# EXHALED GAS AS A NON-INVASIVE MARKER FOR AIRWAY INFLAMMATION IN PATIENTS WITH CYSTIC FIBROSIS

by

Jennifer L. Berkowitz

B.A., Arcadia University, 2004

Submitted to the Graduate Faculty of

The Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2007

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Jennifer L. Berkowitz

It was defended on

April 10, 2007

and approved by

Thesis Director:
Elizabeth Gettig, MS, CGC, Associate Professor
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh

Thesis Co-Director:
Joseph M. Pilewski, MD, Assistant Professor
Division of Pulmonary, Allergy and CCM
Secondary Appointment: Department of Cell Biology and Physiology
School of Medicine
University of Pittsburgh

Susanne M. Gollin, PhD, Professor
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh

EXHALED GAS AS A NON-INVASIVE MARKER OF AIRWAY INFLAMMATION IN PATIENTS WITH CYSTIC FIBROSIS

Jennifer L. Berkowitz, M.S.

University of Pittsburgh, 2007

Cystic Fibrosis (CF) is the most commonly inherited, life-shortening genetic condition amongst Caucasians, with an incidence of about 1 in 3,800 newborns and currently affecting about 30,000 Americans. It is chronically debilitating and the annual cost of medical care per person makes it a serious public health concern. Airway inflammation contributes to progressive pulmonary disease, the leading cause of morbidity and mortality in patients with Cystic Fibrosis. The mechanism by which the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene affects airway inflammation has not been fully elucidated to date; however, several mechanisms have been proposed. Despite the need for continued study in determining this mechanism, we do know that mutations in the CFTR gene ultimately result in bacterial colonization in the lungs, reduced mucociliary clearance and airway inflammation. Chronic airway inflammation results in continued assault on the lungs and progresses the course of the disease. Airway inflammation can be monitored through the use of bronchoalveolar lavage to evaluate the influx of neutrophils; however, routine bronchoscopy is an invasive procedure and is less than ideal for routine assessment. Exhaled gas as a marker for airway inflammation is useful in that it is minimally invasive and relatively easy to obtain. Some of the data on the clinical utility of exhaled gas measurements has been conflicting with regard to its efficacy in assessing airway inflammation. If exhaled gas measurements can be used to assess airway inflammation, they could provide a non-invasive alternative to monitor inflammation and do so more frequently than invasive

methods, with the ultimate goal of being able to detect inflammation earlier with the intent of earlier treatment and possible reduction in progression of lung disease.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## PREFACE

I would like to take this opportunity to express my deepest gratitude to the following individuals:

Dr. Joseph M. Pilewski, whose research, experience, time, resources and continued support made this project possible and whose vast knowledge of Cystic Fibrosis has proven immeasurably valuable. Ms. Elizabeth Gettig, for her unwavering support, mentoring and feedback, all of which helped me to develop and pursue this project, as well as my genetic counseling skills. Dr. Susanne Gollin, who greatly facilitated my presentation and project development skills, for her time and energy towards this project. Dr. Robin E. Grubs, whose guidance, support and thoughtful feedback have really cultivated my personal, professional and academic development.

I'd also like to thank my genetic counseling class and the department of human genetics, all of who greatly contributed not only to my academic progress but also to my personal experiences in Pittsburgh. Finally, I'd like to thank my family and friends for their unconditional love, encouragement, care packages and personal guidance. It has been their immense generosity of time, energy and spirit that has made my educational experience possible. Thank you.

# 1.0 INTRODUCTION

This literature review of research that has been conducted on the use of exhaled gas as a non-invasive marker of airway inflammation in patients with Cystic Fibrosis (CF) is supplemental to a much larger project studying the use of exhaled gas in patients with CF. The primary project is a three-phase study that is currently underway and involves the continuous collection of exhaled gas measurements as well as sputum cultures in order to provide more information on inflammatory markers in patients with CF. This data is still being collected and further research is needed in order to produce preliminary results. In order to more fully understand the relevance of using non-invasive markers to assess airway inflammation, it is important to understand what the disease is and how mutations in the CFTR gene cause the disease as well as the inflammatory process. The diagnosis, progression, management, genetics and inheritance of CF will be discussed with special attention on the role of airway inflammation in the progression of lung disease. The need for monitoring airway inflammation to better manage the disease will also be emphasized. Studies looking at the clinical utility of using non-invasive exhaled gas measurements to assess airway inflammation in CF have not been extensively studied, so this project was designed to highlight the most relevant or substantial research currently available.

Cystic Fibrosis (CF), also called Mucoviscidosis, is often described as the most common, lethal, genetic condition in Caucasians. It is a chronic, progressive disease that affects multiple

systems of the body including the respiratory, digestive, genitourinary and excretory system. With 30,000 Americans currently diagnosed with CF and an annual cost of medical care estimated to be about 20,000 dollars per person, or 600 million nationally, it is a serious public health concern. CF, which was first described by Dr. Dorothy H. Andersen in 1938, results from mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. This gene was located in 1989 on the long arm of chromosome 7 by positional cloning by Collins, Riordan, Tsui and colleagues (2,3). To date, over 1400 CFTR mutations have been identified, but the majority of these do not confer disease status.

The CFTR gene codes for the production of CFTR protein which functions as a channel to transport ions across cell membranes. This protein is located in the membranes of cells that line the lungs, pancreas, liver, intestines, reproductive tract, as well as the skin. Mutations in this gene result in impaired functioning of these channels, which conduct chloride to facilitate movement of chloride out of the epithelial cell to the covering mucus. This gene also helps regulate other ion channels. Impaired functioning of these ion channels reduces chloride transport out of the cell, altering the electrical gradient and contributing to an increase of sodium reabsorption. Without the ability to secrete chloride, and consequent to excess sodium reabsorption, the airway surface becomes dehydrated. In normal airway, regulated movement of sodium and chloride maintains an optimal gradient for water to leave the cell and enter the extracellular fluid (ECF), however in CF, this lack of salt regulation and the resulting lack of water movement causes the mucus lining the ducts and airways to become dehydrated, sticky and viscous. This thickened mucus then aggregates and causes blockages with various consequences depending upon the location of the ducts involved.

If the blockages are in the ducts of the pancreas, they prevent digestive enzymes from leaving the pancreas, resulting in an inability to properly digest and break down food, most notably fat. This is the cause of the malnutrition, poor growth and digestive issues often seen in patients with CF. The lack of pancreatic enzymes is the cause of the bulky, oily, greasy stools and excessive gas that are common amongst patients with CF.

When this blockage occurs in the reproductive tract, it may result in azoospermia, a common finding in men with CF. It should be noted that CF accounts for a significant percentage of men with congenital bilateral absence of the vas deferens, a common finding in men with CF. In women, increased cervical mucus has also been noted.

These duct blockages can also occur in the hepatobiliary tract, causing chronic hepatobiliary disease. An estimated 20-50% of patients express symptoms of hepatobiliary disease. Typically liver involvement presents within the first year of life with increased mucus in the bile ducts and ductules. This may lead to focal biliary fibrosis that is present in about one fifth of CF adolescents. This inflammation and fibrosis is progressive and eventually some of these patients, about 5%, develop cirrhosis of the liver [1].

In the lungs, the increase in mucus viscosity obstructs airflow, contributing to pulmonary decline. It is also responsible for the reduced mucociliary clearance, which when functioning properly, clears the airways of debris and particulate matter, as well as bacteria. Additionally, this thickened mucus is sticky and increases the adherence of bacteria, leading to colonization of the airways, which is thought to potentially increase the prevalence of respiratory infections [2]. These recurrent pulmonary infections may result in more inflammation and progressive damage to the lungs, causing a cyclic pattern of pulmonary decline. However it is unclear if the inflammation is caused solely by bacterial colonization of the lungs since studies, which will be

detailed later, indicate that airway inflammation is present in the lungs of newborns that do not have any indication of bacterial infection [3].

When bacteria enter the lungs and cause an infection, neutrophils (white blood cells) attack the infection. When the neutrophils die, they release DNA and elastase, which continues to thicken the mucus. This further obstructs the airways as well as increases infections and inflammation, which will be described in more detail in the Airway Inflammation Section.

## 1.1 INCIDENCE/PREVALANCE

It is estimated that about 30,000 Americans and 70,000 individuals worldwide are living with CF. Each year, approximately 3,200 babies born in the United States will be affected with CF. The incidence of CF is about 1 in 2900 in the U.S., making it more common than phenylketonuria (PKU) and Galactosemia. The number of annual deaths attributed to CF is roughly 360 deaths each year. The disease is the second most common life-shortening, childhood-onset, inherited condition and has a median age of survival of about 37 years. Although CF is panethnic and affects both men and women, the disease-causing mutations are more prevalent in Caucasians and individuals of Ashkenazi Jewish descent [4]. The approximate carrier frequency of a disease-causing mutation as well as the incidence of CF vary and are based upon ethnic background (Table 1).

**Table 1. CF Carrier Risks and Incidence based upon Ethnic Background [5]**

| Ethnicity | Carrier frequency | Incidence[1] |
|---|---|---|
| Caucasian | ~1/29 | ~1/3,300 |
| Ashkenazi Jewish | ~1/29 | |
| Hispanic | ~1/46 | ~1/8,000-9,000 |
| Native American | | ~1/1,500-3,970 |
| African American | ~1/60-65 | ~1/15,300 |
| Asian American | ~1/90 | ~1/32,100 |

## 1.2 DIAGNOSIS

The median age at diagnosis for CF is between 6-8 months with the majority of patients being diagnosed within the first year of life. A diagnosis is based upon having at least one phenotypic feature characteristic of the disease in addition to one of the following: identification of two disease-causing mutations, two abnormal sweat chloride tests, or an abnormal transepithelial nasal potential difference (NPD) [6].

[1] Incidence estimations obtained from the Centers for Disease Control and prevention http://www.cdc.gov/mmwR/preview/mmwrhtml/rr5313a1.htm

Clinical phenotype is variable but the percentage of patients affected with the common symptoms can be approximated (Table 2).

**Table 2. Percentage of CF patients affected with common symptoms [5-7]**

| Patients Affected | Symptoms |
|---|---|
| ~50% | Acute or persistent respiratory symptoms (includes chronic cough, persistent wheezing, and pulmonary infiltrates |
| >33% | Growth failure |
| <33% | Obvious symptoms of malabsorption |
| ~20% | Neonatal intestinal obstruction by meconium ileus |
| ~100% of men | Congenital bilateral absence of the vas deferens |

Additional symptoms include digital clubbing of the fingers and toes, recurrent sinusitis, nasal polyposis, electrolyte abnormalities (including dehydration or persistent metabolic alkalosis), rectal prolapse, pneumothorax (rupture of lung tissue causing air to be trapped between the lungs and the chest wall), coughing up blood, abdominal pain, excessive gas, enlarged heart and obstructive jaundice. Some but not all patients with CF also have diabetes, pancreatic inflammation, gallstones or liver disease. It should also be noted that virtually all patients present with chronic respiratory infections[8].

## 1.3 TESTING

Testing for CF can be conducted in a variety of ways. Diagnostic testing, carrier testing, population screening (newborn screen), pre-implantation genetic diagnosis and prenatal testing are all available. Diagnostic testing may also include chest x-rays, CT scans, lung function tests,

an upper GI and small bowel series, pancreatic functioning tests, as well as sputum culture and stool examination (fecal fat test, trypsin and chymotrypsin) to assist in confirming a diagnosis. The commonly used molecular and biochemical testing is outlined briefly below [9].

