.007.

11 ANNEXES

JID: JCF [m5G;August 25, 2022;19:26]

Journal of Cystic Fibrosis xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Impact of antibiotic eradication therapy of Pseudomonas aeruginosa on long term lung function in cystic fibrosis

Isabel Gascon Casaredi[a,b], Michelle Shaw[c], Valerie Waters[c,d], Ryan Seeto[c], Ana C. Blanchard[d], Felix Ratjen[a,b,c,*]

[a] Division of Respiratory Medicine, Department of Pediatrics, Hospital Sant Joan de Déu de Barcelona, Universidad de Barcelona, Barcelona, Spain
[b] Division of Respiratory Medicine, Department of Paediatrics, University of Toronto, Toronto, Canada
[c] Translational Medicine, Research Institute, The Hospital for Sick Children, Toronto, Canada
[d] Division of Infectious Diseases, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Canada

article info

Article history:
Received 24 March 2022
Revised 10 August 2022
Accepted 10 August 2022
Available online xxx

Keywords:
Antibiotic therapy
Cystic fibrosis
Eradication
Pseudomonas aeruginosa

abstract

Introduction: While antibiotic eradication therapy (AET) of early *Pseudomonas aeruginosa* infection is considered standard of care, its long-term effect on the subsequent course of cystic fibrosis (CF) lung disease remains unclear.

Methods: CF patients who were *P. aeruginosa*-free for at least a year and had a minimum of 10 years of pulmonary function measurements were included. Subjects were categorized as Never if they never had *P. aeruginosa* isolated from a respiratory tract sample. Subjects changed to the Eradicated group if they had a *P. aeruginosa* infection, were treated with AET, and subsequently cleared their infection. Subjects changed to the Chronic group if AET did not clear their *P. aeruginosa* infection. The primary outcome was absolute FEV_1 decline over time, with age as the time variable. Mixed-effects linear regression models were used to account for the repeated lung function measurements over time within each patient.

Results: 205 CF subjects (48% female) were included; the median (IQR) age at first infection was 9.6 (5.6, 14.6) years. The median (IQR) follow-up was 10.2 (5.7, 14.7) years for the Eradicated group, 8.8 (4.5, 14.9) years for the Chronic group and 2.8 (1.0, 5.7) years for the Never group was among those patients that had at least one *P. aeruginosa* infection over the study period, annual lung function decline of FEV_1 was significantly less (-1.11% predicted/year; 95% CI: -1.18, -1.04) in the Eradication group compared to the Chronic group (-1.57%; -1.64, -1.50) ($p<0.001$).

Conclusions: AET against *P. aeruginosa* improves lung function trajectory in CF patients.

1. Introduction

Cystic fibrosis is a life-limiting autosomal recessive disorder with highest prevalence in Europe, North America and Australia [1]. Around 30,000 individuals are affected in the United States and 45,000 in Europe [2]. Over the last few decades, the median age of survival has increased substantially due to optimised treatment of secondary effects of CFTR dysfunction such as airway infection [3].

Pseudomonas aeruginosa is one of the common pathogens of CF lung disease. *P. aeruginosa* infection begins as intermittent isolation of typically non-mucoid *Pseudomonas* strains from the airways, which, unless treated with targeted antibiotic therapy, will establish chronic infection in most individuals [4]. Early infection with *P. aeruginosa* is a major predictor of mortality and morbidity in young children with CF [5]. Given the potential negative impact of *P. aeruginosa* infection, antibiotic treatment protocols have been developed to aim for eradication early on before chronic infection has been established. Treatment protocols include inhaled therapy alone or combined with systemic antibiotics; the success rate of different protocols is high with negative cultures being accomplished in more than 80 percent of patients [6–9].

While antibiotic eradication therapy (AET) of *Pseudomonas aeruginosa* infection is now considered the standard of care [6–10], and clearance of *P. aeruginosa* can be achieved in most patients, the longer term impact of eradication therapy is less clear. The EPIC trial showed that a failure to eradicate was associated with a higher risk of exacerbation [11] and studies have demonstrated that treatment lengthens the time to chronic infection, but a positive impact on lung function measures has not been established. Since most patients are asymptomatic when a new infection with *P. aeruginosa* is detected in respiratory cultures, early infection is not usually associated with a deterioration in lung function and treatment has no immediate effects on the most commonly used lung function measure, forced expiratory volume in 1 second (FEV_1) [12]. Recent data suggest that this is also the case for sensitive outcome measures such as LCI [13]. We have previously demonstrated that CF patients in whom *P. aeruginosa* infection was cleared had similar FEV_1 trajectories over subsequent years compared to children who were never infected [14]. However, this previous study had limited follow-up data, so long term effects of AET on the subsequent course of lung disease therefore remains unclear [15].

