A retrospective investigation of the risk factors for chronic *Pseudomonas aeruginosa* infections in children with cystic fibrosis in Canada from 2000 – 2020

by

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Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

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Abstract

Rationale: Cystic Fibrosis (CF) is a genetic disorder that causes viscid mucous within the lungs, predisposing people with CF to infections. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a gram-negative bacterium that is common in people with CF. Previous research has found that early eradication with antibiotic treatment can successfully eliminate *P. aeruginosa* from the lungs, but eventually these infections return and can become chronic. Chronic *P. aeruginosa* infection is detrimental to the health of people with CF and often leads to greater morbidity and mortality. This thesis investigated three objectives: (1) What are the characteristics of children with CF who develop chronic *P. aeruginosa* infections; (2) What is the association between number of eradication attempts and time to chronic *P. aeruginosa* infection; and (3) Does the association between the number of eradication attempts and time to chronic *P. aeruginosa* infection differ between children diagnosed with CF clinically and those diagnosed with CF by newborn screening (NBS).

Methods: This study used a retrospective paediatric cohort from prospectively collected data within the Canadian CF Registry (CCFR) from 2000 – 2020. Descriptive statistics were used to summarise the characteristics of children with chronic *P. aeruginosa* (Objective 1). A multivariable time dependent Cox proportional hazards regression was performed to investigate the association between number of previous infections and chronic infection (Objective 2), and the results were stratified by NBS to test effect modification (Objective 3).

Results: Of the 2098 children eligible for this study, 817 (38.9%) developed a chronic *P. aeruginosa* infection. The risk of developing a chronic infection increases by 9% for each new infection acquired (hazard ratio (HR) 1.09; 95% confidence interval (CI) [1.07, 1.12]) and was not attenuated after adjusting for potential confounding variables (HR 1.08; 95% CI [1.06, 1.10]). A non- linear association between the number of previous infections and chronic infection was observed; there was an increased hazard of chronic infection with each additional infection, with the hazard increasing to two times the baseline level (one infection) after 4 repeated *P. aeruginosa* infections. Children diagnosed through NBS were less likely to develop a chronic infection compared with children clinically diagnosed with CF, but the association between number of previous infections and chronic infection was similar. In a sensitivity analysis, limited to children diagnosed after the year 2000, the hazard for chronic infection was 1.17 (95% CI [1.13, 1.21]), suggesting that either the exposure or outcome (or both) were misclassified.

Discussion: With each new *P. aeruginosa* infection children with CF face an increased risk of developing chronic *P. aeruginosa*. The prevention and early eradication of infection is necessary to minimize the risk of chronic *P. aeruginosa* infection.

List of abbreviations used

BMI	Body mass index
CF	Cystic fibrosis
CCFR	Canadian cystic fibrosis registry
CFTR	Cystic fibrosis transmembrane conductance regulator gene
CI	Confidence interval
FEV_1%	Forced expiratory volume in one second percent predicted
HR	Hazard ratio
NBS	Newborn screening
P. aeruginosa	*Pseudomonas aeruginosa*

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Chapter 1: Introduction

Cystic fibrosis (CF) is a genetic disorder caused by a mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7 (1–4). A mutation in the CFTR gene impacts the body's cellular chloride channel, which affects the airways, pancreas, small intestine, liver, reproductive tract and sweat glands in particular (1,2,5). A malfunctioning chloride ion channel results in build-up of thick and sticky mucous that predisposes the lungs to respiratory infections (1,2,6). Infection leads to respiratory tract inflammation, which has been found to be a cause of morbidity and mortality in people with CF (1,2,6–8).

In people with CF, *Pseudomonas aeruginosa* (*P. aeruginosa*) is a common bacterial infection (2,5,7). *P. aeruginosa* is a gram-negative bacterium that thrives in moist environments (9). In healthy individuals, the lungs clear infection through mucociliary clearance, but the viscid mucous in the lungs of people with CF impedes mucociliary clearance (6). The toxins pyocyanin and hydrogen cyanide are produced by *P. aeruginosa*, and both directly impact ciliary function (6). Antibiotic treatment has been shown to be effective at eradicating the *P. aeruginosa* bacteria if the infection is both detected and treated early (2,5).

Repeated *P. aeruginosa* infections after the initial eradication are very common (6). With each subsequent infection, the chances of the *P. aeruginosa* infection developing a resistance to the antibiotics used to treat the infection increase. Once a *P. aeruginosa* infection stops responding to antibiotic treatment, it is deemed chronic and can no longer be successfully eradicated. People with chronic *P. aeruginosa* are at greater risk of morbidity and mortality

(10–12); therefore, it is important to understand how to prevent chronic *P. aeruginosa* in people with CF. In a recent survey of the Canadian CF community, prevention and treatment of chronic *P. aeruginosa* were identified as top research priorities (13).

Chapter 2: Background

2.1 Cystic fibrosis

2.1.1 What is cystic fibrosis?

CF is a genetic disorder that shortens lives (1,2). CF occurs as the result of a genetic mutation on the CFTR gene located on chromosome 7 (3,4). Mutations in the CFTR gene result in synthesis/activation defects in the CFTR protein, resulting in various forms of protein dysfunction or lack thereof (1,2). Dysfunction ranges from improper synthesis to premature degradation or inactivation of the CFTR protein (1,2). The disease-causing variants are grouped into five classes depending on their functional consequences on the CFTR protein (1,2). Clinical disease manifests as a result from the dysfunction of the CFTR protein within the apical membrane of epithelial cells (1,2). The dysfunctional protein, or lack thereof, impacts the chloride channel which affects the airways, pancreas, small intestine, liver, reproductive tract and sweat glands (1,2,5). As a consequence, the airways within the lungs are lined with viscid mucous that has an adhesive and thick consistency, predisposing people with CF to respiratory infections (6). Thus, CF manifests clinically with repeated respiratory infections, which lead to inflammation within the lungs and structural damage to the airway (2,5).

CF has traditionally been considered as a paediatric disease. In the 1960s the median age of survival was reported to be five years of age (14). Advancements in care and treatment have led to dramatic improvements in outcomes. In 2019, the Canadian Cystic Fibrosis Registry (CCFR) estimated the

median age of survival for someone born with CF to be 54.3 years (15). This notable increase in survival from the 1960s to 2019 can also be observed in other countries with CF data registries (14). Survival of people with CF has improved due to several factors such as early diagnosis, aggressive treatment of lung pathogens, and more recently, novel CFTR modulator therapies (14). One notable improvement in the 2000s was the use of aerosolized antibiotics to treat infections for people with CF (16). Early diagnosis and aggressive treatments of lung pathogens has been essential for improved outcomes for people with CF (17,18). Thus early diagnosis of CF is important.

2.1.1.1. Newborn screening

CF was traditionally diagnosed in early childhood based on a series of presenting symptoms (19,20). Children who presented with failure to thrive, recurrent respiratory infection, or malnutrition would be tested for CF using the sweat chloride test, at which point a formal diagnosis could be made (19,20).

Newborn screening (NBS) was developed in the 1970s and was originally designed to test for pancreatic proteases in a stool sample from newborns when they were five days old (19). The technique was later refined to test serum samples for immunoreactive trypsinogen which, if found to be elevated, suggests pancreatic inflammation and the possibility of CF (20,21). Newborns who are found to have elevated immunoreactive trypsinogen go on to have genetic screening performed, which tests for a panel of the most common CFTR mutations (20,21).

The CF screening protocol varies across Canada, but generally includes this two-step process: serum immunoreactive trypsinogen measurement (which is sometimes repeated) followed by genetic screening (21). If a newborn is positively screened for a CF mutation, they are then referred to a CF clinic where they will undergo sweat chloride testing to confirm the diagnosis and, if positive, begin treatment (21).

NBS programs have been implemented in many countries in Europe and North America, as well as in New Zealand and Australia (17,22). In Canada, NBS programs were first introduced in Alberta in 2007, and Ontario followed in 2008 (17,18). Other Canadian provinces followed (17,18) and Quebec was the final province to implement NBS in 2017 (18).

NBS programs allow for early diagnosis of CF and have been shown to play a large role in improving long-term outcomes (17,18). One of the leading motivators for implementing NBS is that it helps diagnose CF earlier, which in turn results in dedicated care by a CF clinic, early detection of respiratory infections, and aggressive treatment of those infections (17).

2.1.2 The impact of cystic fibrosis on the respiratory system

The main line of defence for the lungs to combat environmental injury is a thin layer of mucous that coats the airways (23,24). This protective mucous layer is comprised mainly of water and mucins, which are heavily glycosylated proteins (23,24). In healthy lungs, the mucous layer lies atop a periciliary layer, which has a depth of approximately 7 µm and is known for playing a role in mucociliary

clearance of mucous (23). In lungs affected by CF, the hypersecretion of mucins and subsequent dehydration of the mucous layer leads to a thick viscid mucous layer (23). Viscid mucous in turn impairs mucociliary clearance and the thick stagnant mucous promotes bacterial growth (23,25). For successful mucociliary clearance, the depth of the periciliary layer is crucial, and in persons with CF, this layer is reduced because of the dysfunctional CFTR protein and chloride ion channel (1,2,5,6,9,23).

In healthy lungs mucous gets cleared from the lungs through ciliary movement and sputum cleared by coughing (24). This function is impaired in people with CF, so mucolytic medications are used to help reduce the overall elasticity and viscosity of the mucous in the lungs (24,25). These therapeutics have been demonstrated to have a positive impact to manage mucous accumulation and long-term clinical outcomes in people with CF (25).

2.1.3 Pulmonary infections

The viscid mucous that builds up in the lungs of someone living with CF creates an optimal breeding ground for infections, making people with CF more susceptible to lung infections than people whose lungs are healthy (5,23). Thus, regular clinical surveillance and prompt treatment of respiratory infections are part of routine clinical care. As per the CF standard of care, people with CF are typically seen in clinic every 3-4 months, at which time a microbiological sample is obtained (either oropharyngeal swab, or a sputum sample).

Two common infections that occur in people with CF as identified in the 2019 CCFR annual report are *Staphylococcus aureus* and *P. aeruginosa,* found in 53% and 38% of the Canadian CF population, respectively (15). *S. aureus* is most prevalent in children whereas *P. aeruginosa* is more prevalent in adults (Figure 2.1). Other common pathogens include *Aspergillus fumigatus, Strenotrophomonas maltophilia, Haemophilus influenzae, Methicillin*-resistant *Staphylococcus aureus, Achromobacter* species, *Burkholderia cepacia* complex, and atypical mycobacteria (15).

Pulmonary infections threaten the overall lung health in people with CF. A common cause of death for people with CF is respiratory failure, which is why early detection of pulmonary infections is so important (7–9).

2.2 Cystic fibrosis and *Pseudomonas aeruginosa*

2.2.1 What is *P. aeruginosa?*

P. aeruginosa is a gram-negative bacterium that thrives in moist environments (9), which makes the lungs of people with CF more susceptible to infection. Unlike *S. aureus* which is more prevalent, *P. aeruginosa* infections are associated with worsening lung function, higher rates of hospitalizations and increased mortality (9–11,26–28).

P. aeruginosa can be found in many different environments, so for people with CF, the exact location where an infection is contracted is often difficult to ascertain (2,9). Jackson and Waters (9) highlighted several common environmental sources of *P. aeruginosa* infection, some of which were hospitals,

nebulizers, home environments and cross-infection between patients and families. Additionally, *P. aeruginosa* is a resourceful pathogen due to its ability to continually adapt to its environment and its numerous cellular defenses to protect itself from the immune system. These defenses include multiple virulence factors, highly motile cilia, and various exopolysaccharides, which serve various functions including: biofilm formation (a key characteristic of chronic *P. aeruginosa* infections), secretion of exoproducts (which impair host cellular phagocytosis), and adhesion mechanisms (for optimal cell attachment) (9).

The toxins, pyocyanin and hydrogen cyanide, produced by *P. aeruginosa,* directly impact ciliary function, thus exacerbating the already impaired mucociliary clearance (6). *P. aeruginosa* exists as two distinct phenotypes: nonmucoid and mucoid (12). A nonmucoid *P. aeruginosa* phenotype is likely to respond to antibiotic treatment (12,29,30), whereas the mucoid phenotype results in the formation of a biofilm layer within the lungs that makes the pathogen less responsive to antibiotics, thereby increasing the risk of the infection becoming chronic and persistent (30). With initial detection of *P. aeruginosa* in sputum culture, aggressive protocols are used to both eradicate the infection and postpone the conversion of nonmucoid to mucoid *P. aeruginosa* phenotypes.

In their 2020 review, Jackson and Waters (9) noted that the prevalence of *P. aeruginosa* infections in people with CF has decreased over the years. This decrease has also been noted in the 2019 CCFR annual report (15,16). Some hypothesize that the decrease in the prevalence of infections can be attributed to the development and use of early eradication techniques within the CF treatment

centres (9). The earlier an initial infection is treated, the higher the chances of successful eradication (5,7,31). Additionally, early eradication techniques have been hypothesized to be a factor in the prolongation of time to chronic *P. aeruginosa* infections in patients with CF (5,7,31).