### 1.3.1 Newborn screen

The following states have implemented universal newborn screening (NBS) for Cystic Fibrosis in the United States: Alaska, Colorado, D.C., Delaware, Georgia, Iowa, Kentucky, Maryland, Minnesota, Mississippi, Nebraska, New Hampshire, New Jersey, New Mexico, New York, North Dakota, Ohio, Oklahoma, Oregon, Rhode Island, South Carolina, Virginia, Washington, Wisconsin and Wyoming. Massachusetts and South Dakota are universally offering NBS for CF but it is not yet required. Connecticut, Montana and Pennsylvania offer NBS for CF to select populations or by request. Arizona, California, Florida, Michigan, Missouri, and Texas are requiring NBS for CF but it has not yet been implemented [10]. The Centers for Disease Control and Prevention recommend CF screening for all newborns due to the benefits from early diagnosis and treatment. Babies diagnosed earlier in life can get earlier intervention to reduce pulmonary infections and exacerbations as well as better nutrition, reduced hospitalizations and improved survival [11].

### 1.3.2 Sweat Test

The sweat test has long been considered the “gold standard” in diagnosing CF. It is a diagnostic test that quantifies the amount of sodium and chloride in the sweat. Pilocarpine, a colorless, odorless chemical that causes sweating, is applied on the arm or leg. An electrode is

attached to stimulate a weak electrical current to the area. The sweat from this area is then collected on filter paper or in a plastic coil and analyzed. Increased amounts of salt are indicative of CF (normal range is less than 40 millimoles per liter (mmol/L), while a positive result is above 60 mmol/L. A result between 40-60 mmol/L is considered a borderline sweat test (Handbook of genetic counseling). High levels on a sweat test generally indicate cystic fibrosis, however they can also indicate adrenal insufficiency, Addison's disease, hypothyroidism and kidney failure [12-14].

Sometimes, babies don't produce enough sweat to perform the sweat test, in which case the Immunoreactive Trypsinogen (IRT) test is performed. IRT is a screening test that is used as part of the newborn screen, and identifies pancreatic insufficiency. IRT is performed by immunofluorometric assay on whole blood, and can identify the cationic and anionic forms of trypsinogen (neonatal immunoreactive trypsinogen). In the case of newborn screen, IRT is performed within the first few days of life. The IRT is substantially increased in babies with CF; however, the relatively low specificity of this test requires that all abnormal results be followed by sweat tests and other more specific diagnostic tests [15].

### 1.3.3 Nasal Potential Difference

Nasal Potential Difference is used in patients with borderline sweat tests. It measures the voltage across the epithelial surface in the nasal passageways. This voltage is an indicator of the amount of salt transported across the cell membrane [16].

Mutations in the CFTR gene result in abnormal transport of chloride ions across secretory epithelial cells. Normally, these ions use chloride channels to enter the cell and exit the cell via the basolateral membrane and apical surface, respectively. They cross the cell membrane via an electrochemical gradient. The ions follow an electrochemical gradient and the channels are regulated by phosphorylation that occurs after an increase in cyclic adenosine monophosphate (cAMP).

Sodium ions are transported from the channel through the apical surface of the cell creating a voltage or potential difference (PD). Knowles *et al.* first developed technique of using NPD to measure the potential difference across the nasal epithelium. Their work showed that patients with CF have increased basal potential difference, exaggerated inhibition with amiloride, and perfusion with chloride-free and isoproterenol solutions does not affect the potential difference [16, 17].

### 1.3.4 Carrier testing

Mutation analysis is used to determine carrier status in asymptomatic individuals. In 1997, a panel from the National Institutes of Health (NIH) issued a statement recommending all couples planning a pregnancy or seeking prenatal care should be offered genetic testing for CF [5]. In 2001, the American College of Obstetricians and Gynecologists (ACOG) and The American College of Medical Genetics (ACMG) issued similar statements recommending a 25 mutation panel be offered [18, 19]. More than 10 million people nationwide are carriers of a CFTR gene mutation [4, 5, 18]. Detection rates based on carrier testing and for the 87-mutation panel vary based upon ethnicity (Table 3).

**Table 3. CF Carrier Detection Rates based upon ethnic background [5]**

| Ethnicity | Carrier detection rate | 87 mutation detection rate |
|---|---|---|
| Caucasian (non-Hispanic) | 78% | 85-90% |
| Ashkenazi Jewish | 97% | 97% |
| Hispanic | 58% | 57-85% |
| Native American | 4% | 81-94% |
| African American | 75% | 60-80% |
| Asian American | 33% | 30-38% |

### 1.3.5 Mutation Analysis

Molecular genetic testing is used to detect mutations in the CFTR gene in order to establish or confirm a diagnosis, to detect asymptomatic carriers and in prenatal testing of at-risk

babies. There are a few options in mutation panel testing (23, 25, 32, 87, 97) offered by labs worldwide that perform clinical testing. In-house testing in various sites range from 21-32 mutations. Genzyme laboratory now offers a sequencing test that quotes detection of about 98% of 1,200+ disease-causing mutations identified. Ambry Genetics offers a sequencing that is promoted to detect over 99% of 1,500+ known mutations in patients of all ethnic backgrounds. Mutation detection rates vary based upon ethnicity as previously described. Molecular genetic testing is performed on whole blood in a yellow-top ACD-A or lavender-top EDTA tube. For adults, 20cc are drawn and for children 5-7 cc are drawn. Testing can also be performed using buccal swabs, which involves obtaining samples from two cheek brushes (one from each cheek). Prenatal analysis is performed on 10 cc of amniotic fluid in a 15 ml orange screw-top polyprophylene tube or 10-15 mg of chorionic villi in screw-top tubes with transport medium [12, 19, 20].

Prenatal testing is available and offered in cases where there is a 25% chance of having a child with CF based upon family history or previous mutation analysis. Prenatal tests can be performed on either amniotic fluid or chorionic villi samples. Pre-implantation genetic diagnosis is also available for couples interested in pursuing assisted reproduction via in vitro fertilization [9].

## 1.4 GENETICS/INHERITANCE

CF is inherited in an autosomal recessive pattern. *Autosomal* refers to the disease-causing genes residing on autosomes, or the first 22 pairs of chromosomes that do not designate sex. Both men and women share these chromosomes equally, which is consistent with the

incidence of disease in both sexes. *Recessive* refers to a condition that requires two mutations to be present in order to confer disease status. This means that in order for a child to have CF, both parents would need to carry a CF-causing mutation and both would have to pass on that mutation to the child (Figure 1). This would result in the child having two mutated copies of the CFTR-gene. Individuals with only one mutation are called carriers and are typically asymptomatic. These individuals do not have the disease CF, but are at a fifty percent risk of passing on their mutation to each of their children [6].

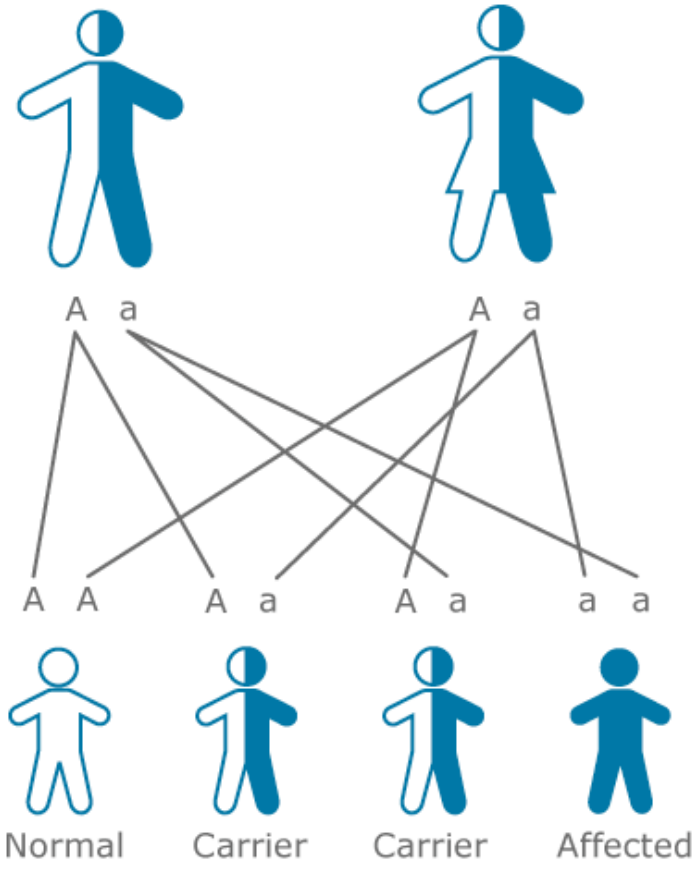


**Figure 1. Autosomal Recessive Inheritance[21]**

Cystic Fibrosis is caused by mutations in the CFTR gene, which is responsible for coding a membrane protein (glycoprotein) that is 1,480 amino acids long and is a chloride channel of epithelial cells[22]. The gene is located on the long arm (q) of chromosome 7 and is 230-kilobases long, includes 27 exons, and produces a 6 Kilobase mRNA product[23]. (Figure 2)

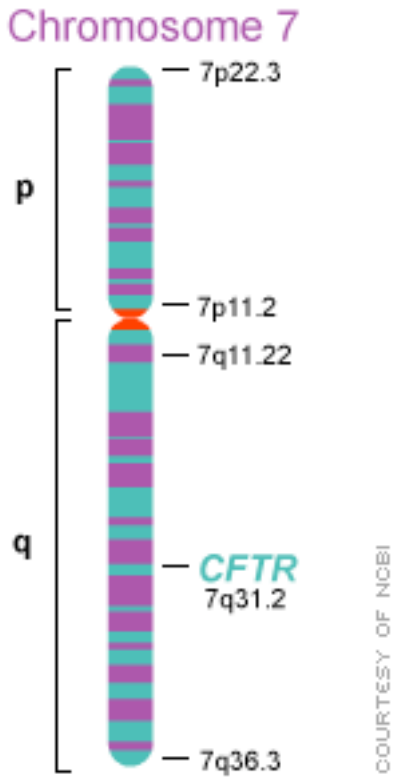


**Figure 2. Chromosome 7 location of the CFTR gene[24]**

At this time, over 1400 mutations have been identified in the CFTR gene, however the vast majority do not predict disease severity. Most of these mutations are point mutations or small deletions that are smaller than 84 base pairs. The most common disease-causing mutation is the delta F508 mutation [23]. This deletion is depicted in figure 3, showing that phenylalanine is deleted. It is interesting to note that although the last nucleotide for the second Isoleucine is deleted (the C from ATC), that ATT also codes for Isoleucine (Figure 3).

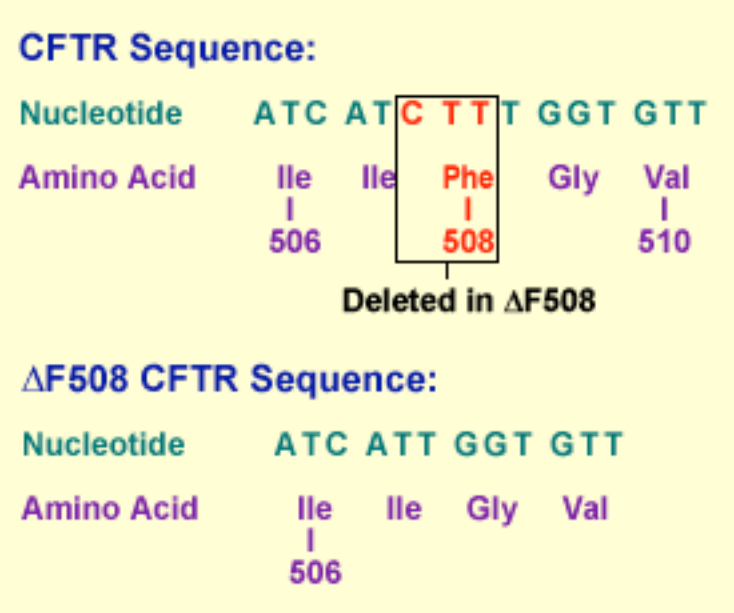


**Figure 3. Delta F508 mutation in the CFTR gene[25]**

The CFTR gene is an ABC transporter-class protein. It is comprised of five different domains. There first two are called membrane-spanning domain 1 (MSD1) and membrane-spanning domain 2 (MSD2), also called transmembrane regions. These two domains each have six spans of alpha helices and are responsible for the chloride ion channel. They are connected to the second pair of domains are called the nucleotide-binding domain 1 (NBD1) and nucleotide-binding domain 2 (NBD2). These two cytoplasmic nucleotide-binding domains bind adenosine triphosphate (ATP). The delta F508 mutation occurs in the sequence of DNA that codes for NBD1. The nucleotide binding folds (NBF) are attached to the fifth domain, which is a regulatory (R) domain. This R domain is unique because most ABC proteins only have four domains, while CFTR is the only transporter to contain this fifth, regulatory domain. It is this fifth domain that controls the influx of chloride across the epithelial membrane. This is controlled by the phosphorylation (addition or removal of phosphates).

