CLINICAL PRACTICE GUIDELINE IMPLEMENTATION
FOR
ALPHA-1 ANTITRYSIN DEFICIENCY TESTING:
EVALUATION OF AN INNOVATIVE METHOD

By
Priscilla Torres Steffen

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As members of the Practice Inquiry Committee, we certify that we have read the practice inquiry prepared by Priscilla Torres Steffen entitled CLINICAL PRACTICE GUIDELINE IMPLEMENTATION FOR ALPHA-1 ANTITRYPSIN DEFICIENCY TESTING: EVALUATION OF AN INNOVATIVE METHOD and recommend that it be accepted as fulfilling the practice inquiry requirement for the Degree of Doctor of Nursing Practice.

Sally J. Reel, PhD, RN, FNP, BC, FAAN, FAANP

Leslie Ritter, PhD, RN

Ted Rigney, PhD, RNP, CCRN, ACNP-BC, FAANP

Final approval and acceptance of this practice inquiry is contingent upon the candidate’s submission of the final copies of the practice inquiry to the Graduate College.

I hereby certify I have read this practice inquiry prepared under my direction and recommend it be accepted as fulfilling the practice inquiry requirement.

Sally J. Reel, PhD, RN, FNP, BC, FAAN, FAANP
Practice Inquiry Director
STATEMENT BY AUTHOR

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SIGNED: Priscilla Torres Steffen
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DEDICATION

To my Mom

You were right!
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ABSTRACT

Purpose/Aims: The American Thoracic Society (ATS) published recommendations for alpha-1 antitrypsin deficiency (AATD) testing in 2003. This descriptive project evaluates the outcomes of ATS AATD guideline use in the setting of the pulmonary function testing (PFT) lab.

The specific aims met by this descriptive project describe the prevalence of AATD cases and carriers in the sample, examine to what degree the established clinical guideline promoted accurate patient selection for the alpha-1 test in the sample, and aimed to determine whether alpha-1 antitrypsin blood levels are reduced in current smokers compared to former or never smokers.

Background: Alpha-1 antitrypsin prevents lung tissue breakdown by attenuating excess elastase released from neutrophils during the inflammatory response. Smoking impairs alpha-1 antitrypsin protection at the site of lung inflammation promoting emphysema development. In the case of genetic mutation, protective alpha-1 antitrypsin levels are reduced, causing emphysema even in non-smokers. Significantly reduced protective levels of alpha-1 antitrypsin increase the odds for morbidity and early mortality from emphysema. The literature provides support for targeted testing in the population most affected.

Sample/Methods: The sample population included adults 21 through 79 years completing pulmonary function testing over 18 months in a metropolitan pulmonary medicine practice and was retrospectively reviewed.
Of the 521 in the sample, 190 were tested for AATD, and 24 were found to carry an abnormal genotype. However, using Table 11 from the ATS CPG failed to provide structured, consistent guidance in selecting patients for AATD testing. Still, the prevalence of the abnormal genotypes MS, MZ, SZ, and ZZ was increased in this pulmonary population compared to the published estimated prevalence for the general population.

A structured decision-tree, developed from the original guideline for diagnostic testing, may provide superior guidance for AATD test patient selection in this setting. Increased case finding by targeted testing of patients in the setting of the pulmonary function lab can serve to integrate this clinical practice guideline in a consistent streamlined fashion.

In this sample, no difference between AAT blood levels among ever, never, and current tobacco smokers was detected. A more powerful sample is needed.
CHAPTER ONE: INTRODUCTION

Since first described (Laurel & Erikkson, 1963), alpha-1 antitrypsin deficiency (AATD) was considered uncommon, however we now understand that the condition is not rare, but is under diagnosed (Campos, Wanner, Zhang, & Sandhaus, 2005). In fact, de Serres (2003), reports that AATD is actually “one of the most common serious hereditary disorders in the world, as it affects all major racial subgroups worldwide” (p. 382).

According to the American Thoracic Society (ATS)("American Thoracic Society/European Respiratory Society Statement: Standards for the Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency,” 2003a), the prevalence estimate in 2003 ranged between 1 in 1,600 individuals to 1 in approximately 5,000 individuals(p 823).

The purpose of alpha-1 antitrypsin is to prevent lung tissue breakdown during the inflammatory response. The protective effect of alpha-1 antitrypsin may be absent due to a genetic deficiency causing overall low blood levels or due to its destruction at the tissue level. A deficient blood level of alpha-1 antitrypsin is associated with hepatic dysfunction and early onset of airway obstruction and emphysema in the absence of tobacco smoking. Chronic airway inflammation and subsequent increased presence of macrophage protease can destroy any protective alpha-1 antitrypsin that is normally present to attenuate the effect of excess neutrophil elastase on lung tissue at the site of inflammation, and this mechanism has been proposed as a factor in tobacco smoke induced emphysema. AATD raises the odds for increased morbidity and early mortality from emphysema.
This project is a descriptive review and outcomes evaluation of the implementation of clinical practice guideline based targeted testing for AATD in the setting of the pulmonary function lab. The result describes incident cases and carriers, evaluates whether the established clinical guidelines promote correct patient selection for the AATD test in the PFT lab in a pulmonary office-based setting (American Thoracic Society – European Respiratory Society Recommendations for AATD Testing, 2003), and examines whether blood AAT levels differ by smoking status in cases and carriers. The ATS clinical practice guideline (CPG), specifically implemented during pulmonary function testing (PFT) in an office-based pulmonary practice is innovative and not described in the literature.

The integration of guidelines into the clinical setting as represented by the fourth point in the ACE Star Model of Knowledge Transformation (Stevens, K., 2002) is discussed as related to this project. It is this fourth point in the process of bringing evidence to daily practice that may be the most difficult as the integration of an innovation, such as consistent use of a CPG, may be slow to diffuse into customary use for a variety of reasons.

The theoretical model known as Diffusion of Innovations (Rogers, 2003) is described to assist in the understanding of this type of change, and may improve our ability to adapt a CPG in an effort to enhance integration into practice and broaden diffusion of CPG use.

Purpose
The American Thoracic Society made recommendations for alpha-1 antitrypsin deficiency testing in 2003. This descriptive project evaluates the outcomes of implementation of the ATS guideline in the setting of the pulmonary function test lab. Specific aims were to describe the prevalence of AATD cases and carriers in the sample, to examine to what degree the established clinical guideline promoted accurate patient selection for the alpha-1 test in the sample, and to determine if alpha-1 antitrypsin blood levels are lower in current smokers compared to former or never smokers.

Relevance to Advanced Practice Nursing

Fundamental to the role of advanced practice nurses, including both Family Nurse Practitioners (FNP) and Acute Care Nurse Practitioners (ACNP) is knowledge of research and research methods as well as evidence-based practice (EBP) as an outcome of this knowledge.

Advanced practice nurse activities for family nurse practitioners include core competencies that mandate practice based on scientific rationale and evidence-based standards of care (National Organization of Nurse Practitioner Faculties, 2002). Practice guidelines for acute care nurse practitioners include core competencies such as efforts to contribute to research driven knowledge that promote evidence-based guidelines and practice for patients with complex acute and chronic illness (National Panel for Acute Care Nurse Practitioner Competencies, 2004).

The American Thoracic Society (Larson, et al., 2006) has published recommendations addressing the need for nursing research related to respiratory nursing priorities “to correlate biochemical and biological markers with clinical variables to
address issues of susceptibility, onset, natural history, and response to treatment and to facilitate clinical trials of new therapies” (2006, p. 472).

This project meets the nurse practitioner competencies as described, as well as the ATS recommendations for respiratory nursing priorities.

Knowledge Gap

Prior to the implementation of this project, a review of the literature did not reveal documentation specific to the implementation of the ATS AATD testing guideline in the clinical setting. No clinical studies describing a relationship or the lack of a relationship between alpha-1 antitrypsin blood levels and smoking status were found in the literature. In addition, no studies or reports describing implementation of this guideline in conjunction with pulmonary function testing were present in the literature.

Aims

(1) Describe the prevalence of AATD cases and carriers in the sample, (2) Examine to what degree the established clinical guideline promoted accurate patient selection for the alpha-1 test in the sample, and (3) Determine if alpha-1 antitrypsin blood levels are lower in those that smoke compared to former or never smokers.

Hypotheses

(1) Testing 300 individuals will yield case finding of at least 27 individuals with carrier or AATD status. (2), The table format as published for established clinical guidelines promotes correct patient selection for the alpha-1 test in the PFT lab in a pulmonary office-based setting, and (3) Blood levels of AAT are lower for current smokers compared to never, or ever smokers in the population evaluated.
Definitions

**Diffusion Test:** To measure diffusion of gases into the blood, the patient breathes a specific gas concentration. The concentration of this gas in the exhaled air is measured. The difference between the concentration of gas inhaled and exhaled provides an estimate of how quickly gas can move from the lungs into the blood. The patient’s hemoglobin value is required for the calculations involved in the determination of diffusion. This test is one of those performed during pulmonary function testing.

**FEV1:** Forced Expiratory Volume in One Second: Represents the volume of air forcibly exhaled from the lungs in the first second of a forced expiratory effort. This important measure of obstruction is measured by spirometry during pulmonary function testing.

**FVC:** Forced Vital Capacity: After a maximum inhalation, this volume of air is forcibly and maximally exhaled until no more can be expired is then measured. This important measure of restriction is evaluated by spirometry during pulmonary function testing.

**FEV1/FVC Ratio:** The ratio of FEV1 to FVC indicates what percentage of the total FVC was exhaled during the first second of forced exhalation. This important measure of obstruction is evaluated by spirometry during pulmonary function testing.

**Lung Volumes:** Term denotes measurement of various lung volumes and capacities taken during pulmonary function testing that characterize lung function and help define lung disease. Measures may include Tidal Volume (volume of air in one resting breath cycle), Inspiratory Reserve Volume (volume of air subject is capable of
inhaling over and above Tidal Volume), Expiratory Reserve Volume (volume of air subject is capable of exhaling beyond Tidal Volume), and Residual Volume (volume of air retained in the lung that cannot be exhaled. Various capacities are calculated based upon these volumes.

**Obstruction**: Airway patency is estimated by measuring the flow of air as the patient exhales as hard and as fast as possible. Obstructed airflow represents a problem with exhaling. FEV1 and FEV1/FVC Ratio are measures of obstruction. FEV1 equal to or less than 80% is characteristic of obstruction.

**Pack Years**: The sum of number of packages of cigarettes smoked daily multiplied by the number of years smoking cigarettes, eg. One pack/day x 20 years= 20 pack years, and three packs/day x 15 years= 45 pack years.

**Pulmonary Function Test(s)**: A group of tests that measure how well the lungs take in and release air and how easily oxygen crosses into the blood. Spirometry for FVC, FEV1, and FEV1/FVC Ratio, lung volumes and diffusion are examples of tests performed during pulmonary function testing.

**Residual Volume**: The volume of air left in the lung at the end of forced expiration.

**Restricted Airflow**: The volume of air that can be inhaled evaluates lung elasticity and chest wall mechanics. Restriction represents a problem with inhalation.

**Spirometry**: The patient breathes into a mouthpiece connected to an instrument called a spirometer. The spirometer records the volume and flowrate of air inhaled and exhaled. FVC, FEV1, and FEV1/FVC Ratio are measured.
Total Lung Capacity: The total volume of air the lung can hold. The composite of Tidal Volume (volume of air in one resting breath cycle), plus Inspiratory Reserve Volume (volume of air subject is capable of inhaling over and above Tidal Volume), plus Expiratory Reserve Volume (volume of air subject is capable of exhaling beyond Tidal Volume) plus Residual Volume (volume of air retained in the lung that cannot be exhaled).

Background

Alpha-1 antitrypsin (AAT) is an anti-protease (or anti-proteinase), and its function is to block the action of the protease, neutrophil elastase (NE), on tissue. The proteinase inhibitor (PI) gene on chromosome 14 encodes the AAT protein (Protease Inhibitor. OMIM). Hepatocytes synthesize then secrete AAT. AAT is normally present in serum, tissue fluids, and macrophages (Kumar et al. 2005). Identification of other sites and mechanisms of action is the target of current research. This ongoing research is increasing our understanding of both the sites and the mechanisms of action of AAT on the molecular and cellular levels. These insights are aiding in an increased appreciation for the role of AAT as an anti-protease and its interactions with both other anti-proteases and proteases (Abboud & Vimalanathan, 2008; Ruta Aldonyte, et al., 2008; R. Aldonyte, Jansson, Ljungberg, Larsson, & Janciauskiene, 2004; Cox & Cox, 2009; Djekic, et al., 2009).

As currently understood, the primary role for AAT is to attenuate the effect of excess neutrophil elastase secreted in lung tissue by activated neutrophils. When AAT is
deficient or dysfunctional, excess neutrophil elastase is not attenuated and emphysematous tissue destruction results.

Emphysema and Other Obstructive Clinical Entities

Note that the term “pulmonary emphysema” describes architectural change seen in the lung whereas the alveolar walls experience degradation and destruction with progressive development of enlarged air spaces and loss of the alveolar-capillary membrane. This disease process may manifest with signs such as an increased anterior-posterior diameter and flattened diaphragms on chest x-ray, parenchymal emphysema on computed tomography (CT) of the chest, reduced FEV1/FVC Ratio on spirometry, increased total lung capacity (TLC) and residual volume (RV) on lung volume determinations, impaired diffusion due to loss of alveolar-capillary membrane surfaces, and hypoxia. Symptoms may include dyspnea, air hunger, and breathlessness. These symptoms are variably present in other obstructive lung conditions.