2.2.2. Chronic *P. aeruginosa*

One of the leading causes of morbidity and mortality of people with CF is chronic *P. aeruginosa* infections (27,31). Approximately 80% of adults living with CF have a chronic *P. aeruginosa* infection (5,31,32). A chronic infection is one that can no longer be treated with eradication therapy and is detrimental to the health of people with CF (30). People with CF that are chronically infected with *P. aeruginosa* are more vulnerable to future pulmonary diseases and face a faster decline in their overall pulmonary function (26). The reasons that someone becomes chronically infected with *P. aeruginosa* are not well understood (6).

2.3 Management of *P. aeruginosa*

2.3.1 Eradication therapy

The key to successful eradication of a non-mucoid *P. aeruginosa* infection is early detection and treatment (2,5) and in some cases, multiple eradication attempts (28). Children diagnosed with CF at birth through NBS are more likely to have their first *P. aeruginosa* infection detected within the first five years of their life, and therefore more likely to have their infections eradicated early (6,12). Li et al. (12) followed 56 cases of children with CF identified through NBS and found

that the median age for contracting a non-mucoid *P. aeruginosa* infection was one year.

The most common aerosolized treatment used to effectively treat a *P. aeruginosa* infection is inhaled tobramycin (28,33). Tobramycin as a single treatment course of 28 days has been shown to effectively treat an infection but is not always successful in eradication and may require subsequent treatment courses if the repeat post treatment culture of *P. aeruginosa* is still positive (28). The effectiveness of a multistep eradication protocol for *P. aeruginosa* infection treatment in people with CF was explored in 2017 (28). Findings showed that the overall success of the treatment improved by 12% (95% confidence interval (CI): 18.6, 50.9) if the first course of inhaled tobramycin was followed by a multistep treatment plan (28). The multistep plan was employed if the individual's cultures for *P. aeruginosa* infection were still positive after the first inhaled tobramycin treatment course (28). Other forms of treatment for *P. aeruginosa* infections are inhaled colistin or inhaled colistin with oral ciprofloxacin (29). Overall, successful *P. aeruginosa* eradication is possible, but a 100% eradication success cannot be guaranteed (12,29,30).

The likelihood of contracting a *P. aeruginosa* infection again is high even after a successful eradication (6). With each subsequent infection, the chances of the infection developing resistance to antibiotics increases (6). Antibiotic resistance to *P. aeruginosa* occurs in infections where mucoid phenotypes are present and is more commonly observed with increasing age (12). Even with these risks, it is imperative to treat any new non-mucoid *P. aeruginosa* infection

because without treatment, the first positive *P. aeruginosa* culture will become chronic in approximately 20% of all people with CF (10,30). It is speculated that in children, early eradication of *P. aeruginosa* impact clinical outcomes later in life (12), which is why timing of the *P. aeruginosa* treatment is so important.

2.3.2. Defining chronic *P. aeruginosa* infection

In broad terms, a chronic *P. aeruginosa* infection is one that no longer responds to antibiotic treatments (6). The most notable adaptation of *P. aeruginosa* that allows clinicians to discern between chronic and non-chronic infections is its resistance to antibiotics (6). A more indepth way to determine when a *P. aeruginosa* infection has become chronic is by examining the phenotypes of a positive culture for mucoid strains. A mucoid phenotype is defined based on the overproduction of the polyanionic exopolysaccharide alginate (27). Mucoid phenotype classification (12,30,34) is not always available in secondary data sources.

To date, there is no gold standard for defining a chronic *P. aeruginosa* infection, but the two criteria are commonly used in research using secondary data sources are known as the Copenhagen criteria and the Leeds criteria (12,30,34,35). These criteria are known to consider the number of previous positive *P. aeruginosa* cultures an individual has within a specified time period (12,30,34). Further considerations such as the presence of two or more precipitating antibodies against *P. aeruginosa* identified in an individual's regular sputum samples taken during clinic visits or the time period from a patient's first

ever *P. aeruginosa* isolate to when they developed a chronic infection are adaptations of the criteria and formulated based on the available data for individual studies (12,30,34).

The first known study to employ the Copenhagen criteria was in 1974, where a chronic *P. aeruginosa* infection was classified when an individual presented with six consecutive monthly sputum samples that all tested positive for *P. aeruginosa* (36–38). These criteria, developed at the Copenhagen CF Centre, where individuals with CF are evaluated on a monthly basis (36–38), have been used consistently there since (37). This definition relies on data being uniformly collected and is not a universal definition because not all countries follow the same clinic schedule (36).

The Leeds criteria were developed in 2003 and define a chronic infection as positive when *P. aeruginosa* has been identified in 50% or more cultures within a 12 month period obtained from monthly sputum swabs (35). The Leeds criteria can predict future *P. aeruginosa* infection status and have high face validity (35). Lee et al. (35) tested the face validity based on accurately classifying chronic *P. aeruginosa* infections in people with CF and how it related to clinical scores and investigations. The accuracy of this definition is higher because of the frequent sampling of cultures; cough or sputum swabs were collected at every monthly clinic visit (a maximum of 12 weeks between visits) (35).

2.4 Previously identified risk factors of *P. aeruginosa*

Recognizing the importance of chronic *P. aeruginosa* infections, several investigators have examined the potential risk factors associated with developing a chronic infection. Pressler et al. (27) studied 225 people with CF at the Copenhagen clinic, 89 of whom did not have any chronic lower respiratory tract infection at the start of the study (27). Using the Copenhagen criteria to define a chronic infection, they identified elevated levels of anti-pseudomonal antibodies of IgG1 and IgG4 subclasses to have the strongest association with chronic *P. aeruginosa* infection (27). Mucoid phenotype of the bacteria and the occurrence of an *Aspergillus* infection were also identified as risk factors for chronic infection (27).

A longitudinal study of 53 children diagnosed with CF via NBS found that the risk of developing a mucoid *P. aeruginosa* infection for people with a non-mucoid *P. aeruginosa* infection who received anti – *P. aeruginosa* treatment was significantly lower compared with children who did not receive treatment (hazard ratio (HR) 0.09 (95% CI: 0.02, 0.39)) (12). This study was performed in 2005, and was the first longitudinal analysis of the stages of *P. aeruginosa* in CF until acquisition of mucoid *P. aeruginosa* (12). Children were likely to acquire their first *P. aeruginosa* infection within their first year of life and develop a mucoid *P. aeruginosa* infection by age 16 years (12).

A second longitudinal analysis that examined the stages of *P. aeruginosa* infection in people with CF used a modified Leeds definition of chronic *P. aeruginosa* infection and adjusted for sex, age at diagnosis and genotype (11).

This study used the CF Foundation Patient Registry from the United States with a total sample size of 5592 (11). Their investigation found that the risk of developing a chronic *P. aeruginosa* infection increased 2.59 times (95% CI: 2.11, 3.19) if a mucoid *P. aeruginosa* infection had been contracted first (11). An additional finding from this study was that children diagnosed with CF who have contracted at least one *P. aeruginosa* infection face an increasing risk of developing a chronic *P. aeruginosa* infection as they age (11).

Levy et al. (39) examined a cohort of 542 children with CF at Children's Hospital Boston to identify specific biomarkers and factors associated with initial detection of mucoid *P. aeruginosa* infection. A mucoid status was diagnosed clinically (39). They showed that female sex, presence of at least one ΔF508 allele, lower forced expiratory volume in one second percent predicted (FEV_1%), and a negative *S. aureus* culture were reported to be predictive with of mucoid *P. aeruginosa* infection (39).

Research has shown an association between eradication failure and mucoid status of a *P. aeruginosa* infection (28). Although host factors have not been shown to be associated with *P. aeruginosa* eradication failure, microbiological factors such as antimicrobial resistant initial *P. aeruginosa* strains, diminished twitch motility (surface movement of the *P. aeruginosa* bacterium) and mucoid status have been identified as risk factors for eradication failure (28). Risk factors of reoccurrence of *P. aeruginosa* infections have been found to mirror the risk factors identified for initial *P. aeruginosa* infection (28).

Taccetti et al. (31) examined the time period between early antibiotic therapy for *P. aeruginosa* and when the bacteria re-cultured in a patient's respiratory tract, as well as attempted to differentiate if the re-cultured bacteria was an identical strain or a different organism. Their study population included 58 individuals with CF followed in a CF centre in Florence, Italy (31). People with CF were free of *P. aeruginosa* after undergoing between 3-weeks to 3-months of eradication therapy and could remain free of *P. aeruginosa* for up to 18 months (31). Genotyping of *P. aeruginosa* isolates was only available for 16 of the participants who had a combined overall total of 50 *P. aeruginosa* isolates. Findings in this group showed that the *P. aeruginosa* isolates were different 73% of the time, indicating successful eradication of the initial infection (31). These findings help to emphasize the importance of early initiation of eradication therapy. Further investigation is needed to determine if any risk factors of chronic *P. aeruginosa* are preventable or modifiable.

2.5 Summary and objectives

P. aeruginosa is one of the most common pathogens found in the lungs of people with CF. Chronic infection exacerbates clinical outcomes and has a poor prognosis. Past research has identified risk factors for initial *P. aeruginosa* infection, but knowledge surrounding risk factors for chronic infection is lacking.

NBS is still new in many provinces in Canada (17,18) and it is not known whether early diagnosis, and therefore early eradication of *P. aeruginosa* delays chronic infection.

This thesis will address these gaps by investigating:

1. What are some characteristics of children with CF who develop a chronic *P. aeruginosa* infection?
2. What is the association between the number of eradication attempts and time to chronic *P. aeruginosa* infection?
3. Does the association between the number of eradication attempts and time to chronic *P. aeruginosa* infection differ between children diagnosed clinically and those diagnosed by newborn screening?

2.6 Figures and tables

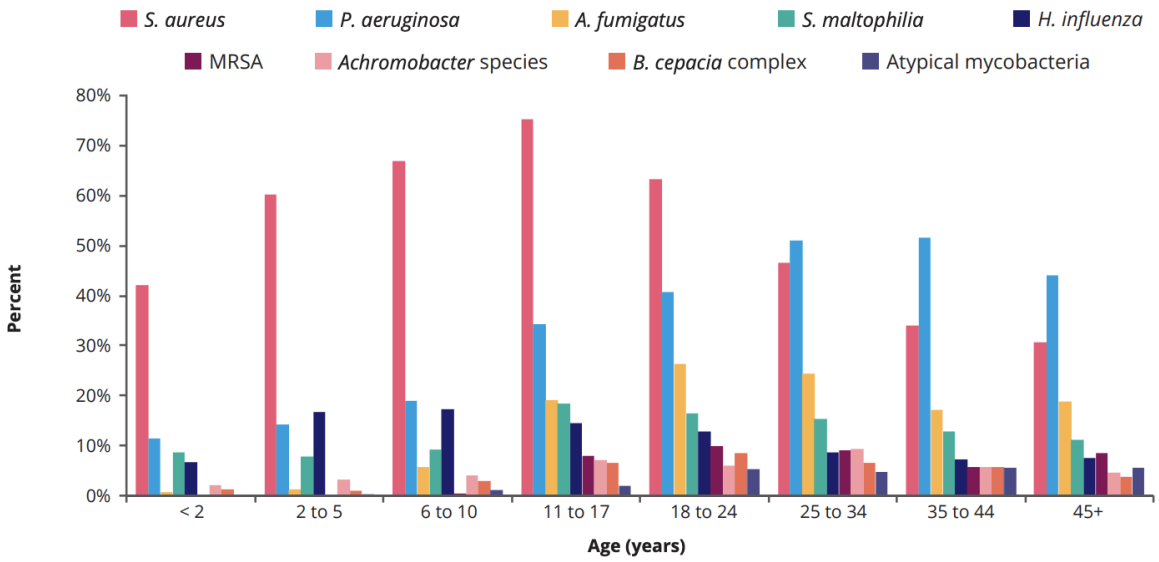

Figure 2.1: Age-specific the prevalence of respiratory infections routinely monitored in people with CF reported from data collected in 2019 by the CCFR. Obtained from the 2019 registry report (Cystic Fibrosis Canada 2020).

Chapter 3: Methods

3.1 Study Overview

3.1.1 Study design

This study was a prospective cohort study that used data compiled within the CCFR.

3.1.2 Study population and exclusion criteria

The population of interest comprised of Canadians under 18 years of age who had been diagnosed with CF, had provided consent to their data being included in the CCFR, and had at least one recorded *P. aeruginosa* infection between 2000 and 2020. The years from 2000 and later were selected because early eradication therapy for *P. aeruginosa* was only introduced in Canada in the early 2000s. The dataset included individuals born between 1982 – 2020 who met the above criteria and were reported within the period of 2000 – 2020. For individuals born between 1982 – 1999, two additional variables were requested: 1) the cumulative number of previous records of *P. aeruginosa* infections that an individual has had in the five years prior to 2000 and 2) a summary number of microbiology samples taken in the five years prior to 2000 (1995 – 1999). The cumulative number of previous infections was divided by the summary number of microbiology samples and converted to a percent. If the percent was greater than 50% in a calendar year, the individual was deemed to be chronic and excluded from the study (12). The paediatric population was included because adults were

likely to have already developed chronic infection before these treatments were introduced.

3.1.3 Ethics and data storage

A data access application was approved by CF Canada in July 2022. The Research Ethics Board at Dalhousie University approved this study (REB # 2022-6109). For security purposes, all data were housed on a secure, password-protected network. Access to the data was limited to only the individuals granted data access by CF Canada in accordance with Dalhousie University's policies. Data were stored on a secured, password protected network for five years following the publication of results (after which it will be destroyed by deleting the raw data and written confirmation will be given to the CCFR).