As AET of *P. aeruginosa* has been performed at our center for more than 2 decades, we utilized this data to assess the effect of AET on long term pulmonary trajectories in pediatric CF patients.

* Corresponding author at: 555 University Avenue, Toronto, Ontario M5G 1X8.
E-mail address: felix.ratjen@sickkids.ca (F. Ratjen).

https://doi.org/10.1016/j.jcf.2022.08.007

2. Methods

2.1. Study population and data collection

This was a retrospective analysis of individuals with CF followed at the Hospital for Sick Children and St. Michael's Hospital (Toronto, ON, Canada), captured in the Toronto CF Database from 1998 to 2018. Data was extracted from the database housed at the Hospital for Sick Children which contains information on patients' pulmonary function tests (PFT), microbiology, and medications. The data was supplemented with specifics on AET treatments and outcomes through systematic review of health records by study investigators (I.G.C., A.B.). This study was approved by the Research Ethics Board at the Hospital for Sick Children (REB#1,000,027,683)

Patients included in this study were younger than 18 years of age at cohort entry and had a confirmed diagnosis of CF. The diagnosis of CF was defined by the presence of clinical features consistent with CF or a positive family history of CF plus either two documented sweat chlorides of > 60 meq/L by quantitative pilocarpine iontophoresis test, a genotype showing two well-characterized disease-causing mutations, or a nasal potential difference consistent with CF. For patients who entered the study cohort in 1998, a full year of negative *P. aeruginosa* cultures (minimum 1 culture) of respiratory tract specimens prior to cohort entry was required to be deemed eligible. Patients had to have at least 10 years of spirometry measurements in order to qualify for inclusion and could not enter the cohort if chronic *P. aeruginosa* infection was already present at that time point. Data was included until the last patient encounter in the time frame, until lung transplantation or death.

2.2. Pseudomonas aeruginosa *eradication protocol and outcomes*

Early infection for *P. aeruginosa* was defined as either the first positive respiratory culture for *P. aeruginosa* within the study period or a positive after at least one year of negative cultures whilst not on antibiotics with activity against *P. aeruginosa*. The details of the AET protocol used at our center have been published before [16] Initial treatment of *P. aeruginosa* in asymptomatic patients consisted of tobramycin inhalation solution (TIS) (either 1 year of tobramycin 80 mg/2 ml or 28 days of inhaled tobramycin 300 mg/5 ml). Symptomatic patients with *P. aeruginosa* infection were given 14 days of intravenous antibiotics and a subsequent course of inhaled tobramycin [16].

2.3. *Statistical analyses*

Due to the long-term nature of the follow-up of this study, the majority of the covariates of interest were non-time-varying, defined based on data from their first encounter in the cohort. These included sex, CFTR functional class (classes I-III vs classes IV-V),

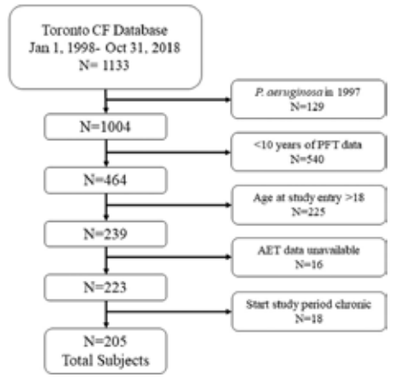


Fig. 1. CONSORT Diagram.

age at diagnosis (<= 2 years versus > 2 years), pancreatic status, and starting FEV_1% predicted measurement.

The exposure of interest in this study was the time-varying categorization of *P. aeruginosa* status. Subjects are categorized in the **Never** group if, over their lifetime documented microbiology samples or since cohort entry, they never had *P. aeruginosa* isolated from a respiratory tract sample. Subjects changed to the **Eradicated** group if they had a *P. aeruginosa* infection, treated with the AET protocol, and subsequently cleared their infection at first post-AET regimen clinic visit. Finally, subjects changed to the **Chronic** group upon having a full AET regimen which did not clear their *P. aeruginosa* infection at first post-AET regimen culture, resulting in a clinical categorization of chronic *P. aeruginosa* infection. This could have occurred on their first AET regimen, representing a shift from the **Never** to **Chronic** group, or after a previously successful AET attempt, representing a change from the **Eradicated** to the **Chronic** group.