The condition termed “chronic bronchitis” is defined by the presence of cough productive of sputum for at least three months of two consecutive years. Patients with chronic bronchitis may experience dyspnea, have abnormal spirometry, and demonstrate obstruction to airflow.

“Bronchiectasis” refers to the condition of airway pocketing and dilatation resulting in impaired clearance mechanisms and retention of secretions with development of bacterial colonization and exacerbations of purulent sputum and recurrent infection. Patients with bronchiectasis cough, may experience dyspnea, have abnormal spirometry, and demonstrate obstruction to airflow.
The Global Initiative of Chronic Obstructive Lung Disease (Rabe, et al., 2007, p. 2) defines “Chronic Obstructive Pulmonary Disease” (COPD) based upon airflow limitation. Airflow limitation with the ratio of FEV1/FVC being 70% or below that predicted by age, height, gender, and race defines mild airflow obstruction (Rabe, et al., 2007, p. 4). This obstruction to airflow may be present in emphysema, chronic bronchitis, or asthma. A lack of response to short-acting beta-agonist (SABA) differentiates COPD from the airflow limitation of asthma that is characteristically responsive and reversible after SABA administration.

Overlap of these obstructive pulmonary problems is often encountered in clinical practice, wherein patients may have any combination of mixed disease. The Venn diagram (Figure #1) depicts this overlap.

![Figure 1. Venn Diagram of Obstructive Lung Disease](http://emedicine.medscape.com/article/297664-media)

A given individual may have features of chronic bronchitis and asthma, may have emphysema and features of chronic bronchitis, may have a history of asthma and
emphysema, or may present simply with the diagnosis of COPD. A patient may carry the
diagnosis of emphysema, but have essentially normal pulmonary function test results.
These mixed patterns may make classification of patients for AATD testing challenging
with respect to decision-making for compliance with AATD CPG purposes.

Physiology, Genetics, and Pathophysiology

*The Protease-Antiprotease Imbalance Hypothesis*

The concept of an imbalance between protective and destructive forces at the
molecular level and manifest on the cellular level is proposed in the Protease -
Antiprotease Imbalance Conceptual Model for the pathogenesis of emphysema. The
diagram depicted in Figure 2 demonstrates the conceptual framework proposed by Tuder
et al (2006) of the potential actors and mechanisms felt to be responsible for the alveolar
tissue destruction seen in this disease. According to this framework, once triggered, a self
perpetuating cycle leads to progressive tissue damage (Tuder, et al., 2006). If a genetic
deficiency of AAT is present, the additional protease- antiprotease imbalance induced by
tobacco smoke inhalation is additive.
Cigarette smoke causes a progressive disruption of alveolar maintenance and variable degrees of inflammation, driven by the cigarette smoke itself, oxidative stress, or alveolar cell damage. After years of relentless attack by this underlying process counterbalanced by potential repair, susceptible individuals would activate molecular and cellular processes involved in alveolar destruction, namely protease/antiprotease imbalance, apoptosis, and oxidative stress. These processes are mutually interactive, and promote amplifying feed-forward loops. Endogenous mediators such as ceramide would promote the amplification of these processes, increasing inflammatory cell injury and hampering repair processes. Several of these elements are also present in organismal aging (Tuder, et al., 2006).

The protease-antiprotease relationship is complex (Abboud & Vimalanathan, 2008). It is known that several enzymes and the inhibitor of each are involved. Neutrophil elastase is inhibited by alpha-1 antitrypsin, macrophage protease is inhibited by metalloproteinase-1, and a third type of elastolytic enzyme, macrophage cathepsin is inhibited by an antiprotease cystatin C (Abboud & Vimalanathan, 2008). The model proposed by Abboud and Vimalanathan (2008) is depicted in Figure 3. Notice the

Figure 2. Conceptual Framework of Emphysema.

(Tuder, et al., 2006)
relationship of metalloproteinase (MMP) on AAT. This relationship will be explored more fully with respect to the impact of macrophage protease.

![Protease Antiprotease Imbalance](image)

**Figure 3. Model of Protease Antiprotease Imbalance**

(ABBoud & Vimalanathan, 2008)

Diagram showing the pathways leading to smoking-induced protease-antiprotease imbalance in the lung.

Smoking induces epithelial cells to produce cytokines that stimulate neutrophils and macrophages. Cigarette smoke also acts directly on neutrophils and macrophages to activate them. Cigarette smoke has oxidants that can inactivate antiproteases, in addition to antiprotease inactivation by oxidants released by macrophages and neutrophils. The stimulated neutrophils and macrophages release proteolytic enzymes. Neutrophil elastase can activate MMPs, while MMPs can inactivate alpha1 antitrypsin. Not shown in the diagram is the role of MMP-12 in releasing TNF, which amplifies the inflammatory reaction. These processes lead to a protease-antiprotease imbalance, which can degrade lung elastin and connective tissue; if sustained, this will lead to emphysema. IL = interleukin; LTB = leukotriene B; NE = neutrophil elastase; MMP = matrix metalloproteases; TIMP = tissue inhibitor of metalloproteases; TNF = tumor necrosis factor alpha. (ABBoud & Vimalanathan, 2008, p. 363).
Neutrophil Elastase

Elastase is a proteinase (or protease) found in azurophil granules of neutrophils. Activated neutrophils secrete neutrophil elastase during the inflammatory response. Normally, neutrophil elastase acts extracellularly to degrade the virulence factors of bacteria.

The basic role of alpha-1 antitrypsin is to block the action of excess elastase. Neutralization of excess elastase by alpha-1 antitrypsin (AAT) prevents degradation of normal tissues (Abboud & Vimalanathan, 2008)Kumar et al. 2005). The presence of excess elastase will damage collagen, basement membrane, fibrin, elastin, and cartilage causing tissue destruction unless attenuated by alpha-1 antitrypsin.

In addition to neutrophil elastase, other proteases exist that can damage lung tissue. A second important protease involved in the lung is macrophage protease. Macrophage protease has its own inhibitor. Neutrophil elastase is inhibited by alpha-1 antitrypsin but is not inhibited by the inhibitor of macrophage protease. Neutrophil elastase can actually break down the inhibitor of macrophage protease (Shapiro, et al., 2003).

Macrophage Protease

As stated, a second enzyme capable of lung tissue destruction derives from macrophages. The action of this macrophage protease is stimulated in the lung by exposure to tobacco smoke (Kumar et al. 2005). Macrophage protease is not inhibited by alpha-1 antitrypsin, and can actually enzymatically digest alpha-1 antitrypsin (Kumar et al. 2005)(Shapiro, et al., 2003). This phenomenon is felt to possibly account for the
functional deficiency induced by cigarette smoking and the emphysema that ensues (Abboud & Vimalanathan, 2008). Interestingly, some pathology studies have demonstrated an affinity of the macrophage for the respiratory bronchiole in smokers that correlates with the centrilobular emphysema seen in smokers (Abboud & Vimalanathan, 2008, p. 362).

Excess macrophage protease is attenuated by metalloproteinase-1 similarly to the attenuation of neutrophil elastase by alpha-1 antitrypsin (Shapiro, et al., 2003). Captivating research has found when lung tissue is broken down by macrophage protease, the presence of residual elastin fragments appears to promote recruitment of monocytes that mature into macrophages and a positive feedback loop is thus formed; a destructive one that results in pulmonary emphysema (Cox & Cox, 2009).

**Alpha-1 Antitrypsin Deficiency**

Alpha-1 antitrypsin deficiency (AATD), as currently understood, can occur as the result of (1) a genetic mutation and/or (2) as an acquired functional deficiency that occurs as the result of tobacco smoking (Abboud & Vimalanathan, 2008).

**Genetic Mutations Deficiency**

Genetic deficiency mutations transmit in an autosomal recessive fashion. One abnormal allele transmits to carriers. Genetic alterations involving single nucleotide polymorphisms (SNP) of the Pi gene locus are said to number over 100 and may result in abnormal gene expression, gene translation, and intracellular protein processing (DeMeo & Silverman, 2004; Luisetti & Seersholm, 2004). A number of these mutations have clinical implications (Snyder, et al., 2006).
The wild-type allele is labeled as “M.” A deficiency state of AAT is not associated with the PiMM phenotype with the exception of three extremely rare deficiency-associated alleles with behavior similar to M when tested by isoelectric focusing (DeMeo, 2004, p.260). In general, PiMM is considered the “normal” phenotype. Carriers are characterized by PiMS and PiMZ alleles. Here the M allele is normal and the second allele of the pair is abnormal. The most common homozygous mutations with clinical implications are PiSS, and PiZZ. Heterozygous deficiency combinations such as PiSZ and PiZNull are possible. A “Null allele” represents complete lack of protein gene transcription. Persons with the PiSZ defect, and in particular, those who smoke, are at increased risk for lung dysfunction that includes recurrent infection and loss of lung function (Alpha-1 Antitrypsin Deficiency, ND). The PiZNull individual would not exhibit detectable AAT (DeMeo & Silverman, 2004). It is important to recognize the fact that Null alleles yield undetectable protein levels as, again, there is no AAT protein gene transcription and no protein is produced (Snyder, et al., 2006). This situation has important implications related to the biomethods used to test for deficiency as discussed later.

When mutated forms of alpha-1 antitrypsin are produced, retained, and then degraded, low serum levels, such as 15% of the normal level of alpha-1 antitrypsin results (Abboud & Vimalanathan, 2008, p. 362). The S allele is said to be a deficiency allele due to intracellular degradation (Snyder, et al., 2006). The presence of homozygous S alleles may result in up to 20% reduction of functional alpha-1 antitrypsin, however, this deficiency may not be clinically manifested (Snyder, et al., 2006).
Some mutations produce abnormal forms of polymerized alpha-1 antitrypsin proteins that become retained in the endoplasmic reticulum during production in the hepatocyte. These polymerized forms experience slowed degradation, and as a result, an alternate degradation pathway is triggered. This alternate pathway produces toxins that cause hepatocyte damage. This liver damage is seen in the PiZZ phenotype of AATD. The PiZZ phenotype also results in manifest lung disease due to both deficient, as well as circulating dysfunctional AAT that does not adequately inhibit elastase (Snyder, et al., 2006).

In the genetically induced form of AATD, there is a chronic accumulation of neutrophils in airways and emphysematous destruction of lung tissue as elastase action proceeds unchecked. In the PiZZ phenotype, polymerization of the small amount of alpha-1 antitrypsin that is indeed present in the lung acts to attract additional neutrophils and the production of more elastase (Abboud & Vimalanathan, 2008). Genetic AATD tissue destruction and emphysema appears to affect the lower lung zones preferentially in contrast to tobacco smoke induced emphysema that has a more apical or centilobular distribution (Abboud & Vimalanathan, 2008).

**Acquired Functional Deficiency**

An acquired functional deficiency of alpha-1 antitrypsin may be present in tobacco smokers. Smoking creates an inflammatory state. Activated neutrophils release reactive oxygen species (ROS) that cause oxidative injury. Tobacco smoke inhalation increases the neutrophil population in lung tissue, increases neutrophil elastase release, increases macrophage protease production and impairs alpha-1 antitrypsin function. This
chain of events promotes lung tissue destruction. According to Abboud (2008, p. 362) “smoking may cause a protease-antiprotease imbalance in the lung by reducing the functional activity of alpha-1 antitrypsin in the lung interstitium and ‘alveolar’ lining fluid, and by increasing the amount of elastolytic proteases released in the lung. Tobacco smoke [is] reported to inhibit the anti-elastase activity of alpha-1-antitrypsin.” We recognize this tissue destruction as pulmonary emphysema related to tobacco smoking (Kumar et al. 2005).

**Natural History of AATD**

Lung function may remain within normal limits in AATD for twenty years or longer with a progressive decline that manifests after thirty to forty years. The Larsson (1978) study found age 40 as the median age of death in smokers and age 60 as the median age of death in never-smokers among PiZZ homozygotes.

**High Risk Phenotypes**

An individual carrying the Z allele is at increased risk related to non-protective levels of circulating alpha-1 antitrypsin. The protective level of circulating alpha-1 antitrypsin is 11 µm/l or above. As depicted in Figure #4, alpha-1 antitrypsin blood levels in persons homozygous for the Z allele fall below this protective level. The PiSZ and PiMZ heterozygote phenotypes demonstrate deficient circulating levels, and the PiSZ heterozygote may indeed have a significant deficiency, as circulating levels are typically borderline.
Risk, Morbidity, and Mortality

With respect to risk for PiZ heterozygotes, an increased risk for COPD does exist for persons carrying the PiMZ alleles, with this risk increased due to multi-factorial influences including those from genetic, environmental, and tobacco use related factors ("American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency," 2003b, p. 834). The increased risk for smoking-related morbidity in the PiSZ heterozygote is highly significant. The influence of smoking on pulmonary function is similar to that seen in PiZZ homozygotes, however if exposure to tobacco smoke is avoided, the risk for pulmonary emphysema is not increased significantly in the non-smoking PiSZ heterozygote ("American Thoracic Society/European Respiratory Society statement: \[\text{Figure 4. Lung Disease Risk by Genotype}\]

(Fairmam & Malhotra. 2009)
standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency,” 2003b, p. 834).