3.2 Data

3.2.1 The Canadian cystic fibrosis registry

The patient organization, CF Canada, was established in the 1960s through funding provided by parents of children diagnosed with CF (15). The CCFR was first created in the early 1970s and focused on monitoring demographics and mortality of people with CF (15). CF data registries play integral roles in both identifying the population with the disease and tracking the population's clinical outcomes (40). The CCFR represents the life course of a person with CF and is extensively used for research to better understand the epidemiology and evolution of CF (40).

In many countries, people with CF receive care from multiple specialists who practice together at accredited CF treatment centres. In Canada, there are

42 accredited CF treatment centres where 98% of the Canadian CF population receive treatment and care (15,41). All 42 accredited CF clinics collect and submit data of patients who have provided consent to have their data stored in the CCFR (15).

CF Canada has taken several steps to minimize ascertainment bias and loss to follow-up (42). CF Canada provides incentives to CF clinics across Canada to encourage them to collect and submit data to the CCFR (42). There are also incentives to encourage people with CF to attend CF treatment centres. Many CF medications are covered financially under provincial drug plans if patients get their medications from a pharmacy associated with an accredited CF treatment centre (42). CF Canada provides financial support to each treatment centre that submits data to the CCFR (42). It is estimated that less than 1% of individuals diagnosed with CF decline consent to have their data captured in the CCFR (42). As a result, the CCFR captures almost all people with CF in Canada (42). To further ensure that the CCFR is devoid of any errors, the database undergoes routine validation checks, as well as cross-referencing any irregularities with the CF treatment centre that first reported the data (40).

Demographic and clinical data are collected as part of the CCFR, including sex, date of birth, genotype, lung function, and bacterial infections. Overall, the CCFR contains approximately 90 variables (40). Not all variables have been collected since the inception of the registry; for example, genotype was not recorded by CF Canada until 1991, approximately two years after the CFTR gene was discovered (15).

3.2.2 Estimated study population size

At the time of project design, the 2019 CCFR annual report was used to inform the sample size estimates. There were 1660 children less than 18 years of age reported on (15). In this report, 13-35% of children had at least one infection with *P. aeruginosa* (15). Therefore in 2019, it was estimated that there were as few as 215 and at most 580 children with at least one infection of *P. aeruginosa* reported on in the CCFR.

An assumption was made at the time of project design that there were approximately 100 new CF diagnoses each year, and approximately 80 of these were diagnosed during childhood. Therefore, it was also assumed that 80 individuals would transition to adulthood each year on their 18th birthday. Since the study period was anticipated to span from 2000 to 2019, the sample size of children included those that were less than 18 years between 2000 and 2019. Therefore, at the time this study was being planned, it was anticipated that the total sample size of children with at least one *P. aeruginosa* infection between 2000 and 2019 would range from a minimum of 400, and a maximum of 1100. The above estimated study population was determined under the assumption that the study would span from 2000 – 2019, but since data from 2020 was available at the time of the data request, the final study period spanned from 2000 – 2020.

3.3 Variables

3.3.1 Outcome variable

The outcome of interest was chronic *P. aeruginosa* infection (Table 3.1). For this thesis, a chronic *P. aeruginosa* infection was defined through an adaptation of the Leeds criteria: if within one calendar year, more than 50% of an individual's *P. aeruginosa* cultures were positive and there was more than one clinic visit or microbiology sample reported, the infection was deemed chronic.

In Canada, microbiology samples (sputum sample, throat swab, or bronchoalveolar lavage) are obtained approximately every three months and results are recorded by calendar year (as opposed to by actual date). Therefore, the total number of cultures and the number that are positive for *P. aeruginosa* were available separated by calendar year in the CCFR. It was not known if the positive microbiology samples were consecutive, which is why the Copenhagen criteria could not be used (34,35,43).

3.3.2 Primary exposure variable

The primary exposure variable was the cumulative number of previous *P. aeruginosa* infections that were not deemed chronic since study entry (Figure 3.1). Historical data from 1995 – 1999 were obtained with the dataset from the CCFR and used to determine an approximate number of previous *P. aeruginosa* infections each individual born between 1982 – 1999 had prior to the start of the study. Individuals whose historical data was obtained would have been less than 18 years of age for some or all of the years between 2000 – 2020 (aging out

once they turned 18 years old) but they would have acquired their first *P. aeruginosa* infection prior to the study start in 2000. This followed a similar approach as previously used by Lillquist et al. (26). A definition can be found in Table 3.1.

The CCFR records both the number of microbiology cultures taken and the number of positive cultures. However, the process to determine previous *P. aeruginosa* infections as done by Lillquist et al. (26) could not be used in this study because the registry did not have three cultures for *P. aeruginosa* obtained within a six-month period. As the CCFR does not record the date the microbiology cultures are taken, it was not possible to know how many months separate two positive cultures, only that two positive cultures were recorded. Therefore, a modified definition was used: if there were less than or equal to 50% of positive *P. aeruginosa* cultures within a one-year period (i.e., it is not chronic) but at least one positive *P. aeruginosa* culture, then that was be counted as a cleared *P. aeruginosa* infection (Figure 3.2).

As NBS is relatively new in Canada, there were individuals who were diagnosed with CF later in childhood and were thus likely to have had undetected and untreated *P. aeruginosa* infections that were not captured in the registry. To account for this left truncation, age was used as the time variable.

3.3.3 Confounding variables

The analysis adjusted for both time-varying and time-invariant confounders. The time-varying confounders, disease severity and nutritional

status, were measured at each time interval (i.e., each year) (Figure 3.1). The time-invariant confounders were age at baseline, sex, region, year of study entry and genotype. These variables were based on the baseline values (first observation for each individual in the study period) and remained the same for the duration of the study (Figure 3.1). Definitions for all confounders can be found in Table 3.1, and they are described in detail in the following sections.

3.3.3.1 Disease severity

Disease severity was measured using FEV_1% standardized for age, height, sex, and ethnicity (44). The first clinically stable (that is an FEV_1 taken from a clinic visit, not a hospitalization) FEV_1% measurement within the specified calendar year was used.

3.3.3.2 Nutritional status

Nutritional status was measured using body mass index (BMI), which was expressed as percentiles for sex and age. Percentiles were obtained from the CCFR, so no further adaptations were required. For descriptive purposes, BMI percentiles were categorized into underweight (< 13^{th} percentile), adequate weight (13^{th} – 85^{th} percentile), and overweight (> 85^{th} percentile).

3.3.3.2 Sex

Sex was coded as the individual's recorded biological sex (male or female).

3.3.3.3 Region

Region was defined as either Eastern Canada, Quebec, Ontario or Western Canada as stated in the dataset provided by CF Canada or was reported as a mixed region if an individual was reported to have gone to clinics in two or more regions within a single report year.

3.3.3.4 Genotype

The CF genotype was described using the mutation group (homozygous ΔF508, heterozygous ΔF508, other, and missing).

3.3.4 Effect modification by newborn screening

NBS was an indicator variable, which was selected if the infant was identified as having CF through a provincial NBS program.

3.3.5 Other descriptive variables

Ethnicity was a categorical variable pre-defined in the CCFR as Asian, Black, White, Indigenous, South Asian, and Other. Pancreatic status and CF related diabetes were indicator variables. Pancreatic status was selected if an individual had insufficient pancreatic status and CF related diabetes was selected if an individual was diagnosed with CF related diabetes. For height and weight, each clinically stable measurement for each report year per individual was converted to a percentile using the World Health Organization's specifications for

individuals aged 2 years or less and the Centers for Disease Control and Prevention for individuals aged 2 – 18 years (45,46).

3.4 Statistical analysis

3.4.1 Data preparation

All statistical analyses were completed using Stata/SE 16.1 (StataCorp., College Station, TX, United States). The variables were first renamed, if necessary, for consistency and clarity. Data were then cleaned, and individuals who met the exclusion criteria were removed. Individuals who did not experience the event of interest were right-censored (when they turned 18, or on December 31, 2020). Next, the data were reviewed for any missing values and outliers. Missing values were dealt with on a case-by-case basis, based on which variable had missing values (explained in the following section).

Data were investigated for biologically implausible outliers, that is values that are impossible or extremely rare. Outlying values that exceeded the biologically plausible range were set to missing, if any. If an outlier was found to be biologically plausible, it was retained, and the analyses were run with and without the outlier it to see if the value was influential to the results.

Cross tabulation of all categorical variables (region, genotype, sex, and NBS) against one another was done to investigate if multicollinearity existed between any of the variables. To estimate if multicollinearity existed between the continuous variables (age at baseline, year of study entry, FEV_1%, BMI percentile, and number of previous *P. aeruginosa* infections), variance inflation

factors of all continuous variables were analyzed. Had the variance inflation factor exceeded 10, one of the variables would have been excluded from the analysis.

3.4.2 Missing values

Due to the relatively small sample size estimated, the risk of Type I error from a high proportion of missing data could have been increased when only complete cases were included in the analysis. To be conservative, variables with 25% or fewer missing values were imputed. Any confounding variables that were found to be missing more than 25% of values were not included in the analysis models due to insufficient data (47).

The time-varying variable, FEV_1%, was expected to be missing often before an individual turned 7 years old (because this measurement is not frequently done on children less than 7 years of age) or for unknown reasons such as an error in inputting data into the CCFR. For those less than 7 years of age, FEV_1% values were imputed randomly, drawing from a normal distribution where FEV_1 ranged from 72 – 112 % (48). For the missing FEV_1% values (such as those missing at random due to data entry) the patterns and proportions of missing data were explored. As there was less than 5% missing data, two simple methods (last observation carried forward/first observation carried back and linear interpolation) were performed.

All individuals missing pancreatic status values (n=6; approximately 0.29% missing) were assumed to have insufficient pancreatic status. There was only

one individual with missing values for CF related diabetes (n=1; approximately 0.05% missing). This individual did not have a CF related diabetes diagnosis date, so their missing values for all observations were imputed as 0. Children with a missing CF diagnosis date were assumed to have had CF since birth, and their date of diagnosis was imputed as their date of birth plus 30 days.

3.4.3 Objective 1

Descriptive statistics for each variable were summarised by chronic *P. aeruginosa* infection status. For continuous variables, the median and interquartile range were presented. For categorical variables, the count and proportion of each level within the variable were presented.

3.4.4 Objective 2

Survival analysis using time-dependent Cox proportional hazards regression was performed to estimate the association between the number of previous *P. aeruginosa* infections and risk of developing a chronic *P. aeruginosa* infection. To achieve this objective, the following steps were performed:

1. The survival function of interest, time to chronic *P. aeruginosa* infection, was examined by plotting the Kaplan-Meier survival estimate and a Nelson-Aalen cumulative hazard estimate.
2. A crude Cox proportional hazards regression model was estimated using chronic *P. aeruginosa* infection as the outcome and only the exposure variable (number of previous *P. aeruginosa* infections).

3. To check the model for a linear association between the number of previous *P. aeruginosa* infections and chronic *P. aeruginosa* infection, the exposure variable was categorized into 1, 2, 3, 4, and ≥5 previous infections, and then plotted as a Kaplan-Meier estimate and used in a multivariable Cox proportional hazards regression model.
4. The proportional hazards assumption for the crude model was checked using Global tests and Schoenfeld residuals.
5. The goodness-of-fit of the crude Cox proportional hazards regression model was evaluated using partial Cox-Snell and Martingale residuals.
6. A multivariable Cox proportional hazards regression model was estimated using chronic *P. aeruginosa* infection as the outcome and the following covariates: number of previous *P. aeruginosa* infections, FEV_1%, sex, baseline age, year of study entry, genotype, region, and BMI percentile.
7. The proportional hazards assumption for the multivariable Cox proportional hazards regression model was checked using Global tests and Schoenfeld residuals.
8. The goodness-of-fit for the multivariable Cox proportional hazards regression model was evaluated using partial Cox-Snell and Martingale residuals.
9. Any potential outliers were identified using dfBeta analysis and the influential outliers were further investigated.

3.4.5 Objective 3

The same steps as described in section 3.4.4 were performed except this time, there were two approaches: (1) a multivariable Cox proportional hazards regression model stratified by NBS and (2) a model that included an interaction term between the previous number of *P. aeruginosa* infections and NBS.

3.5 Sensitivity analysis

To address possible limitations from left truncation of the data that could bias the results, the analyses for objectives 2 and 3 as described in section 3.4.4 were re-run including only individuals who experienced their first *P. aeruginosa* infection within the study period, 2000 – 2020. This excluded all individuals from the main analysis born from 1982 – 1999.

3.6 A priori sample size and power

At the time that this study was planned, only two studies had evaluated risk factors for chronic *P. aeruginosa* (11,12). The smallest HR estimated for an association between infection with *P. aeruginosa* and the risk of chronic *P. aeruginosa* infection in these studies was 2.59 (11). The prevalence of chronic infection in children was between 13 – 17% (12).

With an estimated minimum sample size of 400 children for the proposed study, a power of 80%, alpha level of 0.05, and after accounting for censoring and a range of the probability of event from 10 – 20%, the minimum HR that was estimated as being detectable using the CCFR was 2.51 (Figure 3.3).

3.7 Tables and figures

Table 3.1: Description of outcome, exposure, and confounding variables used in the analyses.