The primary outcome for this study was the absolute FEV_1 decline over time, with age being used as the time variable. This accounts for both the amount of time in the study cohort and simultaneously adjusts for age. FEV_1 was reported as percent predicted using the GLI reference equations [17]. Mixed-effects linear regression models were used to account for the repeated lung function measurements over time within each patient. Subjects could change exposure group from Never to Eradicated, Never to Chronic, or Eradicated to Chronic. Outcome slopes represent the absolute change in FEV_1% predicted per year, adjusted for age or any other specified covariates.

3. Results

3.1. Study population

A total of 205 patients were included in the analysis (Fig. 1), of which 48% were female, 88% had a Class I-III mutation, 74% were diagnosed before the age of 2, and 90% were pancreatic insufficient (Table 1). The median (IQR) age at entry into the study was 5.6 years (5.1, 10.5 years) with a mean (SD) first reported FEV_1% predicted within the study period of 83.4% predicted (19.4%).

Of the 205 patients, 23 (11%) were censored at transplant, and 18 (9%) were censored at death. Mean (SD) follow-up time within the study was 17.3 years (2.7 years) (Table 1). During the study pe-

Table 1
Patient characteristics at study entry.

Patient Characteristic	Total $N = 205$
Age at study entry (years), median (IQR)	5.6 (5.1, 10.5)
Female, n (%)	98 (48%)
Age at diagnosis, n (%)	
<=2 years	152 (74%)
>2 years	53 (26%)
CFTR Functional Class, n (%)	
I-III	181 (88%)
IV-V	17 (8%)
Missing	7 (3%)
Ethnicity, n (%)	
Caucasian	184 (90%)
Black	10 (5%)
Asian	2 (1%)
Other	9 (4%)
Pancreatic Insufficient, n (%)	185 (90%)
Age at first *P. aeruginosa* infection (years), median (IQR)	9.6 (5.6, 14.6)
FEV_1% predicted at study entry, median (IQR)	83.6 (68.7, 98.3)
Follow-up time in study (years), median (IQR)	18.3 (15.9, 19.3)
Follow-up time in each group (years), median (IQR)	
Never ($n = 165$)	2.8 (1.0, 5.7)
Eradicated ($n = 172$)	10.2 (5.7, 14.7)
Chronic ($n = 119$)	8.8 (4.5, 14.9)
Number of patients in each group at selected age snapshots	
Age: 10 years	
Never	44
Eradicated	92
Chronic	22
Age: 15 years	
Never	31
Eradicated	107
Chronic	57
Age: 20 years	
Never	10
Eradicated	60
Chronic	59
Age: 25 years	
Never	2
Eradicated	21
Chronic	48

IQR: interquartile range; CFTR: CF transmembrane conductance regulator; FEV_1% predicted: forced expiratory volume in one second, as measured by spirometry and standardized by GLI [16].

riod, patients had a median (IQR) of 68 (51, 93) reportable PFTs performed, and 74 (47, 99) microbiology samples assessed. This represents a mean (range) of 5.9 (1, 35) PFTs per year and 5.2 (1, 27) microbiology samples per year.

Of the total 205 patients, 165 (80.5%) entered the study period as having Never had *P. aeruginosa* and 40 (19.5%) entered as Eradicated, due to either a recorded history of previous *P. aeruginosa* eradication, or because their entry into the study was on an infection leading to *P. aeruginosa* clearance. These 165 patients contributed a median (IQR) 2.8 years (1.0, 5.7 years) to the Never group. Among patients that had at least one *P. aeruginosa* infection over the study period ($n = 203$), the median (IQR) age at first infection was 9.6 years (5.6, 14.6 years). One year of 80 mg inhaled tobramycin was the treatment course for 448 *P aeruginosa* infections among 186 patients in our cohort whereas 28 days of 300 mg tobramycin was used for 280 eradication attempts among 96 participants. The median (IQR) duration of time contributing to the Eradicated group was 10.2 years (5.7, 14.7 years) ($n = 172$), and 8.8 years (4.5, 14.9 years) for the Chronic group ($n = 119$).

3.2. Lung function trajectories

Due to the long-term nature of this analysis, we considered non-time-varying covariates when developing the explanatory multivariable model. Covariates were attempted forward-stepwise based on univariable association with FEV_1 decline over time, and

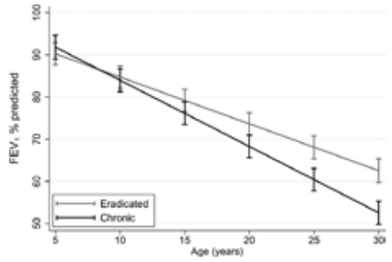


Fig. 2. Progression of FEV_1% predicted among those who successfully eradicate *P. aeruginosa* after AET initiation, compared to those who develop chronic *P. aeruginosa**.

kept in the model if they were statistically significant. While FEV_1% predicted at study entry and pancreatic status were kept in the final model, neither covariate substantially changed the effect estimate of the overall decline in FEV_1 by *Pseudomonas* infection group.