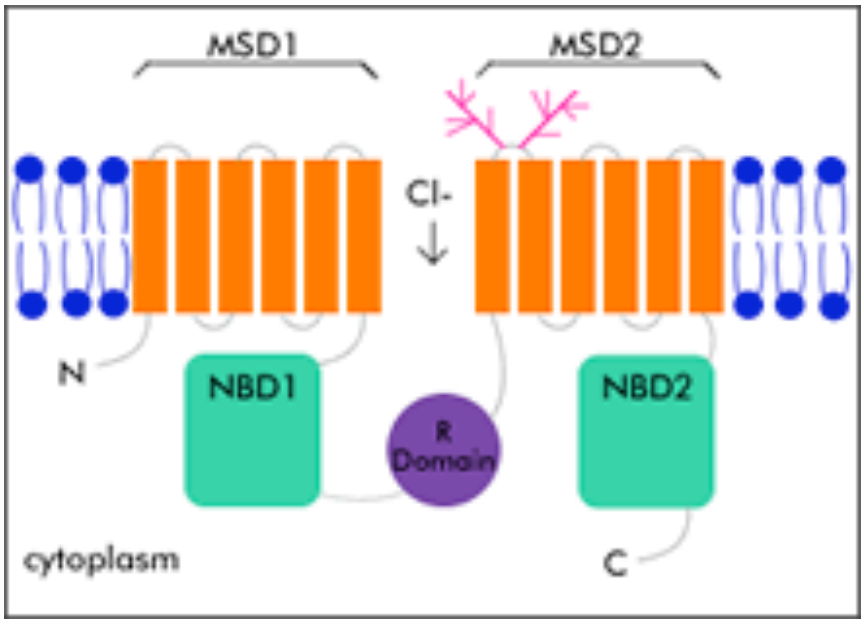


**Figure 4. Five domains of the CFTR membrane protein[25]**

There are four classes of CFTR mutations. (Table 3)

**Table 4. Four Classes of CFTR Mutations**

| Class of Mutation | Type of mutation | Examples |
|---|---|---|
| Class 1 mutation: defective production | Results in a premature termination of the CFTR mRNA translation in the nucleus- resulting in a significant reduction or absence of CFTR protein production | R542X |
| Class 2 mutation: defective processing | Results in the degradation of the protein within the endoplasmic reticulum, resulting in very little or no functional protein being transferred into the cell membrane | Delta F508 (associated with exocrine pancreatic insufficiency) |
| Class 3 mutation: defective regulation | The protein is located in the correct place in the cell membrane but it does not respond normally to the regulatory signals | R117H (strongly associated with pancreatic sufficiency) |
| Class 4 mutations: defective conduction | The protein is located in the correct place in the cell membrane but the transport properties do not function properly. | R347P |

### 1.4.1 Genotype/Phenotype correlation:

Pancreatic sufficiency is the best example of genotype/phenotype correlation in patients with CF. Some mutations are classified as pancreatic-sufficient (PS) or pancreatic-insufficient (PI). In general, patients with pancreatic sufficiency have a milder clinical course as well as an increased median survival rate. These patients have a median survival of about 56 years, however they only make up about ten percent of the CF population. While no distinct genotype-phenotype correlations have been made in regards to pulmonary function and disease, research is still being conducted to investigate potential correlations. One study conducted by deGracia et al. identified an association between more severe respiratory disease and lower survival and having a class I or II mutation on both genes [26]. Another conducted in Europe identified an association between the G85E mutation and a severe phenotype [27].

## 1.5 BRIEF REVIEW OF MANAGEMENT

Discussing the management of a multi-system disease with a lot of phenotypic variability in terms of disease severity and symptoms cannot possibly be comprehensively covered in the scope of this paper, however some of the major management strategies and procedures will be briefly discussed. Issues regarding pulmonary function tests will be discussed in the following section entitled Pulmonary Function.

### 1.5.1 Airway clearance

Airway clearance is one of the most critical components in maintaining the health of the lungs and slowing the progression of lung disease. Airway clearance can be performed through a variety of methods including manual chest therapy (percussion), physical therapy including aerobic exercises to help loosen mucus, and gravity drainage that is conducive to moving secretions from the lower airways (postural drainage), mechanical vests to vibrate the chest, and hand-held breathing devices (flutter) [28].

### 1.5.2 Nutrition

Most patients with CF hav pancreatic insufficiency and rely on the daily use of pancreatic enzyme supplements at every meal to aid in food digestion. Since malnutrition is a major problem with this disease, pancreatic enzyme replacement and fat-soluble vitamin supplements (ADEK) are essential. These patients follow a high-calorie diet due to the malabsorption (even with pancreatic enzymes). Many patients struggle with weight-gain or maintenance. Women with CF will sometimes also have complications with irregular menstrual cycles due to malnutrition and weight-loss [6, 28].

Monitoring of growth by plotting height and weight in children and weight in adults as well as annual screening labs for vitamin A and E levels helps ensure patients are maintaining their weight and vitamin levels. Diabetes screening is performed and BMI is followed in adults; another indicator of nutritional status is skin fold thickness, obtained through the use of anthropometric skin fold calipers. Generally, ketoacidosis is rare, however, decreased glucagons

release increases the risk for hypoglycemia. Some patients require gastrostomy feeding (g-tube) to meet their nutritional needs and maintain or gain weight [6, 28].

### 1.5.3 Medications (inhaled aerosols, salines, steroids)

Antibiotics, corticosteroids, inhaled bronchodilators such as albuterol (Proventil, Ventolin) are used to help open the airways. DNAse enzyme replacement therapy helps thin mucus secretions and preserve lung function [29]. Over the counter anti-inflammatory medications are thought to help slow disease progression. Anti-inflammatory medications will be further described in section 2.2 "Treatment For Airway Inflammation."

### 1.5.4 Transplantation for CF:

Approximately 3% of patients with CF have severe liver disease caused by hepatobiliary duct blockages that ultimately results in cirrhosis. About one fourth of patients have focal areas of cirrhosis. These transplants are generally viewed as a last resort for patients. Additionally heart-lung and bilateral lung transplants are also performed on patients with end-stage lung disease, and are again viewed as a last resort. While individual results may vary greatly in prognosis and outcome, the median survival of transplant patients is currently estimated to be about 5 years for lung transplant recipients, and longer for liver recipients [28].

## 1.6 PROGNOSIS

The overall prognosis for patients with CF is highly variable and depends on the severity and extent of their disease. The median survival in 2005 was estimated to be about 37 years of age, with pulmonary disease being the major cause of morbidity and mortality [6]. Individuals with less severe disease can live significantly longer.

## 2.0 PULMONARY FUNCTION

Cystic Fibrosis affects numerous organs and systems, including the gastro-intestinal tract, liver and reproductive system (vas deferens-obstructive azoospermia, cervical mucus, etc), however, the primary scope of this paper will be focused upon the pulmonary system and airway inflammation. Although CF is not associated with immune deficiency, as previously discussed, mutations in CFTR result in changes in ion transport. These changes lead to the increased susceptibility of the lungs to endobronchial infections. Endobronchial infections are usually caused by bacteria such as Pseudomonas aeruginoas. The airway inflammatory response also ultimately leads to progressive lung damage. Pulmonary function is affected by several things including, recurrent respiratory infection, bacterial colonization, airway inflammation and oxidative stress, all of which contribute to the progressive pulmonary disease in CF [30-32]

Assessing pulmonary function can be performed by measuring oxygen saturation (SaO2), lung volumes such as functional residual capacity (FRC), mixing index (MI), total respiratory system compliance (Crs), and maximal flow at FRC (VmaxFRC). The most common method of measuring pulmonary function is a test called Spirometry [33, 34].

Oxygen saturation has been studied to determine its usefulness as a non-invasive marker to assess patient progress. In one study by Betancourt *et al*., in 1991, the median SaO2 in 50 patients with cystic fibrosis was 94.0%, which was significantly lower than the median SaO2 that that of the 50 controls (patients with stable asthma), which measured 97.0% [33]. A study by

Chetta *et al.,* in the 2001 Respiratory Medicine showed that adults with mild or moderate pulmonary disease showed no significant difference in the 6-minute walk distance but experienced a significant decrease in oxygen saturation as well as perceived dyspnea [35].

Spirometry is considered one of the most important ways to monitor pulmonary status and is used to measure the amount and speed of airflow into and out of the lungs. It is often used to monitor pulmonary conditions such as CF, asthma and Chronic Obstructive Pulmonary Disease (COPD). The volume and flow rates of air that a patient is able to produce are measured by the raised-volume, rapid thoracoabdominal compression technique. The test is performed on a machine called a spirometer, while the nose is pinched closed with nose clips. Spirometry measures slow vital capacity (SVC), forced vital capacity (FVC), forced expiratory volume (FEV), forced expiratory flow (FEF) and Maximal voluntary ventilation (MVV). Functional residual capacity (FRC) can be measured by a plethysmograph [36, Schluchter, 2006 #67, 37].

*Tidal volume* refers to the amount of air that is expelled during normal exhalations. This measurement is obtained when the patient is breathing normally. Typically this number reflects much less than eighty percent of the lungs total capacity, which is sometimes referred to as Total Lung Capacity (TLC). The *residual volume* (RV) refers to the amount of air that remains in the lungs after a full exhalation. *Forced vital capacity* measures the amount of air exhaled after inhaling as much as possible. It is obtained by taking a measurement of the patient breathing in fully and exhaling as quickly and forcefully as possible [36, 38, Vilozni, 2007 #31, Nixon, 2002 #5, 39].

*Forced expiratory volume (FEV)* measures the amount of air exhaled in timed intervals. FEV-1 is the amount of air exhaled in one second and is the most frequently used FEV measurement. *Forced expiratory flow (25-75%)* averages the flow between 25 and 75% of vital

capacity. In other words, the FEF25-75 is the rate of air flow in liters exhaled per second between these two time intervals and is a good indicator of the condition of the medium and small sized airways. [36, 38, Nixon, 2002 #5, Vilozni, 2007 #31, 39]

*Maximal voluntary ventilation (MVV)* measures the volume of ventilation while the patient is breathing as quickly and forcefully as possible for 15 seconds. This test is used to monitor the respiratory strength and stamina [34]. These measurements are then specified in liters as well as percent predicted. The latter is the percent of the predicted values for patients of the same growth (height and weight), sex, age and occasionally race. Percentages ranging from around 80-100% are generally considered within the normal range, and the level of impairment is classified based upon % predicted, however this varies from physician to physician and based upon individual circumstances and health issues. The general level of impairment corresponds to the percent predicted of FVC and FEV1 [35]. (Table 5)

**Table 5. Pulmonary Function and Impairment Classification**

| | Mild | Moderate | Severe |
|---|---|---|---|
| FVC % pred | 60-79 | 40-59 | 40 or less |
| FEV1 % pred | 60-79 | 40-59 | 40 or less |
| FEV1/FVC % pred | 60-69 | 40-59 | 40 or less |

Spirometry does have limitations however, that include requiring the full and active participation of the patient. Additionally, it is only accurate on patients that are old enough to follow instructions, meaning that young children are typically excluded. Additionally, patients that are sedated, unconscious or are unable to exert the energy to perform the test cannot be

monitored. The recommendations for the frequency of conducting pulmonary tests are set based upon age. (Table 6). These tests may be repeated after administration of a bronchodilator such as Albuterol, and is called a reversibility test or post bronchodilation test (post BD) [36] [40].