Variable Name	Definition used for this thesis	Justification	Variable Type
Chronic *Pseudomonas aeruginosa*	Number of positive cultures was greater than 50% of the total number of cultures taken within the one-year time period (present or absent).	(35)	Outcome – time to event
Number of previous *Pseudomonas aeruginosa* infections	Number of positive cultures up to that year (not including those meeting the definition for chronic infection).	(11,26)	Primary exposure – Continuous, time varying
Baseline age	Age at study entry for individuals who have had their first *Pseudomonas aeruginosa* infection before the year 2000 or age at first *Pseudomonas aeruginosa* infection (years) during the study period.	(11,12)	Covariate - Continuous
Year of study entry	The year that an individual first enters the study (calendar year).	(1,41)	Covariate - Continuous
Sex	Biological sex (male or female).	(11,12,27,39)	Covariate - Binary
Disease Severity	The first clinically stable FEV_1% value from within the specified calendar year.	(39)	Covariate – Continuous, time varying
Genotype	Mutation group (homozygous ΔF508, heterozygous ΔF508, other and missing).	(11,12,27,39)	Covariate - Categorical
Region	The region of cystic fibrosis treatment centre (Eastern Canada, Quebec, Ontario and Western Canada) where an individual was reported attending, or mixed region if two or more regions were reported.	(12,40)	Covariate - Categorical
Nutritional status	Body mass index expressed as percentiles for sex and age.	(27,40)	Covariate – Continuous, time varying
Newborn screening	Indicates if an individual was diagnosed with CF clinically of through newborn screening techniques.	(12)	Covariate - Binary, interaction

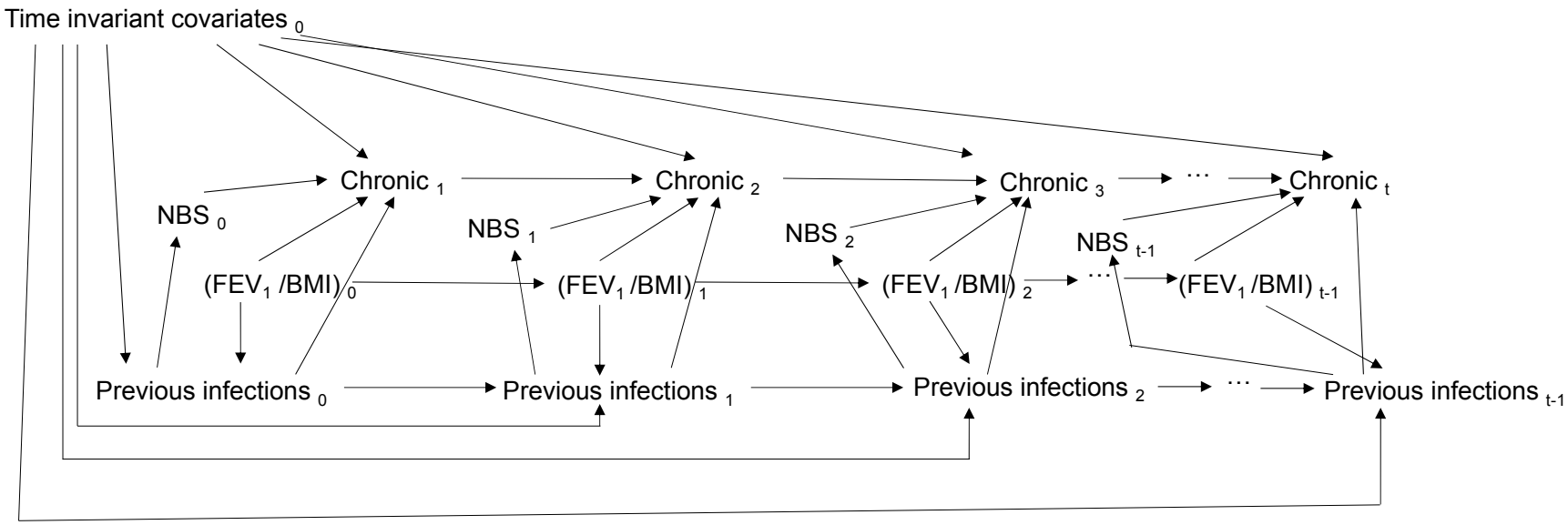

Figure 3.1: Directed acyclic graph depicting how the time-varying and time-invariant confounders were anticipated to act on both the primary exposure variable (previous P. aeruginosa infections) and the outcome variable (chronic P. aeruginosa infection). The time-invariant confounders were measured once at baseline and are age at baseline, sex, region, year of study, and genotype. The time-varying confounders measured annually were FEV_1 percent predicted (FEV_1 in figure) and body mass index (BMI).

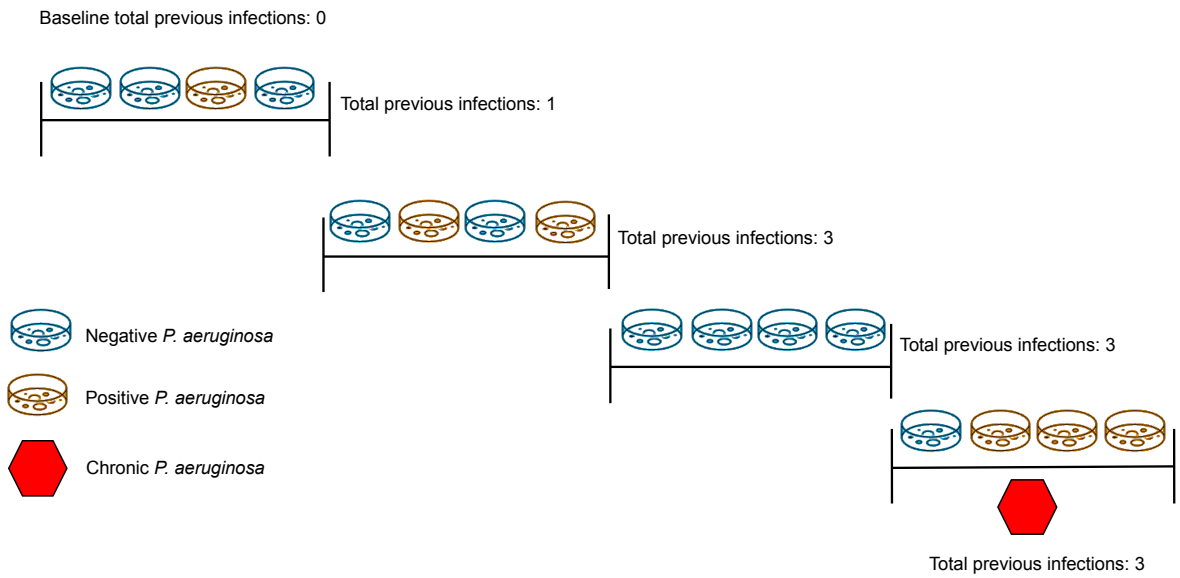

Figure 3.2: Diagram depicting how the primary exposure variable was measured for both the primary and sensitivity analyses. Each grouping of four petri dishes represents the approximate four microbiology cultures taken from this individual with CF in one year. Based on the figure, the individual develops a chronic *P. aeruginosa* infection 4 years after their first *P. aeruginosa* infection.

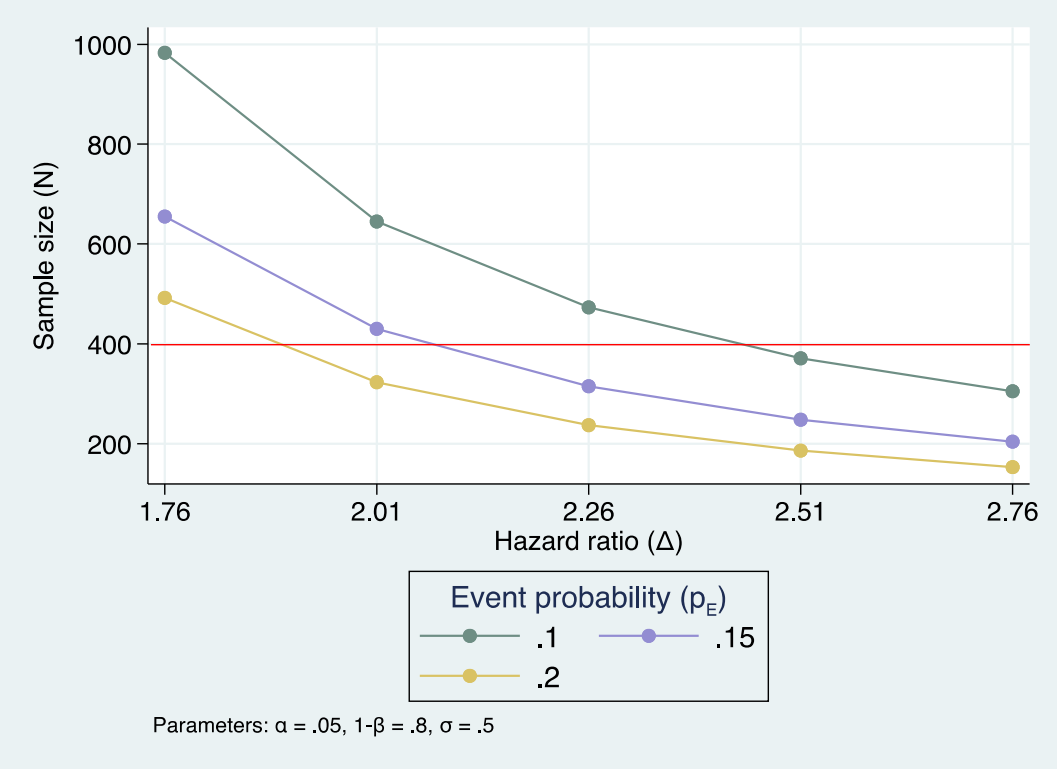

Figure 3.3: Sample sizes required to detect hazard ratios between 1.76 – 2.76 with 80% power and an alpha of 0.05 and event probabilities between 10 – 20%. The red line indicates the anticipated minimum sample size.

Chapter 4: Results

4.1 Study population

A summary of the study population that met the eligibility criteria is presented in Figure 4.1. A 5-year look back window from 1995 – 1999 of the total count of previous *P. aeruginosa* infections for individuals born between 1982 - 1999 found 866 individuals born prior to the study start had already acquired a chronic infection using the pre-specified modified Leeds criteria; where an individual was chronic if more than 50% of all of their microbiology samples within a single report year were positive for *P.* aeruginosa. This left 2135 children eligible for this study. An additional 37 children were excluded due to missing data. The final analytical sample consisted of 2098 individuals. Within the analytical sample, 817 children (38.9%) developed a chronic infection during the study period.

4.1.1 Missing data

Both the time-varying variables, FEV_1% and BMI percentile, had missing values. For individuals younger than 7 years of age, missing FEV_1% values were imputed assuming that all children had a 'healthy' value of lung function drawing from a normal distribution where FEV_1% could range from 72 – 112 % (48). That left 5.3% (n= 879/16 663 observations) with missing FEV_1 values. The percent of missing values for BMI percentile was 4.9% (812/16 663 observations). Both these variables were then reshaped to a wide format (where each individual had one row of data instead of the multiple rows observed in long format) to examine

the patterns of missingness. It was observed that the missing values for both variables were randomly missing throughout all years. To confirm whether complete case analysis would be appropriate for these data, two imputation methods were performed (last observation carried back / first observation carried forward and a linear interpolation) to compare how much the distributions of the variables and the HR from the crude regression model for the association between the previous number of *P. aeruginosa* infections and chronic infection differed between the complete case and imputed datasets. Both imputed datasets had an overall sample size of 2135 individuals each, compared with the complete case dataset which excluded the missing values for an overall sample size of 2098 individuals. The differences between the population characteristics were minor (+/- 1 or 2%) and the preliminary crude HR produced in each regression model, where the previous number of infections was the exposure and chronic infection was the outcome with no confounding variables included, were similar. These results confirmed that the complete case analysis would be appropriate for this study.

4.2 Participant demographics

Characteristics of the 2098 children with CF who were included in the study cohort are presented in Table 4.1. The majority of children in the cohort were diagnosed with CF through a clinical diagnosis (84.8%); in other words, they presented with symptoms of CF. Most of the study population was white ethnicity (90.9%) and attended a CF clinic in either Ontario (35.1%) or Quebec

(30.6%). Over 50% of children with CF in the study population were homozygous for the ΔF508 genotype (50.3%), and 92.5% were pancreatic insufficient.

4.3 Objective 1

Among the 2098 children free from chronic infection at baseline, 817 children developed a chronic *P. aeruginosa* infection over the follow-up period of the study (n = 817; 38.9%; Table 4.1). A full summary of characteristics for the 817 children who developed a chronic infection are presented in Table 4.1. Relative to the study cohort, the sub-set of children with chronic infection had a higher prevalence of CF related diabetes (26.7% compared with 18.8%), were more likely to be diagnosed clinically and not through newborn screening (96.1% compared with 84.8%), and more likely to have an adequate BMI percentile (74.2% compared with 62.6%). The most notable differences observed in the sub-set of children with a chronic infection compared with the study cohort were fewer individuals with a height greater than the 85th percentile (8.6% compared with 16.3%) and fewer individuals with an underweight BMI percentile (17.3% compared with 28.1%). All remaining demographics of the sub-population were similar to the study cohort.