The decline for the Never group was −0.77% per year (95% CI −0.94, −0.60%). In the final model (Table 2), the Eradicated group had an overall annual decline in FEV_1 of −1.11% predicted/year (95% CI −1.18, −1.04) and the Chronic group had a decline of −1.57% predicted/year (95% CI −1.64, −1.50). This represented a 0.46% predicted per year less decline for subjects who successfully eradicated compared to those where AET failed to eradicate *P. aeruginosa* and they developed chronic infection (95% CI 0.37, 0.55; $p<0001$) (Fig. 2).

4. Discussion

To the best of our knowledge, this is the first study demonstrating the effect of early eradication of *Pseudomonas* infection on long term lung function decline in CF patients. In this 20 year retrospective study, we showed that there is less deterioration in FEV_1 in those in whom *P. aeruginosa* eradication was successful compared to those who in whom it was not and who developed chronic infection.

AET of initial *P. aeruginosa* infection is considered standard of care in the management of CF patients. Several prior studies have shown that early eradication of *P. aeruginosa* infection can successfully clear the organism from airway secretions and can also postpone chronic *P. aeruginosa* infection [6–9,18]. Given that chronic *P. aeruginosa* infection is known to be associated with poor clinical outcomes, one would assume that successful *P. aeruginosa* eradication would translate into benefits for subsequent lung disease, but this has been harder to demonstrate. Most patients with early *P. aeruginosa* infection are asymptomatic and have stable lung function, highlighting the necessity of routine surveillance cultures to identify incident infection. Given the lack of acute symptoms and deterioration in lung function with initial *P. aeruginosa* infection, it is not surprising that AET has not been shown to be associated with immediate improvements in lung function. Thus, assessing its effect on subsequent long-term lung function decline is a more suitable measure for the effect of AET on lung disease.

There are few previous studies examining the effect of AET of *P. aeruginosa* infection on measures other than microbiology in CF. Zemanick et al. [19] evaluated clinical outcomes associated with initial isolation of *P. aeruginosa* in a large CF cohort. *P. aeruginosa* acquisition was associated with an increased rate of pulmonary exacerbations, more frequent detection of crackles or wheeze on

Table 2
FEV_1 decline over time by eradication status, adjusted for associated covariates.

Covariate	Slope (95% CI)	p-value
Age (years)	−1.11%/year (95% CI −1.18, −1.04)	<0.001
*Eradication Status**		
Eradicated	0.46%/year (95% CI 0.37, 0.55)	<0.001
Chronic	Reference	
FEV_1% predicted at study start**	0.50 (95% CI 0.38, 0.61)	<0.001
Pancreatic status		
Insufficient	Reference	
Sufficient	11.38 (95% CI 3.86, 18.89)	0.003

* Eradication status was entered in the model as an interaction with age; the slope refers to the additional decline in FEV_1 per year on top of that of eradicated individuals, which would be the decline per year in the age row.

** Represents the difference in FEV_1 for each unit increase in FEV_1% predicted at the study start.

physical exam and increased risk for emergence of methicillin-resistant *Staphylococcus aureus, Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* on respiratory cultures. However, there was no association between initial acquisition of *P. aeruginosa* and more rapid decline in lung function or change in growth parameters. Similarly, Mayer-Hamblett et al. [20] in their 5 year follow-up study of over 200 children with CF who underwent *P. aeruginosa* eradication treatment, observed increased antimicrobial usage in individuals who failed AET versus those who successfully cleared *P. aeruginosa*, but there was no association between eradication status and clinical outcomes including lung function decline over 5 years following treatment. Both of the aforementioned studies did not find an association between AET and FEV_1 decline, consistent with our previous findings [19,20], but had shorter follow up periods than the current study. In a study with a follow up period of several decades, Frederiksen et al. [21] compared CF patients treated with an intensive protocol consisting of colistin inhalations and oral ciprofloxacin at the time of initial *P. aeruginosa* colonization with historic controls who had never participated in any early-treatment trials. They found that aggressive treatment maintained or increased pulmonary function during the year after inclusion, compared with the control group. While these data are consistent with our current findings, the use of historic controls limits a direct comparison of a treatment effect.