According to data summarized in the ATS Guideline (2003, p. 834), the FEV1 can be predictive in terms of prognosis. Once the FEV1 drops to the 35% predicted level, the mortality rate rises exponentially in concert with the decline in FEV1. The cited data indicate an average survival for AATD patients with FEV1 less than 25% predicted to be 6.3 years. For individuals whose FEV1 is above 25% predicted, life expectancy has been estimated to be 10.5 years while for those at 50% predicted FEV1 have a life expectancy described as approximately 14.2 years (p. 831).

If AATD is present and detected, affected individuals may take action, such as avoidance of tobacco smoke and exposure to other pulmonary irritants and toxins, to avoid further risk for damage to lung tissue.

In general, screening is indicated if a condition is serious, has important consequences, is progressive, has a preclinical phase, is prevalent, has a long duration in the population to be screened, and can be impacted by avoidance measures and/or therapy (Aschengrau, 2006, p.415). AATD fits this profile. As we are becoming increasingly aware of the potential widespread prevalence of AATD, implementation of programs aimed at targeted testing are indicated to promote risk reduction among individuals at risk, and to effect augmentation therapy for PiZZ homozygotes.

AATD Detection Bio-methods

*Dried Blood Spot Specimens*
A variety of studies have documented the fact that alpha-1 antitrypsin blood level results determined from samples collected as dried blood spots are valid and reliable (Bals, et al., 2007; Braun, Meyer, Cleve, & Roscher, 1996; Costa, et al., 2000; Rodriguez, Jardi, Costa, Cotrina, Galimany, Vidal, & Miravitlles, 2002; Wencker, Marx, Konietzko, Schaefer, & Campbell, 2002). The DBS method of blood specimen collection is useful to simplify office-based testing.

**Biomethods**

A variety of bio-methods can detect alpha-1 antitrypsin biomarkers. These methods include nephelometry, enzyme-linked immunosorbent assay (ELISA), isoelectric focusing (IEF), and polymerase chain reaction (PCR). Nephelometry, isoelectric focusing and PCR are the relevant methods discussed here.

**Nephelometry and DBS Specimen Level Correlations**

Circulating levels of alpha-1 antitrypsin can be measured by nephelometry. Nephelometry measures the quantity of the alpha-1 antitrypsin protein by quantification of antigen-antibody complex formation. Specific commercial antibodies are mixed with serum and the scatter of applied light measures the complexes that form. As increasing numbers of protein complexes are formed, light scatter increases. Standards are used for comparison of light scatter to determine the protein level present in the specimen (Nephelometry Principles, ND).

The nephelometry specimen can be obtained from a dried blood spot (DBS). Bals (2007) explains how to obtain the sample from the DBS: “the circular disk [is] punched out and incubated with 200 ml PBS overnight at 4°C. The samples [are] applied to
nephelometry (Nephelometer Analyzer 2, Dade Behring, Frankfurt, Germany) and the result [is] reported as mg/ml.”

Costa et al described the use of nephelometry in conjunction with the DBS method of sample collection in 2000. In this study, DBS specimens were tested for alpha-1 antitrypsin concentration using a modified nephelometric assay and phenotyped with an isoelectric focusing method. Genetic diagnosis was established by deoxyribonucleic acid sequencing using a simple purification procedure to remove contaminants. The nephelometric method showed a detection limit of 0.284 mg x dL(-1), corresponding to a serum concentration of 13 mg x dL(-1). The correlation coefficient between alpha1-AT concentrations in DBS versus serum samples was R2=0.8674 (p<0.0001). All 200 healthy individuals had DBS [alpha-1 antitrypsin] concentrations >1.9 mg x dL(-1), corresponding to 114 mg x dL(-1) in serum samples. One hundred and twenty-five COPD patients (42%) showed AAT values <1.8 mg x dL(-1). Twenty patients with the PIZ phenotype had alpha1-AT values lower than 0.64 mg x dL(-1). On the basis of genotyping, one COPD patient was classified as heterozygous (PIMM(heerlen)). Selective elution of contaminants resulted in optimal alpha-1 antitrypsin genotyping.

Because of its sensitivity and excellent correlation with the standard method, the dried blood spot quantitative assay is a reliable tool for routine measurement of alpha1-antitrypsin.

Polymerase Chain Reaction (PCR)

“Rapid screening for A-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using dried blood specimens” (Rodriguez, Jardi, Costa,
Cotrina, Galimany, Vidal, Miravitlles, et al., (2002) has been employed using nephelometry for determination of serum alpha-1 antitrypsin levels coupled with the use of PCR to determine genotype for low alpha-1 antitrypsin levels found upon nephelometry. The researchers sought to find an accurate but convenient method to determine alpha-1 antitrypsin status. They condone the use of DBS for the convenient method of specimen collection and transport for large-scale screening-type initiatives. They report a cost savings by this method of 80% per sample for processing DNA by way of DBS elution compared to serum DNA isolation by use of their current kit. The small DNA sample produced by eluted DBS specimens is recognized and acknowledged. However, the researchers report excellent results with the LightCyclerPCR System (LightCycler; Roche Diagnostics Canada, Montreal, Quebec, Canada) by use of highly sensitive fluorescent probes, use of an integrated computer system for reading results, and the fact that this system involves less manipulation of sample and the risks to accuracy inherent to excessive specimen handling.

The use of a primer to mark the region of DNA to be amplified allows PCR to amplify the target region to a quantity that can be recognized. The heat required to denature the protein, and allow double-stranded DNA to separate, requires the use of polymerized enzyme that is itself resistant to becoming denatured at the temperature required to melt the target genetic material (Heid, Stevens, Livak, & Williams, 1996). Taq DNA polymerase, derived from Thermis aquaticus, is one product that is widely used for this reason. The cycling of temperature to optimize denaturing and annealing of amplification product is maintained at optimal levels to produce amplification or
progressive accumulation of copied genetic material that can be identified by fluorescence. Several preliminary cycles provide baseline data at the beginning of the process and a threshold level is determined based upon a standard deviation value. Once the amplification process generates a level of fluorescence that crosses this threshold (termed “Ct”), a baseline level of the quantity of product can be determined (Heid, et al., 1996). This concept marked the advent of improved real-time PCR (RT-PCR) and resolved the problems associated with control of initial “quantitation of the initial target sequences” (Heid, et al., 1996)(p. 986). According to the information described for Real-Time PCR, “Ct is defined as the fractional PCR cycle number at which the reporter fluorescence is greater than the threshold. The Ct is a basic principle of real time PCR and is an essential component in producing accurate and reproducible data” (Real-Time PCR, ND).

A system that simultaneously measures the fluorescence of 96 wells in the thermal cycler reduces error as a result of reduced sample handling and prevents potential PCR product carry-over contamination (Heid, et al., 1996). Heid (1996, p. 989) explains that the determinants of the efficiency of PCR amplification: [include] magnesium and salt concentrations, reaction conditions (i.e., time and temperature), PCR target size and composition, primer sequences, and sample purity. All of the above factors are common to a single PCR assay, except sample-to-sample purity. If equal amounts of nucleic acid are analyzed for each sample and if the amplification efficiency before quantitative analysis is identical for each sample, the internal control (normalization gene or competitor) should give equal signals for all samples (Heid, et al., 1996).
Rodriguez et al. (2002) found the derivative melting curves were highly reproducible on analysis of various samples with different amplification efficacies. They explain, “melting curves are converted to melting peaks by software, allowing easy distinction of the wild-type from the mutant by the different melting temperature.”

In the Rodriguez et al study (2002), the within- and between-run melting peaks for the same allele differed by less than 0.3_C for both the PIZ and PI S mutations. Thus, melting curve analysis allowed easy and unambiguous assignment of genotyping for PI S and PI Z alleles.” They report, “total concordance between PI S and PI Z genotype for results obtained from DBS samples with the LightCycler-PCR and with the PCR-DNA sequencing method” (p. 815).

Andolfato et al. (2003) confirmed the accuracy of PCR for detection of abnormal alpha-1 antitrypsin alleles for specimens taken from whole blood versus serum and validated the PCR results via the use of isoelectric focusing.

Kaczor et al. (2007) reported the validation of real-time PCR using dual-labeled, target specific fluorescent probes to type PI*S and PI*Z alleles. Concordance was reportedly achieved with serum levels and restriction fragment length polymorphisms. Accuracy was confirmed via external inter-laboratory validation for approximately 1200 samples.

An extremely in-depth discussion regarding results of their study, the potential sources of error, and the specificity of real-time PCR technical attributes is discussed at length by Leong, DT, Gupta, A, Bai, HF, Wan, G, Yoong, LF, and Too, HP, et al. (2007). They conclude, “absolute quantification with PCR standards in a real-time PCR protocol
gave better accuracy than using end-point PCR methodology. By using this method of aqPCR, there is no need for incorporating molecular cloning steps into the existing real-time PCR protocol to quantify gene expression” (p. 209).

As indicated by these studies, the valid and reliable use of real-time PCR for alpha-1 antitrypsin genotyping is demonstrated.

*Isoelectric Focusing (IEF)*

Isoelectric focusing involves “mutation detection by which proteins are separated according to the pH at which their net charge is zero (isoelectric point; pI).” (Isoelectric Focusing #1). The process involves placing a pH gradient using a mixture of ampholytes, or different molecules designed to have range of isoelectric points, which are first electrophoresed on the gel to form the pH gradient. Following the development of the pH gradient, the proteins are applied, the electric field is initiated, and each protein moves to the position (pH) at which its net charge is zero. (Isoelectric Focusing #2,ND). The concept that “DNA sequence alteration [results] in an amino acid substitution [and] can change the isoelectric point of a protein” is the basis for the use of IEF. It is extremely important to remember however, that no gene transcription for alpha-1 antitrypsin protein occurs in the Null phenotype, and as such, Null is not identifiable for phenotyping via isoelectric focusing (IEF) because no protein is present (DeMeo & Silverman, 2004; Ferrarotti, et al., 2007).

**Currently Recommended AATD Detection Biomethods**

While various biomethods can be used to determine the presence of AATD, IEF has been considered the gold standard as the bio-method used for AATD testing. This
situation is changing as we learn more about AAT and genetic factors related to it as the bio-method used for testing has implications related to the result.

An excellent evidence-based laboratory methods paper (Snyder, et al., 2006) describes the combination of quantification and genotyping, with a reflex to phenotyping, as the optimal strategy for the laboratory evaluation of alpha-1 antitrypsin deficiency. At this point, it is important to point out that “although the term is not used correctly in AATD, phenotyping refers to identifying [alpha-1 antitrypsin] variants with isoelectric focusing (IEF)” (Rachelefsky, G., & Hogarth, D. K., 2008, p. 835). Genotyping is performed via RT-PCR. Snyder (p. 2238) emphasizes, “genotype assay [is] interpreted with respect to the Z and S alleles. An individual with a Z/Z genotype is homozygous for the Z variation and homozygous for the wild-type allele at the codon associated with the S allele. A similar interpretation is made for an individual with an S/S genotype. If neither the Z nor the S allele is detected, it is likely that the individual possesses two wild-type alleles. However, the result is termed a non-Z/non-S genotype because it is not possible from the genotype assay alone to determine if any rare or disease-causing variants are present outside the two regions that are assessed by S and Z genotyping. If one Z allele is detected, the individual is interpreted as having a Z/non-Z genotype, otherwise referred to as a Z heterozygote and consistent with carrier status. This individual is heterozygous for the Z variation at codon 288 and homozygous for the wild-type allele at the codon associated with the S allele. A similar interpretation is made for an individual in whom 1 S allele is detected.”
As shown in Figure #5, alpha-1 antitrypsin quantification and genotyping are performed as a response to healthcare provider order. If serum level of alpha-1 antitrypsin agrees with that expected for the genotype, testing is complete. If discordance exists between serum alpha-1 antitrypsin level and genotype reported, phenotype is performed.

![Snyder Bimethods Protocol](image)

Figure 5. Snyder Bimethods Protocol

(Snyder, et al., 2006)

An Italian report in the journal Translational Research (Ferrarotti, et al., 2007) describes the successful use of DBS and the biomethods protocol (Figure #6) in use there. The Ferrarotti (2007) protocol, depicted in Figure #6, agrees with the protocol depicted in Figure #5, as described by Snyder (2006) but is far more detailed:
The rationale for the development of these biomethods protocols to detect AATD is clearly explained by Brantly (2006). He states that the protocol reported by Snyder et al. [validates] an efficient and accurate algorithm for the diagnosis of [alpha-1 antitrypsin] deficiency using a combination of assays. Synder et al. present evidence that the use of a PCR based assay system that identifies the Z and S alleles coupled with a measurement of serum or plasma [alpha-1 antitrypsin] concentration accurately identifies 96% of all subjects when compared with the gold standard of isoelectric...
focusing-based phenotyping. In individuals in whom there is discordance between the [alpha-1 antitrypsin] concentration and the genotype, reflex phenotyping is performed. The strength of this approach is that genotyping is straightforward and provides unequivocal identification of the most common alleles associated with [alpha-1 antitrypsin] deficiency. The addition of [alpha-1 antitrypsin] concentration determination identifies those samples with rare deficiency alleles not recognized by genotyping for the S and Z alleles. These samples would prompt the use of phenotyping as a reflex test. While this approach will not identify the rare normal AAT variants, these variants [eg. variants of PiMM] are not germane to clinical care. Rare [alpha-1 antitrypsin] deficiency variants not readily recognized by phenotyping would be referred for more advanced diagnostic methods such as DNA sequencing. The systematic and less complex approaches to the laboratory diagnosis of [alpha-1 antitrypsin] deficiency should provide for greater penetration of [alpha-1 antitrypsin] genetic testing into the hospital laboratory settings and clinics. When coupled with increased awareness of this disorder, perhaps the early diagnosis of [alpha-1 antitrypsin] deficiency alleles will allow clinicians to prevent rather than treat a disease.