4.4 Objective 2

Figure 4.2 shows that as children get older and their CF disease progresses, their likelihood of developing a chronic *P. aeruginosa* infection increases. For every new *P. aeruginosa* infection, the hazard of developing a

chronic *P. aeruginosa* infection was estimated to increase by 1.09 times ((95% CI: 1.07, 1.12); Table 4.2 and Table 4.3). The association between previous infection and time to chronic infection was not attenuated after adjusting for the potential confounding variables, FEV_1%, age at baseline, sex, clinic region, year of study entry, genotype, and BMI percentile (HR 1.08 (95% CI: 1.06, 1.10); Table 4.3).

To investigate for a linear association between the previous number of *P. aeruginosa* infections and the time to chronic infection, the exposure variable was summarised as five categories (1 infection, 2 infections, 3 infections, 4 infections, and 5+ infections). The Kaplan Meier estimates are presented in Figure 4.3. In a Cox proportional hazard model, the HRs for individuals with 2 or 3 previous *P. aeruginosa* infections compared with individuals who only had 1 previous infection, were similar (Table 4.4). Compared with individuals who only had 1 previous infection, the hazard was 1.42 times for individuals with 2 previous infections (95% CI: 1.14, 1.77), 1.54 times for individuals with 3 previous infections (95% CI: 1.21, 1.97), 2.41 times greater for those with 4 previous infections (95% CI: 1.88, 3.11) and 2.95 times greater ((95% CI: 2.42, 3.60); Table 4.4) for those with 5 or more previous infections. There was a small step change in the HR observed for individuals who had 4 or 5+ previous *P. aeruginosa* infections which suggests that overall, there is no linear association between the previous number of infections and chronic infection.

4.5 Objective 3

The sub-set of individuals diagnosed with CF through NBS was much smaller (n = 319) than those clinically diagnosed with CF (n = 1779). Individuals diagnosed through NBS were at lower risk of developing a chronic infection compared with individuals clinically diagnosed (Figure 4.4).

In the group that was diagnosed with CF through NBS, the hazard for developing a chronic infection was 18% higher with each new *P. aeruginosa* infection (HR 1.18 (95% CI: 1.05, 1.33); Table 4.3). In the clinically diagnosed group, the hazard for developing a chronic infection was 9% higher for each new *P. aeruginosa* infection (HR 1.09 (95% CI: 1.07, 1.11); Table 4.3). The primary association between previous infection and time to chronic infection was not attenuated after adjusting for potential confounding variables (NBS group: HR 1.16 (95% CI: 1.03, 1.31) or those diagnosed clinically: HR 1.08 (95% CI: 1.06, 1.10); Table 4.3).

In a separate model, the interaction term between previous *P. aeruginosa* infections and NBS was not statistically significant; NBS did not modify the relationship between previous infections and chronic infection (interaction coefficient 1.09 (95% CI: 0.96, 1.22)).

4.7 Sensitivity analysis

4.7.1 Exclusions

The analytical sample was further restricted to include only individuals born on or after January 1, 2000, and who acquired their first *P. aeruginosa*

infection during the study period (2000 – 2020); 1679 individuals (55.9%) were excluded (Figure 4.5). An additional 37 individuals were excluded due to missing data. The analytical sample for the sensitivity analysis included 1285 individuals. Within this sample, 360 (28.0%) individuals developed a chronic *P. aeruginosa* infection between the years of 2000 – 2020.

4.7.2 Objective 2

The overall Kaplan Meier estimate was similar in the contemporary sub-set (Figure 4.6). With each additional *P. aeruginosa* infection, the hazard of chronic infection was estimated to increase by 18% (univariate HR 1.18 (95% CI: 1.14, 1.22); Table 4.3), and this association was not attenuated after adjusting for potential confounding variables (HR 1.17 (95% CI: 1.13, 1.21); Table 4.3).

Further, the linearity of the association was assessed (Figure 4.6) as in the main analysis; the risk of developing a chronic infection was increased by 33% for individuals with 2 previous *P. aeruginosa* infections compared with individuals who only had 1 previous infection (HR 1.33 (95% CI: 0.96, 1.83); Table 4.5). This risk remained similar for individuals with 3 previous infections with only a 37% increased risk compared with individuals who only had 1 previous infection (HR 1.37 (95% CI: 0.92, 2.03); Table 4.5). A step change in the HR was observed in the contemporary sub-set for individuals who had 4 or 5+ previous *P. aeruginosa* infections. Compared with individuals who only had 1 previous infection, the hazard was 2.17 times greater for those with 4 previous infections (95% CI: 1.42, 3.34) and 3.99 times greater for those with 5 or more previous infections ((95% CI: 2.93, 5.43); Table 4.5). This suggests that overall, there was no linear

association between the previous number of infections and chronic infection within the contemporary sub-set.

4.7.3 Objective 3

The Kaplan Meier estimate of the probability of not having a chronic *P. aeruginosa* infection stratified by NBS was similar in the sensitivity analysis (Figure 4.8). In the group that was diagnosed with CF through NBS, the hazard for developing a chronic infection was 19% higher for each new *P. aeruginosa* infection (HR 1.19 (95% CI: 1.05, 1.34); Table 4.3). In the clinically diagnosed group, the hazard for developing a chronic infection was 17% higher for each new *P. aeruginosa* infection (HR 1.15 (95% CI: 1.13, 1.21); Table 4.3). The univariate association between previous *P. aeruginosa* infections and time to chronic infection was not attenuated after adjusting for potential confounding variables for either sub-set (diagnosed through NBS (HR 1.18 (95% CI: 1.05, 1.34)) versus those clinically diagnosed (HR 1.17 (95% CI: 1.13, 1.21)); Table 4.3).

The interaction term was not statistically significant indicating that NBS did not modify the relationship between previous *P. aeruginosa* infections and chronic *P. aeruginosa* infection (interaction coefficient 1.07 (95% CI: 0.95, 1.20).

4.8 Model assumptions and goodness of fit checks

For all analyses performed, none of the models violated the Cox proportional hazards assumption based on Global tests and Schoenfeld residual plots. Poor model fit was indicated (observed probabilities compared with

predicted probabilities) such that the Nelson Aalen cumulative hazard function deviated from the partial Cox-Snell residual function.

4.9 Tables and figures

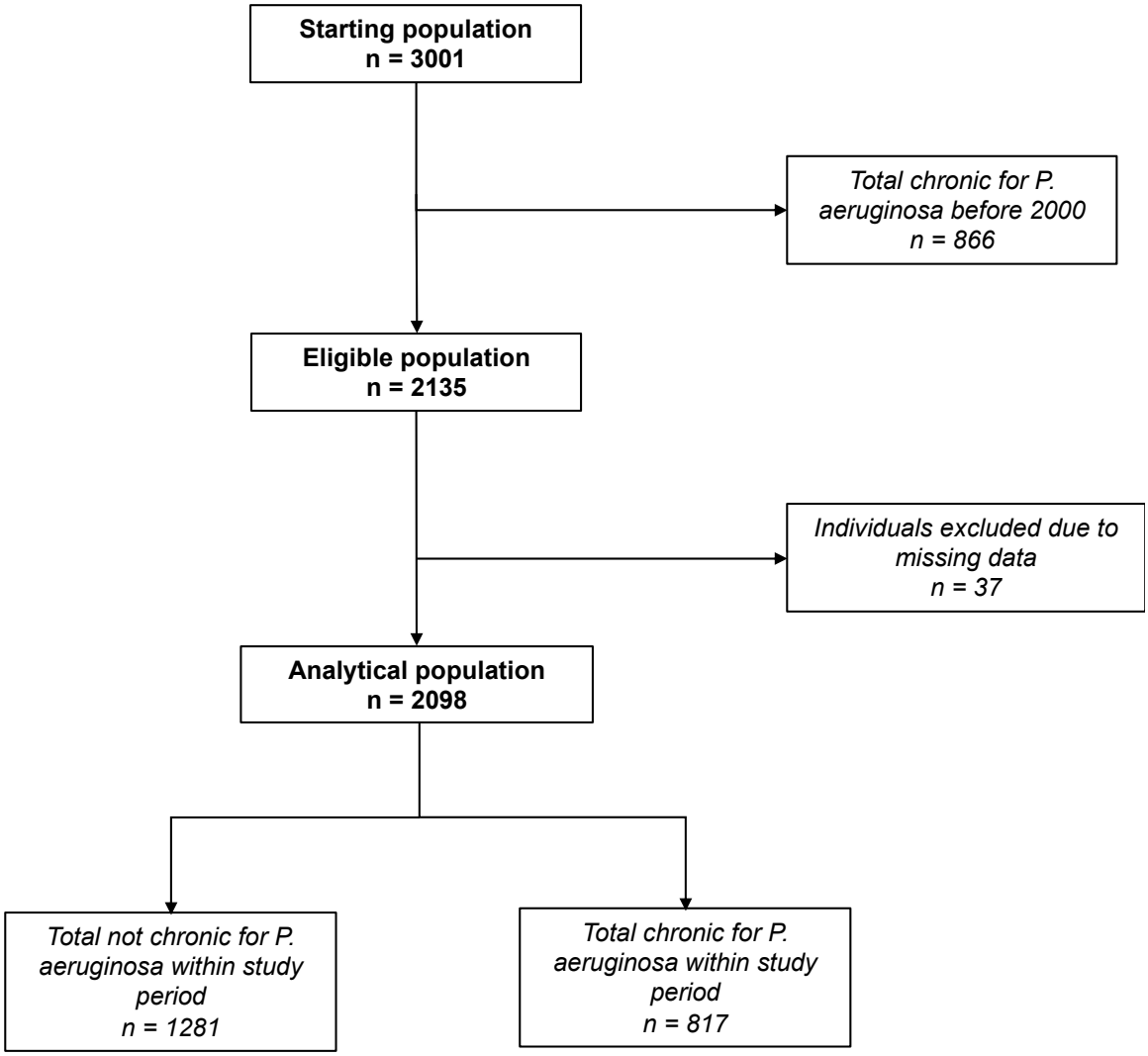

Figure 4.1: Flow chart of study population.

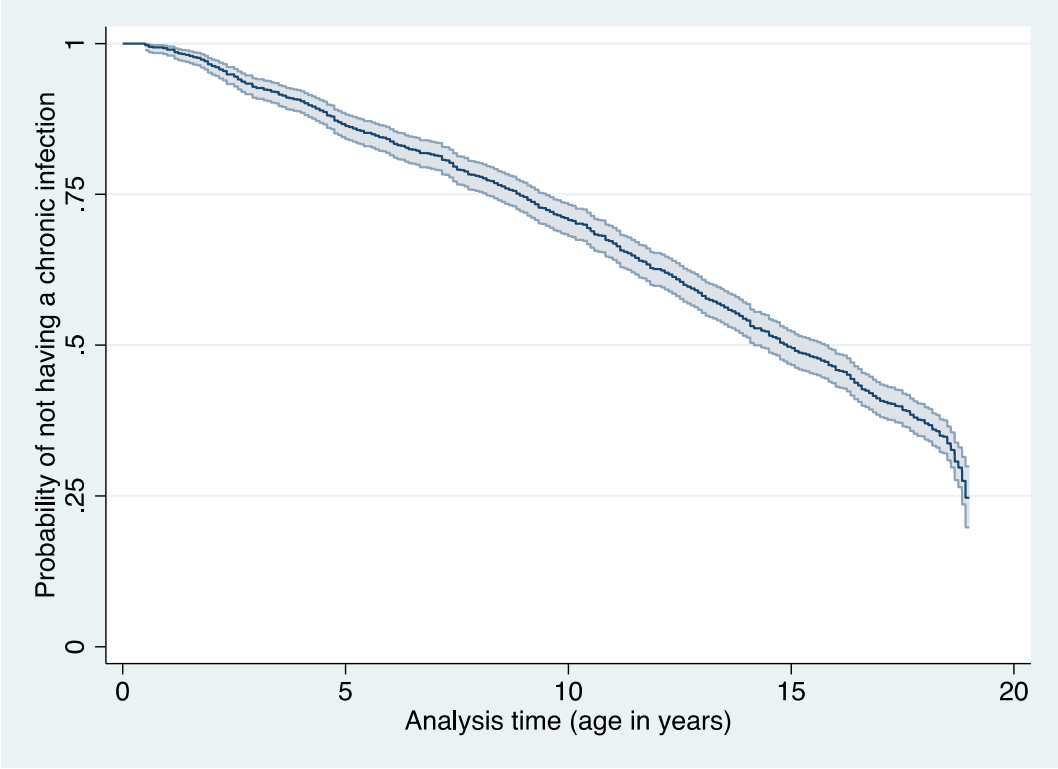

Figure 4.2: Kaplan Meier estimate of the probability of not having a chronic *P. aeruginosa* infection by analysis time (age in years) (n = 2098). The median age of chronic infection was 10 years.