There were several strengths as well as limitations to our study. The main strength is the 20 years of follow up data that allowed us to detect the change in long-term lung function decline. Furthermore, our detailed antibiotic database permitted accurate classification of eradication treatment success and failures. The limitations were the retrospective nature of the study, not allowing direct comparison of treatment versus no treatment for early *P. aeruginosa* infection, which would not be possible given that AET is standard of care for CF patients. The change in care over time was accounted for by using time as a covariate in the model. While we used a linear model in the analysis, nonlinear models have been proposed to better capture changes over long observation periods in CF [23]. There were some changes in the AET protocol over the 20 year study period, namely the switch from 1 year of TIS (80 mg/2 ml) to 28 days of TIS (300 mg/5 ml) but we have previously shown these 2 regimens to be comparable in terms of AET efficacy. Given the retrospective nature of the study we cannot prove causality between AET and improved lung function trajectory, and other factors could be responsible for the observed group differences. We did not assess pulmonary exacerbation as an outcome which has previously reported to differ between individuals with successful P. aeruginosa eradication and those that fail AET. Different time points rather than the initial culture end of AET could be chosen of an endpoint for successful eradication. Finally, the success of an eradication protocol can be influenced by multiple factors including P. aeruginosa phenotypes as described before [22], host factors affecting bacterial clearance and adherence to treatment that we cannot control for in this analysis.

In conclusion, we have demonstrated for the first time that successful *P. aeruginosa* eradication therapy is associated with improved long term lung function, supporting the current standard of care in the management of CF patients. Thus, AET can not only result in improved microbiological outcomes, but clinical outcomes as well.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

All authors have nothing to disclose.

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

CRediT authorship contribution statement

Isabel Gascon Casaredi: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Michelle Shaw:** Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Valerie Waters:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Ryan Seeto:** Formal analysis, Data curation, Visualization. **Ana C. Blanchard:** Investigation. **Felix Ratjen:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

References

[1] Elborn JS. Cystic fibrosis. The Lancet 2016;388(10059):2519–31.
[2] Cystic Fibrosis Foundation. 2018 Patient registry annual data report. Cystic fibrosis foundation patient registry 2018 annual data report. 2018;92.
[3] Burgel PR, Bellis G, Olesen HV, Viviani L, Zolin A, Blasi F, et al. Future trends in cystic fibrosis demography in 34 European countries. Eur Respiratory J 2015;46(1):133–41.
[4] Bendiak G, Ratjen F. CFTR, Mucins, and Mucus in Cystic Fibrosis. Semin Respir Crit Care Med 2009;30(05):587–95.
[5] Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol 2002;34(2):91–100.
[6] Blanchard AC, Horton E, Stanojevic S, Taylor L, Waters V, Ratjen F. Effectiveness of a stepwise Pseudomonas aeruginosa eradication protocol in children with cystic fibrosis. J Cystic Fibrosis 2017;16(3):395–400.
[7] Ratjen F, Moeller A, McKinney ML, Asherova I, Alon N, Maykut R, et al. Eradication of early P. aeruginosa infection in children <7 years of age with cystic fibrosis: the early study. J Cystic Fibrosis 2019;18(1):78–85.

[8] Ratjen F, Munck A, Kho P, Angyalosi G. Treatment of early Pseudomonas aeruginosa infection in patients with cystic fibrosis: the ELITE trial. Thorax 2010;65(4):286–91.

[9] Early T. Comparative efficacy and safety of 4 randomized regimens to treatearly. Arch Pediatric Adol Med 2011;165(9):847–56.

[10] Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, Khan U, Kulich M, Kronmal R, et al. Comparative efficacy and safety of 4 randomized regimens to treat early Pseudomonas aeruginosa infection in children with cystic fibrosis. Arch Pediatric Adolescent Med 2011;165(9):847–56 Sep.

[11] Mayer-Hamblett N, Kronmal RA, Gibson RL, Rosenfeld M, Retsch-Bogart G, Treggiari MM, et al. Initial Pseudomonas aeruginosa treatment failure is associated with exacerbations in cystic fibrosis. Pediatr Pulmonol [Internet] 2012;47(2):125–34. Feb [cited 2022 Jun 6]Available from: https://pubmed.ncbi.nlm.nih.gov/21830317/ .

[12] Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF, et al. Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis. J Pediatrics 2001;138(5):699–704.

[13] Stanojevic S, Davis SD, Retsch-Bogart G, Webster H, Davis M, Johnson RC, et al. Progression of lung disease in preschool patients with cystic fibrosis. Am J Respir Crit Care Med 2017;195(9):1216–25.