Dr. Brantly is the lead investigator at the University of Florida Alpha-1 Antitrypsin Genetics Laboratory in Gainesville.
Intravenous infusion of purified, pooled human alpha-1 antitrypsin has been recommended for individuals of the PiZZ phenotype with serum alpha-1 antitrypsin deficiency below 11 µm (equivalent to 50 mg/dL by nephelometry) by the ATS since 2003 ("American Thoracic Society/European Respiratory Society Statement: Standards for the Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency,"). Augmentation therapy is advocated especially for a subgroup of this population with moderate obstruction to airflow, as demonstrated by a reduced Forced Expiratory Volume in 1 second (FEV1) in the 31% to 65% predicted range. Intravenous therapy is recommended for this specific group because in observational studies, this group of individuals experienced a significantly slower decline in FEV1 over time if receiving augmentation therapy (p. 835). Dosing is weight-based and should maintain nadir serum levels of alpha-1 antitrypsin greater than 15 µm/L at day #7 prior to the next infusion ("American Thoracic Society/European Respiratory Society Statement: Standards for the Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency," 2003a).

“A Cautionary Note” was recently published (Sandhaus, et al., 2008) citing a lack of definitive evidence for efficacy and the unwise use of scarce resources for augmentation therapy for PiMZ heterozygotes. In this advisory, The Medical and Scientific Advisory Committee of the Alpha-1 Foundation strongly advised against augmentation therapy for other than PiZZ homozygotes. In their note, they urged insurance companies to observe for this practice and the Federal Drug Administration to provide labeling to this effect.
Prevalence

According to Brantley (2006), 97% of the United States population is indeed homozygous for the M allele however; this leaves 3% of the population homozygous for the S or Z allele or as carriers with heterozygous status. Of those individuals with true deficiency of circulating [alpha-1 antitrypsin], approximately 95% are homozygous for the Z allele (p. 2180). Among persons with diagnosed chronic obstructive pulmonary disease (COPD), up to 10 percent possess the Z allele, according to Brantley (2006), and the frequency of AATD in persons with COPD is 10 times that of the normal population (p. 2180). The risk ratio for COPD is increased 12 times for those homozygous for the Z allele and variably increased from 1.5 up to 12 times risk for heterozygous combinations (2006, p 2180). According to DeSerres (2003), “estimates of the weighted Pi gene prevalences using Hardy–Weinberg equilibrium statistics indicate that there is 1 carrier or deficiency allele combination phenotype for every 11.3 individuals in the American population, with 1 in 17 of phenotype PiMS, 1 in 36 of phenotype PiMZ, 1 in 1058 of phenotype PiSS, 1 in 1124 of phenotype PiSZ and 1 in 4775 of phenotype PiZZ.”

Brantley reports that based upon population estimates, 15 million persons in the United States have COPD and of these, it is likely that 1.5 million have at least one Z allele. He points out that the implication of these numbers is one that suggests that as COPD is the fourth leading cause of death in the United States, we should be testing for the only known genetic link to the disease (p. 2180). Finally, of the estimated 100,000 persons estimated to be homozygous for the Z allele, Brantley reports that only 6,000 have been identified.
Conclusion

This project is a descriptive review and outcomes evaluation of the implementation of clinical practice guideline based targeted testing for AATD in the setting of the pulmonary function lab.

This paper has thus far described the prevalence, physiology, genetic basis, and pathophysiology of alpha-1 antitrypsin deficiency. Relevant biomethods for both quantitative and qualitative evaluation of serum alpha-1 antitrypsin include nephelometry, PCR, and isoelectric focusing. The collection of a DBS for measurement of blood alpha-1 antitrypsin level correlates closely with circulating serum levels. A conceptual framework that represents the pathophysiology in pulmonary emphysema has been presented, and the conceptual model that portrays Protease--Anti-protease Imbalance in the Lung has been discussed.

The rationale for AATD testing includes case finding for interventions aimed at risk-factor reduction and implementation of augmentation therapy for those of the PiZZ phenotype.

The relevance of this project to the Family Nurse Practitioner or the Acute Care Nurse Practitioner working in the specialty of pulmonary medicine is supported by the core competencies for each advanced practice nurse specialty respectively. In addition, the ATS mandate for respiratory nursing research priorities supports this relevance.

The specific aims met by this descriptive project were to describe the prevalence of AATD cases and carriers in the sample, examine to what degree the established clinical guideline promoted accurate patient selection for the alpha-1 test in the sample,
and to determine whether alpha-1 antitrypsin blood levels are reduced in current smokers compared to former or never smokers.
CHAPTER TWO: CONCEPTUAL FRAMEWORK AND LITERATURE REVIEW

Institute of Medicine Mandate

The 2001 Institute of Medicine (IOM) mandate describes six core needs for healthcare. These include the need for effective care such that we are “providing services based on scientific knowledge to all who could benefit, and refraining from providing services to those not likely to benefit” (p. 3). As such, we seek to provide care that is consistent with the best current evidence. The IOM mandate is one that promotes the current trend toward evidence-based practice (EBP) (p. 4). With reference to testing for AATD, the IOM mandate makes it clear that targeted testing should be the goal, as we should test in the population that is most affected. The idea of targeted testing would suggest that in the clinical arena, we would perform AATD testing in a setting of patients with chronic pulmonary disease rather than a setting of general medicine patients.

ACE Star Model of Knowledge Transformation

The Academic Center for Excellence (ACE) Star Model of Knowledge Transformation (Stevens, K., 2002) provides a framework for understanding the processes involved in the evolution of health-related knowledge as it transitions through five stages to provide a basis for EBP. A five-point star conceptualizes the five stages in the Star Model. Each point on the star represents a stage of health-related knowledge as progressively refined. The representative points are “(1) knowledge discovery, (2) evidence summary, (3) translation into practice recommendations, (4) integration into
practice, and (5) evaluation. Evidence-based processes and methods vary from one point on the Star Model to the next” (Stevens, K., 2002). Figure #7 depicts these relationships in this conceptual model. Simplified, these stages correspond with (1) research, (2) the clarification and summarization of research findings through processes such as evidence synthesis, systematic review, meta-analysis, integrative review, review of literature, state of the science review, (3) translation of the evidence into guideline form, (4) integration of the guideline to clinical use, and (5) evaluation of outcomes (Stevens, R, 2002).

The fourth point in the model, Integration, may be challenging in the clinical setting as resistance to change often poses a barrier to implementation of research findings and clinical practice guidelines.
The ATS Guideline

After comprehensive and careful review of the literature related to alpha-1 antitrypsin, AATD, and all related research, the American Thoracic Society and European Respiratory Society (ATS) issued a position statement in 2003 related to testing for AATD. This position statement represents the third point in the ACE Star Model: Translation. In the statement, the ATS makes specific graded recommendations for AATD testing. The guideline presents categories for diagnostic, predisposition, and carrier state testing. The recommendations for testing involve various potential testing situations in individuals of differing age groups with obstruction to airflow on spirometric testing, and risk.

Algorithms or Protocols and Guidelines

The difference between what constitutes a guideline versus an algorithm, or protocol is distinguished by the general nature of a guideline and the more specific nature of an algorithm, protocol (Morris & Morris, 2003). Algorithms or protocols can help with guideline interpretation and “are schematic models of the clinical decision pathway described in a guideline” (American College of Physicians, 2009). It is important to understand that “adequately explicit protocols [algorithms, decision-trees], but not guidelines, have the attribute of eliciting the same decision from different clinicians” (Morris & Morris, 2003). These attributes are relevant to the evaluation of the tool used in the PFT lab for this project as well as recommendations made following the evaluation.

Innovative Guideline Implementation in the PFT Lab
The tool used in the PFT lab for identification of patients for AATD testing was Table 11 from the ATS guideline (2003, p 893). Type A recommendations include, (1) symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible and (2) asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g., cigarette smoking, occupational exposure). Type B recommendations relate to adults with bronchiectasis without evident etiology (p. 893).

A search for reports describing current use of the ATS guideline was conducted via OVID at the University of Arizona Health Science Center Library to determine how extensive the use of the guideline has been to date, however, no reports were identified that specifically discuss implementation of the ATS guidelines in the United States. Several recent reports however (Bals, et al., 2007; Brantly & Brantly, 2006; de la Roza, et al., 2005; de Serres, Blanco, & Fernandez-Bustillo, 2006; Hogarth & Rachelefsky, 2008; K. M. Shermock, T. R. Gildea, M. Singer, & J. K. Stoller, 2005) have been identified related to targeted testing for AATD.

Targeted Testing

A targeted Spanish study (de la Roza, et al., 2005) was conducted testing all patients carrying the clinical diagnosis of COPD. This study was conducted in two phases with the first phase taking patients from respiratory centers and the second phase taking patients from both primary care and specialty centers. Results found a differential in case-finding between the two phases, and the authors concluded that the higher rates of PiZZ
case-finding in the first phase related to the more targeted sample population in that phase.

A hypothetical model constructed to determine the probable distribution of deficiency alleles in a Caucasian COPD population “demonstrated that 1,829,673 alpha-1 antitrypsin deficiency patients would be detected by testing 19.3 million white COPD patients and 536,033 in white non-COPD concurrent controls” (de Serres, et al., 2006). Odds ratios (OR) were calculated to determine the odds of the various phenotypes of AAT in white COPD patients. Findings from these calculations indicate that there is a significant decreased OR for the PiMM phenotype, no significant increased or decreased OR for PiMS or PiSS phenotypes, but highly significant increased odds ratio for PiMZ, PiSZ, and PiZZ phenotypes in Caucasian COPD cases. As a result, the authors found significant support for targeted testing for AATD in COPD patients (de Serres, et al., 2006).

A European study (Bals, et al., 2007) was conducted over 37 months to increase awareness of AATD and involved the distribution of free test kits that resulted in the testing of 2722 patients with 355 persons identified with severe AATD and among those detected 16 persons with very rare phenotypes. The authors concluded that the targeted distribution of free test kits resulted in the identification of a large number of cases and improved case finding.

Using a Markov-based analytic decision model, investigators at The Cleveland Clinic (Kenneth M. Shermock, Thomas R. Gildea, Mendel Singer, & James K. Stoller, 2005) determined that while broad-based screening for AATD is not a cost-effective
intervention, “cost-effectiveness criteria could be satisfied when case-finding in a high prevalence population is undertaken” (p. 411).

The findings in all of these studies do provide support for targeted testing for AATD.

Barriers to Guideline Implementation

In general, barriers to guideline implementation are varied. Barriers cited in the literature include lack of time, lack of skills, impractical recommendations, unclear guidelines, and associated cost (Carlsen, et al., 2007). Brantley (2006) wonders why guideline adoption for AATD testing has not been widely implemented and postulates that perhaps lack of genetics training in residency programs is one factor or that alpha-1 antitrypsin experts have not specified allele detection over serum concentration as a means of detection (p. 2180).

The Theory of Diffusion of Innovations

We can turn to the Diffusion of Innovations theory (Rogers, 2003) to aid our understanding of successful adoption of innovations or changes to the status quo such as guidelines adoption or implementation of processes or programs by both individuals or systems (fourth point of the ACE Star: Integration). We ask ourselves, why does there appear to be relative apathy in the widespread uptake of guideline acceptance and usage?

An innovation is something new or different. It may be a product, a process, or an idea. By way of what process do we integrate an innovation into customary use and practice? How does the innovation diffuse into relatively widespread use? The theory
helps us understand that our perceptions of specific characteristics of a specific innovation dictate whether we adopt the innovation early, late, or not at all (p. 15).

Rogers (p. 15) lists the characteristics, or “perceived attributes” of an innovation that influence an individual or system to consider or adopt the innovation: (1) Relative advantage, (2) Compatibility, (3) Complexity (4) Trialability and (5) Observability.

The relative advantage attribute relates to whether or not the innovation improves on the status quo. A number of factors will influence whether or not a perceived relative advantage does indeed exist. Examples include economic factors, social prestige, convenience, and satisfaction. Rogers (p. 15) explains that the rate of adoption increases with increasing relative advantage perception.

The degree to which the innovation is compatible involves whether or not the existing values, norms, belief systems, past experiences, and needs of the individual or system constitute a match with those of the innovation (p. 15). Hampered diffusion of an innovation occurs when values and norms of the individual or system are incompatible with the aims and/or outcomes of the innovation.

The magnitude of complexity involved in adoption, use, and maintenance of the innovation will dictate the diffusion of an innovation to individuals, groups and systems. An innovation that is difficult to understand or cumbersome to implement and use will require more time to diffuse (p. 16).

The concept of trialability is important to the adoption of an innovation. Rogers (p. 16) clarifies this concept. Diffusion of the innovation may proceed at an increased rate if an innovation can be broken down into trial phases, samples, or smaller parts.
Trialability of an innovation may mean the difference between accepting versus complete rejection of an innovation.

