Exhaled Nitric Oxide in Patients with Interstitial Lung Disease: A Pilot Study

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Idiopathic pulmonary fibrosis (IPF) and sarcoidosis have an unknown etiology and require, periodic monitoring due to the insidious, unpredictable, and irreversible nature of disease progression. Exhaled nitric oxide (NO) has been used as a non-invasive marker of monitoring airway inflammation in patients with asthma and may have utility in monitoring airway inflammation in patients with IPF and sarcoidosis.

The purpose of this pilot study was to explore the utility of exhaled NO in monitoring disease progression and response to therapy in patients with IPF and sarcoidosis. Individuals with IPF (n=15) and sarcoidosis (n=43), and healthy non-smokers (n=20) underwent single breath end-tidal NO (FeNO) measurement at 7 flow-rates (50, 100, 150, 200, 250, 300, & 400 ml/s) using a chemiluminescence analyzer (LR1800; Logan Research, UK) following ATS/ERS guidelines (2005). Alveolar NO concentration ($C_{Alv}NO$) and airway NO flux ($J_{AW}NO$) were estimated using the model by Tsoukias, et al. (1998). In individuals with active sarcoidosis, follow-up measurements were performed after being on treatment

The findings in patients with IPF were: 1) FeNO was not significantly different from that of controls for the 7 flow rates; 2) while there was no significant difference in $J_{AW}NO$ compared with controls, $C_{Alv}NO$ was significantly higher, and 3) $C_{Alv}NO$ showed significant negative correlations with FEV_1% and FVC%. In patients with sarcoidosis,: 1) FeNO at a flow rate of 50 ml/sec was lower than that of controls with marginal statistical significance (p=.05); 2) $J_{AW}NO$, was significantly lower in patients with sarcoidosis compared to controls; there was no

significant difference in $C_{Alv}NO$; 3) $C_{Alv}NO$ showed significant negative correlations with FVC% and D_LCO%. The subset of patients with active sarcoidosis (n=8) had significantly lower $C_{Alv}NO$ compared with those with inactive sarcoidosis (n=35), but no significant difference in FeNO and $J_{AW}NO$. In six patients with active sarcoidosis who completed follow-up at various intervals, exhaled NO (FeNO, $C_{Alv}NO$ and $J_{AW}NO$) did not change significantly as a result of treatment. Due to a large inter-subject variability in FeNO, confounding from medications used to manage this disease and variable concentrations of ambient NO, exhaled NO does not appear to be effective in detecting changes in airway inflammation in this population.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

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1.0 INTRODUCTION

Interstitial lung disease (ILD) encompasses a broad range of heterogeneous disease groups, which share common pathologic characteristics, including scarring of the lung and progressive deterioration of alveolar gas exchange capacity (Lindell & Jacobs, 2003; G Raghu, 1998; Strieter, 2001). Idiopathic pulmonary fibrosis (IPF) and sarcoidosis are two examples of commonly occurring ILD (King, 2007). Although relatively rare, IPF and sarcoidosis cause substantial patient burden due to the insidious, unpredictable, and irreversible changes in lung function associated with these conditions (G Raghu, 1998). Although medications can decrease symptoms, there is no therapy that reverses lung damage. Lung transplantation is the only known option for cure (Lindell & Jacobs, 2003). For these reasons, timely monitoring and symptom management are important in order to slow disease progression and improve quality of life (Lindell & Jacobs, 2003).

An important component of the management of IPF and sarcoidosis involves periodic monitoring of symptoms and pulmonary function tests (American Thoracic Society, 1999b; Demedts & Costabel, 2002). These methods are non-invasive, but often not specific enough to identify changes consistent with disease progression. Therefore, it would be beneficial to identify a non-invasive technique that is easy to use and provides timely information on disease progression and response to treatment.

Exhaled nitric oxide (NO) was first discovered in the exhaled breath of rabbits, guinea pigs, and humans by Gustaffson et al. in 1991 (Gustaffson, Leone, & Persson, 1991). This discovery was followed by intense interest in the potential use of exhaled NO as a noninvasive marker of inflammation and oxidative stress in the lung (S. Kharitonov & Barnes, 2001). The technique used to monitor exhaled NO is easy to perform, non-invasive, inexpensive, and requires minimal cooperation from patients (Choi, Hoffman, Rodway, & Sethi, 2006). Testing can be performed in multiple settings, age, and disease groups (S. Kharitonov & Barnes, 2001).

In early studies, exhaled NO was measured using a single breath technique wherein patients exhaled at a constant flow rate over a defined time interval (American Thoracic Society, 2005). Recently, a multiple flow-rate technique has been advocated as a more promising method. When multiple flow rate measurements are used, it is possible to estimate NO levels from two lung compartments, i.e., the conducting airways and alveoli (American Thoracic Society, 2005; Tsoukias & George, 1998). The multiple flow-rate measurement technique is considered more appropriate for ILD patients because disease typically involves both airway compartments (Strieter, 2001).

The use of exhaled NO in detecting and monitoring airway inflammation has been extensively studied in patients with asthma, cystic fibrosis, and lung transplant (S. Kharitonov & Barnes, 2000). However, few studies were identified that tested the utility of exhaled NO as a monitoring method in patients with ILD with either the single-breath (Moodley, Chetty, & Lalloo, 1999; O'Donnell et al., 1997; Paredi et al., 1999; Riley et al., 1997; Wilsher, Fergusson, Milne, & Wells, 2005; D.H. Yates, Kharitonov, & Barnes, 1997; Ziora, Kaluska, & Kozielski, 2004) or multiple flow-rate technique (Brindicci, Goh, Wells, & Barnes, 2005; Girgis, Gugnani, Abrams, & Mayes, 2002; Lehtimaki et al., 2001).

According to findings from studies using the single-breath measurement in patients with ILD, exhaled NO levels are positively correlated with inflammatory cell count in bronchioalveolar lavage (BAL) during active inflammation (Paredi et al., 1999). However, once fibrotic changes develop exhaled NO levels may not be different (Wilsher et al., 2005) or lower than healthy controls (Paredi et al., 1999), with no correlation with clinical data (Wilsher et al., 2005). Each study had several limitations (see Table 1-3). Data were collected cross-sectionally (O'Donnell et al., 1997; Paredi et al., 2003; Riley et al., 1997; Wilsher et al., 2005; Ziora et al., 2004). The sample size was small and a heterogeneous group of ILD patients was recruited rather than those with one type of ILD (Paredi et al., 1999; Riley et al., 1997). Because the single breath measurement technique was used, findings did not provide information about differences in the conducting airways (flux of airway NO, $J_{AW}NO$ [nl/sec]) or alveoli (alveolar NO concentration, $C_{Alv}NO$ [ppb]).

Several more recent studies used the multiple flow-rate technique in patients with ILD (Brindicci et al., 2005; Girgis et al., 2002; Lehtimaki et al., 2001). Again, there were differences in patient diagnoses that might impact study findings, e.g., IPF and hypersensitive pneumonitis (Lehtimaki et al., 2001), scleroderma with and without pulmonary hypertension (Girgis et al., 2002). Nevertheless in all studies alveolar NO was higher in ILD patients than controls (Brindicci et al., 2005; Girgis et al., 2002; Lehtimaki et al., 2001) especially when the disease involved active inflammation in the lungs, such as usual interstitial pneumonia (Brindicci et al., 2005). In addition, there was a significant negative relationship between alveolar NO and clinical data, such as diffusing capacity for carbon monoxide (D_LCO), and vital capacity (VC) (Girgis et al., 2002; Lehtimaki et al., 2001). As in studies using a single breath measurement, there were

limitations including a small sample size, mixed diagnoses, and insufficient data regarding correlations with clinical variables.

1.1 PURPOSE

The purpose of this pilot study was to determine if exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) could be used to identify changes in disease progression and response to therapy in patients with idiopathic pulmonary fibrosis (IPF) and sarcoidosis diagnosed using current international guideline (American Thoracic Society, 1999b; Demedts & Costabel, 2002). Healthy non-smokers were recruited as a control group (Control). Results were also examined to determine if a correlation existed between exhaled NO levels and other clinical variables (dyspnea and pulmonary function tests) and disease activity.

1.2 SPECIFIC AIMS

The specific aims were:

1) To compare FeNO levels measured at 7 flow rates (50, 100, 150, 200, 250, 300, and 400 ml/s) between patients with IPF or sarcoidosis and healthy non-smoking subjects (controls);
2) To compare calculated airway wall NO flux ($J_{AW}NO$) and alveolar NO concentrations ($C_{Alv}NO$) between patients with IPF or sarcoidosis and healthy non-smoking subjects (controls);

3) To examine the relationship between exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) and selected clinical variables (dyspnea, pulmonary function tests) in patients with IPF or sarcoidosis.