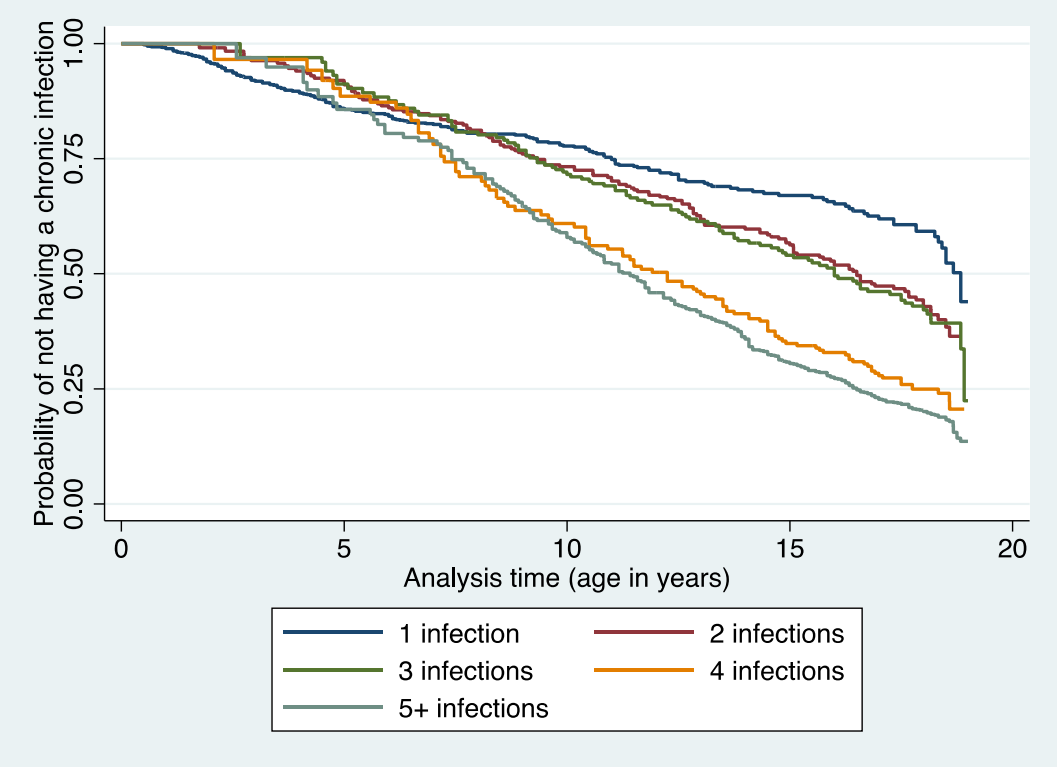

Figure 4.3: Kaplan Meier survival estimates of the probability of not having a chronic *P. aeruginosa* infection by analysis time (age in years) from the crude model of the main analysis (n = 2098). The exposure variable, the previous number of *P. aeruginosa* infections, was summarised as categories.

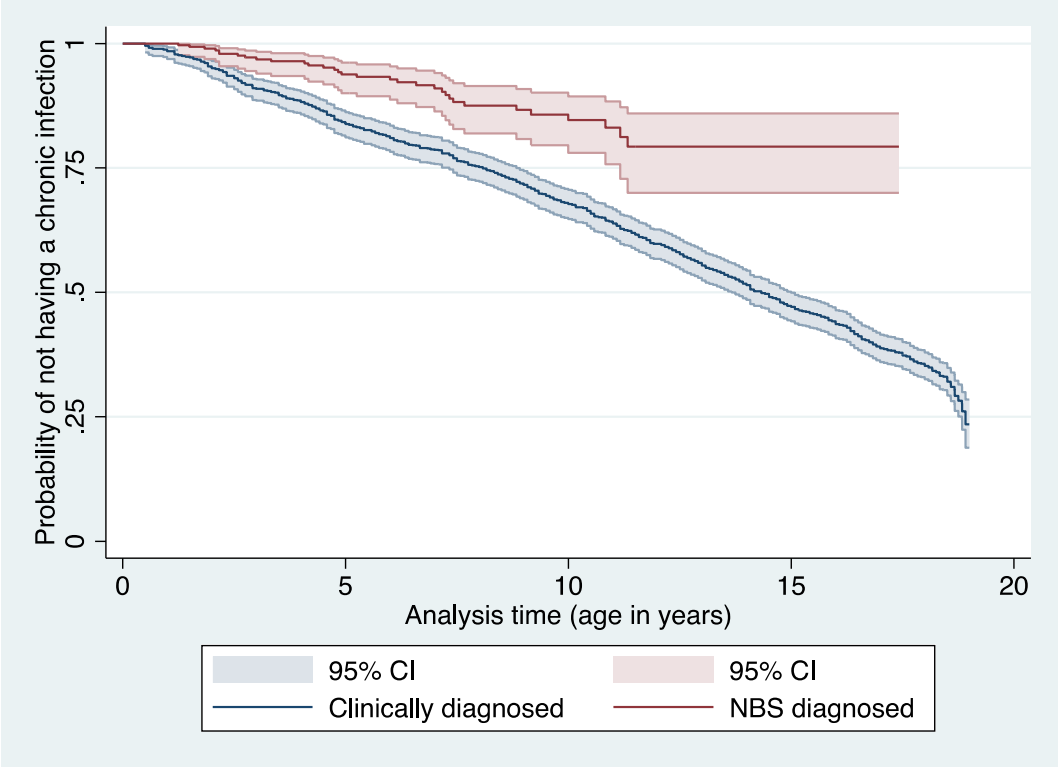

Figure 4.4: Kaplan Meier estimate of the probability of not having a chronic *P. aeruginosa* infection by analysis time (age in years) of the analytical sample stratified by cystic fibrosis diagnosis: clinically diagnosed (n = 1779), diagnosed through newborn screening (NBS) (n = 319).

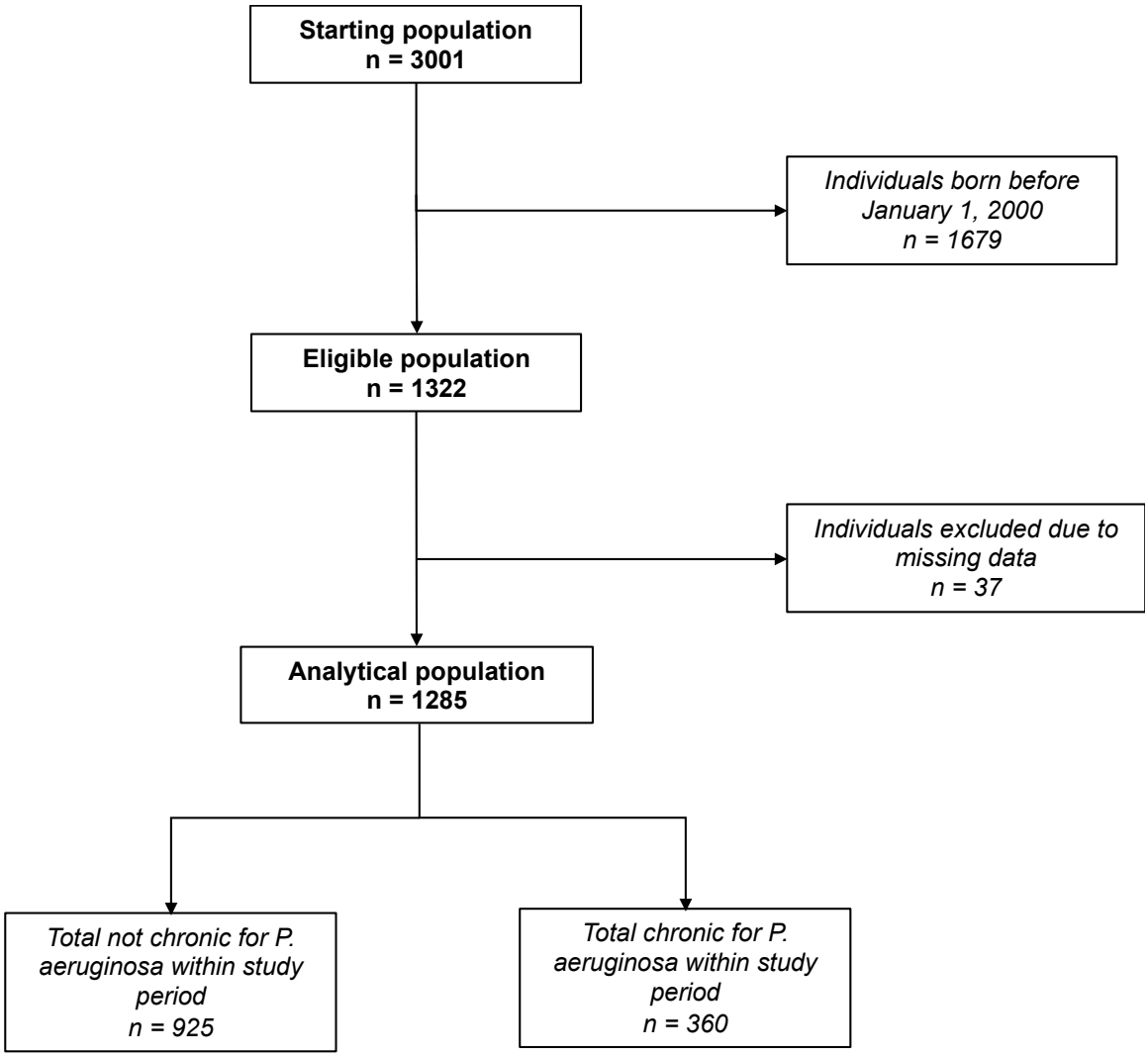

Figure 4.5: Flow chart of study population for the sensitivity analysis, where everyone enters from the year 2000 onward and after they acquire their first *P. aeruginosa* infection.

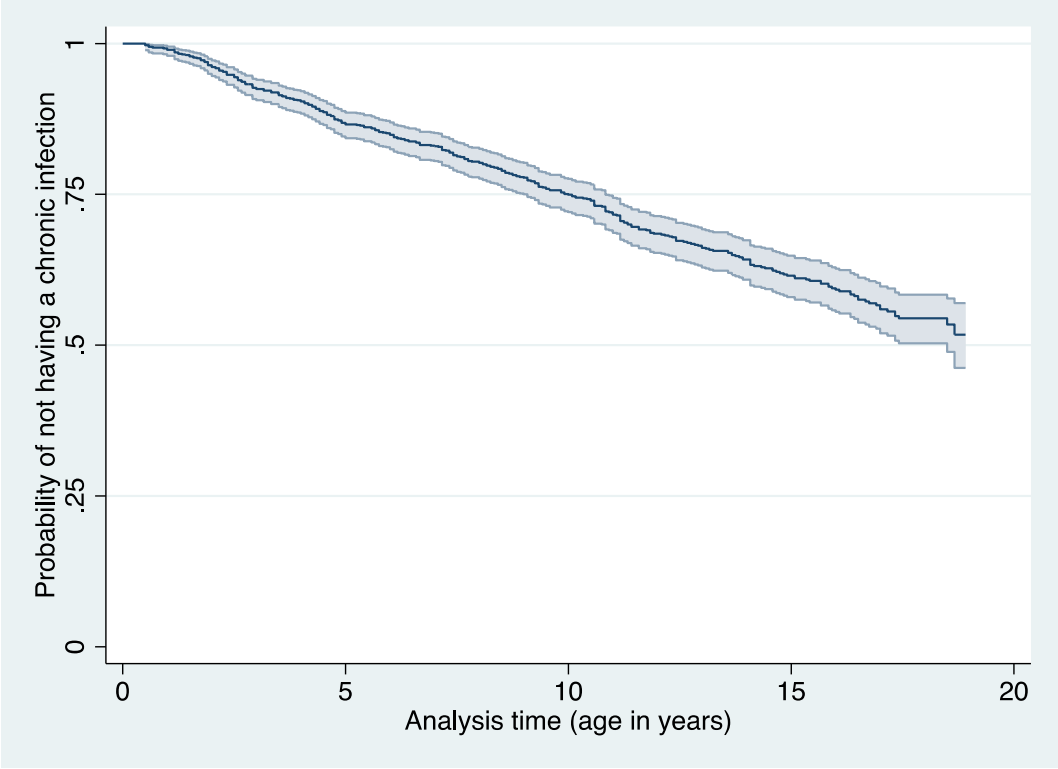

Figure 4.6: Kaplan Meier estimate of the probability of not having a chronic *P. aeruginosa* infection by analysis time (age in years) based on the crude model from the sensitivity analysis, which only included individuals who experienced their first infection within the study period (n = 1285). The median age of chronic infection was 6 years.