**Table 6. Pulmonary test/frequency based upon age[34]**

| Age | Pulmonary test/frequency |
|---|---|
| 0-3 | Infant pulmonary function test |
| 3-5 | Spirometry based upon ability |
| 6-7 | Spirometry each visit |
| 8+ | Spirometry each visit, lung volumes as needed |

Serum, sputum and other markers can be used to measure airway inflammation, and the literature indicates that exhaled gas measurements may provide a non-invasive way to determine early indications of airway inflammation, resulting in the possibility of earlier treatment and reduction of respiratory infection[41].

## 2.1 AIRWAY INFLAMMATION

Airway inflammation is a significant factor in the development and progression of lung disease in patients with Cystic Fibrosis. The increased susceptibility of the lungs to endobronchial infections may occur via several possible mechanisms. The first is that mutation in the CFTR gene causes altered chloride secretion and increased sodium re-absorption, altering

the amount of water in the airway secretions resulting in dehydration. This dehydration causes the mucus to become viscous and sticky and also prevents cilia from functioning properly to clear mucus [42]. The outcome of altered mucus clearance is recurrent bacterial infections and airway inflammation.

A second mechanism suggests defects in the host defense. The airways are lined with what is called airway surface liquid (ASL). This thin fluid layer has antibacterial properties, which functions to prevent inhaled bacteria from causing infections. Studies have indicated that the airway surface liquid in CF patients does not have the antibacterial properties, which are normally present in the ASL. This lack of antibacterial properties is attributed to the increased salt content in the ASL [39, 43]. Further investigation suggested that the antibacterial factors present in ASL were lysozyme and lactoferrin. More recent studies have failed to confirm this mechanism, and the prevailing consensus is that depletion of airway liquid volume, rather than altered salt composition, is the major cause of infection in CF.

A third mechanism suggests that mutations in CFTR are associated with increased Pseudomonas and Staphylococcus aureus adherence in the airway epithelium. Pseudomonas aeruginosa produces large quantities of alginate (a polysaccharide matrix), which adheres to the damaged epithelial cell surfaces of the airway. This adherence makes it very difficult for the lungs to clear the organisms after an infection [44, 45]. Inability to effectively clear the lungs results in chronic inflammation and continued damage to the epithelial cell surface [46] .

Fourth is the characterization of CFTR as a key membrane receptor, which acts in binding and killing P. aeruginosa, which is compromised in patients with CF.

The literature indicates that patients with CF are born with healthy lungs and that endobronchial colonization of organisms including Staphylococcus aureus, Haemophilus

influenzae, Klebsiella pneumoniae and Escherichia coli does not occur until after birth. The fact that these patients are born with healthy lungs indicates that a "first line of defense" against infection, called the "innate immune system," is compromised in CF [40, 47].

The inflammatory process involves continuous assault on the airways by neutrophils. **Neutrophils r**elease substances including reactive oxygen species (destructive oxidases) and proteolytic enzymes (proteases). Both of these noxious mediators cause damage the lungs, with the elastase degrading the structural proteins.

The innate defense system includes "alveolar macrophages, neutrophils and epithelial cells". "Neutrophils" are white blood cells that are filled with granules (tiny sacs of enzymes) that aid in killing and digesting microorganisms the cell has engulfed via phagocytosis [47].

Mucociliary clearance is the mechanism by which pathogenic particles are cleared. The first step in this process occurs when mucus functions to trap particles, which can then be moved by cilia. The cilia along with help from aerodynamic filtering and airway reflexes (such as coughing and sneezing) aid in clearing these particles from the lungs [47].

There are several methods employed for measuring airway inflammation. Bronchoalveolar lavage (BAL) is used in some centers routinely, however, it is an invasive procedure. High-resolution CT may also be used, however because it uses radiation, its use must be limited and this technique has not been validated for routine monitoring of airway inflammation[48]. Various methods currently being used to examine airway inflammation and infection will be discussed.

## 2.2 TREATING AIRWAY INFLAMMATION

Airway inflammation is often treated using corticosteroids, macrolides, or non-steroidal anti-inflammatory drugs (NSAID), and further research investigating the safety and efficacy of leukotriene antagonists is also being conducted [49].

### 2.2.1 Corticosteroids (Prednisone)

Corticosteroids are widely known for their anti-inflammatory properties and are commonly used for patients with Cystic Fibrosis in order to treat allergic bronchopulmonary aspergillosis (ABPA), steroid-responsive wheezing, refractory airflow obstruction and to reduce the local inflammatory response. Corticosteroids are thought to work either by inhibiting the production of neutrophils or inhibiting the migration of neutrophils by releasing chemotaxins like IL-8. Auerbach et al, conducted a study over four years and found that patients treated with corticosteroids had fewer respiratory infective exacerbations, lower IgG levels and better nutrition. Other studies following this research revealed side effects from treatment including diabetes and growth suppression [7]. Treated patients with chronic P. aeruginosa infection upon entry of the study had better respiratory function as measured by FVC. Growth suppression appeared to be irreversible, especially if treatment begins in males before adolescence [50]. Another study by Greally et al. indicated treated patients had an increase in respiratory function and reduction in IgG and cytokines [51]. Additional side effects include adrenal suppression/insufficiency and osteoporosis [52].

### 2.2.2 NSAIDS (Ibuprofen)

Non-steroidal anti-inflammatory drugs act as inhibitors to neutrophil migration, adherence and lysosomal enzymes when taken in high doses. Studies have indicated that this treatment can lessen the progression of lung disease, as well as weight and chest radiographic scores. Since this improvement was really only seen in patients who were under the age of 13, it is thought that the timing of treatment is critical and that children between the ages of 5-12 would benefit the most. Side effects include toxicity and plasma levels need to be carefully regulated[48].

### 2.2.3 Macrolides (Erythromycin, Clarithromycin and Azithromycin)

Macrolides are antibiotics commonly used to treat Mycoplasma pneumonia, Chlamydia pneumonia and legionella, however recently they have been suggested to act as an anti-inflammatory for patients with CF as well as reduce sputum viscosity and airway adhesion of P. aeruginosa[28].

### 2.2.4 Defensins and cathelicidins

Defensins and cathelicidins are peptides produced in airway epithelial cells, macrophages and neutrophils and found in higher quantities in the airway surface liquid of patients with CF[53]. It has been suggested that the increase in sodium in the airway surface fluids of patients with CF results in the reduced ability to kill bacteria[54].

### 2.2.5 Leukotriene receptor antagonists

Leukotrienes are released as part of the inflammatory response and are found in high quantities in the sputum of patients with CF. In patients with CF, inhibiting leukotrienes has been associated with a reduced need for antibiotics, fewer inflammatory markers, less dyspnea as well as overall improved well-being and a reduction in eosinophilic inflammation, in one small study [55], however further research needs to be conducted to support this therapy.

### 2.2.6 Alpha-1 antitrypsin and secretory leukoprotease inhibitor (SLPI)

Airway cells produce Alpha-1-antitrypsin (a-1AT) and Secretory Leukoprotease Inhibitor (SLPI), two antiproteases that function to protect the lungs from damage caused by Elastase enzyme. Research involving the use of aerosol-administered supplemental antiproteases is underway, and needs to be conducted in order to establish the safety and efficacy of this treatment[28].

## 2.3 MARKERS FOR AIRWAY INFLAMMATION

The most commonly used markers for airway inflammation include serum C-reactive protein (CRP), serum immunoglobin (immunoglobulin G), and blood neutrophil cell count[46].

#### 2.3.1.1 Sputum

Increased concentrations of pro-inflammatory cytokines including IL-1, tumour necrosis factor-alpha, and IL-6, have been detected in CF sputum and are known to increase airway inflammation. Sputum can also be obtained to measure the increased amounts of deoxyribonucleic acid (DNA) that accumulates when neutrophils decompose, indicating pulmonary exacerbations. High levels of active collagenase (a neutrophil protease) have been reported in CF sputum and are most likely inversely related to the severity of the disease. Increased amounts of oxidants (chloramines, taurine and myeloperoxidase) and chemoattractants (LTB4 and pro-inflammatory cytokines) have also been increased in CF sputum. Cytokines are chemical signals made up of proteins and peptides and used by cells to communicate with each other. They play a central role in regulating innate immune responses and are involved in inflammatory diseases [51, 56-58].

Although induced sputum is used to help assess the presence of inflammation, it requires the use of inhaled hypertonic saline, which can cause coughing and bronchoconstriction. For this reason it is considered minimally invasive. It is also challenging to obtain sputum on younger children. Additionally, this procedure is limited by the fact that it causes an inflammatory response, which can't be repeated within a 24-hour time period [41, 48].

### 2.3.2 Serum/Plasma and Urine

Serum is used to measure C-reactive protein and antioxidant levels, which may indicate inflammation (oxidative stress) [46, 59]. Additionally, lipid peroxide levels are also studied because severe lung disease in CF has been associated with increased levels of lipid peroxides.

Biomarkers like eosinophil granule proteins (EGP) are measured in the blood and urine of patients with CF, and other lung diseases. Increased serum ECP as well as EGPs suggests eosinophil participation in any chronic inflammatory process, however, these eosinophil markers in peripheral blood do not seem to be disease-specific. Serum ECP levels correlate with ECP levels in BAL fluid from patients with asthma and ECP concentrations in the sputum of patients with cystic fibrosis. This finding suggests that eosinophil activity in the blood correlates to eosinophilic inflammation in the lungs [46, 48].

Peripheral blood may also used to measure the level of myeloperoxidase (MPO), lactoferrin and human neutrophil lipocalin (HNL), which are proteins that are produced by neutrophils. The levels of MPO in the serum have correlated with levels in sputum, indicating that serum levels reflect inflammation levels in patients with CF. HNL proteins appear to be able to differentiate between chronic and acute infections in CF much more accurately than MPO and lactoferrin. This is advantageous in that serum markers for neutrophil activity are easier to process than serum for EGPs. This measurement is limited in that bacterial infections in other parts of the body can also increase the levels of these serum proteins, meaning that raised levels do not necessarily indicate that the infection is confined to or involving the airways [58].

Peripheral blood can be used to measure cytokines and adhesion molecules in order to monitor airway inflammation, however interpreting the levels is difficult. Peripheral blood is also used to monitor interleukin 1, 4, 5, 6 and 8 as well as interferon, granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor (TNF)[58].

Urine is used to study oxidative stress by looking at urine TNF receptor 1. The Oxidative Stress Analysis detects urinary salicylates, which are markers of hydroxyl radical activity. Urine lipid peroxides, glutathionine peroxidase, superoxide dismutase and reduced glutathione are also

measured to help identify potential sources of cellular damage that may exacerbate the disease[41, 46, 58].

Glutatione is the major antioxidant in the lungs, and has been found to be decreased in the epithelial lining fluid of the lungs and in the plasma of patients with CF. This is thought to be due to the CFTR mutation. This becomes problematic when recurrent infections lead to the influx of activated neutrophils, which release free radicals. As a result of the decrease in glutathione, the other antioxidants that are present are not capable of counteracting this increased amount of free radicals that have been produced [31].