The exploratory aims were:

1) To compare exhaled NO (FeNO, C_{AlvNO}, $J_{AW}NO$) between patients with active sarcoidosis and patients with inactive sarcoidosis;
2) To examine changes in exhaled NO (FeNO, C_{AlvNO}, $J_{AW}NO$) over time (from initial clinic visit to follow-up visit) in patients with active sarcoidosis completed follow-up.

1.3 DEFINITION OF TERMS

1) IPF (Idiopathic Pulmonary Fibrosis): a distinctive type of chronic fibrosing interstitial pneumonia of unknown cause limited to the lungs confirmed by histologic diagnosis (Demedts & Costabel, 2002).
2) Sarcoidosis: a systemic granulomatous disease that primarily affects the lungs and lymphatic systems diagnosed by histological evidence of noncaseating epithelioid cell granulomas due to an unknown cause or not the result of local sarcoid reactions (American Thoracic Society, 1999b).
3) Active sarcoidosis (active pulmonary sarcoidosis): Active pulmonary sarcoidosis is diagnosed as present when the patient meets 3 or more of following criteria over 6-12 weeks: a) complaints of progressive respiratory symptoms, such as shortness of breath, cough, dyspnea on exertion; b) exercise desaturation tests that indicate deterioration of 10% or greater in arterial oxygen saturation by pulse oximetry (SpO_2) or an increase in flow requirement of supplemental oxygen during exertion; c) pulmonary function test results

(FVC, D_LCO) that indicate a deterioration of 10% or greater, or d) evidence of worsening radiographical change (American Thoracic Society, 1999b).

4) FeNO (ppb): Fraction of NO in exhaled breath measured at various flow rates (50, 100, 150, 200, 250, 300, and 400 ml/sec).
5) $C_{Alv}NO$ (ppb): Steady state NO concentration in alveolar air estimated by the multiple flow rate model of Tsoukias (George, Hogman, Permutt, & Silkoff, 2004; Tsoukias & George, 1998).
6) $J_{AW}NO$ (nl/sec): Airway wall NO flux. The quantity of NO transferred from bronchial wall to luminal air per unit time estimated by the multiple flow rate model of Tsoukias (George et al., 2004; Tsoukias & George, 1998).

1.4 CONCEPTUAL FRAMEWORK

The conceptual framework shown in Figure 1 illustrates the proposed relationships among variables examined in this study. As conceptualized, exhaled NO levels (FeNO, $C_{Alv}NO$, $J_{AW}NO$) will be influenced by the presence of IPF and sarcoidosis. There may be correlations between NO levels (FeNO, $C_{Alv}NO$, $J_{AW}NO$) and selected clinical variables: 1) dyspnea; 2) pulmonary function tests (FEV1%, FVC%, and D_LCO%); and/or 3) disease activity.

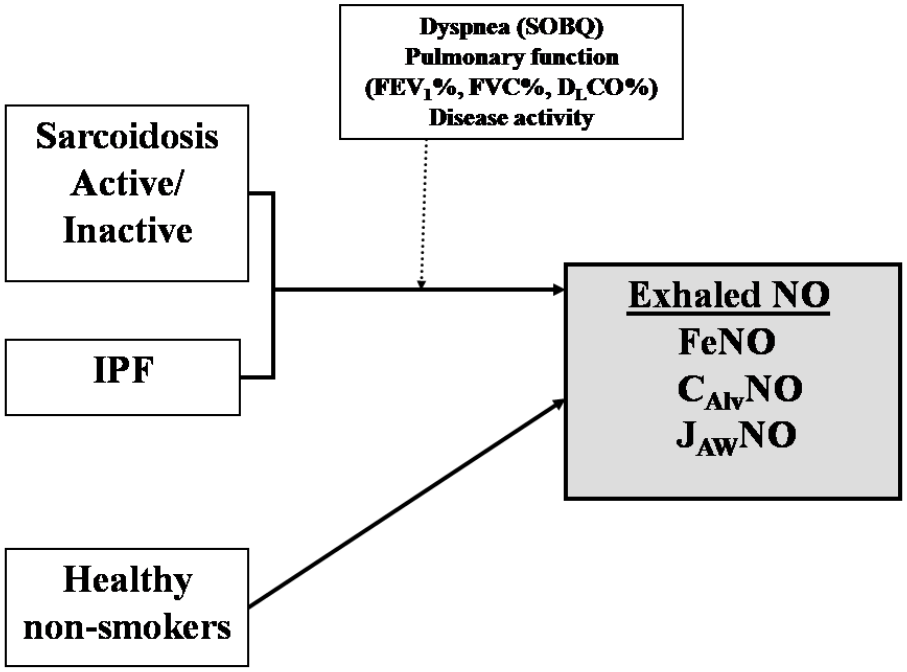

Figure 1. Conceptual Framework

Note: SOBQ, Shortness of Breath Questionnaire

2.0 BACKGROUND AND SIGNIFICANCE

2.1 INTERSTITIAL LUNG DISEASE (ILD)

Interstitial lung disease (ILD), a diffuse parenchymal lung disease, consists of a heterogeneous group of diseases that share common characteristics, including scarring of lung tissue and progressive loss of the normal gas exchange capacity of the alveolar capillary membrane (G Raghu, 1998). In many cases, ILD affects both alveolar and airway tissue (Strieter, 2001). By etiology, there are three groups of ILD. The first group includes conditions that result from occupational or environmental exposure, mainly organic or inorganic dusts, e.g., asbestosis, silicosis, hypersensitivity pneumonitis also known as farmers' lung. Second, ILD can develop as a consequence of systemic connective tissue diseases, such as systemic lupus erythematosus, rheumatoid arthritis, or scleroderma. The third group includes the many types of ILD with an unknown etiology (King, 2007). IPF and sarcoidosis are included in this category.

2.1.1 Idiopathic Pulmonary Fibrosis (IPF)

Idiopathic Pulmonary Fibrosis (IPF) is also known as cryptogenic fibrosing alveolitis (Dempsey, Kerr, Gomersall, Remmen, & Currie, 2006) or in histopathologic diagnosis, as "usual interstitial pneumonia (UIP)" (Demedts & Costabel, 2002). IPF is characterized by interstitial inflammation with fibrosis, which results in chronic irreversible scarring with honeycombing

changes mainly in the lung parenchyma (Dempsey et al., 2006). In the US, approximately 15,000 new cases are diagnosed every year (Coultas & Hughes, 1996). The estimated incidence rate is 20 to 30 per 100,000 and the condition occurs more commonly in men than in women (Coultas & Hughes, 1996). Typically, at the time of presentation, patients are between 40 and 70 years (American Thoracic Society, 2000). About two-thirds of IPF patients are older than 60 years (American Thoracic Society, 2000). Although ethnic variation has not been clearly established, IPF related mortality is known to be higher in white Caucasians than other ethnic groups (American Thoracic Society, 2000). Various risk factors have been suggested including cigarette smoking, use of antidepressants, chronic aspiration secondary to gastroesophageal reflux disease, occupational or environmental exposure, viral infection, and genetic predisposition (Lindell & Jacobs, 2003).

The exact causative mechanism of IPF remains under investigation. It is believed that an aberration of wound healing mechanisms in the lung results in subsequent irreversible fibrotic changes in scattered areas of lung parenchyma (Dempsey, 2006). The consequent chronic inflammation and scarring cause a progressive decline in the gas exchanging capacity of alveolar capillary membrane (Demedts & Costabel, 2002). Patients typically present with complaints of non-specific respiratory symptoms, such as cough, shortness of breath, decrease in exercise tolerance, fatigue, etc. (Lindell & Jacobs, 2003). The diagnosis is established by suggestive findings, including evidence of restrictive lung disease, a decrease in the D_LCO, and changes in high resolution computed tomography (HRCT) (Lindell & Jacobs, 2003). The differential diagnosis of IPF is a challenge because it is necessary to rule out conditions which cause similar symptoms. In order to confirm the diagnosis, lung biopsy is required (American Thoracic Society, 2000). Due to heterogeneous pathological changes in IPF, it is recommended that

biopsies be taken from multiple lobes (Dempsey et al., 2006). Nevertheless, due to frailty, advanced age, or co-morbidities, surgical biopsy may not be recommended due to the risk of complications (Dempsey et al., 2006).

Management of IPF is directed toward minimizing symptoms and improving quality of life. Conventional pharmacologic management such as corticosteroids, other immunosuppressive drugs (e.g. azathoprine, cycpophosphamide), and antifibrotic agents (e.g. colchicines) are used either individually or in combination (Lindell & Jacobs, 2003). A number of additional agents are under investigation but, to date, there is no curative therapy. Although survival varies depending on age at diagnosis and the number of risk factors, median survival after initial diagnosis is less often than three years (Bjoraker et al., 1998). Lung transplantation is the only treatment that has the potential to improve survival (Alalawi, Whelan, Bajwa, & Hodges, 2005; Lindell & Jacobs, 2003).