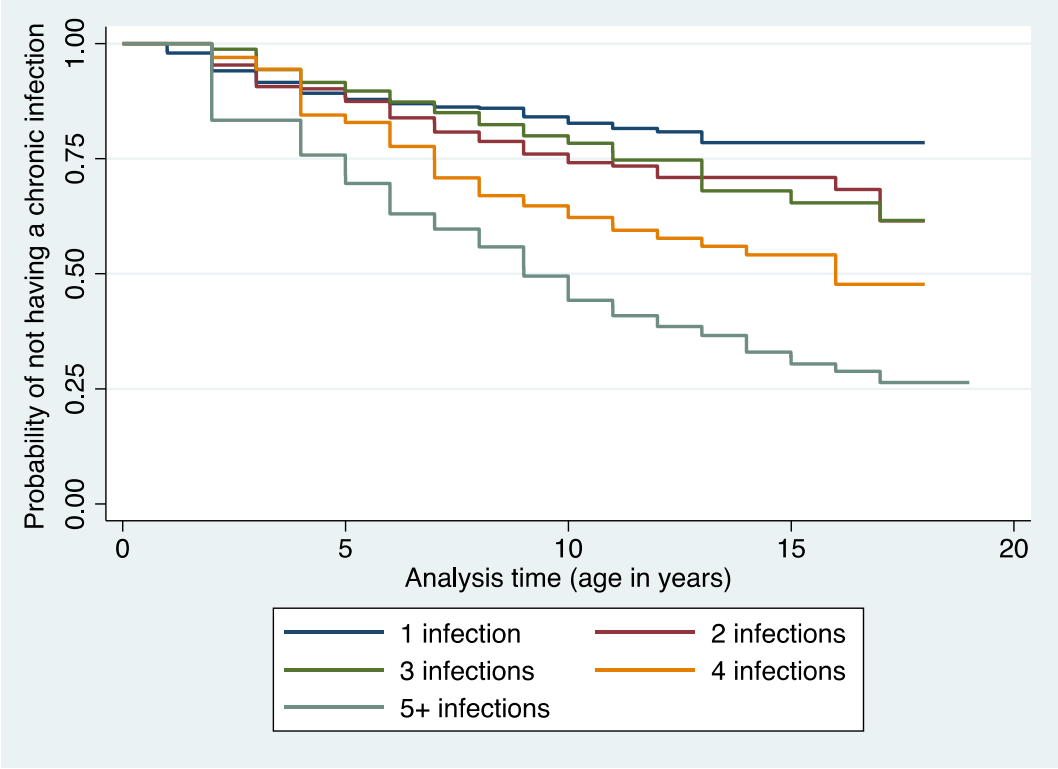

Figure 4.7: Kaplan Meier estimates of the probability of not having a chronic *P. aeruginosa* infection by analysis time (age in years) from the crude model of the sensitivity analysis, where individuals enter the study after their first *P. aeruginosa* infection (n = 1285). The exposure variable, the previous number of *P. aeruginosa* infections, was summarised as categories.

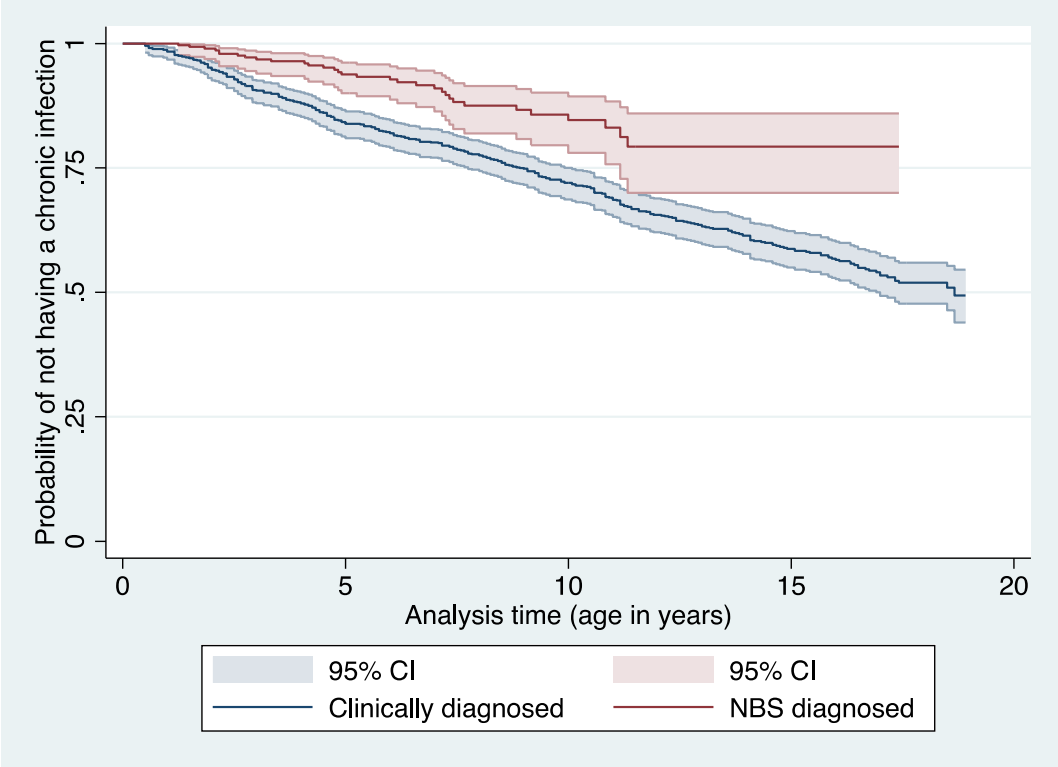

Figure 4.8: Kaplan Meier estimates of the probability of not having a chronic *P. aeruginosa* infection by analysis time (age in years) from the sensitivity analysis, stratified by cystic fibrosis diagnosis: clinically diagnosed (n = 966), diagnosed through newborn screening (NBS) (n = 319).

Table 4.1: General characteristics of all individuals who meet the inclusion criteria measured at baseline (where the first observation for each individual was used to calculate the demographics) (n = 2098) and for all individuals who develop a chronic *P. aeruginosa* infection within the study period measured at baseline (n = 817).

		Demographics	
		Eligible sample	***Chronic infection***
Number of patients (n)		2098	817
General			
Sex			
	Female, n (%)	1056 (50.3)	410 (50.2)
Ethnicity			
	Asian, n (%)	8 (0.4)	4 (0.5)
	Black, n (%)	24 (1.1)	10 (1.2)
	White, n (%)	1907 (90.9)	737 (90.2)
	Indigenous, n (%)	32 (1.5)	14 (1.7)
	South Asian, n (%)	21 (1.0)	10 (1.2)
	Other, n (%)	105 (5.1)	42 (2.0)
Pancreatic status			
	Insufficient, n (%)	1940 (92.5)	775 (94.9)
CFRD			
	Yes, n (%)	395 (18.8)	218 (26.7)
Clinic region			
	East, n (%)	150 (7.15)	59 (7.2)
	Quebec, n (%)	642 (30.6)	273 (33.4)
	Ontario, n (%)	736 (35.1)	280 (34.3)
	West, n (%)	559 (26.6)	200 (24.5)
	Two or more, n (%)	11 (0.5)	5 (0.61)
Diagnosis			
Newborn screening			
	Yes, n (%)	319 (15.2)	32 (3.9)
Genotype mutation group			
	Heterozygous F508del, n (%)	807 (38.5)	320 (39.2)
	Homozygous F508del, n (%)	1056 (50.3)	411 (50.3)
	Missing, n (%)	22 (1.1)	10 (1.2)
	Other, n (%)	213 (10.2)	76 (9.3)

	Demographics	
	Eligible sample	***Chronic infection***
Number of patients (n)	2098	817
Diagnosis		
Age at diagnosis (years)		
Overall, median (IQR 25%, 75%)	0.3 (0.0, 1.5)	0.3 (0.0, 1.8)
Nutritional markers at baseline		
Height (percentiles), n (%)		
12	665 (31.7)	231 (28.3)
13 - 85	1090 (52.0)	516 (63.2)
> 85	343 (16.3)	70 (8.6)
Weight (percentiles), n (%)		
12	656 (31.3)	202 (24.7)
13 - 85	1158 (55.2)	553 (67.7)
> 85	284 (13.5)	62 (7.6)
BMI at baseline (percentiles), n (%)		
Underweight	590 (28.1)	141 (17.3)
Adequate Weight	1314 (62.6)	606 (74.2)
Overweight	194 (9.2)	70 (8.6)
FEV_1 %		
Median (IQR 25%, 75%)	91.6 (79.6, 102.2)	85.5 (72, 98)
Death		
Total deaths within study period, n (%)	10 (0.5)	0 (0.0)
Age at death (years), median (IQR 25%, 75%)	14.2 (12.3, 16.2)	n/a

*n/a - cannot report due to insufficient numbers

Table 4.2: Univariate Cox regression hazard ratios (HR) and 95% confidence intervals (95% CI) for the association of the main exposure (previous *P. aeruginosa* infections) and confounding variables with the development of a chronic *P. aeruginosa* infection (n = 2098).

		Univariate	
Characteristic		**HR**	**95% CI**
Previous P. aeruginosa infections		1.09	(1.07, 1.12)
FEV_1%		0.99	(0.986, 0.99)
Age at baseline		0.99	(0.96, 1.02)
Sex			
	Female	1.00	Reference
	Male	0.99	(0.86, 1.13)
Region			
	Ontario	1.00	Reference
	East	1.24	(0.93, 1.64)
	Quebec	1.17	(0.99, 1.38)
	West	0.84	(0.70, 1.01)
	Mixed	1.46	(0.60, 3.54)
Year of study entry		1.00	(0.9996, 0.9997)
Genotype			
	Homozygous ΔF508	1.00	Reference
	Heterozygous ΔF508	1.08	(0.93, 1.25)
	Missing	1.62	(0.86, 3.03)
	Other	0.90	(0.71, 1.15)
Body mass index percentile		1.00	(0.9943, 0.9993)

Table 4.3: Crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) for all univariate (crude) and multivariable (adjusted for FEV_1%, age at baseline, sex, region, year of study entry, genotype, and BMI) Cox proportional hazards regression models and the analytical population for each model used for objectives 2 and 3 for both the main and sensitivity analyses.

	Crude model		Adjusted model		Analytical population
	HR	**95% CI**	**HR**	**95% CI**	**n**
Objective 2					
Analytical sample	1.09	(1.07, 1.12)	1.08	(1.06, 1.10)	2098
Sub-set born in 2000 or later	1.18	(1.14, 1.22)	1.17	(1.13, 1.21)	1285
Objective 3 (by mode of diagnosis of cystic fibrosis)					
Analytical sample - clinically diagnosed	1.09	(1.07, 1.11)	1.08	(1.06, 1.10)	1779
Analytical sample - NBS	1.18	(1.05, 1.33)	1.16	(1.03, 1.31)	319
Sub-set born in 2000 or later - clinically diagnosed	1.17	(1.13, 1.21)	1.17	(1.13, 1.21)	966
Sub-set born in 2000 or later - NBS	1.19	(1.05, 1.34)	1.18	(1.05, 1.34)	319

Table 4.4: Univariate hazard ratios (HR) and 95% confidence intervals (95% CI) for the exposure variable, previous *P. aeruginosa* infections, when summarised as five categories (1 infection, 2 infections, 3 infections, 4 infections, 5+ infections) in the main analysis (n = 2098).

Number of previous infections	HR	95% CI
1 infection	1.00.	Reference
2 infections	1.42	(1.14, 1.77)
3 infections	1.54	(1.21, 1.97)
4 infections	2.41	(1.88, 3.11)
5+ infections	2.95	(2.42, 3.60)

Table 4.5: Univariate hazard ratios (HR) and 95% confidence intervals (95% CI) for the exposure variable, previous *P. aeruginosa* infections, when summarised as five categories (1 infection, 2 infections, 3 infections, 4 infections, 5+ infections).

Number of previous infections	HR	95% CI
1 infection	1.00.	Reference
2 infections	1.33	(0.96, 1.83)
3 infections	1.37	(0.92, 2.03)
4 infections	2.17	(1.42, 3.34)
5+ infections	3.99	(2.93, 5.43)

Chapter 5: Discussion

5.1 Main findings

In this cohort of 2098 children who had at least one acute *P. aeruginosa* infection and were followed for the development of a chronic *P. aeruginosa* infection from 2000 – 2020, the overall findings showed that characteristics of children who developed a chronic *P. aeruginosa* infection were similar to those without chronic infection. With each new *P. aeruginosa* infection, children and young people with CF are at an increased risk of developing chronic *P. aeruginosa.* The overall association between the number of previous infections and chronic infection was not linear, such that the risk was increased after 4 previous infections when compared with individuals who only had 1 previous infection. Children diagnosed with CF through NBS (a more contemporary population) were less likely to become chronically infected compared with children clinically diagnosed, but had a similar relative risk of chronic infection for each increasing *P. aeruginosa* infection compared with those diagnosed clinically. No confounding variables were found to attenuate the association between the number of previous infections and chronic infection in any of the adjusted models.

The HRs from the main analysis (n = 2098) and sensitivity analysis (n = 1285) indicate that there is a discordance between the two analytical samples. Due to the ambiguity of the modified Leeds criteria, where a chronic infection was determined by if an individual was positive for *P. aeruginosa* in more than 50% of all their microbiology samples taken within a single report year, misclassification

of chronic infection for any individuals born between 1982 – 1999 may have occurred. These individuals may have been classified as not chronic when they were or vice versa. Due to this misclassification, the results from the sensitivity analysis, restricted to children born in 2000 or after in whom the first *P. aeruginosa* infection could be determined, more likely reflects the true association between the number of previous *P. aeruginosa* infections and the risk of developing a chronic infection.

5.2 Findings in context of the previous literature

Compared with the previous literature, the population in the current study was more diverse and included children who were clinically diagnosed as well as those diagnosed through NBS. Previous research focused on identifying risk factors of initial *P. aeruginosa* acquisition (39), reasons or factors for eradication failure of *P. aeruginosa* treatment (28), and the time between early antibiotic *P. aeruginosa* therapy and when a *P. aeruginosa* infection re-cultures within an individual's lungs (31). Therefore, this study adds to existing knowledge surrounding the risk of chronic *P. aeruginosa* infection and builds onto the findings by using the number of previous infections to investigate the time to chronic infection using a population that includes individuals both clinically diagnosed with CF and diagnosed through NBS.