Finally, the observability of an innovation in action serves to improve its diffusion (p. 16). The decision to adopt an innovation improves when an individual or system can observe the affect and effects of an innovation. Observability prompts discussion, debate, and improved communication about the innovation and its merits. Observability and word-of-mouth effect change.

Application of Theory: Innovative Implementation

So how do we apply the diffusion of innovation theory to the integration of clinical practice guidelines and recommendations at the fourth point on the ACE Star Model?

a. The provider or practice must perceive a relative advantage for adoption of the guideline within the boundaries of cost, time, and clinical outcomes.

b. The guideline must be compatible with the population and setting.

c. The complexity for guideline implementation must be tolerable in terms of provider and staff understanding and ease of use.

d. Specific guideline implementation may require a trial period or piloting for a given location.

e. Observation of ongoing use of the guideline in a specific population or cohort should demonstrate positive outcomes and feedback to those considering implementation.
In consideration of these concepts, the innovative integration of the ATS guideline with pulmonary function testing achieves these goals. The use of the ATS guideline in the PFT lab setting promotes a relative advantage to the pulmonary practice by the consistent pursuit of guideline use at no direct cost to the patient (if no-cost DBS test kits are utilized) and reduces interruption to patient flow during clinic hours induced by frequent requests by the provider to staff for spontaneous sporadic AATD testing. The protocol is compatible with the population and setting. The use of the ATS guideline in the PFT lab should simplify AATD testing as well as reduce the complexity of guideline compliance. The protocol allows a trial period for evaluation. Finally, the protocol provides a means for observation of outcomes for providers.

Conclusion

A review of the Institute of Medicine mandate for evidence-based practice provides a basis for implementation of clinical guidelines. The ACE Star Model describes the stages of knowledge transformation from research to summarization of research findings to the evolution of this knowledge and its packaging into clinical guidelines to the clinical implementation of guidelines and the subsequent evaluation of guideline implementation. As discussed, the fourth point in the ACE Star Model, implementation of guidelines, can be difficult to achieve for reasons cited, and the theoretical model known as Diffusion of Innovations helps explain this difficulty as well as offer insight into ways to overcome these barriers to change. These models illustrate how an innovative strategy to guideline implementation can help achieve success in applying a guideline in the clinical setting.
CHAPTER THREE: METHODS

Introduction

The purpose of this retrospective descriptive project was to describe and evaluate the outcomes of implementation of the ATS AATD guideline in the setting of the pulmonary function testing lab, primarily to describe the prevalence of AATD cases and carriers in the targeted population, to examine how the established clinical guideline promoted accurate patient selection for the AATD test in the sample, and secondarily to determine whether alpha-1 antitrypsin blood levels are lower in those that smoke compared to former or never smokers.

In the clinical setting, options for AATD testing require the provider to identify patients that meet CPG criteria for testing, and to either (1) draw blood during the office visit and send it out for testing, (2) order the test be done in an outside lab, or (3) use one of the no-cost test kits for AATD to take a dried blood spot (DBS) sample during an office visit. Taking any blood sample for AATD during an office visit, and doing this repeatedly through the course of the clinic day is a time-consuming process that slows patient flow by keeping the exam room tied up and requires additional medical assistant time to complete. These two time factors may be at odds with the provider’s need to keep patient flow moving.

During the sample period, Table 11 from the ATS CPG was utilized at each PFT session to guide patient selection for collection of a dried blood spot (DBS) specimen for the AATD test.
Selection for AATD testing should be improved in this setting because specific AATD testing indicators evolve (e.g. non-reversible obstruction to flow is demonstrated upon spirometric testing) and are identified during the course of the PFT session. In addition, since a finger-stick blood specimen for hemoglobin is routinely taken for diffusion studies during the PFT session, this is the logical opportunity to collect a DBS specimen for AATD testing. No-cost test kits are available that use the DBS specimen collection process. These kits are simple and convenient for blood sampling in the PFT lab. This opportunity for specimen collection should reduce the need to interrupt office visits and slowed patient flow for the additional procedural burden that AATD testing can represent during office hours. These no-cost test kits were used, during the period reviewed for this project, to collect dried blood spot specimens. Table 11 from the ATS CGP (p 893) was in use throughout the 18-month sample period in the PFT lab to guide patient selection.

Study Design

This descriptive project was a retrospective review of data obtained from records spanning an 18-month period.

Stated Aims

Specific aims were to (1) describe the prevalence of AATD cases and carriers in the sample, (2) examine to what degree the established clinical guideline promoted accurate patient selection for the alpha-1 test in the sample, and (3) determine whether alpha-1 antitrypsin blood levels are lower in those that smoke compared to former or never smokers.
Hypotheses

(1) Testing 300 individuals will yield case finding of at least 27 individuals with carrier or AATD status, (2) The table format as published for established clinical guidelines promotes correct patient selection for the alpha-1 test in the PFT lab in a pulmonary office-based setting, and (3) Blood levels of alpha-1 antitrypsin are lower for current smokers compared to never, or ever smokers in the population evaluated.

Setting

The setting for this project was a pulmonary function laboratory in a private metropolitan pulmonary medicine practice.

Inclusion Criteria

Inclusion criterion was solely patients 21 to 79 years of age who completed PFT's.

Sampling Plan

Medical records of patients with completed pulmonary function testing were located by the presence of a red sticker on the chart edge. Chart review took place during non-business hours. The PI was the sole reviewer. Chart data were reviewed only for the specified variables. No element of the chart was photocopied. The PI input data to the statistical software data sheet. No personal identifying information was collected, and no linking data was isolated or kept. Once data was collected from the chart, a small mark was made across the red sticker to indicate data collection was complete to avoid data duplication, and the chart was re-filed. The database was maintained on a laptop computer. The computer is stored inside a locked, fireproof safe in Phoenix Arizona to protect the database and maintain confidentiality.
Sample

The convenience sample included all patients completing pulmonary function testing during the 18-month period of March 15, 2008 through September 14, 2009. After excluding 31 cases by age criteria, and 5 cases for extensive missing data, the result was a total sample size of n=521. The principle investigator retained two cases in the data set even though blood level data was missing. Although blood level data was missing, these two cases were indeed tested and a genotype recorded. As a result, the two cases were retained for inclusion in prevalence counts.

Measures

Specific variables collected for each case included age; gender; pre-existing diagnosis of emphysema, chronic bronchitis, COPD, bronchiectasis, or asthma; FEV1 pre bronchodilator; FEV1%, FEV1 post bronchodilator; post bronchodilator FEV1 change in milliliters; FEV1/FVC ratio; respiratory symptoms of cough, dyspnea, recurrent infection, or asymptomatic; alpha-1 tested yes, or no; alpha-1 antitrypsin genotype MM, MS,MZ, SS, SZ, or ZZ; alpha-1 antitrypsin blood level; smoking status as current, ever, never; and pack years tobacco use.

AIM #1 was evaluated with frequency counts by genotype detected in the sample. These counts were compared to prevalence rates reported in the general population as well as those reported for the pulmonary population.

Aim #2 was examined to critique the utility of Table 11 of the ATS CPG for promoting accurate patient selection for the AATD test by evaluating case summary
results in SPSS to determine what fraction of patients were correctly tested for AATD based upon the presence of, or incorrectly tested for AATD based upon the absence of the following criteria:

1. Symptomatic adults with a pre-existing diagnosis of emphysema, chronic bronchitis, or COPD.
2. The pre-existing diagnosis of bronchiectasis of unknown etiology.
3. The pre-existing diagnosis of asthma with persistent obstruction and less than 200 ml post SABA reversibility.
4. A history of tobacco exposure and FEV1/FVC ratio 70% predicted or lower and asymptomatic for dyspnea, SOB, cough, and sputum production.

In addition, the fraction of patients correctly NOT tested for AATD or incorrectly NOT tested for AATD based upon these same criteria was determined.

Aim #3 was evaluated with analysis of variance (ANOVA) to determine if any significant difference exists in alpha-1 antitrypsin blood levels in current smokers compared to ever or never smokers.

Procedures

The Innovation: Testing for AATD in the PFT Lab

Testing for AATD in this PFT lab was implemented in March 2008 (APPENDIX C) and continued using the recommendations as published in ATS guideline Table 11 (APPENDIX D) and summarized in Figure 8.
Selection of Patients for Collection of a DBS Specimen

During the PFT appointment, the pulmonary function technician reviews the patient chart, and based upon the diagnosis in the chart and the FEV1 and FVC measures obtained during spirometry (Figure 8.), the technician makes a decision concerning whether the patient meets criteria for collecting a dried blood spot specimen for AATD testing or not. Every pulmonary function testing session generates the requirement for a log sheet entry, and the decision to collect or not collect the DBS specimen is recorded. If the decision is made not to collect a DBS specimen, the reason for this decision is recorded (APPENDIX G).

Identified patients meeting AATD testing criteria are provided an informative brochure regarding AATD, and are offered the AATD test. After consenting (APPENDIX E), the patient receives testing at no cost using a DBS test kit. This kit is provided by Talecris Biotherapeutics, Inc, Research Triangle Park, North Carolina 27709 USA for the alpha-1 antitrypsin testing program administered by The University of

<table>
<thead>
<tr>
<th>Symptomatic adults with persistent obstruction and</th>
</tr>
</thead>
<tbody>
<tr>
<td>o Emphysema</td>
</tr>
<tr>
<td>o Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>o Asthma with airflow obstruction that is incompletely reversible</td>
</tr>
</tbody>
</table>

| Bronchiectasis without identifiable etiology |
| Asymptomatic individuals with |
| o obstruction on pulmonary function tests with identifiable risk factor such as tobacco use |

Figure 8.

Summary of Criteria PFT Lab for DBS Collection
Florida in its alpha-1 antitrypsin genetics laboratory. There has been no involvement or financial support from Talecris Biotherapeutics Inc. nor the University of Florida genetics laboratory in or for this project.

The pulmonary practice elected to use this particular no-cost test kit due to the simplicity of the required paperwork (APPENDIX E), positive previous experiences with timely test result reporting by the associated testing lab, and previous experiences with two other free test kits that resulted in negative experiences such as more complex, time-consuming paperwork and delayed test results reporting. Note that at any time, a practitioner could order AATD testing for a patient at any community or hospital lab; however, given the availability of the no-cost test kits, they are used at this facility for the pilot study that this innovative guideline implementation represents.

Testing candidates are made to understand that the test is a screening test for a genetic condition that can cause COPD. A dried blood spot specimen is collected once verbal consent is obtained (APPENDIX F).

Collection of Blood Sample in the Clinical Setting

In the pulmonary function lab setting, the timeline does not exceed three hours for collection and processing of the DBS sample utilizing the no-cost test kit (APPENDIX F). The time required for finger stick and application of blood to three filter paper spots is less than five minutes including time to prepare associated paperwork. Once blood is applied to the filter paper, the sample is placed standing on its side in a drying rack for a minimum of two hours. Once drying time is complete, the specimen is placed in a pre-
addressed envelope and mailed to the lab via standard U.S. mail service. Turnaround time for receipt of results is approximately ten days.

Potential sources of error for sample collection in the clinical setting include errors in specimen labeling and mislabeling, inadequate collection of blood, incorrect application of sample to the filter paper, e.g. incomplete saturation of the three circular specimen areas on the filter paper with blood, incorrect processing of specimens during drying such as allowing specimens to touch other objects, allowing specimens to contact other specimens with accidental comingling of sample, and failure of staff to mail the specimens to the lab for processing. In addition, errors outside of the control of the clinic could include problems with mail transport and delivery to the lab as well as errors on the part of the lab that could involve processing incoming mailed specimens.

Data Collection

Medical records of all patients having presented for pulmonary function testing between March 15, 2008 and September 15, 2009 were reviewed for the variables as described. Data points were recorded for each de-identified individual on a SPSS (Grad Pack Version 15, 2006) spreadsheet (APPENDIX F) beginning with subject number 1 and continued until all records for patients meeting inclusion criteria were reviewed. The principle investigator performed the data collection.

Data Management

Data was input directly into the SPSS spreadsheet without identifying information. After data input to the database, the database was scrutinized to identify any duplication of cases, and none were identified.
Data was examined for missing values. Five cases did have PFT data missing completely, and these cases were removed from the dataset. It is likely that this data was missing due to “chart splitting” in that these charts had been divided into volume #1 and volume #2 etc. due to increasingly large size. The missing PFT data is may be present in an unavailable, stored volume.

Missing ratio data was present for seven of the pre-existing COPD cases, likely due to error in data recording. Two abnormal genotype cases did have blood levels missing and these two cases were retained for preservation of prevalence counts. Two asthma cases did have obstruction but post SABA data was missing as these cases did not have SABA administered or post-SABA spirometry performed. Data related to history of recurrent infection was missing for one bronchiectasis case however this case was retained in the dataset given the low frequency of cases with pre-existing diagnosis of bronchiectasis.

Due to the discovery of missing data, an upgrade to SPSS 18 was completed to capitalize on the feature of missing values analysis (MVA).

Data Analysis

Evaluation of data utilized descriptive statistics. The database was analyzed using SPSS Grad Pack Version 15 (2006). The sample is described with frequencies. Case summaries for frequencies describe the data for Aim 1. Frequencies in Case Summaries were scrutinized for Aim 2. Analysis of variance (ANOVA) was utilized to detect any potential differences between groups for serum alpha-1 antitrypsin levels by Never, Former, and Current smoking status for Aim 3.
Human Subjects Protection

The College of Nursing Departmental Review Committee evaluated and approved the project proposal. Subsequently, the Human Subjects Protection Program of the University reviewed and approved the project proposal.