2.1.2 Sarcoidosis

In the US, the annual incidence of sarcoidosis ranges from approximately 5 to 40 out of 100,000, depending on ethnicity and gender. According to the only population-based study by Rybicki et al. (1997), African Americans tend to have a higher incidence rate (35.5 per 100,000) than white Caucasians (10.9 per 100,000) (Rybicki, Major, Popovich, Maliarik, & Iannuzzi, 1997). Females also have a slightly higher incidence rate (6.3 per 100,000) than males (5.9 per 100,000) (Rybicki et al., 1997). Sarcoidosis can occur across the lifespan, but most patients are diagnosed between the ages of 25 to 40 years (American Thoracic Society, 1999b).

Sarcoidosis is characterized by the presence of noncaseating granulomas in various organs resulting from uncontrolled cell-mediated immune reactions (Nunes, Soler, & Valeyre,

2005). The disease can affect multiple organs but predominantly involves the skin, eyes, lymph nodes, and chest (Nunes et al., 2005). Both genetic susceptibility and environmental factors are believed to be involved. Genetic susceptibility affects the presentation, progression and prognosis (American Thoracic Society, 1999b; Nunes et al., 2005). A variety of environmental factors including infectious agents (mycobacteria, parasites, and fungi), inorganic agents (beryllium, zirconium, and zluminum), and organic particles are suspected as potential triggers (American Thoracic Society, 1999b; Nunes et al., 2005).

The clinical presentation and progression of pulmonary sarcoidosis is highly variable (Costabel, 2001). In more than 50% of cases, diagnosis is made incidentally by radiographic abnormalities while patients are asymptomatic (Costabel, 2001). The diagnosis requires histologic confirmation of the presence of noncaseating granulomas and exclusion of other conditions that produce similar symptoms (American Thoracic Society, 1999b). The goal at the time of diagnosis include: 1) histologic confirmation of the disease; 2) determining the extent and severity of organ involvement; 3) determining if the disease is inactive or active, and 4) determining the potential benefit of initiating therapy (American Thoracic Society, 1999b). Pulmonary involvement occurs in more than 90% of sarcoidosis patients (American Thoracic Society, 1999b). In advanced stages of pulmonary sarcoidosis, fibrotic changes involve the entire lung parenchyma and may also involve the larynx, trachea and bronchi. Patients experience a progressive loss of functional ability and eventually die from acute respiratory failure (Nunes et al., 2005).

Similar to IPF, management for patients with sarcoidosis focuses on symptom management and pharmacologic treatment (Nunes et al., 2005). Corticosteroids are the first line of treatment (Nunes et al., 2005). As a second line, immunosuppressive agents, e.g.,

hydroxychloroquine, methotrexate or azathioprine, are recommended (Nunes et al., 2005). The long-term benefits of those medications remain under investigation (Nunes et al., 2005). Spontaneous remission may occur with no treatment and the appropriate time of initiating treatment is therefore still controversial (Costabel, 2001).

In summary, the exact causative mechanisms of IPF and sarcoidosis are still under investigation. With the exception of lung transplantation, there are no effective current treatment options (American Thoracic Society, 1999b, 2000). The progress of both diseases is insidious, unpredictable and irreversible. In order to slow disease progression and improve quality of life, the timing of therapy is important. Hence, it would be beneficial to develop a non-invasive monitoring method that can be frequently and easily used to detect changes in lung function and monitor response to therapy.

2.2 EXHALED NITRIC OXIDE (NO)

2.2.1 Early History of NO

Nitric oxide (NO) was first discovered by Joseph Priestly in 1772 (Chinard, 1995). NO was viewed as a "non-respirable " poisonous gas for over 200 years, because the focus was on its adverse actions, such as a cause of smog, acid rain and cancer (Kreuzer & Patel, 1971; Norman & Keith, 1965; Terrell & Schmeltz, 1968; Weissbecker, Creamer, & Carpenter, 1971). In 1980, Furchgott and Zawadzki reported that the vascular endothelium contained an endothelium derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980; Vallance & Chan, 2001) and later it was suggested that EDRF may be NO (Furchgott, 1996; RM Palmer, Ferrige, & Moncada

1987). In 1987, Palmer et al. (RM Palmer et al., 1987) and Ignarro et al. (Ignarro, Buga, & Wood, 1987) reported that the actions and characteristics of NO in arteries and veins were identical to those of EDRF. Following this report, scientists began to investigate the physiologic role of NO. In 1998, the Nobel Prize for Physiology or Medicine was awarded for these discoveries (The Nobel Foundation, 1998).

2.2.2 NO Synthesis

Endogenous NO is formed via the action of the enzyme, NO synthase (NOS) which converts L-arginine into L-citrulline and NO (Bateman, Sharpe, & Ellis, 2003; Ignarro, Fukuto, Griscavage, & Rogers, 1993; Moncada & Higgs, 1993; R Palmer, Ashton, & Moncada 1988). This reaction requires oxygen, and cofactors, such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin (BH_4)(Adding & Gustaffson, 2003; S. Kharitonov & Barnes, 2001; Ricciardolo, 2003).

There are three isoforms of NOS, termed neural NOS (nNOS), endothelial NOS (eNOS), and induced NOS (iNOS). The isoforms are the product of three different genes located on three different chromosomes (chromosome 7, 12, and 17, respectively) (Ricciardolo, 2003). The two constitutive isoforms (nNOS and eNOS) require influx of calcium (Ca^{2+}) and calmodulin for activation (Bredt & Snyder, 1990). The third isoform, iNOS, is ready for physiologic activity immediately after translation, because Ca^{2+} and calmodulin are already tightly bonded (Belvish, Mitchell, & Yacoub, 2003). Therefore, activation of iNOS is Ca^{2+} independent (Bateman et al., 2003; Ignarro et al., 1993; Murad, 1996).

nNOS is released from nerve cells (Belvish et al., 2003) and is known to be up-regulated by heat, electrical activation (Reiser, Kline, & Vaghy, 1997), light, ischemic injury (Prabhakar et

al., 1996; Zhang, Chopp, Gautam, Zaloga, & Schmidt, 1994), and sex hormones, including estradiol and testosterone (Luckman, Huckett, Bicknell, Voisin, & Herbison, 1997; Reily, Zamorano, Stopper, & Mills, 1997). Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), may reduce the expression of nNOS (Fosterman, Boissel, & Kleinert, 1998; Reiser et al., 1997). eNOS is released by endothelial cells (Mitchell et al., 1991), which may be up-regulated by shear stress and proliferation of tissue (Corson et al., 1996; Xino, Zhang, & Dramond, 1997), and down-regulated by endotoxin or cytokines (Belvish et al., 2003). iNOS, the Ca^{2+}- independent isoform, is normally not expressed or expressed only very low levels and induced after several hours of exposure by pro-inflammatory cytokines, such as TNF-α, interleukin-1β (IL-1β), and interferon-γ (IFN-γ) (Belvish et al., 2003). In the respiratory system, when these proinflammatory cytokines or oxidants activate nuclear factor κB (NF-κ B), the most important transcription factor for regulating iNOS induction (Xie, Kashiwabara, & Nathan, 1994), iNOS is expressed in various cells: bronchial epithelial cells (Guo et al., 1995; Kobzik et al., 1993; Watkins, Peroni, Basclain, Garlepp, & Thompson, 1997), alveolar macrophages (Kobzik et al., 1993; Tracey et al., 1994; Wang et al., 1998), nasal vascular epithelial cells and nasal ciliated epithelial cells (Furukawa et al., 1996). iNOS has a dual nature: it involves cell protection as well as cell damage (Bateman et al., 2003; Brune, Knethen, & Sandau, 2000; Wink & Mitchell, 1998).

2.2.3 Physiologic Actions of NO

Once formed, NO exists for a brief time (6-10 seconds) before being converted into other substances (Myron, 1995). NO is an essential biological molecule in the human body that is involved in various functions: selective vasodilator, bronchodilator, neurotransmitter, and

inflammatory mediator (Adding & Gustaffson, 2003; Barnes & Belvish, 1993; Culotta & Koshland, 1992; Vallance & Chan, 2001).