### 2.3.3 Bronchoalveolar lavage fluid (BALF)

Increased concentrations of pro-inflammatory cytokines (IL-1, tumour necrosis factor-alpha, and IL-6) have been detected in the BALF of CF patients and are also associated with airway inflammation. The number of neutrophils in the BALF is also an indicator of the inflammatory process[60]. Increased levels of IL-8 and neutrophils have been found in the BALF of newborns with no other signs or symptoms of infection, suggesting that inflammation is present before infection[61]. IL-8 is produced by macrophages and endothelial cells and target neutrophils, and is important for white cell migration (chemotaxis). In one study comparing the cytokine response in airway epithelial cells, the CF phenotypic line had significantly more IL-8, IL-6 and granulocyte-macrophage colony-stimulating factor[3].

Bronchoalveolar lavage fluid is obtained by performing a bronchoalveolar lavage, which is a medical procedure that involves inserting a bronchoscope into the lungs through the mouth or nose. Fluid is instilled into a small part of the lung and then collected for examination. This procedure is typically performed in order to diagnose lung disease, or to identify infection. One

of the main limitations in using bronchoalveolar lavage is the wide range of normal values for each parameter, which makes this sample insensitive in detecting disease. Additionally, abnormal results are not disease-specific, making interpretation difficult. Some individuals will have abnormal BALF results and not have any indication of disease, while others with known disease have normal BALF results. Additionally, the process of removing BALF may damage some of the cells and change the fluid from the epithelial lining [46, 48].

### 2.3.4 Exhaled Gas

Exhaled Gas is used as a non-invasive way to measure the amount of both end-tidal and sometimes mixed expired gas in the lungs. Levels of various exhaled gas mediators have been correlated with different pulmonary conditions. This measurement will be discussed in the section entitled Exhaled Gas Markers[62] [63]. (Table 7)

**Table 7. Profile of different exhaled gas mediators[64, 65]**

| | NO | CO | 8-isoprostane |
|---|---|---|---|
| Asthma | Large increase | Modest increase | Moderate increase |
| COPD | Little/no increase | Large increase | Large increase |
| Cystic Fibrosis | Decrease | Large increase | Large increase |

### 2.3.5 Other biochemical markers

Although the focus of this project is to explore the use of exhaled gas measurements as markers for inflammation in the airways of patients with CF, it is important to acknowledge other non-invasive measurements for inflammation as well as additional uses of exhaled breath. Exhaled breath condensate is also being studied extensively as a potential marker for inflammation. This is another completely noninvasive marker for inflammation that uses exhaled air and cools or freezes it for condensation. It does not affect airway function or induce an inflammatory response, so it can be repeated without limitation. Non-volatile substances like proteins and pulmonary surfactants as well as inflammatory markers like oxidants and ions have been collected and measured from breath condensate. The basic idea behind breath condensate is that airway surface liquid becomes aerosolized and that the condensate collected reflects the composition of the airway surface liquid [65]. Additionally, exhaled breath condensate enables us to look at the eicosanoid profile in patients. This is advantageous because eicosanoids are inflammatory mediators and are thought to control vasodilation, vasoconstrction, plasma exudation, mucus secretion, bronchoconstriction, bronchodilation, cough and inflammatory cell recruitment [64].

Exhaled breath analysis can also be used to detect levels of volatile hydrocarbons. The primary source of these exhaled hydrocarbons is the liver, and they are excreted in the breath and used to monitor lipid peroxidation. Additional volatile gases including ethane and pentane are also studied. Exhaled ethane has been found to be elevated in correlation with the exhaled CO and airway obstruction found in patients with CF. These findings indicate that oxidative stress and lipid peroxidation are both increased in patients with CF. Measurements of hydrogen peroxide have been significantly lower in patients with exacerbations of CF (including bacterial

infection) when treated with antibiotics [65, 66]. Although these exemplify the various uses of exhaled gas, the use of these measurements are really beyond the scope of this project and will not be further elaborated upon.

# 3.0 EXHALED GAS MARKERS

Exhaled gas as a marker for airway inflammation is useful in that it is non-invasive, relative easy to obtain and can measure several different exhaled gases, including carbon monoxide, carbon dioxide and nitrogen oxide. Exhaled gas measurements are often used in various arenas not limited to pulmonary disease. End tidal exhaled gas measurements are often used as standards of care because they are safe to perform. The Intensive Care Society requires end tidal carbon dioxide monitoring for all patients being transported[67]. Additionally, the American Heart Association recommends that all patients undergoing intubation are confirmed with an end tidal carbon dioxide measurement[68]. End-Tidal Carbon Monoxide (ETCO) is commonly used to help assess and diagnose children with haemolytic diseases [69]. Exhaled CO has also been used to monitor cardiovascular disease, diabetes, nephritis, bilirubin production, lung diffusion capacity as well as to help identify current and passive smokers, further illustrating its varied use in the medical field.

## 3.1 CARBON MONOXIDE

Exhaled Carbon Monoxide (CO) has different sources, including that produced through enzyme-induced heme catabolism, non-heme-related release of CO and exogenous CO. Non-heme-related release of CO includes what is released through lipid peroxidation, and from

xenobiotics and bacteria. The vast majority of endogenous CO is produced when heme is catabolized by the enzyme heme oxygenase-1 (HO-1), and also results in the production of the antioxidant bilirubin, the induction of ferritin and the removal of iron [31]. HO-1 can be up regulated by cytokines and oxidants during the inflammatory response in the lungs. This up-regulation has a protective effect against oxidative stressors, and is thought to increase the amount of exhaled CO. It is estimated that about eighty percent of the CO that is produced from heme catabolism is exhaled [65].

Elevated CO levels are seen in individuals with inflammatory conditions such as upper respiratory infections, asthma and bronchiectasis. These findings suggest that there is more oxidative stress in these conditions. Some studies indicate that CF patients have elevated CO levels that would also indicate oxidative damage. Thus we would expect to see markers of this oxidative damaged increased during exacerbations of CF [59].

Alveoli are thought to be the primary source of exhaled CO because the amount of CO is not affected by airflow. Additionally, the amount of CO at the very end of an exhalation is approximately that measured by a bronchoscope. The level of exhaled CO peaks at the end of the breath and is typically measured by using electrochemical sensors, although laser spectrophotometers and near-infrared CO analyzers are also used[31].

In asthma, and perhaps in other pulmonary diseases that are associated with chronic airway inflammation, the amount of inducible nitric oxide synthase (iNOS) producing nitric oxide (NO) and inducible heme oxygenase (HO-1) releasing carbon monoxide (CO) correlates with other markers of airway inflammation. Concentrations of exhaled NO and exhaled CO are also higher in patients with bronchiectasis, which may reflect the iNOS and HO-1 expression in macrophages and neutrophils in the airways of these patients [70]. In one study conducted by

Horvath et al, the levels of exhaled and nasal NO and CO in patients were compared. The amount of exhaled NO in patients with primary ciliary dysinesia (PCD) and CF was found to be significantly lower than the levels found in healthy subjects [70].

As previously mentioned, End-Tidal Carbon Monoxide (ETCO) is produced through the catabolism of heme and then diffused to the air in the alveoli. This is important because the diffusion capacity of CO (DLCO) or the surface area of the alveoli may affect the amount of CO that is transferred from the blood stream to the alveoli. Decreases in lung volume and DLCO may occur in patients with CF, affecting the ambient ETCO. Ambient ETCO may be affected by other conditions, which could increase the rate of heme catabolism. These conditions include anemia, hematomas and fasting [71].

One of the major advantages of using CO measurements from exhaled gas is that the quantity of CO is much higher in exhaled air amounts than NO, which is also used as a marker for inflammation. While NO is measured in parts per billion, CO can be measured in parts per million. This is financially advantageous, as the equipment required to measure CO is less expensive than the equipment needed to measure exhaled NO [72]. Often times the equipment used to measure CO relies on electrochemical sensors, which are inexpensive but sensitive to the influence of other gases like hydrogen [62].

Limitations of CO measurements are possible confounders including exposure to cigarette smoke (first or second-hand), car exhaust and various other pollutants, which can increase the amount of exhaled CO [72]. Additionally, the amount of ambient air that is inhaled by the patient is also a potential confounder so correcting for inhaled air is necessary for accurate measurements.

Studies have been conducted that have shown an increase in the End-Tidal carbon monoxide in patients with CF, however since conflicting data indicating no increase in CO has also emerged, some of the studies will be discussed and reviewed[31, 59, 72, 73].

## 3.2 NITRIC OXIDE

Nitric Oxide (NO) is a gaseous chemical compound produced by macrophages, neutrophils and stimulated bronchial epithelial cells. It mediates inflammatory and the immune response in the lungs and regulates the smooth muscle contractility in the airways as well as pulmonary perfusion. Higher levels have been found in the exhaled condensate of asthma patients, suggesting a possible role in the pathophysiology of airway inflammation. NO is produced along the surface of the airways, which secrete it into the lumen where it mixes with the NO from the alveoli [65]. It is also produced more during bacterial infections, therefore we would expect to see higher amounts of exhaled NO in patients with recurrent infections and inflammation, including CF. Contrary to reasoning, patients with CF actually have lower amounts of NO in their exhaled condensate, which may be due to the increase in mucus viscosity. This increase in viscosity and amount of mucus may keep NO from entering the airway or may actually serve to trap and remove NO through oxidative reactions [64].

Ho *et al.* suggested that exhaled NO may not be an accurate indicator of the amount of NO produced in the airways because of the amount of byproducts found (nitrate and nitrite) and that these byproducts from the metabolism of NO may be better indicators of the actual amount of NO being produced[64].

The increase of NO present in exhaled gas is due to the increased production of inducible nitric oxide synthase (iNOS). This iNOS is located in inflammatory cells and epithelial cells, and increases the production of NO. There is a significant increase in the expression of iNOS in the airway epithelium in patients with asthma [72].

According to Matsui, *et al*., "Nitric oxide may regulate airway function in the pathophysiology of inflammatory airway disease. Exhaled NO has been shown to be a marker of inflammation in asthma and bronchiectasis. However, despite chronic airway inflammation in cystic fibrosis, exhaled NO levels appear to be lower than normal possibly because of a reduced expression of the inducible form of NO synthase (iNOS), trapping of NO in the mucous layer, or because NO is metabolized to peroxynitrite making this measurement of little use for monitoring the lung inflammation in cystic fibrosis"[42].

Exhaled NO levels may be affected factors like soft palate closure, expiratory flow, and dead space air. Exhaled NO is also no affected by variables like the age, sex and lung function of the patient. It has been suggested that because the hormone estrogen activates endothelial NOS (NOS3) in airway epithelial cells, that a woman's menstrual cycle may alter her exhaled NO measurements [65]. While external variables including NO ozone and chlorine dioxide can increase exhaled NO levels, factors like alcohol consumption and smoking can decrease exhaled NO. Other factors can also affect exhaled NO like upper respiratory infections, which are known to greatly increase the amount of exhaled NO [65].

Lower NO values are associated with chronic cough that are not caused by asthma. NO is measured using chemiluminescence analyzers. This type of testing used to be limited due to the high cost of the equipment but a number of companies are making machines, and they are now used routinely in many asthma clinics[70, 74].

## 3.3 OBTAINING GAS MEASUREMEMNTS

### 3.3.1 End Tidal

End-tidal exhaled gas measurements are obtained during an exhalation and reflect the gas level at the very end of the breath. End tidal (ET) measurements are obtained by tracking the flow of the air during the exhalation and then measuring the level of the gas at the end of the tidal volume[63].

### 3.3.2 Mixed Expired

Mixed-expired gas measurements are obtained by collecting the gas from the entire exhalation into a vacuumed, airtight bag. The gases have an opportunity to distribute evenly (mix) and the measurement is taken from this mixture and averages what was collected during the exhalation. Mixed-expired gas measurements are thought to reflect the gas coming from the alveolar dead space and the alveoli of the lungs[34].