[14] Amin R, Lam M, Dupuis A, Ratjen F. The effect of early Pseudomonas aeruginosa treatment on lung function in pediatric cystic fibrosis. Pediatr Pulmonol 2011;46(6):554–8 Jun.

[15] Santos S, Perrem L, Shaw M, Hewko S, Sanders DB, Davis S, Stanojevic S, Solomon M, Grasemann H, Sweezey N, Waters V, Ratjen FSantos S, Perrem L, Shaw M, Hewko S, Sanders DB, Davis S, Stanojevic S, Solomon M, Grasemann H, Sweezey N, Waters VRF. LCI response to acquisition and eradication of Pseudomonas aeruginosa in children with CF. North American Cystic Fibrosis Conference, October 2020, Phoenix, USA (virtual conference). Pediatr Pulmonol., 48(S36): S471. Pediatr Pulmonol 2020;55:S38–361.

[16] Stanojevic S, Waters V, Mathew JL, Taylor L, Ratjen F. Effectiveness of inhaled tobramycin in eradicating pseudomonas aeruginosa in children with cystic fibrosis. J Cystic Fibrosis 2014;13(2):172–8.

[17] Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respiratory J 2012;40(6):1324–43.

[18] Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, Khan U, Kulich M, Kronmal R, et al. Comparative efficacy and safety of 4 randomized regimens to treat early Pseudomonas aeruginosa infection in children with cystic fibrosis. Arch Pediatr Adolescent Med 2011;165(9):847–56 Sep.

[19] Zemanick ET, Emerson J, Thompson V, McNamara S, Morgan W, Gibson RL, et al. Clinical outcomes after initial pseudomonas acquisition in cystic fibrosis. Pediatr Pulmonol 2015;50(1):42–8.

[20] Mayer-Hamblett N, Kloster M, Rosenfeld M, Gibson RL, Retsch-Bogart GZ, Emerson J, et al. Impact of sustained eradication of new pseudomonas aeruginosa infection on long-term outcomes in cystic fibrosis. Clin Infect Dis 2015;61(5):707–15.

[21] Frederiksen B, Koch C, Høiby N. Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Pediatr Pulmonol 1997;23(5):330–5.

[22] Jackson L, Waters V. Factors influencing the acquisition and eradication of early Pseudomonas aeruginosa infection in cystic fibrosis. J Cyst Fibros [Internet] 2021 Jan 1;20(1):8–16. [cited 2022 Jun 28]Available from: https://pubmed.ncbi.nlm.nih.gov/33172756/ .

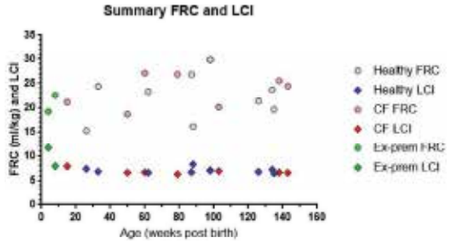
[23] Szczesniak RD, Li D, Su W, Brokamp C, Pestian J, Seid M, Clancy JP. Phenotypes of rapid cystic fibrosis lung disease progression during adolescence and young adulthood. Am J Respir Crit Care Med 2017 Aug 15;196(4):471–8. doi:10.1164/rccm.201612-2574OC.PMID: 28410569.

11 ANNEXES

11 ANNEXES

ANNEX 2

Early *Pseudomonas aeruginosa* treatment and lung function in pediatric cystic fibrosis. Gascón Casaredi I, Klingel M, Waters V, Ratjen F. Ped. Pulmonology. 2019; 54 (2) vol 54, supplement 2:428.



717

EARLY *PSEUDOMONAS AERUGINOSA* TREATMENT AND LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS

Gascon Casaredi, I.[1]; Klingel, M.[2]; Waters, V.J.[4]; Ratjen, F.[1,2,3]
1. Respiratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada; 2. Translational Medicine, The Hospital for Sick Children, Toronto, ON, Canada; 3. University of Toronto, Toronto, ON, Canada; 4. Infectious Disease, The Hospital for Sick Children, Toronto, ON, Canada

Background: While antibiotic eradication therapy (AET) of early *Pseudomonas aeruginosa* infection is considered standard of care, its long-term effect on the subsequent course of lung disease remains unclear. Our aim is to assess the effect of treatment of early infection and eradication of *P. aeruginosa* on pulmonary function in pediatric CF patients.