In the respiratory system, physiologic actions of NO have three different aspects. NO produces regulative, protective, and deleterious effects (Bateman et al., 2003; Brune et al., 2000; Wink & Mitchell, 1998). In the airways, NO regulates tracheobronchial circulation and maintains baseline bronchial caliber (Barnes & Liu, 1995; Higenbottam, 1995; Kuo, Liu, & Barnes, 1992). NO suppresses airway plasma exudation (Bernareggi, Mitchell, Barnes, & Belvish, 1997; Erjefalt, Erjefalt, Sundler, & Persson, 1997) and stimulates mucociliary clearance (Jain, Rubinstein, Robbins, Leise, & Sisson, 1993; Ramnarine, Khawaja, Barnes, & Rogers, 1996; Tamaoki, Chiyotani, Kondo, & Konno, 1995), a primary airway defense mechanism. In the pulmonary circulation, NO acts as a tonic vasodilator (Stampler, Loh, Roddy, Currie, & Creager, 1994) by altering pulmonary vascular resistance (Albert et al., 1997; Cooper et al., 1996) and ventilation-perfusion (V/Q) matching during hypoxic pulmonary vasoconstriction (Archer, Tolins, Raij, & Weir, 1989; Barnes & Liu, 1995; Persson, Gustaffson, Wiklund, Moncada , & Hedqvist, 1990; Sprague, Thiemermann, & Vane, 1992).

Optimal NO production in the pulmonary system requires oxygen (Adding & Gustaffson, 2003). In the well-oxygenated pulmonary blood vessel, active NO production maintains pulmonary vascular tone. In hypoxemic regions, the low oxygen concentration decreases NO production. Decreased NO production results in minimal stretch (vasodilation) of pulmonary vessels, which decreases blood flow to poorly oxygenated lung regions (Adding & Gustaffson, 2003; Grimminger, Spriestersbach, Weissman, Walmrath, & Seeger, 1995; Nelin, Thomas, & Dawson, 1996). This action shunts blood to better oxygenated regions and, thereby, promotes more optimal V/Q matching. NO may also act as a ventilatory depressant by controlling

hyperventilation (Persson et al., 1990) via an inhibitory effect on respiratory neurons (Barros & Branco, 1998) and respiratory muscle force (El Dwairi et al., 1998).

Involvement of NO is "multifaceted" as well as "paradoxical (Wink & Mitchell, 1998). " As a regulator, NO controls bronchodilation, vascular tone and mucus secretion (Ricciardolo, 2003). As a cytoprotective agent, NO defends the body against reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), alkyl hydroperoxides, and superoxide (Wink & Mitchell, 1998). NO is known to neutralize oxidants associated with oxidative stress and attenuate ROS mediated toxicity (Wink et al., 1994). The up-regulation of iNOS activity increases NO production and creates a toxic environment for viruses, bacteria, fungi, parasites, e.g., herpes simplex virus, mycobacterium tuberculosis (Lowenstein, Dinerman, & Snyder, 1994). This activity of iNOS in airway epithelium explains the role of NO in airway host defense (Guo et al., 1995; Shaul et al., 1994).

Although NO itself is not inherently cytotoxic, depending on intracellular redox milieu, NO can react as a cytotoxic or cytoprotective agent. Reaction of NO with oxygen or redox metal complexes produces NO and NO-derived chemical species thereby creating its deleterious effects. The powerful oxidant, peroxynitrite ($ONOO^-$), is formed by the reaction between NO and O_2^-, which inhibits enzyme function, causes damage in DNA, induces lipid peroxydation, and increases cellular susceptibility to radiation, toxic metals, and alkylating agents (Beckman & Koppenol, 1996; Wink & Mitchell, 1998).

2.2.4 Measuring NO in Exhaled Breath

Because of its short half-life and rapid oxidation in biological tissues, NO measurement was a challenge. Initially, researchers tried to indirectly measure NO using different assays, such as

cGMP, nitrites (NO_2^-), or citrulline, which reflect NO activity, NO metabolism, or NO synthesis, respectively (Adding & Gustaffson, 2003). In 1970, environmental scientists discovered that NO could be directly measured using the principle of chemiluminescence (Fontijin & Ronco, 1970). When NO reacts with ozone, it produces energy in the form of light that is proportional to the amount of NO and can be measured with a luminometer (Fontijin & Ronco, 1970). The pioneering NO studies in 1987 by Palmer and colleagues used this chemiluminescence method (RM Palmer et al., 1987). However, because of the difficulty in avoiding oxidation of NO to NO_2^-, they summed the amount of NO and NO_2^- and separated the NO value through calculation. In 1991, Gustafsson et al. reasoned that NO may escape through exhalation, due to its gaseous nature and low solubility in fluid, an observation led their discovery of NO in exhaled breath (Gustaffson et al., 1991). Their work, the first to use the chemiluminescence method to measure NO in exhaled breath, led to an explosion of research focused on identifying the potential role of exhaled NO in diagnosis and monitoring of various respiratory diseases (S. Kharitonov & Barnes, 2001).

Exhaled gas analysis is particularly attractive to the practice of nursing because of many advantages. It is non-invasive, easy to learn and perform (patients perform a slow, steady exhalation into a mouthpiece connected with the machine). Adults and children can easily follow the test and there is no learning effect or systematic error when serial measurements are performed (S. Kharitonov, 2004). The test can be reliably performed in those older than 7 years of age and the majority of children 4 to 7 years of age (S. Kharitonov, 2004). Test results are immediately displayed on the monitor screen and testing requires minimal space and technical support. Accordingly, serial exhaled NO analysis may be helpful in decreasing use of other invasive or expensive tests. Nurses can apply this non-invasive test in research and clinical

practice in populations across the lifespan. When the equipment becomes more compact and less expensive, this technique may be expanded to primary care clinics in the community and, perhaps, even home care settings. However, a search identified a limited nursing literature addressing NO (Cicutto & Downey, 2004). The proposed study will provide important information about exhaled NO in patients with IPF and sarcoidosis that may form the basis for testing future interventions.

2.2.5 Factors Influencing Exhaled NO Levels

Exhaled NO levels may be influenced by several factors. The influence of body mass index (BMI), gender, and age are not yet clear (Ekroos, Tuominen, & Sovijarvi, 2000; Tsang et al., 2001). Cigarette smoking is known to significantly decrease exhaled NO levels, possibly because NO in cigarette down-regulates NOS production (S. A. Kharitonov, Robbins, Yates, Keatings, & Barnes, 1995). Alcohol consumption has been shown to decrease exhaled NO levels in patients with asthma, presumably because ethanol decreases iNOS production (D. H. Yates, Kharitonov, Robbins, Thomas, & Barnes, 1996). Whether similar changes occur in healthy individuals has not been determined. Caffeine consumption significantly decreases exhaled NO levels in healthy volunteers (Bruce, Yates, & Thomas, 2002), but does not produce significant changes in exhaled NO in patients with asthma (Taylor, Smith, Cowan, Herbison, & Taylor, 2004). Although the effect of diet on exhaled NO levels is not clear, there may be some influence from food supplements containing L-arginine, or nitrate (S. A. Kharitonov, Lubec, Lubec, Hjelm, & Barnes, 1995; Olin et al., 2001; Popovic, Zeh, & Ochoa, 2007). Therefore, it is recommended that patients refrain from eating, drinking caffeinated beverages, or smoking for one hour before measurements are made (American Thoracic Society, 1999a). Some studies indicate that ambient

NO alters measured values (Baraldi et al., 1998; Byrnes, Dinarevic, Busst, Shinebourne, & Bush, 1997), whereas others do not (Baraldi et al., 1998; C. Borland, Cox, & Higenbottam, 1993; S. Kharitonov, Logan-Sinclair, Busset, & Shinebourne, 1994; Kimberly, Nejadnik, Giraud, & Holden, 1996; Massaro et al., 1996; Piacentini et al., 1998). Therefore, it is important to measure ambient NO when performing this test. Inhaled corticosteroids (ICS) may also alter exhaled NO levels. Exhaled NO is decreased in response to the use of ICS (S. Kharitonov, Donnelly, Corradi, Montuschi, & Barnes, 2000; S. Kharitonov, Yates, & Barnes, 1996). For this reason, it is important to control for the effect of ICS analyzing results obtained from this measurement.

2.2.6 Exhaled NO measurement

Two techniques are currently used for measuring exhaled NO (American Thoracic Society, 2005). In offline measurement, expired air is collected into a reservoir and the concentration of NO is analyzed from this sample (American Thoracic Society, 2005). The more commonly used method involves analyzing the concentration of end tidal exhaled NO (FeNO) (American Thoracic Society, 2005) (Figure 2).