# 4.0 PRIOR STUDIES

## 4.1 EARLY AIRWAY INFECTION, INFLAMMATION, AND LUNG FUNCTION IN CYSTIC FIBROSIS BY NIXON *ET AL.*

In this study, conducted by Nixon et alForced Expiratory Volume (FEV) and Bronchial Lavage (BAL) were used to assess the role of inflammation and its relationship to lower airway infection in infants and children under the age of three, with CF [39].

Methods: The study looked at 36 children with CF, all of whom were under the age of three. BAL was used to indicate lower airway infection and was performed right after FEVs, which were used to assess lung function.

Results: Lower airway infection as evidenced by BAL, was associated with a decrease in FEV by 10%. Twenty of the subjects had a daily moist cough the week prior to testing and seven had an infection detected. Subjects with a daily cough had lower FEVs than those without.

Conclusions: It appears that airway infection, as well as respiratory symptoms (such as daily coughing) affect lung function, both independently and with an additive effect. No assocationa between airway inflammation and lung function was identified.

Discussion: This study was not hypothesis-driven but was designed to help determine the relationship between lower airway infection and inflammation, respiratory symptoms and lung function. A larger sample size, especially including more subjects that were without moist cough

prior to testing would be helpful in establishing the relationship between respiratory symptoms and lung function. This study was limited in that it only looked at seven patients with infection, so establishing a relationship between infection and pulmonary function requires more data.

## 4.2 EXHALED CARBON MONOXIDE IS NOT ELEVATED IN PATIENTS WITH ASTHMA OR CYSTIC FIBROSIS BY ZETTERQUIST *ET AL*.

Zetterquist et al., conducted a study entitled "Exhaled carbon monoxide is not elevated in patients with asthma or cystic fibrosis" in which the researchers looked at whether or not Carbon Monoxide measurements from exhaled air can be used as a marker of inflammation. The theory is based upon increased CO production in inflammatory states could be due to the induction of Heme oxygenase (HO-1) which would be an indicator of oxidative stress. However studies have not clearly supported the increase in HO-1 in disorders involving airway inflammation[72].

Methods: 32 asthmatic patients without steroid treatment, 24 steroid-treated asthmatics (16 with allergic rhinitis and 9 with CF) were compared to 30 healthy controls (non-smokers). End-tidal CO and NO measurements were taken. Two CO machines were used, the first was a fast-response, nondisperse infrared (NDIR) CO analyzer and the second was an electrochemical sensor. The researchers opted to measure CO and NO after a breath-hold in order to help determine if the molecule originally came from the alveoli or the bronchi. This is distinguished because end-tidal CO or NO from the alveoli should increase after a breath hold whereas end-tidal CO or NO from the bronchi should remain the same after a breath hold.

Results: No increase in the level of CO among patients with allergic rhinitis, steroid-naïve asthma, steroid-treated asthma, or CF. Levels of NO were significantly elevated for pts

with allergic rhinitis and steroid-naïve asthma when compared to controls. Steroid-treated asthma patients did not have a significant increase in NO. Patients with CF showed lower concentrations of NO when compared with controls.

Conclusions: The results from both of the CO analyzers used showed no significant increase in fractional concentration of exhaled CO in patients with airway inflammation disorders including CF, asthma and allergic rhinitis, when compared to healthy controls. A decrease in the NO levels of patients with CF was also noted. As previously indicated, this could be attributed to poor diffusion across the lumen. It could also be attributed to reduced production of NO from the epithelial cells or even a reduction in nasal NO contamination.

Discussion: This study was not hypothesis-driven but instead, aimed to evaluate the previous research findings that suggested that exhaled carbon monoxide is increased in patients with inflammatory airway conditions. The study design was limited in that it included very few patients with CF, despite the amount of participants. Utilizing patients with inflammatory airway disease is useful, although having more subjects with CF from which to collect data would strengthen the study. Additionally, controlling for ambient levels would have further strengthened the results as to help reduce possible confounders that vary based upon where the participants live (rural vs. urban areas). It is of note that it was really one of the first to suggest that exhaled CO was not increased in patients with CF. Contrary to these findings; several prior studies and reports had indicated that exhaled CO was increased in patients with upper respiratory tract infections. This is important because it questioned whether or not exhaled CO could be used as a marker for airway inflammation and whether or not the methods in obtaining and assessing the measurements should be re-evaluated [31, 59, 62, 71, 74].

Of interesting note, the levels of CO did not appear to be flow-dependent, indicating that the airway epithelium does not contribute to the amount of CO in exhaled air, or else an increase in the concentration would be noted upon decrease airflow.

## 4.3 EXHALED ETHANE IS ELEVATED IN CYSTIC FIBROSIS AND CORRELATES WITH CARBON MONOXIDE LEVELS AND AIRWAY OBSTRUCTION BY PAREDI, *ET AL.*

Exhaled Ethane is Elevated in Cystic Fibrosis and Correlates with Carbon Monoxide Levels and Airway Obstruction by Paredi, et al., 1999. This study looked at the volatile gas, ethane and compared it to levels of exhaled CO and NO [66].

Methods: The researchers looked at 23 patients with CF, including 10 males and 13 females, with a mean age of 21 years and mean FEV1 of 62 (4% of predicted). Of these patients, 10 were steroid treated; however, lung function was tested and was similar between the two patient populations. All of the patients were tested for unusual CF pathogens like Burkholderia cepacia, methicillin-resistant Staphylococcus aureus and patients found to be colonized with these bacteria were excluded. Patients with signs of symptoms of acute chest infection, pancreatic insufficiency or pulmonary exacerbation or a medical history of diabetes, lung cancer, liver disease or substance abuse were also excluded. 14 controls were used. The subjects were tested and found to be non-smokers, and all of who were life-long non-smokers except for one patient who had quit one year prior to the study. Any individuals with second-hand exposure to smoke (more than a half hour a day) were excluded. Airway obstruction was measured by using residual volume and total lung capacity [66].

In order to help control for various ambient CO levels, each subject rested for an hour before testing. End-tidal air was tested using gas chromatography for ethane. CO was measured by electrochemical sensor and the mean of two measurements was taken with ambient CO levels subtracted from the values. No was also measured and lung function tests were performed using spirometry on all of the subjects.

Results: The results showed that the levels of exhaled ethane were higher in the patients with CF that were not treated with steroids or the controls. In patients with CF that were not treated with steroids, exhaled ethane levels correlated with CO concentrations and airway obstruction. Exhaled CO levels were higher in patients that were not treated with steroids than those that were treated with steroids as well as the controls. Exhaled NO levels did not appear to be affected by steroid treatment status and were lower in the patients with CF than in the controls.

Conclusion: This data and results are significant in that they further support the use of exhaled CO as a marker for oxidative stress and inflammation. This is based upon the role of ethane as a marker for lipid peroxidation. Since increased ethane levels correlated with increased levels of CO in CF patients not treated with steroids, this provides further evidence that exhaled CO is increased in patients with CF due to increases in oxidative stress.

Discussion: This study was very carefully designed to reduce the amount of possible confounders, including ambient gas levels, smoking status and age. It was not hypothesis-driven but was designed to compare exhaled ethane with other markers of oxidative stress and inflammation (NO and CO) as well as compare the relationship between exhaled ethane and disease severity. Separating patients based upon steroid-treatment status also was a strength of the study. These results are consistent with the previous literature indicating that exhaled NO is

lower in patients with CF. These lower findings may reflect an increase in NO metabolites, or an inhibition of inducible NO synthase due to increased CO levels reducing overall production. Most importantly, this study may help establish a pattern of markers indicating that increased ethane and CO along with decreased NO may be used to help assess and monitor disease progression.

## 4.4 INCREASED CARBON MONOXIDE IN EXHALED AIR OF PATIENTS WITH CYSTIC FIBROSIS BY PAREDI, *ET AL*.

In the study entitled, "Increased carbon monoxide in exhaled air of patients with cystic fibrosis" by Paredi et al. done in 1999, exhaled CO was measured to determine if it can be used as a marker for airway inflammation and oxidative stress.

Methods**:** 29 patients, including 15 men and 14 women with CF were studied to measure the levels of exhaled CO and NO concentrations. Patients were tested for Burkholderia cepacia and methicillin resistant Staphylococcus aureus. Any individuals found to be colonized with either of these were excluded from the study. Additionally, any patients with severe respiratory infections or symptoms of exacerbation were also excluded. The mean age of the patients with cystic fibrosis was 25 years, and they had on average an FEV of 43. Of these 14 were being treated with steroids. These patients were compared to a control group of 8 men and 7 women with a mean age of 31. All of the subjects were tested and confirmed to be non-smokers and free of smoke exposure, and they all appeared to be free of pancreatic insufficiency.

A modified analyzer was used to measure the exhaled CO in the subjects. The maximum value obtained from two breath exhalations was used and ambient CO levels were recorded prior to exhalation. Exhaled NO was also measured using a chemiluminescence analyzer.

Results: The results of this study are summarized in the table below. (Table 8)

**Table 8. Results comparing Exhaled CO and NO levels in controls, CF patients (with and without steroid treatment and homo and heterozygous for the deltaF508 mutation)**

| | Exhaled CO | NO levels |
|---|---|---|
| CF patients | 6.7 (0.6) ppm | 3.2 (0.2) ppb |
| Controls | 2.4 (0.4) ppm | 6.8 (0.4) ppb |
| CF w/ steroid | 5.1 (0.5) ppm | |
| CF no steroids | 8.4 (1.0) ppm | |
| dF508/dF508 | 7.7 (1.8) ppm | 4.1 (0.5) ppb |
| Heterozygous | 4.0 (0.6) ppm | 1.9 (0.7) ppb |

Conclusion: These findings support that exhaled CO is increased in patients with CF, which could reflect an increase in the amount of induced HO-1, which could be a direct result of oxidation and inflammation. This theory is further supported by a reduction in exhaled CO measurements in patients that were treated with steroids. This decrease in CO production could be attributed to the reduction in inflammation from steroid treatment. This would lower the amount of oxidants released by inflammatory cells, and limit cytokine production, thereby decreasing the amount of HO-1 expressed. Reduced HO-1 subsequently results in decreased production of CO, as seen in the steroid-treated subjects. This study further supports the use of

CO a marker for airway inflammation [31]. This is of clinical importance because the measurement of exhaled CO may be useful to assess levels of oxidation and inflammation, which may allow us to better understand the pathophysiology as well as treat the progression of lung disease.

Discussion: This study was not hypothesis-driven, but instead was designed to further investigate whether levels of exhaled CO would be higher in patients with CF and whether the levels of exhaled CO and NO would be affected by the use of corticosteroids. The separate analysis of patients who were treated with steroids and those who were not, greatly contributed to the strength of this study. The use of ambient CO levels taken prior to each measurement also further strengthened the results by reducing potential confouners.

## *4.5* END-TIDAL CARBON MONOXIDE CORRECTED FOR LUNG VOLUME IS ELEVATED IN PATIENTS WITH CYSTIC FIBROSIS BY TERHEGGEN-LAGRO, *ET AL.*

The study entitled "End-Tidal Carbon Monoxide Corrected for Lung Volume Is Elevated in Patients with Cystic Fibrosis" conducted by Terheggen-Lagro, et al., in 2003 looked levels of exhaled end-tidal CO in order to determine if it is an appropriate marker for airway inflammation[71].

Methods: These researchers obtained twenty patients with CF who were considered to be clinically stable and thirty control subjects in order to compare the levels of end-tidal CO from exhaled breath.