All data collected was de-identified and no information with identifying attributes toward a specific individual was recorded. Compliance with procedures for protection of human subjects was maintained. No adverse or unexpected events were encountered that mandated reporting for compliance with the University procedures for protection of human subjects.

Materials and Data Security

Throughout all project phases, the database was stored in a locked, fireproof safe at the residence of the researcher when not in active use.

Conclusion

The methods for this project have been discussed and described. Data was gathered and recorded as proposed. The database was reviewed for duplicate and missing cases. The dataset was maintained in a secure location as described. The data was analyzed in SPSS and case summaries provided counts for evaluation of Aim #1 and Aim#2. Data was analyzed by ANOVA for Aim#3 and the Levene Test for homogeneity of variance detected no significance though the group sizes were not equal.
CHAPTER FOUR: RESULTS

Introduction

Data was gathered and recorded into SPSS as proposed. Variable frequencies are displayed in Table 9.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n)</th>
<th>Mean (M)</th>
<th>Standard Deviation (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Range 22-79 yrs, M=62.24 yrs, SD 12.69)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (Male n=244, Female n=277)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-existing diagnosis of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma (n=59)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emphysema (n=35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Bronchitis (n=1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD (n=103)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchiectasis (n=18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (n=480, M=72.0, SD=21.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post bronchodilator FEV1 change in ml (n=471 M= -0.09, SD=0.26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC Ratio (n=486 M=69.10 liters, SD=15.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough (Yes n=285) or Dyspnea (Yes n=435)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent Infection (Yes n=27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic (Yes n=37, No n=483)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-1 Tested (Yes n=190, No n=331)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-1 Antitrypsin Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS n=13</td>
<td>MM n=167</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ n=9</td>
<td>SS n=0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ n=1</td>
<td>ZZ n=1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-1 Antitrypsin Blood Levels (n=22, M=26.01, SD=8.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Status as Current (n=35)</td>
<td>Ever (n=303)</td>
<td>Never (n=178)</td>
<td></td>
</tr>
<tr>
<td>Smoking Status as Current (n=35)</td>
<td>Ever (n=303)</td>
<td>Never (n=178)</td>
<td></td>
</tr>
<tr>
<td>Pack Years Tobacco Use (n=340, Range 0-220, Mode 40, M= 37.71, SD=29.69)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 9. Variables and Outcome Frequencies
Aim #1

Aim #1 sought to describe case finding through targeted testing. Table 11 from the CPG was used to guide patient selection. Only 63% or 190 of the hypothesized 300 patients to be tested were actually tested.

<table>
<thead>
<tr>
<th>Alpha-1 Antitrypsin</th>
<th>Project</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Population</td>
<td></td>
</tr>
<tr>
<td>MM n=167</td>
<td></td>
<td>1 in 17</td>
</tr>
<tr>
<td>MS n=13</td>
<td>1 in 15</td>
<td>1 in 17</td>
</tr>
<tr>
<td>MZ n=9</td>
<td>1 in 21</td>
<td>1 in 36</td>
</tr>
<tr>
<td>SS n=0</td>
<td>0 in 191</td>
<td>1 in 1058</td>
</tr>
<tr>
<td>SZ n=1</td>
<td>1 in 191</td>
<td>1 in 1124</td>
</tr>
<tr>
<td>ZZ n=1</td>
<td>1 in 191</td>
<td>1 in 4775</td>
</tr>
</tbody>
</table>

Figure 10.

Aim #1 Project Genotype Frequencies Results
Compared with General Population Genotype Frequencies

AIM #2

Aim #2 sought to examine the degree to which the established clinical guidelines promote correct patient testing in the PFT lab in a pulmonary office-based setting for the patients that should be offered testing while providing effective guidance for not testing
those individuals that should not be tested for AATD. It was hypothesized that Table 11, as published for the established clinical guideline promotes correct patient selection for the alpha-1 test in the PFT lab in a pulmonary office-based setting.

Reasons to offer test:

1. Symptomatic adults with a pre-existing diagnosis of COPD, Emphysema, or Bronchiectasis and evidence of persistent obstruction on spirometry (FEV1/FVC Ratio <=70%).

2. Pre-existing diagnosis of Bronchiectasis with no known etiology.

3. Symptomatic adults with a pre-existing diagnosis of Asthma with evidence of persistent obstruction and FEV1 post-SABA improved < 200 ml.

4. No pre-existing diagnosis COPD, Emphysema, Chronic Bronchitis, Bronchiectasis, or Asthma; asymptomatic for cough, dyspnea, sputum production; persistent obstruction on spirometry; and a history of smoking exposure.

Counts were performed to evaluate the data for Aim #3 relative to the reasons to offer the test as outlined above, and the results were as follows:

**COPD:**

Of the 103 patients with a pre-existing diagnosis of COPD, 79 had a FEV1/FVC ratio below 70%. Overall, the mean ratio for those with pre-existing COPD was 52.11%. The pooled mean ratio for those with pre-existing COPD calculated after imputation of missing values was 51.43 and still below 70%, the commonly recognized value for an obstructive defect. Of the 79 individuals with documented obstruction, 40 were correctly tested and 39 were incorrectly not tested. Of the 103 persons with a pre-existing
diagnosis of COPD, 17 had a FEV1/FVC ratio above 70% and six of these were correctly not tested while 11 were incorrectly tested when they should not have been. Missing ratio data was present for seven of the pre-existing COPD cases likely due to error in recording data. Of these seven, six were tested for AATD, and one was not. Overall, it is estimated (taking into account missing ratio data for seven cases) that 45% of those with a pre-existing diagnosis of COPD were correctly tested based upon data that was available.

**Emphysema:**

Of the 35 individuals with a pre-existing diagnosis of emphysema, 25 did have documented persistent obstruction. Overall, the mean ratio for those with pre-existing emphysema was 49.58%. The pooled mean ratio for those with pre-existing emphysema calculated after imputation of missing values was 57.80% and still below 70%, the commonly recognized value for an obstructive defect. Sixteen of these test candidates were correctly tested, while nine were incorrectly not tested. Eight emphysema cases were identified that did not demonstrate persistent obstruction. Five of these cases were incorrectly tested and should not have been tested, based upon the ATS CPG while three were correctly not tested. The missing ratio data for two emphysema cases were likely due to an error in recording data. Overall, it is estimated (missing ratio data for two cases) that 54% of those with a preexisting diagnosis of emphysema were correctly tested.

**Chronic Bronchitis:**

One patient in the entire sample carried a pre-existing diagnosis of chronic bronchitis and did indeed have documented persistent obstruction. This individual was
incorrectly not tested. As a result, zero percent of test candidates with Chronic Bronchitis were tested.

**Bronchiectasis:**

Of the 18 individuals with a pre-existing diagnosis of bronchiectasis, 10 were not tested. Seven of these 10 did have an obstructive ratio below 70% and three did not. Three of these did not have a history of recurrent infection, which could be an etiology for their bronchiectasis. As a result, three of these ten should have been tested but were not. One of the 18 was missing ratio data for unknown reasons. Seven of the 18 with a pre-existing diagnosis of bronchiectasis were tested for AATD. Four of these had a ratio below 70%, one has data missing regarding recurrent infection as this information could not be gleaned from the chart, and two had no history of recurrent infection. Overall, for those with a pre-existing diagnosis for bronchiectasis with persistent obstruction and without evident etiology, patient selection for the AATD test by use of Table 11 for this group was inconsistent.

**Asthma:**

Overall, the mean ratio for those with pre-existing asthma was 68.40% and the mean FEV1 was 71.09%. The pooled mean ratio for those with pre-existing asthma calculated after imputation of missing values was 68.41% and the pooled mean FEV1 was 71.08, virtually unchanged. Of the 59 individuals with a pre-existing diagnosis of Asthma, 32 did have documented persistent obstruction as well as less than 200 ml post SABA change in FEV1. Seventeen of these test candidates were correctly tested while 15 were incorrectly not tested. Two cases did have obstruction but post SABA data was
missing. The post SABA data was missing because these two cases did not have post-bronchodilator testing performed. Sixteen of the 59 individuals with a pre-existing diagnosis of asthma were correctly not tested based upon a lack of persistent obstruction. Nine were incorrectly tested, as obstruction was absent. Overall, 56% of those with pre-existing Asthma were correctly tested.

**No Pre-existing Diagnosis of Pulmonary Disease, Asymptomatic, Documented Persistent Obstruction, and Smoking Exposure:**

Six individuals with no prior diagnosis of pulmonary disease were actual test candidates by virtue of their being asymptomatic for cough and dyspnea, having documented evidence of persistent obstruction, and a history of prior smoking exposure. Of these six test candidates, three were correctly tested for AATD, while three were incorrectly not tested.

**Aim #3**

Aim #3 sought to evaluate the relationship of blood Alpha-1 Antitrypsin levels with smoking status. Twenty-two blood levels were reported for the 190 cases tested. Twenty-four cases and carriers were detected during the reviewed period. Blood level data was missing for two cases as the report from the testing lab was missing from these two charts, however, the genotype was recorded on the daily AATD testing log sheet for these two cases. Figure 11 details the mean blood level by genotype and smoking status.
In the location where this review was performed, the testing lab only reports blood levels for cases and carriers. No blood level is reported by the testing lab for those found to carry the MM genotype. Of the 190 tested, 167 were of the MM genotype, and for the MM genotype, no blood level data is available.

Consistent with Aim #3, the 22 reported blood levels were examined via analysis of variance (ANOVA) with reference to smoking status for never, ever, or current cigarette smoking. Group sizes were unequal. The Levene Statistic for homogeneity of variance was insignificant (Figure 12).
The reported blood levels of alpha-1 antitrypsin were not significantly lower in current smokers. Blood levels by ever, never, or current smoking status $F(2,19)=0.433, p=.655$, did not differ significantly (Figure 13).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLOOD LEVEL * SMOKE EVER/NEVER/CURRENT Between Groups</td>
<td>60.600</td>
<td>2</td>
<td>30.300</td>
<td>.433</td>
<td>.655</td>
</tr>
<tr>
<td>Within Groups</td>
<td>1328.131</td>
<td>19</td>
<td>69.902</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1388.730</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 13. Aim #2. ANOVA Blood Level by Smoking Status

Conclusion

The College of Nursing departmental review committee as well as the University Human Subject Protection Program committee approved this descriptive retrospective review in advance.

Records were reviewed and confidentiality was maintained as per the proposal. At the conclusion of data collection, the total sample size was $n=521$. Of the total sample, 190 individuals were tested for AATD. Of these patients tested, 24 individuals were found to be carriers or cases.
No difference in blood levels of AAT were detected between groups of ever, never, or current tobacco smokers based upon available data as reported for 22 of the 24 incident carriers and cases.

The use of Table 11 taken directly from the ATS CPG resulted in less than ideal accuracy in terms of structured selection for testing as well as promoting discrimination for not testing a given case.
CHAPTER FIVE: IMPLICATIONS AND RECOMMENDATIONS

Introduction

The Doctor of Nursing Practice (DNP) is specifically prepared to implement the fourth and fifth points in the ACE Star Model. Testing evidence-based clinical guidelines through their implementation is one role for the DNP and represents the fourth point in the ACE Star model as research transitions from discovery data to a more formatted structure. Evaluation of the outcomes of guideline implementation, as represented by this retrospective review embodies the fifth point in the ACE Star model. The competence of the DNP in the area of translational practice allows for assessment, diagnosis, planning, intervention, and evaluation, at the advanced level but still within the construct of the nursing process. Through this project, the nursing process moves again forward from implementation and evaluation, to transition back through the discovery, summary, and translation points in the ACE Star model. The assessment and diagnosis of areas for improvement in the ATS clinical guideline, by evaluation for potential clarification and refinement in established guidelines, and to planning recommendations for guideline modification are the outcomes of this project.

This project was a descriptive review and outcomes evaluation of the ATS CPG for alpha-1 antitrypsin testing in the novel setting of the pulmonary function lab in a metropolitan pulmonary medicine practice. The targeted testing of pulmonary patients as they complete pulmonary function testing is unusual and not previously described in the literature.
The implementation of a guideline in clinical practice can be a challenge. The theoretical change model known as “Diffusion of Innovations” as described by Rogers (2003) helps us understand obstacles encountered in practice when modifications to the status quo are proposed and the theory offers insight into ways to overcome those barriers. The change model is useful in the context of promoting clinical guideline use and finding ways to apply the principles of the guideline.

Project Strengths and Limitations

The large overall sample size strengthened this project, as did the pioneering setting that was evaluated, however the small sub-sample size for Aim 3 was a limitation as was the retrospective design. The presence of some missing values increased risk for biased results.

Implication of the Results

Aim #1 intended to evaluate whether the alpha-1 antitrypsin cases and carriers discovered during the sample period were similar to the prevalence cited in the literature. Targeted testing in the PFT lab, using Table 11 from the ATS CPG for guidance yielded 190 individuals actually tested for AATD.