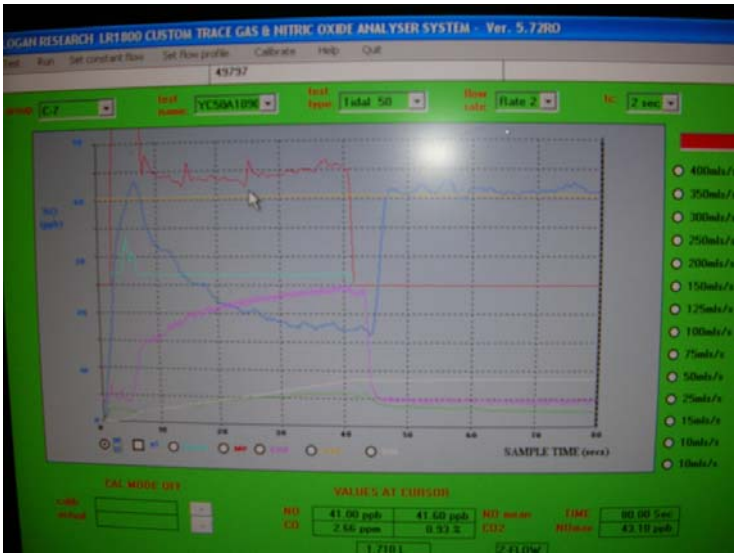

Figure 2. End tidal exhaled NO measurement

The concentration of FeNO is inversely related to flow-rate. Therefore, when patients exhale at lower flow rates, more NO is contributed from the airways relative to the overall concentration in the breath (Tsoukias & George, 1998). This characteristic flow pattern occurs because the slower flow rate allows more time for NO to enter from the airway and be exhaled. (Figure 3).

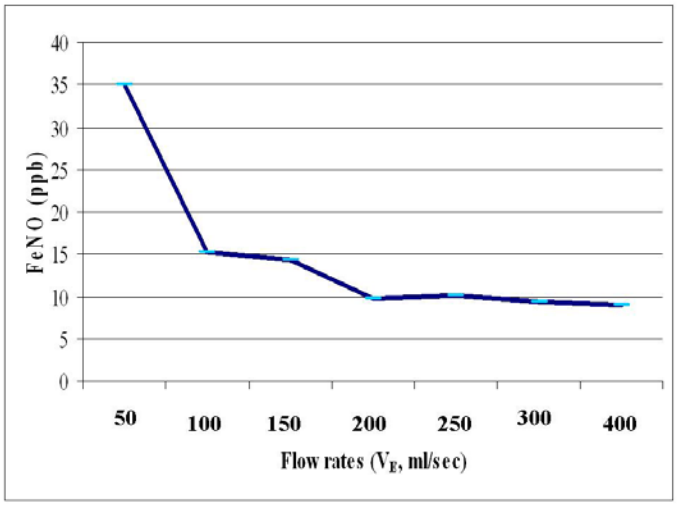

Figure 3. Flow rate dependent characteristics of FeNO

Using this flow-rate dependent characteristic, Tsoukias et al.(1998) developed a two-compartment model that can be used to calculate steady NO levels in alveoli ($C_{Alv}NO$, ppb) and flux of NO from the airway wall ($J_{AW}NO$, nl/sec). According to this model, the lung comprises two separate regions: a rigid and non-expansible airway compartment and an expansible alveolar compartment (George et al., 2004; Tsoukias & George, 1998). Two parameters, $C_{Alv}NO$, and $J_{AW}NO$, define the contributions from each compartment. While alveolar NO ($C_{Alv}NO$) moves through the conducting airways toward the mouth during exhalation, NO diffuses from airway wall ($J_{AW}NO$). $C_{Alv}NO$ and $J_{AW}NO$ are estimated by measuring NO elimination rate (V_LNO, nl/s), which is a product of FeNO and measurement flow-rate. Once FeNO is measured at two or more flow-rates (V_E, ml/s), a linear relationship can be seen between V_E and V_LNO (Figure 4).

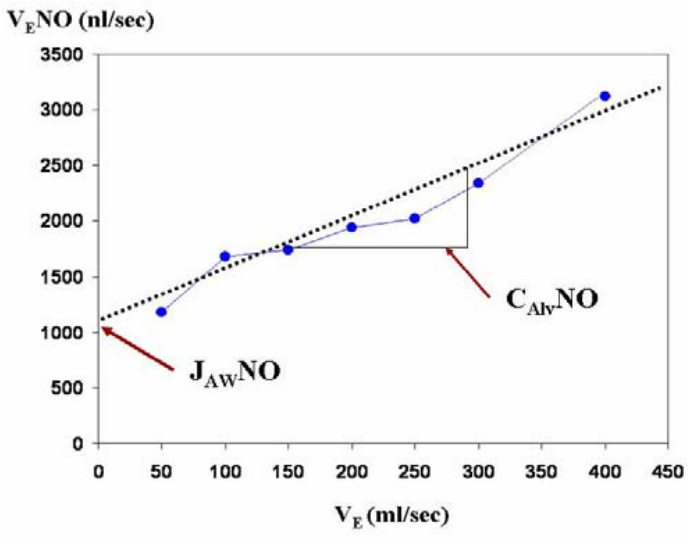

Figure 4. Schematic of Tsoukias technique to estimate the flow-independent NO parameters using a minimum of 2 constant exhalation flow rates

$C_{Alv}NO$ and $J_{AW}NO$ can be estimated from the slope and intercept. This technique has the advantage of providing more specific information about inflammation as contributions of the two compartments can be analyzed separately (American Thoracic Society, 2005). Therefore, this two-compartment model is considered to be a promising research tool.

2.3 EXHALED NO IN ILD

The utility of exhaled NO in diagnosis and monitoring has been extensively studied in patients with asthma, cystic fibrosis, and lung transplant recipients (S. Kharitonov & Barnes, 2001) with utility most strongly established in patients with asthma (Choi et al., 2006). FeNO levels increase during periods of airway inflammation and FeNO monitoring is an FDA approved option for asthma management (US Food and Drug Administrations, 2003).

Relatively few studies have examined the utility of exhaled NO in patients with IPF and sarcoidosis. In 10 human studies, 7 used the single-breath technique (Moodley et al., 1999; Moodley & Lalloo, 2001; O'Donnell et al., 1997; Paredi et al., 1999; Riley et al., 1997; Wilsher et al., 2005; Ziora et al., 2004) and 3 used multiple flow-rate measurement (Brindicci et al., 2005; Girgis et al., 2002; Lehtimaki et al., 2001). All recruited a heterogeneous group of patients, e.g., scleroderma with ILD, scleroderma with pulmonary hypertension (Girgis et al., 2002), pulmonary fibrosis (Riley et al., 1997), IPF (Paredi et al., 1999), progressive systemic sclerosis with ILD and without ILD (Moodley & Lalloo, 2001), IPF and hypersensitive pneumonitis (Lehtimaki et al., 2001), and sarcoidosis (Moodley et al., 1999; O'Donnell et al., 1997; Wilsher et al., 2005; Ziora et al., 2004).

The following tables summarize findings from studies in patients with sarcoidosis which used the single breath technique for measurement of exhaled NO (Table 1), studies which used the same measurement technique in patients diagnosed with various types of ILD (Table 2) and studies which used the multiple flow rate technique (Table 3).

Table 1. Single breath FeNO measurement in sarcoidosis

Author (Year)/ Sample	Treatment	FeNO (ppb)	Correlation with clinical variables	
O'Donnell et al. (1997) •Active sarcoidosis (n=10) •Healthy non-smokers (n=12)	No oral, ICS or other immuno-suppressive treatment	•Patients: 6.9 ± 4.5 •Healthy non-smokers: 6.6 ± 4.0	BAL NO_2^- leukocyte ACE D_LCO	 NS NS NS NS
Moodley et al. (1999) •Newly diagnosed patients (n=12) •Healthy non-smokers (n=21)	•No oral, ICS or other immuno-suppressive treatment at enrollment •8 patients received oral prednisone (40mg/day) for 6 weeks.	•Patients¶: 9.8 ± 0.4 (n=12 enrollment) •Pre vs. post treatment (n=8)*: 9.7 ± 0.5 vs. 5.9 ± 0.7 •Healthy non-smokers: 4.1 ± 0.2	BAL CD_4/CD_8 ACE FEV_1	 NS NS NS
Ziora et al. (2004) •Patients (n=27); 21 active sarcoidosis •Healthy non-smokers (n=11)	No oral or ICS	•Patients: 6.7 ± 0.5 •Healthy non-smokers: 5.2 ± 0.7	BAL lymphocyte Macrophage FVC D_LCO*	 NS NS NS r =.515
Wilsher et al. (2005) •Patients (n=52) •Healthy non-smokers (n=44)	No oral or ICS	•Patients: median 6.8 (2.4-21.8) •Healthy non-smokers: median 6.3 (1.6 – 28)	Serum IgE FEV_1 FVC D_LCO	NS NS NS NS

Note: All studies used single breath end-tidal exhaled NO measurement,
¶ Significantly high FeNO levels compared with healthy controls ($p<.05$); *$p < .05$