Clinically stable was defined as not having any of the following:

- Increase in sputum production
- Increase in cough
- Fever
- Anorexia
- Decline in lung function (FEV1)

The patients had a mean age of 13.5 while controls were a mean age of 22.8. All of the patients with CF were tested and found to have bacterial colonization of two of the most common bacterial infections, Pseudomonas aeruginosa and/or Staphylococcus aureus. An electrochemical sensor was used to measure CO and H2 and end-tidal CO was measured, along with ambient CO levels after each measurement. Airflow rates were used to help distinguish the origin of the ETCO (alveolar or bronchial). Spirometry and plethysmography was used to measure lung functioning in the patients, while multiple-breath helium wash-in was used to help assess the lung volume in the controls.

Results: In this study, no significant increase/decrease in levels of ETCO was seen in patients with CF when compared to the healthy controls. However when assessing the controls, there was a strong correlation between the ETCO and TLC-He levels. When controls were compared to patients with CF, the levels of TLC-He were significantly lower in patients with CF, so the patients ETCO measurements were corrected for the TLC-He percent. After this was completed, the patients with CF were found to have ETCO levels that were significantly higher than those of the controls.

Conclusion: This study was very significant in that although no difference was initially found in the ETCO levels of patients with CF and the controls, by studying physiologic factors that could affect ETCO, the following things were found. Individuals with an increased lung

volume also had increases in ETCO. When corrected for lung volumes, patients with CF had significantly higher levels of ETCO than controls.

Discussion: This study was designed to look at factors that influence end-tidal carbon monoxide levels in patients with CF. It is notable in that it hypothesized that increases in exhaled CO in patients with CF may be present but may not be evident upon initial exam due to differences in lung volumes between the subjects. The design included adequate sample sizes with regard to cases and controls; however, it should be noted that the controls were on average almost a decade older than the cases.

## 4.6 INCREASE IN EXHALED CARBON MONOXIDE DURING EXACERBATIONS OF CYSTIC FIBROSIS BY ANTUNI, ET AL.

The study entitled "Increase in exhaled carbon monoxide during exacerbations of cystic fibrosis" by Antuni et al., was conducted in 2000 and looked at whether exhaled CO measurements were increased in CF patients with respiratory exacerbation as compared to those with clinically stable disease[59].

Methods: The researchers looked at 44 patients with CF, some with clinically stable disease status and others who were considered to be in respiratory exacerbation, along with 12 healthy controls (non-smokers). They were studied in order to compare exhaled CO concentrations amongst the three populations to see if patients with exacerbation would have an increase in exhaled CO. The average age of the controls was 37 years with FEV1 % predicted of 95, and the average age of the CF patients was 29 with an FEV1 % predicted of 56.

The controls were individuals without respiratory disease who had also been clear of any upper or lower airway infections for at least a month prior to the start of the study. They also needed to have normal spirometry levels. Individuals for the CF patient population were screened for severe exacerbation, liver disease, haemolytic anaemia, and asthma. Individuals with these conditions were excluded from the study. Additionally, any smokers, individuals with upper airway infections within the past month, and patients unable of performing the exhaled breath measurement were also excluded.

Respiratory exacerbation was defined as having at least one of the following symptoms:

- Increased cough
- Increased sputum
- Change in sputum quality (more purulent, increased viscosity, presence of blood)
- Sensation of increased chest congestion
- Increased dyspnea
- Decreased exercise tolerance
- Worsening FEV1

An electrochemical sensor was used to measure exhaled CO, along with NO and CO2. The level of CO in the ambient air was recorded prior to each exhaled CO measurement. Two measurements were used and the mean of the two measurements was used after subtracting the ambient CO level. A t-test was used to compare the populations for statistical significance.

Results: Of the 44 patients with CF, 15 were considered to have exacerbations, while the rest were considered stable. The results of exhaled CO are listed in parts per million and are based upon CF disease status (exacerbation vs stable) as compared to the controls. Exacerbation was defined as having increased coughing, increased amount and change in quality of sputum, perceived shortness of breath and chest congestion as well as a decreased FEV1. (Table 9)

**Table 9. Results of exhaled CO measurement comparing controls with CF patients in stable condition and with respiratory exacerbation.**

| Subjects | Exhaled CO in ppm |
|---|---|
| Controls | 2.0 |
| CF patients-stable | 2.7 |
| CF –exacerbation | 4.8 |

Conclusion: The results of this study showed that levels of exhaled CO were higher in patients with CF than in controls and that within the patient population, individuals with signs and symptoms of respiratory exacerbation (as defined above) had significantly higher exhaled CO levels than that of patients who were considered clinically stable. This is significant in that it indicates that exhaled CO can potentially be used to distinguish between patients who are clinically stable and patients who have disease exacerbation.

Discussion: This study was hypothesis-driven, with the working hypothesis having been that the HO-1 activity may be increased as a result of inflammation and oxidative stress in patients with CF who also had infective exacerbations, which would ultimately result in an increase in exhaled CO. The findings supported this hypothesis, and the sample size of 44 patients with CF was adequate. The study was limited in that it did not separate patients who were being treated with steroids from those who were not being treated with steroids. This additional information could potentially have strengthened the study design.

## 4.7 DISCUSSION OF OVERALL FINDINGS

This literature review was originally intended to discuss the application of the exhaled gases CO and NO, in assessing airway inflammation in patients with cystic fibrosis. Although CF has been researched and studied for decades, a surprising amount is still not fully elucidated or understood. Over the past decades, medical advances have improved the quality and care of these patients, resulting in the significant increase in median survival and quality of life. The specific mechanism by which CFTR mutations result in bacterial colonization and inflammation are still being dissected. Overall, after reviewing the available literature and studies conducted on exhaled gas levels in patients with CF, the resulting patterns emerged. The majority of evidence indicates that exhaled CO is, in fact, elevated in patients with CF and even further increased in patients with signs and symptoms of pulmonary exacerbation. This increase in exhaled CO is attributed as a byproduct of heme catabolism, which is part of the inflammatory response and appears to be a result of oxidative stress. In addition, the majority of literature indicates that exhaled CO measurements are independent of airflow, suggesting that the CO from breath measurements is alveolar in origin and not from the bronchial airways, which is consistent with previous studies. [30, 31, 39, 47, 49, 56, 57, 59, 60, 62, 63, 65, 73-77].

Furthermore, exhaled NO levels seem to be consistently decreased in patients with CF, which helps to further establish a pattern of high CO and low NO in patients with CF. This can be used to help distinguish between inflammation, steroid treatment efficacy and also potentially be used in conjunction with other markers including volatile gases from exhaled breath (ethane) and 8-isoprostane to help establish additional biomarker patterns. These patterns could potentially be used to differentiate between different disease severities and rates of progression, and allow us to assess inflammation prior to other clinical signs and symptoms of exacerbation,

perhaps allowing for earlier medical intervention and ultimately, reduce disease progression[42, 64, 65, 70, 73].

## 4.8 APPLICATION TO GENETIC COUNSELING AND PUBLIC HEALTH

Genetic Counseling incorporates not only the genetic testing but also the management and follow-up of patients with genetic conditions. Cystic Fibrosis, one of the most commonly inherited genetic conditions is certainly no exception. Monitoring pulmonary disease status through non-invasive monitoring of airway inflammation is a critical component in the management and care of patients with Cystic Fibrosis. As with all genetic conditions, genetic counselors should be familiar with the medical management of patients with CF. Additionally, the use of exhaled gas measurements could potentially help patients comply with airway clearance habits, as well as better predict pulmonary exacerbation and aid in the treatment of pulmonary infection, allowing for better patient care and overall improvement of well-being. The significant cost of medical care for these patients, in conjunction with the incidence of the disease and its chronically disabling nature warrant both continued research as well as public health priority. Cystic Fibrosis has been studied for several decades but the need for continued research to better treat symptoms, manage disease, reduce and slow progression and ultimately develop a cure, is evident[4-6, 45].

# 5.0 EXHALED GAS STUDY

As previously discussed, this literature review was conducted in context to a much larger exhaled gas study. The exhaled gas study is a 3-phase study that is still currently underway and with which I have participated for the past two years in data collection. The full IRB protocol is available for further review; however, it is much too extensive to include here. The participating patients were selected based upon their ability to perform reliable PFTs and the majority was voluntarily consented prior to my participation, however I have consented patients after IRB updates were made to the original protocol. Patients were recruited from both the pediatric and adult clinic from the University of Pittsburgh Medical Center and I obtained exhaled gas measurements from patients at two separate clinic sites, Falk Clinic in Oakland and the Children's North Clinic in Wexford.

Data collection has involved monitoring patient FEV1's as well as obtaining exhaled CO measurements on the pediatric patients and exhaled CO, NO and CO2 measurements on the adult patients. The primary goal of the study are to see if the levels of exhaled gas, primarily exhaled CO, can be used as markers for airway inflammation in patients with CF. Tracking these levels both before patients get sick as well as when patients are sick in order to monitor them as they improve, will help provide further data to determine the clinical utility of exhaled gas. These measurements may allow us to better monitor airway inflammation and exacerbation in patients and perhaps provide earlier indicators of bacterial infection and colonization or pulmonary

decline. Earlier markers of exacerbation, colonization or pulmonary decline would enable us to begin earlier treatment and avoid more invasive monitoring techniques, which have long been the standard of care.

# 6.0 PERSONAL REFLECTION

Participating in this research has provided me with a lot of insight in terms of caring for patients with this disease. Working with both the pediatric and adult populations has allowed me to see the disparities between the needs of the patient populations, with disease progression being a primary factor. Since this condition is chronically debilitating, I have established rapport with patients that have frequent clinic visits and have experienced first-hand some of the frustrations and emotional issues that come with chronic illness.

Working with the pediatric patients I have seen how the disease affects the family dynamic, as parents are generally very involved in the care of their children. This includes monitoring daily enzyme dosages, antibiotics, assisting and performing manual chest percussion for airway clearance, financial issues, taking time off for doctor's appointments, transportation issues and a vast array of other issues and challenges that this disease causes.

The adult patients still have some of the same concerns and issues, however they are facing additional challenges, including maintain weight, slowing lung disease progression, preserving lung function and ultimately fighting for increase survival. Variation is disease severity is evident when some of these individuals are on disability and unable to work or do many of the things they would like to do, while others have full time jobs and physically active.

The opportunity to work with this group of patients has provided me with greater insight with regard to the complex needs and unique concerns of this particular patient population. It

has given a face to the numbers and statistics and many of these patients have forever touched my life with their struggles, their hope and ambition, their earnest pursuit for the things many people take for granted, like a breath of air. These past two years of experience exposed me to the human side of the disease that medical texts and journal articles cannot possibly provide and have further instilled a passion to utilize the skills I've developed to better meet the needs of my future patients.

## BIBLIOGRAPHY


1. Yang, Y., et al., *An approach for treating the hepatobiliary disease of cystic fibrosis by somatic gene transfer.* Proc Natl Acad Sci U S A, 1993. **90**(10): p. 4601-5.

2. Imundo, L., et al., *Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface.* Proc Natl Acad Sci U S A, 1995. **92**(7): p. 3019-23.

3. Armstrong, D.S., et al., *Lower airway inflammation in infants and young children with cystic fibrosis.* Am J Respir Crit Care Med, 1997. **156**(4 Pt 1): p. 1197-204.

4. FitzSimmons, S.C., *The changing epidemiology of cystic fibrosis.* J Pediatr, 1993. **122**(1): p. 1-9.

5. (NIH), N.I.o.H., *Consensus Development Conference Statement.*

6. Cystic Fibrosis Foundation. [cited 2007 3/2/07]; Available from: http://www.cff.org/.

7. Rosenstein, B.J. and H. Eigen, *Risks of alternate-day prednisone in patients with cystic fibrosis.* Pediatrics, 1991. **87**(2): p. 245-6.

8. Bals, R., et al., *Salt-independent abnormality of antimicrobial activity in cystic fibrosis airway surface fluid.* Am J Respir Cell Mol Biol, 2001. **25**(1): p. 21-5.