Based upon the 190 actually tested, if testing were performed in the general population, we would expect 17 individuals to be identified as carriers or cases. However, in this study of targeted testing in a population of pulmonary patients, 24 individuals were identified as carriers or cases.
The higher prevalence of abnormal genotypes MS, MZ, SZ, and ZZ in this targeted testing sample, compared to the described expected prevalence for testing the general population are shown in Figure 14 (de Serres et al, 2003). The only exception for this difference was for the PiSS genotype.

If we look at this sample of pulmonary patients in terms of the Z allele, six percent (6%) indeed carried at least one z allele. In the seven studies for AATD in populations of “COPD,” or “emphysema,” or “pulmonary patients” cited by the ATS, the prevalence for the presence of the z allele reportedly ranged between 5% and 10% in those populations studied (ATS, 2003, p 842). The seven studies referenced in the ATS report occurred between 1969 and 1986. The prevalence of the Z allele detected during the 18 months of targeted testing evaluated by this project, with reference to prevalence

<table>
<thead>
<tr>
<th>Incident Genotype</th>
<th>Project</th>
<th>General Population Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS n=13</td>
<td>1 in 15</td>
<td>1 in 17</td>
</tr>
<tr>
<td>MZ n=9</td>
<td>1 in 21</td>
<td>1 in 36</td>
</tr>
<tr>
<td>SS n=0</td>
<td>0 in 191</td>
<td>1 in 1058</td>
</tr>
<tr>
<td>SZ n=1</td>
<td>1 in 191</td>
<td>1 in 1124</td>
</tr>
<tr>
<td>ZZ n=1</td>
<td>1 in 191</td>
<td>1 in 4775</td>
</tr>
</tbody>
</table>

Figure 14.
Project Targeted Testing Versus General Population
rates of the Z allele, is similar to the reported frequency for the Z allele in the seven prior studies reported in the ATS CPG for pulmonary patients.

Since 2003, various published studies provide support for targeted testing among pulmonary patients, as does this project. Increased odds ratios for the alpha-1 antitrypsin z allele in Caucasian COPD patients, as well as evidence for the cost effectiveness of targeted testing have been cited. No published reports, however, have been found specifically referring the use of the ATS CPG for targeted testing in the setting of the pulmonary function lab. As a result, this project evaluated ATS guideline use in a unique setting and did find an increased prevalence in that setting.

Aim #2 related specifically to the usefulness of Table 11 taken directly from the ATS CPG as a tool to be used for guidance in the PFT lab for AATD testing. This tool was in use during the 18-month period under review and was less than ideal for promoting consistency in testing for AATD or for its ability to aid in discrimination for not testing a given patient when not indicated.

The significance of the relative ineffectiveness of Table 11 as a tool for promoting structured AATD testing in the PFT lab setting is important in two ways. First, in spite of the ineffectiveness of Table 11 as a tool, testing for AATD in the setting of the PFT lab did uncover prevalent abnormal genotypes at higher rate than that for the general population. As a result of this increased rate of case finding even in the face of an ineffective selection tool, one could theorize that if an effective ATS CPG-based tool was indeed used in the PFT lab to guide patient selection for AATD testing, an improved rate
of case-finding, above and beyond that found in this study, may result. Second, the need for a refined tool to assist in application of the ATS CPG has been highlighted.

The exact reasons for the ineffectiveness of Table 11, as found during this study, are unclear, but it is hypothesized that the Table format is not “user-friendly.” The Table is part of the “guideline.” Guidelines, by definition, are more general, and less specific than “protocols” which are meant to be more structured, provide systematic guidance, and should achieve the same result by all users. The setting of the PFT lab, if useful for furthering targeted AATD testing, requires the availability of a tool with a standardized and structured format to guide patient selection for AATD testing.

Again, the 2001 IOM report encourages us to remember that we should be “providing services based on scientific knowledge to all who could benefit, and refraining from providing services to those not likely to benefit” (p. 3). In this context, a structured decision-tree to aid in patient selection of AATD testing would improve the process in implementing the ATS CPG in the PFT lab.

Aim #3 sought to examine whether a difference exists between groups, in blood levels of current versus never, or former tobacco smokers. The sample size was underpowered, and the underlying hypothesis for Aim #3 needs further exploration.

Implications for Practice

Targeted testing of patients in the pulmonary patient setting, based upon ATS CPG criteria is supported by this project and others (Bals, et al., 2007; Brantly & Brantly, 2006; de la Roza, et al., 2005; de Serres, Blanco, & Fernandez-Bustillo, 2006; Hogarth & Rachelefsky, 2008; K. M. Shermock, T. R. Gildea, M. Singer, & J. K. Stoller, 2005).
Targeted testing promotes objectivity, and the use of the clinical practice guideline should promote accuracy in selection of patients for AATD testing.

Any patient found to have documented significant emphysema onset prior to age 40, or the person with documented evidence of emphysema more dominant in lower lobes both merit testing for alpha-1 antitrypsin blood level.

A blood level for circulating alpha-1 antitrypsin is recommended as the first step with consideration for genotyping if the blood level is found to be below the lower limit of normal range (Snyder et al, 2006, Figure 5; Ferrarotti et al, 2007, Figure 6).

It should be recognized that the no-cost Talecris test kits and administration of actual lab processing by the University of Florida Genetic Lab (UFGL) of the DBS sample that were in use during the period reviewed for this project actually tested genotype first and tested blood level only if an abnormal genotype was detected. This is not the usual recommended order of biomethods processes for testing for AATD and represents the preferences of the University of Florida Genetic Lab.

Finally, AATD screening not indicated in a general population such as primary care clinics unless an individual presents with pulmonary complaints and documented findings suggestive of early onset emphysema. The testing of smokers with normal spirometry is not indicated.

The finding of a low alpha-1 antitrypsin blood level, or abnormal genotype, comes with implications for clinician practice. If the blood level is normal, there is no need for genotyping unless there is suspicion based upon family history for an abnormal genotype. Depending upon the lab processing the blood sample, blood level alone, or
blood level in combination with genotyping may be performed. In the situation of a family history positive for heterozygous genotype or concern for homozygous state, blood level and genotyping should be performed as the results may have implications for family members.

If a low blood alpha-1 antitrypsin level is found, the blood level should be repeated and genotyping should be performed. If a blood level of 11 micromoles or less is confirmed, and a PiZZ genotype is found, the patient is a candidate for augmentation therapy and the family should be tested. If a low blood level is found and confirmed, but a normal genotype (PiMM) is reported and confirmed (discordant results), specialized testing for rare genotype should be completed as there are indeed several exceedingly rare deficiency sub-genotypes that report as PiMM on usual genotyping (see Figure 6 on page 54). In addition, it must be remembered that the various biomethods each have certain sensitivities and specificities such as PCR genotyping specificity for the S and Z alleles, and the inability of isoelectric focusing (aka “phenotyping”) to detect the Null allele (Brantly, 2006, Snyder et al 2006).

As research regarding the physiology and pathophysiology, involving alpha-1 antitrypsin evolves as we move forward, additional implications may continue to unfold. As an example, Yang et al (2008) performed a dual case-control study finding a significantly increased odds ratio for lung cancer in alpha-1 antitrypsin heterozygotes. If these findings were confirmed, this would represent a significant set of implications for heterozygotes. The concept of conformational change in the z alpha-1 antitrypsin protein molecule, long felt to be implicated in damage to the hepatocyte, may have implications
in lung tissue as well. Polymerized alpha-1 antitrypsin has been reported to have been found in and possibly produced by lung cells, appears to act as an attractant for neutrophils, and if confirmed, this situation would have implications related to the effectiveness of augmentation therapy given the potent ongoing inflammatory effect of local polymerized alpha-1 antitrypsin (Mulgrew et al, 2004).

The targeted testing for AATD in a pulmonary medicine office-based PFT lab as reviewed during this project detected AATD carriers and cases with prevalence greater than that expected in the general population even with the use of an ineffective tool. This increased rate of detection indicates that testing for AATD during pulmonary function testing may be an ideal time and place for the use of the ATS alpha-1 antitrypsin CPG. One method to improve targeted testing and the clarity of the ATS AAT CPG in the setting of the PFT lab may be to implement the guideline testing criteria in a structured protocol, flowchart, or decision-tree format.

AATD Decision-Tree Tool for the PFT Lab

A decision-tree tool (Figure 15) was developed based upon ATS CPG diagnostic testing criteria to demonstrate a flowchart or protocol format of guideline recommendations that may be useful to improve clarity of the guideline and promote structured consistency in test candidate selection in the PFT lab setting. Slight modification was made to clarify the CPG recommendations as the terms “persistent obstructive defects,” and “symptomatic” needed clarification to fit into a decision-tree model. The term “obstructive defect” was modified by clarification to a FEV1/FVC ratio
of 70% or below. The term “symptomatic” is meant to indicate cough, dyspnea, or sputum production.

Figure 15. Proposed AATD Test Decision Tree for the PFT Lab
This decision-tree tool begins with the basic question to the user: Is the patient symptomatic and does the patient have persistent obstruction to airflow as evidenced by a FEV1/FVC ratio of 70% or below and a diagnosis of emphysema, chronic bronchitis, or COPD? If so, the decision to offer the AATD test is positive, as this is a type “A” recommendation in the guideline. If the answer is no, the next question is posed: Does the patient have a diagnosis of bronchiectasis? If the answer is yes, and if the patient has no known etiology for his or her bronchiectasis, this indicates a type “A” recommendation. The decision to offer the AATD test in the PFT lab is positive since AATD may be the unknown cause.

If the patient does have a known etiology for the bronchiectasis, this indicates a type “B” recommendation and the decision is made to defer the consideration for AATD testing back to the pulmonary healthcare provider at follow-up.

If the answer is no to a FEV1/FVC ratio of 70% or below with a pre-existing diagnosis of symptomatic emphysema, chronic bronchitis, or COPD, and there is no preexisting diagnosis of bronchiectasis, the next question becomes: Is the patient symptomatic and does the patient have a pre-existing diagnosis of asthma? If the answer is yes, the next question is whether the ratio is 70% or lower indicating persistent obstruction. If yes, the next question is whether there is post short acting beta-agonist (SABA) improvement in the FEV1 of 200 milliliters (ml) or greater. This question is conveniently answered at the time of the pre-post SABA spirometry done during pulmonary function testing. If yes, the patient does not need AATD testing because a 200 ml improvement post SABA indicates reversible obstruction and the guideline assigns a
“C” recommendation that testing is not indicated in this situation. If no improvement greater than 200 ml is seen in the FEV1 post SABA, the decision to offer the AATD test is positive and a type “A” recommendation. If the patient does have a pre-existing diagnosis of asthma, and the ratio is not 70% or lower, the decision is not to offer the patient AATD testing because he or she does not exhibit persistent obstruction. If the answer is no to a FEV1/FVC ratio of 70% or below and a pre-existing diagnosis of emphysema, chronic bronchitis, or COPD, and there is no preexisting diagnosis of bronchiectasis, and no pre-existing diagnosis of asthma, the next question becomes, Does the patient have symptoms of pulmonary disease such as dyspnea, cough, or sputum production? If the answer is yes, the decision whether the patient should have the AATD test would be referred back to the pulmonary healthcare provider, but if the answer is no and the patient is asymptomatic, and the patient does have a FEV1/FVC ratio 70% or below and a smoking exposure history, he or she should be offered the AATD test as this case represents a type “A” recommendation. If the patient does have a FEV1/FVC ratio 70% or below but no history of smoking exposure, the decision to test is referred back to the pulmonary healthcare provider as this represents a type “B” recommendation. If the answer is no to a FEV1/FVC ratio of 70% or below with a pre-existing diagnosis of emphysema, chronic bronchitis, or COPD, and there is no preexisting diagnosis of bronchiectasis, and no pre-existing diagnosis of asthma, and the patient does not have respiratory symptoms and the FEV1/FVC ratio is not 70% or below, the patient is not a candidate for the AATD test.
The results of Aim 1 and Aim 2 of this project indicate that case finding for AATD may be improved through the use of targeted testing in the setting of the pulmonary function lab. A decision-tree tool to assist in application of guideline recommendations should provide a more structured mechanism to promote consistency with the guideline in the selection of patients for alpha-1 antitrypsin testing in the PFT lab. It is recommended that this decision-tree be integrated into the current PFT lab processes at the pulmonary medicine PFT lab where this project was performed and an evaluation of the outcomes of its use be repeated in the future.

Conclusion

This project reflects the role of the DNP as related to the integration of evidence in practice. This evaluation of the use of the ATS CPG in a novel, but relevant setting highlights the importance of targeted testing for AATD as well as the need for a structured tool to assist in the refinement of targeted testing in this setting. In addition, our understanding of the complex pathophysiology related to the natural history of pulmonary emphysema may be improved by evaluation of differences in blood alpha-1 antitrypsin levels by smoking status through the inclusion of all alpha-1 antitrypsin genotypes.
APPENDIX A:

HUMAN SUBJECTS PROTECTION PROGRAM APPROVAL
**HSPP Correspondence Form**

**Date:** 01/06/10  
**Investigator:** Priscilla Torres Steffen, Student  
**Advisor:** Sally Reed, PhD  
**Project No./Title:** 09-1099 Clinical Practice Guideline Implementation for Alpha-1 Antitrypsin Deficiency  
**Testing:** Evaluation of an Innovative Method  
**Current Period of Approval:** NA  

<table>
<thead>
<tr>
<th>IRB Committee Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>☑ Administrative Action</td>
</tr>
<tr>
<td>FWA Number: FWA00004218</td>
</tr>
</tbody>
</table>

**Nature of Submission**

| ☑ New Project |  

**Documents**

| Reviewed Concurrently | Appr: Approved  
|-----------------------| Akr: Acknowledged  
| Rev: Reviewed |  

| ☑ Project Review Form (received 11/24/09) | Appr |  

**Determination**

| ☑ Not Human Subjects Research |  

**Additional Determination(s)**

- **Not Human Subjects Research as defined by 45 CFR 46.102(f):** As presented, the activities described above do not meet the definition of research involving human subjects as cited in the regulations issued by the U.S. Department of Health and Human Services which state that “human subject means a living individual about whom an investigator (whether professional or student) conducting research obtains data through intervention or interaction with the individual, or identifiable private information.”