Table 2. Single breath FeNO measurement in other ILD

Author (Year) Sample	Treatment	FeNO (ppb)	Correlation with clinical variables	
Riley et al. (1997) •PPH (n=9) •PF (n=6: Sarcoidosis [n=3]; scleroderma [n=2]; CFA [N=1]): •Healthy non-smokers (n=20)	PF: 3 patients were on oral prednisone (mean 6.7 mg/day)	•PPH: 13.6 ± 8.3 •PF: 5.9 ± 1.6 •Healthy non-smokers: 13.6 ± 6.0		
Paredi et al. (1999) •IPF (n=11) •Healthy non-smokers (n=13)	IPF: 5 on oral prednisone 30mg/day	•IPF: 9.8 ± 1.0¶ Treatment vs. No treatment*: 9.0 ± 1.0 vs. 13.1 ± 1.0 •Healthy non-smokers: 6.9 ± 0.5		
Moodley & Lalloo (2001) •PSS-ILD (n=12) •PSS- no ILD (n=12) •Healthy non-smokers (n=30)	PSS-ILD: All on treatment with oral prednisone or azathioprine PSS- no ILD: No treatment	•PSS-ILD: 6.2 ± 0.6 •PSS- no ILD: 9.6 ± 0.7¶ •Healthy non-smokers: 6.3 ± 0.2	FEV_1 FVC D_LCO	NS NS NS

Note: The study by Riley et al.(1997) used continuous monitoring of mixed expired NO during exercise intervention (NO production rate [V_ENO, nl/min] was calculated; FeNO was measured at rest and peak exercise. Other 2 studies used single breath end-tidal FeNO measurement.
PPH=Primary Pulmonary Hypertension; PF=Pulmonary Fibrosis; PSS=Progressive Systemic Sclerosis
¶ Significantly high FeNO levels compared with other groups in sample ($p<.05$); * $p<.05$

Table 3. Multiple flow-rate exhaled NO measurement in ILD

Author (Year)/ Sample/ Flow rates (ml/sec)	Treatment	$C_{Alv}NO$ (ppb)	$J_{AW}NO$ (nl/sec)	Correlation with clinical variables
Lehtimaki et al. (2001) •ILD (n=17) - HP (n=7) - IPF (n=10) •Healthy non-smokers (n=57) •100, 175, 370	ILD: 5 on prednisone (1 HP; 4 IPF) Allergen avoidance or medications in 8 patients who were not on treatment at enrollment	•ILD (n=17): 4.1 ± 0.3 •ILD (n=8, pre vs. post treatment)*: 4.0 ± 0.6 vs. 2.4 ± 0.5¶ • Healthy non-smokers: 1.1 ± 0.1	•ILD: 0.7 ± 0.1 •ILD (n=8, pre vs. post tx): 0.5 ± 0.1 vs. 0.6 ± 0.1 •Healthy non-smokervs: 0.7 ± 0.1	$C_{Alv}NO$ & D_LCO *: r=- .55 $C_{Alv}NO$ & VC *: r=- .54
Girgis et al. (2002) •SSc –ILD (n=20) - without PH (n=15) - with PH (n=5) •PPH (n=5) •Healthy non-smokers (n=20) •50, 100, 150, 200	SSc –ILD: 10 on immunosuppressive agents (8 without PH; 2 with PH)	SSc -ILD without PH: 4.3 ± 0.6 with PH: 6.0 ± 0.7 •PPH: 2.3 ± 0.1 •Healthy non-smokers: •1.8 ± 0.2	•SSc ILD without PH: 0.6 ± 0.1 / with PH: 0.6 ± 0.4 •PPH: 0.9 ± 0.3 •Healthy non-smokers 1.2 ± 0.2	$C_{Alv}NO$ & D_LCO**: r= -.66
Brindicci et al. (2005) •IPF (n=12) •Sarcoidosis (n=9) •Healthy non-smokers (n=14) •10, 50, 100, 200, 260	IPF: 2 on immuno-suppressive agents	•IPF: 4.0 ± 0.4 ¶¶, ¶¶¶ •Sarcoidosis: 2.8 ± 0.3¶¶¶ •Healthy non-smokers: 1.4 ± 0.1	•Values not provided •No significant difference among 3 groups	

Note: HP=Hypersensitive Pneumonitis; SSc= Systemic Sclerosis; PH=Pulmonary Hypertension; PPH=Primary Pulmonary Hypertension; ¶ Significantly high $C_{Alv}NO$ levels compared with healthy non-smokers (p=0.008); ¶¶ Significantly high $C_{Alv}NO$ levels compared with sarcoidosis (p = 0.002); ¶¶¶ Significantly high $C_{Alv}NO$ levels compared with healthy non-smokers (p = 0.001); * $p < 0.05$; ** $p <$ 0.005

2.3.1 FeNO in IPF

One study was identified that compared FeNO in patients with IPF (Paredi et al., 1999). Paredi et al. (1999) measured exhaled NO in patients with IPF (n=11) and healthy controls (n=13). In this study, 5 patients with IPF were on oral prednisone of 30 mg/day. FeNO was compared between patients with IPF and healthy controls. In patients with IPF, FeNO was compared depending on the use of oral prednisone. Potential correlations with clinical data, including BAL cell counts and pulmonary function tests, were also examined. Significantly higher levels of exhaled NO were found in patients with IPF (11.2 ± 1.0 ppb) compared with healthy controls (6.9 ± 0.5 ppb). In patients with IPF, exhaled NO was lower in those treated with oral prednisone, compared to those not on treatment (9.0 ± 1.0 ppb, n=5, and 13.1 ± 1.0 ppb, n=6, respectively; $p < 0.05$). BAL cell counts, particularly lymphocyte counts, were significantly higher in treated compared to untreated patients (16.6 ± 1.8 % and 7.2 ± 1.7 %, respectively; $p < 0.05$).

2.3.2 FeNO in sarcoidosis

In studies enrolling patients with sarcoidosis, four studies used the single flow-rate end tidal NO measurement. One study by Moodley et al. (1999) compared FeNO levels before and after corticosteroid treatment, whereas the remaining three studies used a cross-sectional, correlational design (O'Donnell et al., 1997; Ziora et al., 2004; Wilsher et al., 2005). With the exception of Ziora et al. (2004), all used a chemiluminescence analyzer from the same manufacturer (model LR2000; Logan Research, Rochester, UK) and the same sampling flow-rates (Moodley et al., 1999; O'Donnell et al., 1997; Wilsher et al., 2005). The studies were designed to answer three questions: 1) Is there a significant increase or decrease in FeNO levels in patients with ILD

compared with control subjects; 2) Is FeNO significantly correlated with disease activity as determined by pulmonary function tests, histological findings, radiographic findings, etc., and 3) Is there a change in FeNO in response to treatment?

Regarding the first question, contradictory findings were reported. Three of the four studies reported no significant difference when FeNO values were compared in patients with sarcoidosis and healthy controls, regardless of sarcoid disease activtiy. A summary of their findings follows. O'Donnell et al. (1997) measured FeNO in 10 patients with active sarcoidosis who were not on treatment and 12 healthy non-smoking controls (O'Donnell et al., 1997). The mean FeNO in patients with untreated active sarcoidosis was not significantly different from healthy controls (6.9 ±4.5 ppb vs. 6.6 ±4.0 ppb, p=.60). Wilsher et al. (2005) measured FeNO in 59 sarcoidosis patients. Of these, 4 few subjects were stage 0 (n=3) or stage IV (n=1) and the remainder were stage I to stage III (n= 13, 21, and 14, respectively) (Wilsher et al., 2005). None were on oral or ICS for at least 3 months prior to measurement. The median FeNO in untreated sarcoidosis patients (n=59, 6.8 ppb, range 2.4 – 21.8) was not significantly different from healthy controls (n=44, 6.3 ppb, range 1.6-28). Ziora et al. (2004) measured FeNO in patients with sarcoidosis who had been diagnosed for < 2 years prior to enrollment. Among 27 patients, 21 were diagnosed with active sarcoidosis. None were on oral or ICS prior to measurement. Patients with sarcoidosis had higher FeNO levels than controls, with marginal statistical significance (6.7 ± 0.5, 5.17 ± 0.73, respectively, p= 0.05). When FeNO was compared in patients with active sarcoidosis (n=21) with those with inactive sarcoidosis (n=6), there was no significant difference (p=.124). A comparison of patients with stage I, II and III disease showed no statistical significance (p=.985).

In contrast, Moodley et al. (1999) reported significantly higher FeNO levels in 12 patients with newly diagnosed sarcoidosis compared to healthy controls. At enrollment, when none of the patients were prescribed oral or ICS or immunosuppressive agents, FeNO levels were significantly higher than healthy non-smokers (n=21; 9.8 ± 0.4, 4.1 ± 0.2, respectively, p <.001). After baseline measurement of FeNO, 8 patients were prescribed oral prednisone (40mg/day for 6 wks). FeNO was significantly decreased after treatment (from 9.7 ± 0.5 to 5.9 ± 0.7 ppb, p=0.01). This is the only study examined changes in FeNO before and after steroid treatment in sarcoidosis and the only study to report higher FeNO levels in patients with sarcoidosis.