Methods: CF patients either followed from birth or being *P. aeruginosa*-free for at least a year from 1998 onwards who had a minimum of 10 years of pulmonary function measurements were included in this retrospective cohort study. Patients were censored at transplant or *Burkholderia cepacia* complex infection. At each available culture since birth or study start, patients were categorized based on the past 12 months with at least three available cultures as never infected (no cultures positive), intermittent (≤50% cultures positive), or chronic infection (>50% cultures positive). Infections which were eradicated prior to becoming chronic were considered intermittent. Mixed effects linear regression models using an interaction between age and infection group assessed FEV_1 percent predicted decline per year for each group.

Results: 182 CF subjects (42% female) were included, of which 106 (58%) were followed from birth. During the follow-up, 34 (19%) never had a *P. aeruginosa* infection, 68 (37%) had intermittent infection, and 80 (44%) had chronic infection at any point. The median age at first infection was similar between those with intermittent infection (10.2 years) and chronic infection (9.7 years). FEV_1 decline was lowest among those never infected (-0.64% predicted per year, 95% CI -0.85, -0.43). Decline was worse for those chronically infected (-1.50% predicted per year, 95% CI -1.95, -1.05) than intermittently infected (-1.31% predicted per year, 95% CI -0.84, -1.77) (p=0.02).

Conclusions: While any *P. aeruginosa* infection is associated with long-term decline in lung function, decline is less severe among those where AET against *P. aeruginosa* infection was successful.

718

RESIDENTIAL ROADWAY PROXIMITY AND LUNG FUCTION DECLINE IN PEDIATRIC CYSTIC FIBROSIS

Flores, J.K.; Gillespie, S.; Sarnat, S.; Stecenko, A. *Emory University, Chamblee, GA, USA*

Introduction: Air pollution exposure is thought to be detrimental to anyone with lung disease, and this may be particularly true for people with CF. Research indicates that environmental factors account for about half of variations in lung function of CF patients (Collaco JM, et al. J Pediatr. 2010;157(5):802-7). The purpose of this study was to better understand the effects of air pollution on lung function decline in children with CF. Residential roadway proximity was utilized as a proxy for exposure to traffic-related air pollution.

Methods: Demographic and clinical data were collected from pediatric patients at the Emory University + Children's Healthcare of Atlanta CF Care Center (n = 98) for the 2013-2017 period. Residential distance to the closest major roadway was used as a proxy for air pollution exposure. Spirometry test results were used to calculate each subject's annual baseline lung function scores by finding the mean result of the highest percent predicted value of forced expiratory volume in one second (FEV1) from each quarter. Annual rates of decline (ROD) in FEV1 for each subject were determined by calculating the differences between subsequent baseline values. Other collected independent characteristics known to influence CF disease progression were: gender, race, insurance status, income, bacterial acquisition status, and CF-related diabetes (CFRD) status. Subjects were placed into two exposure groups based on evidence that elevated concentrations of traffic-related air pollution persist within 570 meters of roadways (Trasande L, Thurston GD. J Allergy Clin Immunol. 2005;115(4):689-99). Differences in independent characteristics between groups were assessed using parametric (i.e., ANOVA/chi-square) and non-parametric (Wilcoxon/Fisher's) statistical tests to examine exposure associations of ROD with residential roadway proximity.

Results: Individuals living within 570 meters of a major roadway had a mean annual ROD of -2.87% (95% CI: -4.21, -1.53), while subjects residing further away had a mean ROD of -0.94% (95% CI: -1.53, -0.36) (p=0.011). In addition, patients with chronic MRSA infection or diagnosis of CFRD also had significantly higher mean ROD than those without MRSA infection or CFRD diagnosis (p=0.006 and p=0.037, respectively). Self-reported income levels were significantly associated with residential roadway proximity (p=0.048), with lower income levels among patients living closer to major roadways, but not ROD. Sex, race, insurance status, and *P. aeruginosa* infection status were not significantly associated with residential roadway proximity or ROD.

Conclusions: Utilizing residential roadway proximity as a proxy for traffic-related air pollution exposure, we found greater annual lung function decline in pediatric patients with CF who were exposed to elevated concentrations of roadway air pollutants. These results provide evidence that the CF patient population is vulnerable to the effects of air pollution. Further research is warranted to better understand this effect, utilizing a larger cohort and more precise measures to quantify pollution exposure.