9. Cronin, C., *Cystic Fibrosis Exams and Tests.* 2007.

10. (NNSGRC), N.N.S.a.G.R.C., *National Newborn Screening Status Report.* 2007.

11. Grosse, S.D., et al., *Newborn screening for cystic fibrosis: evaluation of benefits and risks and recommendations for state newborn screening programs.* MMWR Recomm Rep, 2004. **53**(RR-13): p. 1-36.

12. Chernecky CC, B.B., eds, *Laboratory Tests and Diagnostic Procedures*. 4th ed. 2004, Philadelphia: Saunders.

13. Fischbach FT, D.M.I., eds, *Manual of Laboratory and Diagnostic Tests*. 7th ed. 2004, Philadelphia: Lippincott Williams and Wilkins.

14. Pagana KD, P.T., *Mosby's Manual of Diagnostic and Laboratory Tests*. 2nd ed. 2002, St. Louis: Mosby.

15. Ranieri, E., et al., *Neonatal screening for cystic fibrosis using immunoreactive trypsinogen and direct gene analysis: four years' experience.* Bmj, 1994. **308**(6942): p. 1469-72.

16. Knowles, M.R., et al., *Measurements of nasal transepithelial electric potential differences in normal human subjects in vivo.* Am Rev Respir Dis, 1981. **124**(4): p. 484-90.

17. Wilschanski, M., et al., *Nasal potential difference measurements in patients with atypical cystic fibrosis.* Eur Respir J, 2001. **17**(6): p. 1208-15.

18. Watson, M.S., et al., *Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel.* Genet Med, 2004. **6**(5): p. 387-91.

19. (ACMG), A.C.o.M.G. *Laboratory Standards and Guidelines for Population-based Cystic Fibrosis Carrier Screening*. 2001 [cited 2007; Available from: http://www.acmg.net/resources/policies/pol-005.asp.

20. (AMA), A.M.A., *Cystic Fibrosis Testing.* 2004.

21. *6.1.gif*, A.R. Inheritance, Editor.

22. De Rose, V., *Mechanisms and markers of airway inflammation in cystic fibrosis.* Eur Respir J, 2002. **19**(2): p. 333-40.

23. Rommens, J.M., et al., *Identification of the cystic fibrosis gene: chromosome walking and jumping.* Science, 1989. **245**(4922): p. 1059-65.

24. NCBI, *Chromosome 7*, cfgene.gif, Editor.

25. Bownas, J., *Delta F508 Mutation in the CFTR gene image*, The U.S. Department of Energy Biological and Environmental Research program.

26. de Gracia, J., et al., *Genotype-phenotype correlation for pulmonary function in cystic fibrosis.* Thorax, 2005. **60**(7): p. 558-63.

27. Decaestecker, K., et al., *Genotype/phenotype correlation of the G85E mutation in a large cohort of cystic fibrosis patients.* Eur Respir J, 2004. **23**(5): p. 679-84.

28. Boas, S.R., *Exercise recommendations for individuals with cystic fibrosis.* Sports Med, 1997. **24**(1): p. 17-37.

29. Fuchs, H.J., et al., *Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group.* N Engl J Med, 1994. **331**(10): p. 637-42.

30. Heeckeren, A., et al., *Excessive inflammatory response of cystic fibrosis mice to bronchopulmonary infection with Pseudomonas aeruginosa.* J Clin Invest, 1997. **100**(11): p. 2810-5.

31. Paredi, P., et al., *Increased carbon monoxide in exhaled air of patients with cystic fibrosis.* Thorax, 1999. **54**(10): p. 917-20.

32. Tirouvanziam, R., et al., *Inflammation and infection in naive human cystic fibrosis airway grafts.* Am J Respir Cell Mol Biol, 2000. **23**(2): p. 121-7.

33. Betancourt, M., G. Slade, and R. Dinwiddie, *Oxygen saturation in cystic fibrosis.* Arch Dis Child, 1991. **66**(9): p. 1075-6.

34. Blonshine, S.B., *Pediatric pulmonary function testing.* Respir Care Clin N Am, 2000. **6**(1): p. 27-40.

35. Chetta, A., et al., *Six-minute walking test in cystic fibrosis adults with mild to moderate lung disease: comparison to healthy subjects.* Respir Med, 2001. **95**(12): p. 986-91.

36. Vilozni, D., et al., *Spirometry in early childhood in cystic fibrosis patients.* Chest, 2007. **131**(2): p. 356-61.

37. Schluchter, M.D., et al., *Classifying severity of cystic fibrosis lung disease using longitudinal pulmonary function data.* Am J Respir Crit Care Med, 2006. **174**(7): p. 780-6.

38. Eigen, H., et al., *Spirometric pulmonary function in healthy preschool children.* Am J Respir Crit Care Med, 2001. **163**(3 Pt 1): p. 619-23.

39. Nixon, G.M., et al., *Early airway infection, inflammation, and lung function in cystic fibrosis.* Arch Dis Child, 2002. **87**(4): p. 306-11.

40. Marostica, P.J., et al., *Spirometry in 3- to 6-year-old children with cystic fibrosis.* Am J Respir Crit Care Med, 2002. **166**(1): p. 67-71.

41. Sagel, S.D., *Noninvasive biomarkers of airway inflammation in cystic fibrosis.* Curr Opin Pulm Med, 2003. **9**(6): p. 516-21.

42. Matsui, T., et al., *Role of nitric oxide in pathogenesis underlying ischemic cerebral damage.* Cell Mol Neurobiol, 1999. **19**(1): p. 177-89.

43. Smith, J.J., et al., *Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid.* Cell, 1996. **85**(2): p. 229-36.

44. Poschet, J.F., et al., *Molecular basis for defective glycosylation and Pseudomonas pathogenesis in cystic fibrosis lung.* Proc Natl Acad Sci U S A, 2001. **98**(24): p. 13972-7.

45. Ratjen, F. and G. Doring, *Cystic fibrosis.* Lancet, 2003. **361**(9358): p. 681-9.

46. Rayner, R.J., et al., *Inflammatory markers in cystic fibrosis.* Respir Med, 1991. **85**(2): p. 139-45.

47. Bals, R., D.J. Weiner, and J.M. Wilson, *The innate immune system in cystic fibrosis lung disease.* J Clin Invest, 1999. **103**(3): p. 303-7.

48. Konstan, M.W., et al., *Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation.* Am J Respir Crit Care Med, 1994. **150**(2): p. 448-54.

49. Peckham, D.G., *Treatment of airway inflammation.* Airway Inflammation, 2003. **online**.

50. Eigen, H., et al., *A multicenter study of alternate-day prednisone therapy in patients with cystic fibrosis. Cystic Fibrosis Foundation Prednisone Trial Group.* J Pediatr, 1995. **126**(4): p. 515-23.

51. Greally, P., et al., *Interleukin-1 alpha, soluble interleukin-2 receptor, and IgG concentrations in cystic fibrosis treated with prednisolone.* Arch Dis Child, 1994. **71**(1): p. 35-9.

52. Skov, M., et al., *Iatrogenic adrenal insufficiency as a side-effect of combined treatment of itraconazole and budesonide.* Eur Respir J, 2002. **20**(1): p. 127-33.

53. Bals, R., et al., *The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface.* Proc Natl Acad Sci U S A, 1998. **95**(16): p. 9541-6.

54. Goldman, M.J., et al., *Lentiviral vectors for gene therapy of cystic fibrosis.* Hum Gene Ther, 1997. **8**(18): p. 2261-8.

55. Conway SP, E.C., Peckham DG, Whitehead A., *Zafirlukast (Accolated) as additional therapy in non asthmatic non-ABPA adult patients with cystic fibrosis.* 24th European Cystic Fibrosis Conference, 2001: p. 71-80.

56. Jones, A.M., et al., *Inflammatory markers in cystic fibrosis patients with transmissible Pseudomonas aeruginosa.* Eur Respir J, 2003. **22**(3): p. 503-6.

57. Kennedy, M.J., *Inflammation and cystic fibrosis pulmonary disease.* Pharmacotherapy, 2001. **21**(5): p. 593-603.

58. Koller, D.Y., *Sampling methods: urine/blood analysis.* Am J Respir Crit Care Med, 2000. **162**(2 Pt 2): p. S31-3.

59. Antuni, J.D., et al., *Increase in exhaled carbon monoxide during exacerbations of cystic fibrosis.* Thorax, 2000. **55**(2): p. 138-42.

60. Konstan, M.W. and M. Berger, *Current understanding of the inflammatory process in cystic fibrosis: onset and etiology.* Pediatr Pulmonol, 1997. **24**(2): p. 137-42; discussion 159-61.

61. Khan, T.Z., et al., *Early pulmonary inflammation in infants with cystic fibrosis.* Am J Respir Crit Care Med, 1995. **151**(4): p. 1075-82.

62. Vreman, H.J., R.J. Wong, and D.K. Stevenson, *Exhaled carbon monoxide in asthma.* J Pediatr, 2000. **137**(6): p. 889-91.

63. MS, B., *End-tidal carbon dioxide monitoring in pediatrics: concepts and technology.* Journal of Postgraduate Medicine, 2001. **47**(2): p. 153-6.

64. Ho, L.P., J.A. Innes, and A.P. Greening, *Nitrite levels in breath condensate of patients with cystic fibrosis is elevated in contrast to exhaled nitric oxide.* Thorax, 1998. **53**(8): p. 680-4.

65. Kharitonov, S.A. and P.J. Barnes, *Exhaled markers of pulmonary disease.* Am J Respir Crit Care Med, 2001. **163**(7): p. 1693-722.

66. Paredi, P., et al., *Exhaled ethane is elevated in cystic fibrosis and correlates with carbon monoxide levels and airway obstruction.* Am J Respir Crit Care Med, 2000. **161**(4 Pt 1): p. 1247-51.

67. Warren, J., et al., *Guidelines for the inter- and intrahospital transport of critically ill patients.* Crit Care Med, 2004. **32**(1): p. 256-62.

68. Guazzi, M., et al., *Pulmonary function, cardiac function, and exercise capacity in a follow-up of patients with congestive heart failure treated with carvedilol.* Am Heart J, 1999. **138**(3 Pt 1): p. 460-7.

69. Chan, G.C., Y.L. Lau, and C.Y. Yeung, *End tidal carbon monoxide concentration in childhood haemolytic disorders.* J Paediatr Child Health, 1998. **34**(5): p. 447-50.

70. Horvath, I., et al., *Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia.* Thorax, 2003. **58**(1): p. 68-72.

71. Terheggen-Lagro, S.W., et al., *End-tidal carbon monoxide corrected for lung volume is elevated in patients with cystic fibrosis.* Am J Respir Crit Care Med, 2003. **168**(10): p. 1227-31.

72. Zetterquist, W., et al., *Exhaled carbon monoxide is not elevated in patients with asthma or cystic fibrosis.* Eur Respir J, 2002. **20**(1): p. 92-9.

73. Paredi, P., et al., *Changes in exhaled carbon monoxide and nitric oxide levels following allergen challenge in patients with asthma.* Eur Respir J, 1999. **13**(1): p. 48-52.

74. Paredi, P., et al., *Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease?* Chest, 1999. **116**(4): p. 1007-11.

75. Stevenson, D.K., et al., *Carbon monoxide detection and biological investigations.* Trans Am Clin Climatol Assoc, 2000. **111**: p. 61-75.

76. Master, V., et al., *Efficacy of once-daily tobramycin monotherapy for acute pulmonary exacerbations of cystic fibrosis: a preliminary study.* Pediatr Pulmonol, 2001. **31**(5): p. 367-76.

77. Conway, S.P., et al., *Antibiotic treatment of multidrug-resistant organisms in cystic fibrosis.* Am J Respir Med, 2003. **2**(4): p. 321-32.