5.2.1 Characteristics of chronic *P. aeruginosa*

Pressler et al. (27) investigated the association between clinical factors and chronic *P. aeruginosa* infection focusing on factors prior to the onset of chronic *P. aeruginosa* infection, bacteriology identified through the collection of sputum or lower respiratory tract secretion and specific IgG anti-*pseudomonas* antibodies. Several factors investigated by Pressler et al. (27) were not available in this thesis (i.e., mannose-binding lectin genotypes and the occurrence of *S. aureus* or *Aspergillus fumigatus* in the lower respiratory tract). Since none of the variables used by Pressler et al. (27) matched the variables used in this thesis, the results are not comparable.

A recent study published in 2022 by Mésinèle et al. (49) examined a cohort of children with CF from France to identify risk factors and genetic modifiers for initial *P. aeruginosa* acquisition, chronic *P. aeruginosa* colonization and the progression from initial *P. aeruginosa* acquisition to chronic *P. aeruginosa* colonization (49). The analytical population included children with CF born between January 1, 2001, to December 31, 2019 (n = 1231) (49). Similar to the analysis performed in this thesis, Mésinèle et al. (49) estimated that children with CF related diabetes had 33% increased risk of chronic *P. aeruginosa* colonization compared with children without CF related diabetes (HR 1.33 (95% CI: 0.47, 3.79)). In the present Canadian study population (n = 2098), children with CF related diabetes were more likely to develop chronic infection (26.7% compared with 18.8%).

5.2.2 Association between previous infections and chronic *P. aeruginosa* infection

Mésinèle et al. (49) presented a cumulative incidence figure that showed an individual's risk of developing chronic *P. aeruginosa* infection increased as a child ages, a finding that was quite similar to the Kaplan Meier estimate presented in Figure 4.2. Unlike Mésinèle et al. (49), this study found a lower median age of chronic infection in the main analysis (10 years compared with 14.7 years). This difference was more noticeable when compared with the median age of chronic infection from the sensitivity analysis cohort (n = 1285, born in 2000 and onward; 14.7 years compared with 6 years). The higher median age of chronic infection in Mésinèle et al. (49) is likely due to a more refined study population that excluded individuals with sufficient pancreatic status. France introduced NBS earlier (in 2001; (49)) compared with Canada (introduced by province from 2007 to 2018; (17,18)), which may also explain why the populations differed. The French study had the ability to more accurately determine a chronic infection using dated microbiology sputum samples (where a chronic infection was determined when an individual had three positive *P. aeruginosa* samples at least one month apart across a 6-month period), whereas in this thesis the dates of microbiology samples are not available before 2015. Consistent with the findings from this thesis, they did not identify any risk factors that would indicate an individual is more susceptible to developing a chronic *P. aeruginosa* infection.

Heltshe et al. (11) described the stages of *P. aeruginosa* infection, characterized the emergence of age-specific prevalence of *P. aeruginosa* in young CF patients, and looked at the relationship between the different stages of *P. aeruginosa* infection and a mucoid status. A mucoid status is a severe form of a *P. aeruginosa* infection that does not respond to antibiotic treatments (6). Heltshe et al. (11) looked at how the acquisition of mucoid *P. aeruginosa* increases the risk of developing chronic *P. aeruginosa* (HR 2.59 (CI: 2.11, 3.19)) after adjusting for CFTR mutation functional class, ethnicity, sex, diagnosis type, and number of respiratory cultures. The HR from Heltshe et al. (11), is much higher than the HR estimated in this thesis but the two are not directly comparable because the CCFR does not differentiate between mucoid and non-mucoid strains of *P.* aeruginosa. As explained above, mucoid strains are much less responsive to antibiotics due to the formation of a biofilm layer within the lungs, thereby increasing the risk of the infection becoming chronic and persistent (30). It is for this reason that often a mucoid infection is used as an indicator of a chronic *P. aeruginosa* infection (6). Further, Heltshe et al. (11), used a stricter definition of chronic infection. They restricted their study population to only individuals who had at least 3 quarters (3-month intervals) with respiratory cultures for a minimum of 2 years prior to entering the study (11), which would have increased the accuracy of determining when individuals developed chronic *P. aeruginosa.* The classification of chronic infection in this thesis could not differentiate the exact dates of infection and was limited to the number of infections in a calendar year, which introduced possible

misclassification in the determination of chronic infection. The sensitivity analysis attempted to correct for some of this bias by further restricting the analytical sample to only include individuals born in 2000 and onward in whom the first *P. aeruginosa* infection could be determined. The data used by Heltshe et al. (11) was less likely to have misclassification bias compared with this thesis.

5.2.3 Time to chronic *P. aeruginosa* infection stratified by newborn screening

Li et al. (12) investigated time to mucoid *P. aeruginosa* infection among non-mucoid *P. aeruginosa* patients who were receiving anti-*P. aeruginosa* treatment compared with those not receiving anti-*P. aeruginosa* treatment. They studied 53 children from Wisconsin, USA, who were diagnosed with CF through NBS and adjusted for CF center, sex, genotype, and pancreatic status in their multivariable model. They found that the hazard of developing mucoid *P. aeruginosa* infection in those receiving anti-*P. aeruginosa* treatment was 0.09 less likely than those not receiving anti-*P. aeruginosa* treatment ((95% CI: 0.02, 0.39); (12)). Since Li et al. (12) looked at time to mucoid status and *P. aeruginosa* treatment and not chronic infection, their findings are not directly comparable to this thesis.

5.3 Strengths

One major strength of this study is that the CCFR captures virtually all Canadian residents living with CF and provides a complete, verified, longitudinal dataset specifically collected for research purposes (42). This study is anticipated

to be the second largest study to date to examine risk factors for chronic *P. aeruginosa* infection (11,49) and this type of research has been identified as one of the top research priorities by the Canadian CF community (13). Another strength is that the sample size used for this study was much larger than the sample size estimated a priori, allowing for the detection of a smaller effect size. The minimum HR estimated to be detectable using the CCFR (HR 2.51; n = 400; Figure 3.3) was much higher than the actual HR produced in this study (main analysis (n = 2098): adjusted HR 1.08 (95% CI: 1.06, 1.10); sensitivity analysis (n = 1285): adjusted HR 1.17 (95% CI: 1.13, 1.21)). Based on the sample size estimates shown in Figure 3.3, it was estimated that if a larger sample size was obtained, the minimum HR detectable could be lower. Another strength of this study was the use of a carefully defined directed acyclic graph and selection of variables for the analysis. The directed acyclic graph allowed for a clearer understanding of how the time-varying exposure variable, previous number of *P. aeruginosa* infections, would be affected by both the time-varying confounders, FEV_1% and BMI percentile, for each measurement of chronic infection at the end of each report year across the study period. The variables selected as potential confounders of the relationship between the previous number of *P. aeruginosa* infections and the time to chronic *P. aeruginosa* infection were chosen with the consideration of their availability in the CCFR and how they could compare to the findings from past studies. Most of the confounders selected were based on the previously identified risk factors of initial *P. aeruginosa* acquisition to see if they also posed a risk to the time to chronic infection. Lastly, the sensitivity analysis

helped to mitigate some of the misclassification bias introduced in the determination of chronic *P. aeruginosa* infection using the Leeds criteria in the main analysis. The analytical sample was restricted in the sensitivity analysis to only include individuals born in 2000 and onward who had experienced their first *P. aeruginosa* infection within the study period of 2000 – 2020. By including the individuals born between 1982 – 1999 in the main analysis but only using a lookback window of 5 years from 1995 – 1999, it is likely that individuals were chronic prior to the study start but misclassified as not chronic and included in the study, misrepresenting the overall non-chronic population within the study. By having everyone in the analytical sample of the sensitivity analysis enter the study at the same time zero (experience their first *P. aeruginosa* infection) the accuracy of the sensitivity analysis was improved and is a better representation of the relationship between the number of previous *P. aeruginosa* infections and the risk of developing a chronic *P. aeruginosa* infection.

5.4 Limitations

There are a number of limitations that need to be acknowledged.

There is risk of misclassification bias through the use of a modified Leeds criteria to identify a chronic *P. aeruginosa* infection. By using a one-year time period with the number of cultures per year being calculated based on whichever reported number was higher, the number of clinic visits or the number of microbiology samples from within one report year, it was not possible to confirm the number of eradicated infections. Consecutive monthly cultures, which are

required for the Copenhagen criteria (34,35,43), would have provided a more accurate determination of a chronic *P. aeruginosa* infection as they allow for a clearer definition of chronic. Prior to 2015, the date of microbiology cultures was not recorded in the CCFR. Ideally, classification of a mucoid strain would be best for the determination of a chronic *P. aeruginosa* infection but was not feasible for this study as the CCFR does not identify mucoid strains.

The CCFR captures the date of death of a person living with CF; therefore, any people who died before the completion of this study would have exited the cohort at their date of death. Deaths before age 18 years among people with CF are less common in contemporary populations but still occur (14,15). Out of the 2098 children measured at baseline, 10 individuals (including both chronic and not) died before the completion of follow-up (0.5%). Death was a competing risk in all 10 individuals who died before they developed a chronic infection. However, with so few deaths occurring before the development of a chronic infection, the competing risk of death would not have substantively affected the results of this thesis.

Measurement error may have been introduced by imputing a 'normal' FEV_1 for children under 7 years of age (48). Obtaining spirometry measurements for children under 7 years, especially infants, is difficult. It is possible that with the random imputation from the pre-specified FEV_1% range for any individuals under 7 years of age who were missing FEV_1% values were assigned values that were not representative of their lung function. By assuming a healthy lung function, FEV_1% would have been overestimated to be better than it may have been for

some children, causing the direction of bias to be towards the null (closer to a HR of 1.0).

Another limitation is that the data were left-truncated, both with respect to late diagnosis of CF (clinical measures are only captured after a formal diagnosis is made) and infections that occurred before the study observation period. Healthy survival bias would have occurred for any individuals who did not have their first *P. aeruginosa* infection until later in childhood resulting in them entering the study at older ages. Left truncation could have disproportionally affected the children who were diagnosed clinically, compared with children diagnosed through NBS. Using age as the time variable helped to account for left truncation but could not eliminate the bias. To address these biases the sensitivity analysis had all study participants enter at the year of their first known infection (i.e. no assumptions were made about number of infections or chronic infection status before this time). When there was more certainty around the exposure and outcome definitions, the HRs observed for both objectives 2 and 3 were noticeably higher than the hazards in the main analysis. It is likely that the sensitivity analysis is a better representation of the actual hazard for time to chronic *P. aeruginosa* infection as there is less likelihood for misclassification of chronic infection. It is possible that the main analysis included people who were chronically infected or in whom the number of previous infections was undercounted.

The analyses performed met the primary assumption of proportional hazards but had poor predictive ability. This was less of a concern since the aim

of this thesis was not to produce a model to predict a chronic *P. aeruginosa* infection but rather to investigate the association between the number of previous *P. aeruginosa* infections and the time to chronic *P. aeruginosa* infection. Poor model fit of the univariate analyses indicated that the exposure alone cannot explain why individuals develop a chronic infection. Poor model fit of the multivariable analyses indicated that after adjusting for all confounders, the model could still not predict why individuals develop a chronic infection.

5.5 Generalizability

The findings from this thesis may be generalized to paediatric CF populations outside of Canada who have a minimum of one *P. aeruginosa* infection. The findings from the main analysis of this thesis may not be as applicable to individuals diagnosed through NBS as the NBS program in Canada is still relatively new. Therefore, the results from the analysis restricted to only children diagnosed with CF through NBS are more appropriate. Lastly, this thesis is not generalizable to the adult CF population as no adult results were reported.

5.6 Knowledge translation

The findings from this study will be presented to the local CF team at respirology rounds at the IWK Health Centre in Halifax, Nova Scotia. As this research aligns with the current research interests of the Canadian CF community (13), an infographic will be created summarizing the major findings and sent to CF Canada for dissemination to the CF community. This infographic is being designed to target parents and teenage audiences, with a focus on the

use of lay terms to help them better understand what factors are associated with chronic *P. aeruginosa* infection. Lastly, the findings are anticipated to be presented at a CF conference and as a peer reviewed manuscript.

5.7 Future work

Microbiology infection acquisition dates have been recorded in the CCFR since 2015, therefore it may be possible re-classification of chronic infection requiring a minimum time interval between consecutive infections would help to identify chronic *P. aeruginosa* infection more accurately. Ideally, this research should be repeated with knowledge of mucoid strains to more accurately determine the risk of chronic infection.

Chapter 6: Conclusion

This thesis examined a cohort of 2098 children with CF who had at least one acute *P. aeruginosa* infection from 2000 – 2020 and followed them to investigate the association between the number of previous *P. aeruginosa* infections and the risk of developing a chronic *P. aeruginosa* infection. It was found that with each new *P. aeruginosa* infection children and young people with CF develop, they face an increased risk of developing a chronic *P. aeruginosa* infection. A non-linear association between the number of previous infections and chronic infection was observed; there was an increased hazard of chronic infection with each additional infection, with the hazard increasing to two times the baseline level (one infection) after 4 repeated *P. aeruginosa* infections. After stratifying by NBS it was found that children diagnosed with CF through NBS are less likely to develop a chronic infection compared with children diagnosed clinically. The hazard for chronic infection for each increasing *P. aeruginosa* infection was found to be similar between the NBS and clinically diagnosed groups. The findings from this thesis could be used by clinicians and provide more specific information regarding the risk of chronic *P. aeruginosa* infection as well as a possibe number of previous *P. aeruginosa* infections that could be used as an indicator for when a child may become chronic with *P. aeruginosa*.

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