719▲

LUNG FUNCTION DETERIORATION IN SCHOOL CHILDREN WITH CYSTIC FIBROSIS IN POLAND

Sands, D.A.[1,2]; Walicka-Serzysko, K.[1,2]; Postek, M.[1,2]; Milczewska, J.[1,2] *1. Cystic Fibrosis Centre, Institute of Mother and Child, Warsaw, Poland; 2. Cystic Fibrosis Centre, Pediatric Hospital, Dziekanow Lesny, Poland*

Introduction: Lung disease in the course of cystic fibrosis (CF) begins early in life but the capabilities for detecting abnormalities of pulmonary dysfunction in children remain limited. Proper assessment and fast interventions during this period are crucial for delaying and minimizing disease progression. In our survey we investigated indicators of pulmonary function useful to monitor functional deterioration during childhood.

Objectives: The aim of the study was to evaluate the early progression of lung function by tracking pulmonary hyperinflation, ventilation inhomogeneity (VI), trapped gas and airway obstruction with the age of the participating patients. We also assessed the accuracy of FRC_{pleth} derived from plethysmography and FRC_{MBNW} obtained from multiple-breath nitrogen washout (MBNW) test in children with CF.

Methods: One hundred CF patients were included in the study. They were aged 7-18 (44 males; 56 females), divided into two groups aged 7-12 (n-40) and 13-18 (n-60). Patients performed MBNW test and plethysmography for measurements of lung clearance index (LCI), functional residual capacity (FRC_{pleth}, FRC_{MBNW}), volume of trapped gas (V_T), total resistance (R_{tot}), effective and specific effective airway resistance (R_{eff}, sR_{eff}). Data were analysed with *STATISTICA* version 13.1. We used Z-transformation to change values into z-scores in our population.

Results: We obtained a positive correlation of FRC_{plet} , FRC_{MBNW}, and LCI with age, as well as negative correlation of R_{tot} (r=-0.5286 p<0.0001) and R_{eff} (r=-0.4763 p<0.0001) with age. A linear correlation between FRC_{MBNW} and FRC_{pleth} (r=0.9184 p<0.0001) was observed but Blant-Altman's analysis showed a significant difference between FRCpleth and FRC_{MBNW} values.

11 ANNEXES

11 ANNEXES

ANNEX 3

Gascon Casaredi I,; Shaw M.; Waters V.; Seeto R.; Blanchard A.; Ratjen F. Impact of *Pseudomonas aeruginosa* antibiotic eradication therapy on lung function decline in cystic fibrosis. ERS International congress September 2022; ERJ (Vol 60, Issue suppl 66).

EUROPEAN RESPIRATORY SOCIETY
INTERNATIONAL CONGRESS 2022
BARCELONA Spain, 4 - 6 September

Impact of Pseudomonas aeruginosa antibiotic eradication therapy on lung function decline in cystic fibrosis

I. Gascon Casaredi (Barcelona, Spain), M. Shaw (Toronto, Canada), V. Waters (Toronto, Canada), R. Seeto (Toronto, Canada), A. Blanchard (Montreal, Canada), F. Ratjen (Toronto, Canada)

Introduction: While antibiotic eradication therapy (AET) of early *Pseudomonas aeruginos*a infection is considered standard of care, its long-term effect on the subsequent course of cystic fibrosis(CF)lung disease remains unclear.

Methods: CF patients who were *P. aeruginosa*-free for at least a year and had a minimum of 10 years of pulmonary function measurements were included. Subjects were categorized as **Never** if they never had *P. aeruginosa* isolated from a respiratory tract sample. Subjects changed to the **Eradicated** group if they had a *P. aeruginosa* infection, were treated with AET, and subsequently cleared their infection. Subjects changed to the **Chronic** group if AET did not clear their *P. aeruginosa* infection. The primary outcome was absolute FEV1 decline over time, with age as the time variable. Mixed-effects linear regression models were used to account for the repeated lung function measurements over time within each patient.

Results: 205 CF subjects (48% were female) were included; the median (IQR) age at first infection was 9.6 (5.6, 14.6) years. The median (IQR) age at entry into the study was 5.6 (5.1, 10.5) years. The median (IQR) follow up for the Never group was 2.8 (1.0, 5.7) years, 10.2 (5.7, 14.7) years for the Eradicated group, and 8.8 (4.5, 14.9) years for the Chronic group. Among those patients that had at least one *P. aeruginosa* infection over the study period, annual lung function decline of FEV1 was significantly lower (-1.11% predicted/year; 95% CI: -1.18, -1.04) in the Eradication group compared to the Chronic group (-1.57%; -1.64, -1.50) ($p<0.001$).

Conclusions: AET against *P.aeruginosa* infection improves lung funtion trajectory in CF patients.

ERS
INTERNATIONAL CONGRESS

11 ANNEXES