---

*Elizabeth A. Boyd*  
01/06/10  
Assistant Vice-President, Research Compliance & Policy  
Office for the Responsible Conduct of Research

EAB:mm  
Cc: Departmental/College Review Committee

---

**Reminders:** Continuing Review materials should be submitted 30–45 days prior to the expiration date to obtain project re-approval  
- Projects may be concluded or withdrawn at any time using the forms available at .  
- No changes to a project may be made prior to IRB approval except to eliminate apparent immediate hazard to subjects.  
- Original signed consent forms must be stored in the designated departmental location determined by the Department Head.
APPENDIX B:

SITE AUTHORIZATION LETTER
October 15, 2009

Re: Priscilla Torres Steffen

To Whom It May Concern:

Priscilla Torres Steffen is a doctoral student at the University of Arizona. She has the permission of the practice (Arizona Pulmonary Specialists, Ltd.) to perform a retrospective chart review of data from pulmonary function test patient records at the Scottsdale office for the time frame of April 15, 2008 through September 14th 2009. Please contact me if you require any further information.

Sincerely,

Vicki Berk Farmer
Practice Administrator
APPENDIX C:

PULMONARY FUNCTION LAB PROCEDURE
Pulmonary Function Testing Procedure

1. Patient will be instructed to arrive 30 minutes prior to scheduled testing in order to complete paperwork.

2. Upon arrival, patient will complete the Pulmonary Functional Status & Dyspnea Questionnaire-Modified (PFSDQ-M).

3. Place completed PFSDQ-M in the chart

4. Place red “dot” on chart so that chart is flagged so that staff can easily identify charts of patients having completed pulmonary function testing. Place red “dot” on all charts of patients completing pulmonary function testing.

5. Complete pulmonary function testing as ordered by licensed health care provider

6. Perform Alpha-1 Anti Trypsin Deficiency (AATD) screening using University of Florida test kit for all patients meeting ATS guidelines.
   a. Screening is indicated for:
      i. Symptomatic adults with emphysema, COPD, or asthma with airflow obstruction that is incompletely reversible
      ii. Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (eg tobacco smoking or occupational exposure)
      iii. Asymptomatic individuals with persistent airflow obstruction
   b. If patient declines AATD screening, mark the red “dot” on the chart with the letter “N.”
   c. Process sample as per test kit instructions

7. Maintain a roster of patients completing pulmonary function testing by date. Indicate on the roster date AATD screening was performed and date specimen was sent to lab.

---

[Signature]
Office
3/6/08
APPENDIX D:

ATS CPG TABLE 11
TABLE 11. RECOMMENDATIONS FOR GENETIC TESTING FOR ALPHA-1 ANTITRYPSIN DEFICIENCY

<table>
<thead>
<tr>
<th>Type of Genetic Testing</th>
<th>Recommendation</th>
<th>Rationale of the Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Diagnostic Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Symptomatic adults with persistent obstructive defects on pulmonary function testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Emphysema</td>
<td>Type A: Testing is recommended (in populations where the prevalence of AAT deficiency is known to be much lower than the prevalence in North America or Europe [e.g., &lt; 1/3,000], a Type B recommendation is made.)</td>
<td>Prevalence of AAT deficiency in individuals with emphysema is not insignificant, and observational studies suggest efficacy of augmentation therapy in such patients. In addition, preventive measures can be employed (e.g., smoking cessation and change of occupation). It is not known, however, if providing knowledge of having a genetic disease can influence smoking quit rates. Beneficial psychological effects may also be gained from testing, due mainly from having an explanation of the disease process. Finally, a diagnosis of AAT deficiency can have economic benefits, as a diagnosis of AAT deficiency can lead to further diagnostic testing for other diseases. These benefits must likely outweigh potential adverse social discriminatory effects.</td>
</tr>
<tr>
<td>b. COPD</td>
<td>Type A: Testing is recommended Type B: Testing should be discussed</td>
<td>Same as above.</td>
</tr>
<tr>
<td>c. Bronchiectasis</td>
<td>Type A: Testing is recommended Type B: Testing should be discussed</td>
<td>In the context of discordant studies about whether bronchiectasis is clearly associated with AAT deficiency, diagnostic testing should be considered because bronchiectasis occurs frequently in individuals with AAT deficiency and because AAT deficiency is clearly underrecognized.</td>
</tr>
<tr>
<td><strong>d. Asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Incompletely reversible airflow obstruction</td>
<td>Type A: Testing is recommended</td>
<td>No evidence that such individuals are more likely to have AAT deficiency.</td>
</tr>
<tr>
<td>ii. Completely reversible airflow obstruction</td>
<td>Type C: Testing is not recommended</td>
<td>Efforts at preventing risky health-related behaviors may be more successful in this age group, for example, efforts at smoking prevention and occupational counseling efforts at a time when adolescents are actively choosing future career opportunities; and adverse psychological effects have not been well established in adolescents. A Type A recommendation is not being made due to (1) low prevalence of persistent obstructive pulmonary disease in adolescents, (2) concern with the future autonomy rights of adolescents, and (3) potential social discriminatory effects.</td>
</tr>
<tr>
<td>2. Adolescents with persistent obstructive pulmonary dysfunction</td>
<td>Type B: Testing should be discussed</td>
<td>Existence of potential adverse psychosocial effects (including individuals identified as being a heterozygote) coupled with low likelihood of any medical benefits obtained from a positive test warrants that testing be merely discussed, but not recommended.</td>
</tr>
<tr>
<td>3. Asymptomatic individuals with persistent obstructive pulmonary dysfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. No risk factors present for promoting AAT deficiency-related lung disease</td>
<td>Type B: Testing should be discussed</td>
<td>A positive test, in conjunction with the efforts of the clinical provider, may lead such individuals to stop smoking. One study, however, showed that although receipt of genetic risk information enhanced motivation to quit smoking, the smoking quit rates were not affected. More research is needed to explore the factors that influence smoking quit rates.</td>
</tr>
<tr>
<td>b. Smoking exposure</td>
<td>Type A: Testing is recommended</td>
<td>A positive test result may influence an individual to change occupation.</td>
</tr>
<tr>
<td>c. Occupational exposure</td>
<td>Type A: Testing is recommended</td>
<td>A positive test result may influence an individual to change occupation.</td>
</tr>
</tbody>
</table>
APPENDIX E:

EXEMPLAR OF CONSENT FORM
**Please fill out completely**

This entire card must be completed for the sample to be processed.
APPENDIX F:

COLLECTION INSTRUCTIONS FOR DBS SPECIMEN
INFORMATION ON FORM must be completed for identification and reporting of results. Use Ballpoint Pen ONLY.

For questions please call 1-866-522-0692

BLOOD COLLECTION INSTRUCTIONS

- Wash hands with warm water and dry thoroughly.
- With Alcohol Prep, clean the tip of the finger and allow to AIR DRY, approximately 12-15 seconds. DO NOT BLOW ON THE FINGER.
- Remove the protective blue tip from the lancet.
- Hold hand palm up. Place tip of lancet slightly off center of the finger tip. Quickly and firmly press the lancet until it "clicks", then release.
- When blood begins to flow, wipe the first drop away with a sterile gauze pad. If blood does not readily flow, apply gentle pressure to the base of the finger and massage toward the tip. The drops should be large and "hanging" before being touched to the center of each circle. DO NOT PRESS THE FINGER TO THE CARD.
- If blood flow begins to diminish, follow the initial blood collection procedures in order to fill the remaining circles.
- Fill each circle with a single large drop of blood. Blood should completely cover the inside of each circle. If a drop is too small to completely fill a circle, additional small drops may be placed next to each other, BUT TRY NOT OVERLAP THE DROPS.
- After all the circles have been filled, use a sterile gauze pad to stop the blood flow and then apply the bandage.

IT IS VERY IMPORTANT TO ALLOW THE SAMPLES TO COMPLETELY AIR DRY AT ROOM TEMPERATURE BEFORE BEING SHIPPED. FOR PROPER DRYING, STAND CARD ON ITS SIDE FOR AT LEAST 2 HOURS (OR OVERNIGHT). DO NOT REFRIGERATE. DO NOT STACK WET CARDS ON TOP OF ONE ANOTHER TO PREVENT CONTAMINATION OF THE SAMPLES.
APPENDIX G:

EXEMPLAR OF LOG SHEET
<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Date test drawn/sent</th>
<th>Date test returned</th>
<th>Significance of test &amp; reason not done</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-15-09</td>
<td>4-23-09</td>
<td>Normal FVC</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>1-19-09</td>
<td>4-23</td>
<td>Normal</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>1-20-09</td>
<td>4-23</td>
<td>N FEV1 improved by 22%</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>1-22-09</td>
<td>4-23</td>
<td>Normal</td>
<td>MM</td>
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<tr>
<td></td>
<td>1-26-09</td>
<td>Already done</td>
<td>Normal</td>
<td>MM</td>
</tr>
</tbody>
</table>
APPENDIX H:

EXEMPLAR OF AATD TEST RESULT REPORT


## Alpha-1 Antitrypsin Genetics Laboratory Results

Dr.
10250 North 92 Street
Suite 102
Scottsdale, AZ 85258
Fax: 480-614-1731
Phone: 480-614-2000

Patient's Alpha-1 Antitrypsin (AAT) Laboratory Results:

<table>
<thead>
<tr>
<th>Patient's Name:</th>
<th>Lab Reference No.:</th>
<th>Reference Range:</th>
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<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>Age (Years):</th>
<th>Specimen Collection Date:</th>
<th>Specimen Received Date:</th>
<th>Approval Date:</th>
<th>AAT Genotype*</th>
<th>Estimated AAT Level **(mg/dL):</th>
<th>Estimated AAT Level ***(mg/dL):</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
<td>2008</td>
<td>2008</td>
<td>MZ</td>
<td>22.1649</td>
<td>114.6406</td>
</tr>
</tbody>
</table>

** AAT Level obtained from the DBS is an estimate of the AAT plasma level.

* Genotypic analysis detects the S and Z alleles which constitute >95% of all mutations that cause alpha-1 antitrypsin deficiency and will not identify rare cases of alpha-1 antitrypsin deficiency. Greater than 95% of the alleles, which are not S or Z, are the normal M alleles. Since genetic variations and other problems can affect the accuracy of direct mutation testing, this result should always be interpreted in light of the clinical and familial data. While the accuracy and specificity of this test is very high, we encourage the submission of a second sample to the laboratory for typing of individuals identified as MZ, SZ, ZZ, or suspected rare deficiency alleles based upon the importance that this diagnosis may have on your patient's future healthcare. A free testing kit is included with this letter (four 7 mL EDTA tubes with postage mailer). If the diagnosis is confirmed by the second test, you are encouraged to offer testing to family members.

Individuals with PI types ZZ, SZ, MZ, and some rare deficiency alleles are at greater risk than the normal population for developing chronic obstructive pulmonary disease and liver disease.

To assist in counseling your patient with an "at risk" allele, we ask that you encourage your patient to read the enclosed brochure(s). For your alpha-1 antitrypsin deficient patient, we request that your patient consider participating in the Alpha-1 Foundation Research Registry and Alpha-1 Foundation DNA and Tissue Bank (see enclosed forms). Alpha-1 Association’s Genetic Counseling Call Center (1-800-765-3177) offers information and resources to patients, family members, and medical professionals. If you have any questions regarding this report, or need more testing or educational materials, please contact our program at (866) 284-2708, FAX at (352) 846-3748, or email us at alpha1lab@alphafone.ufl.edu or visit us at www.alphafone.ufl.edu

Sincerely,

Mark Brantly, M.D.
APPENDIX I:

EXEMPLAR OF SPSS DATA SHEET
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<tr>
<th>AGE</th>
<th>COPD Y/N</th>
<th>CHRONICBRONCHITIS Y/N</th>
<th>EMPHYSEMA Y/N</th>
<th>GENDER</th>
<th>ASTHMA Y/N</th>
<th>BRONCHIECTASIS Y/N</th>
<th>CHRONIC COUGH Y/N</th>
<th>DYSPNEA Y/N</th>
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<th>ASYMPTOMATIC</th>
<th>TESTED Y/N</th>
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<th>GENOTYPE</th>
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<th>FEV1</th>
<th>FEV1 %</th>
<th>ML POST SABA CHANGE</th>
<th>SMOKEEVER/NEVER/CURRENT</th>
<th>PACK YEARS</th>
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APPENDIX J:

EXEMPLAR OF CODE BOOK
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<td>EMPHYSEMA</td>
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<td>0</td>
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<td>ALPHA-1 TESTED?</td>
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<td>GENOTYPE</td>
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