When correlations were examined between FeNO and other clinical variables, except for D_LCO in one study (Ziora et al., 2004), none of clinical variables examined demonstrated a significant correlation with FeNO, e.g., BAL NO_2, BAL leukocyte count (O'Donnell et al., 1997), BAL CD_4/CD_8 ratio (Moodley et al., 1999), serum ACE (Moodley et al., 1999; O'Donnell et al., 1997), total serum IgE (Wilsher et al., 2005), FEV_1 (Moodley et al., 1999; Wilsher et al., 2005), FVC (Moodley et al., 1999; Wilsher et al., 2005). For D_LCO, both a moderate positive correlation (r= 0.515, p =0.03) (Ziora et al., 2004) and no significant correlation were reported (Wilsher et al., 2005). As noted previously, heterogenous sample characteristics may explain these inconsistent results.

2.3.3 $C_{Alv}NO$ and $J_{AW}NO$ in IPF and sarcoidosis

One abstract was identified that compared $C_{Alv}NO$ and $J_{AW}NO$ in patients with IPF (n=12) and sarcoidosis (n=9), and healthy non-smokers (n=15) (Brindicci et al., 2005). Two patients with IPF were on immunosuppressive therapy; use of ICS was not reported. Whereas there was no

difference in $J_{AW}NO$ among three groups, $C_{Alv}NO$ was significantly higher in patients with IPF (4.0 ± 0.4 ppb) compared with patients with sarcoidosis or healthy non-smokers (2.8 ± 0.3 ppb, *p*=0.0002; 1.4 ± 0.1 ppb, *p*=0.001, respectively) (Brindicci et al., 2005). In this study, patients with sarcoidosis showed higher $C_{Alv}NO$ compared with healthy non-smokers, but its statistical significance was not described.

2.3.4 Exhaled NO in other ILD

The following studies examined diverse groups of patients with mixed types of ILD and reported varying findings. Riley et al. (1997) were the first to measure exhaled NO in patients with various ILD using offline measurement. In this study, 6 patients with ILD had various diagnoses, including sarcoidosis (n=3), scleroderma (n=2), cryptogenic fibrosing alveolitis (n=1). Three out of 6 patients with ILD were on oral prednisone (mean 6.7 mg/day, range 5-10 mg/day). In this study, FeNO was significantly lower in PF patients (5.9 ± 1.6 ppb) compared to those with PPH (13.6 ± 8.3 ppb) and healthy controls (13.6 ± 6.0 ppb).

Moodley & Lalloo (2001) measured FeNO in patients with progressive systemic sclerosis (PSS) with and without interstitial lung disease (PSS-ILD, PSS-non ILD, respectively), and healthy controls. All patients with PSS-ILD were diagnosed with pulmonary hypertension whereas all patients with PSS-non ILD had no clinical evidence of pulmonary hypertension. After the initial diagnosis of PSS-ILD, the patients were placed on a regimen of azathioprine and prednisone for 6 months and FeNO was measured before and after treatment. FeNO was significantly higher in the PPS-non ILD group (9.6 ± 0.7 ppb) compared to the PPS-ILD group (6.2 ± 0.6 ppb, p<.001) and controls (6.3 ± 0.2 ppb, p<.001). In PSS-ILD group, there was no significant change in FeNO after 6 months of azathioprine and prednisone (from 6.2 ± 0.6 ppb to

6.4 ± 0.3 ppb). Similar to findings from Riley et al. (1997) that reported no difference in FeNO between patients with PPH and healthy controls (13.6 ± 8.3 ppb, 13.6 ± 6.0 ppb, respectively), this study showed no significant difference in FeNO of patients with PSS-ILD, those who also diagnosed with pulmonary hypertension, and healthy controls (6.2 ± 0.6 ppb, 6.3 ± 0.2 ppb, respectively). Such results are contradictory to the findings from the study that reported significantly lower FeNO in patients with PSS with pulmonary hypertension compared to PSS without pulmonary hypertension and healthy controls (20 ± 6 ppb, 149 ± 19 ppb, 80 ± 7 ppb, respectively) (S. Kharitonov, Cailes, Black, DuBois, & Barnes, 1997). It appears likely that patients with PSS-ILD had ongoing inflammation evidenced by markedly high neutrophil, lymphocyte and eosinophil counts in BAL fluid, which were higher than counts recorded for PSS-non ILD patients. The presence of pulmonary hypertension among the patients with PSS-ILD may be another factor causing a lower FeNO.

Lehtimaki et al. (2001), measured FeNO at 3 flow-rates (100, 175 and 370 ml/s) in ILD (n=17), and healthy controls (n=57). Then, $C_{Alv}NO$ and $J_{AW}NO$ were calculated. In this study, ILD patients included those diagnosed with hypersensitive pneumonitis (n=7, 1 on oral prednisone) and IPF (n=10, 4 on prednisone and 1 on azathioprine). $C_{Alv}NO$ was significantly higher in ILD patients (4.1 ± 0.3 ppb) compared to healthy controls (1.1 ± 0.1 ppb, $p<0.001$), whereas $J_{AW}NO$ was not significantly different (0.7 ± 0.1 nl/s, 0.7 ± 0.1 nl/s, respectively, $p<0.001$). Because effect of treatment with oral prednisone or azathiprine was not controlled at baseline, it is difficult to interpret whether the changes in $C_{Alv}NO$ related to disease activity or medications. For those who were not on treatment at baseline (n=8), follow-up measurements were performed after 2 months of treatment (allergen avoidance, n=3; oral prednisone, n=4; and azathiprine, n=1). After treatment, there was a significant decrease in $C_{Alv}NO$ (from 4.0 ± 0.6

ppb to 2.4 ± 0.5 ppb, p=0.011) and significant improvement in D_LCO (from 59 ± 6 % to 76 ± 3 %, p=0.006), whereas no change was found in $J_{AW}NO$ (from 0.5 ± 0.1 nl/sec to 0.6 ± 0.1 nl/sec, p=NS). Because the focus of the study was on the effect of treatment in patients, it is difficult to determine separate changes resulting from the disease process. Regarding exhaled NO and clinical data, a significant negative correlation was reported between $C_{Alv}NO$ and D_LCO (r=- .55, p=0.02) and vital capacity (r= -.54, p=0.03). Again, due to heterogenous diagnoses and treatment effects, it is difficult to explain the results.

Girgis et al. (2002) measured FeNO at 4 flow-rates (50, 100, 150, and 200 ml/s) in 20 patients with scleroderma (SSc). The SSc group (n=20) was divided into: 1) SSc-ILD without pulmonary hypertension (n=15), and 2) SSc with pulmonary hypertension (n=5). Patients with primary pulmonary hypertension (n=5) and healthy controls (n=20) were also included. Ten patients in SSc were on treatment with immunosuppressive agents at enrollment. Overall, in patients with SSc, $C_{Alv}NO$ was significantly higher than controls (4.7 ± 0.5 ppb, 4.1 ± 0.3 ppb, respectively; p< 0.001), whereas $J_{AW}NO$ was lower (0.6 ± 0.1 nl/sec, 1.2 ± 0.2 nl/sec, respectively; p= 0.01). Except for a significantly higher $C_{Alv}NO$ in SSc-PH group, there was no significant difference in FeNO levels and $J_{AW}NO$. Again, due to the small and unequal sample size among groups and potential effect from medications, it is difficulty to attribute the cause of these findings. As with the study by Lehtimaki et al (2001), there was a significant negative correlation between $C_{Alv}NO$ and D_LCO (r= -.66, p=.002).

2.4 SIGNIFICANCE & INNOVATION

Compared with single breath exhaled NO measurement (FeNO), multiple flow-rate NO measurement offers the potential to differentiate exhaled NO from the alveolar and airway compartments. Because the contributions of the two compartments can be evaluated separately, this technique may provide more specific information about the site of inflammation. e. g, airways or alveoli (American Thoracic Society, 2005). In sarcoidosis and IPF, inflammation may occur in the early stages of the disease. If exhaled NO could be shown to reflect changes in disease activity or response to therapy, it would provide an easily reproducible means of monitoring change over time. In particular, the multiple breath flow measurements may have utility in this regard. More information regarding the inflammatory and fibrosing changes which are typical in patients with these types of ILD may help to increase understanding of the disease progression and the impact of these changes on patient symptoms. Therefore, we choose to conduct a pilot study in an attempt to further clarify the relationships between exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) and clinical information (dyspnea, pulmonary function tests, disease activity) in patients with sarcoidosis and IPF.

3.0 METHODS

3.1 DESIGN

A comparative descriptive correlational design was used. Three groups of subjects were enrolled: 1) patients with IPF (**IPF**); 2) patients with sarcoidosis (**sarcoidosis**), and 3) healthy non-smokers (**control**). For all groups, FeNO was measured at seven separate flow rates (50, 100, 150, 200, 250, 300, and 400 ml/sec), applied in random order. To provide information about dyspnea, patients with sarcoidosis were asked to complete the UCSD Shortness of Breath Questionnaire (**SOBQ**) (See Appendix A). Clinical data were obtained by reviewing the medical record, e.g., diagnosis, years of diagnosis, past smoking history, past medical history, allergy, current medications, and pulmonary function tests (FEV_1%, FVC%, FEV_1/FVC ratio, D_LCO).

3.2 SITE AND SAMPLE

Subjects with **IPF** (n=15) and **sarcoidosis** (n=43) were recruited from the Dorothy P. & Richard P. Simmons Center for Interstitial Lung Disease located in the Comprehensive Lung Center, University of Pittsburgh Medical Center. **Control** subjects (n=20) were recruited by word-of-mouth and from flyers giving a contact number for study information. Prospective control subjects were assessed for study eligibility using a screening questionnaire over the phone or

face-to-face interview. In all groups, informed consent was obtained from each participant prior to data collection. The study protocol was reviewed and approved by the institutional review board (IRB) in University of Pittsburgh.

3.2.1 IPF and Sarcoidosis

For all patients, the **entry criteria** were: 1) ≥ 18 years of age, and 2) diagnosed with **IPF** or **sarcoidosis**. **IPF** was diagnosed as present when a patient met the following criteria: 1) abnormal HRCT that demonstrated classical features of usual interstitial pneumonia (UIP); 2) surgical lung biopsy for patients without a classical pattern of UIP or < 50 years of age; 3) exclusion of other conditions which might cause similar symptoms (Demedts & Costabel, 2002). For patients > 50 years of age, surgical lung biopsy was not recommended if HRCT demonstrated classical features of UIP.

Sarcoidosis was confirmed by histological evidence of noncaseating epithelioid cell granulomas due to an unknown cause or not the result of local sarcoid reactions (Nunes, Brillet, Valeyre, Brauner, & Wells, 2007). Active pulmonary sarcoidosis (**Active sarcoidosis**) was diagnosed as present when the patient met 3 or more of following criteria over 6-12 weeks: 1) complaints of progressive respiratory symptoms, such as shortness of breath, cough, dyspnea on exertion; 2) exercise desaturation of 10% or greater in arterial oxygen saturation by pulse oximetry (SpO_2) or the need to increase the flow rate of supplemental oxygen during exertion; 3) pulmonary function test results (FVC, D_LCO) that indicated a deterioration of 10% or greater, or 4) evidence of worsening radiographical changes (American Thoracic Society, 1999b).

Inactive sarcoidosis was defined as present when a patient with the diagnosis of s**arcoidosos** did not meet these criteria. For the cases of newly diagnosed **sarcoidosis**, disease

activity was determined at the next follow-up visit which typically occurred 6-12 weeks after the initial visit. Sarcoidosis patients were not enrolled if they had been on oral prednisone or methotrexate prior to clinic evaluation.

3.2.2 Controls

Control subjects (n=20**)** were required to meet the following **entry criteria:** 1) ≥ 18 years of age; 2) non-smoker or stopped smoking more than 6 months ago. **Exclusion criteria** were: 1) prior history of heart, lung, liver, kidney, endocrine, or neurological disorders, e.g., heart attack, COPD, cirrhosis, hepatitis, renal failure, diabetes, thyroid disease, stroke or seizure disorders (self-report); 2) symptoms of a respiratory tract infection $\underline{\leq}$ 1 month prior to the study; 3) use of medications 7 days prior to data collection (self-report).

3.3 MEASUREMENT

3.3.1 Demographic & clinical data

Demographic and clinical data were obtained from the interview and medical record. Demographic data included: age, gender, and ethnicity. Clinical data included: diagnosis, years of diagnosis, past smoking history, current use of inhaled corticosteroids, and pulmonary function tests (FEV_1%, FVC%, D_LCO).

3.3.2 UCSD Shortness of Breath Questionnaire (SOBQ)

The SOBQ was completed by asking subjects to rate the severity of shortness of breath on a 6-point scale (*0=not at all to 5=maximal or unable to do because of breathlessness*) while performing 21 activities of daily living (ADLs) associated with varying levels of exertion (Eakin, Resnikoff, Prewitt, Ries, & Kaplan, 1998). There are 3 additional questions that ask daily life limitations due to shortness of breath, fear of over exertion and fear of shortness of breath. If subjects do not routinely perform the activity, they are asked to estimate the shortness of breath anticipated. The score is obtained by summing responses on the 24 items to form a total score (range 0-120) (Eakin et al., 1998). In psychometric testing, internal consistency was $\alpha = 0.96$ (Eakin et al., 1998). Item-total corrections ranged from 0.49 to 0.87 (Eakin et al., 1998). SOBQ scores were negatively correlated with physiologic measures of disease severity (FEV_1, D_LCO), health related quality of life (Quality of Well-Being) and exercise tolerance (6 minutes walk test) (r= –0.41 to -0.68) and positively correlated with ratings of perceived breathlessness (Borg Scale) and depression (Center for Epidemiological Studies-Depression Questionnaire) (r= 0.37 to 0.45) (Eakin et al., 1998). It takes approximately 5 minutes to complete the SOBQ (See Appendix A).

3.3.3 Exhaled NO consists of FeNO, $C_{Alv}NO$, and $J_{AW}NO$

3.3.3.1 FeNO

FeNO was measured using a chemiluminescence analyzer (model LR1800; Logan Research, Rochester, UK) which has sensitivity to NO from 1 to 5000 parts per billion (ppb) by volume and a resolution of 0.3 ppb adapted for on-line recording of exhaled NO concentration. The

measurement technique was consistent with guidelines in the product manual and standards published by the American Thoracic Society/European Respiratory Society in 2005 (American Thoracic Society, 2005). Subjects were sitting in a chair during the entire measurement. When the machine gave a signal to exhale with a green light, subjects were asked to take a full inspiration from room air and then exhale into the machine with putting their lips around the mouthpiece, which was connected to the analyzer. Subjects were asked to make a full exhalation as long as possible without an episode of inspiration.

Prior to each measurement, a constant flow rate was programmed in the machine prior to provide a different level of resistance when subjects exhale and help them to keep the constant flow rates. To ascertain if subjects maintained a constant flow-rate during exhalation, two procedures were used: 1) continuous monitoring of expiratory flow-rate graphically displayed on the monitor screen, and 2) feedback noise (clicking sound) from the machine in the event that the exhalation flow-rate exceeded the programmed limit. If this occurred, the subject was asked to slow their exhalation and not fight the resistance. When a subject exhaled slower than a programmed constant flow rates, as indicated by the graphical change on the screen, faster exhalation was encouraged.

3.3.3.2 $C_{Alv}NO$, and $J_{AW}NO$

These variables were estimated using the model developed by Tsoukias and George (1998). For each subject, FeNO values were obtained at a total of 7 flow rates (50, 100, 150, 200, 250, 300, 400 mL/s). A random order generator was used in order to apply random ordering for the 7 separate flow-rates (Oxford Brooks University). Between each measurement, subjects rested for 5 minutes. The steps of calculation of $C_{Alv}NO$, and $J_{AW}NO$ were made using the formula by Tsoukias and George (1998).

3.4 DATA COLLECTION PROCEDURES

3.4.1 IPF and sarcoidosis

Data collection occurred during subjects' clinic visit. After informed consent, demographic data and SOBQ (sarcoidosis only) were obtained. FeNO was measured at 7 separate flow-rates. $C_{Alv}NO$, and $J_{AW}NO$ were calculated using measured FeNO values. Clinical data were obtained from electronic medical records. For patients newly diagnosed with sarcoidosis, data collection occurred twice within a 6-12 week interval (time 1 and time 2) if possible depending on scheduled appointments. Depending on disease activity, each subject was allocated to either **Active sarcoidosis** or **Inactive sarcoidosis**.

3.4.2 Controls

Data collection occurred once. After informed consent, demographic data and anthropometric data (height and weight) were obtained. Then FeNO was measured at 7 separate flow-rates. $C_{Alv}NO$, and $J_{AW}NO$ were estimated using measured FeNO values.

3.5 STATISTICAL ANALYSIS

Data were analyzed using the SPSS software version 15.0 (SPSS, Inc.; Chicago, IL, USA) and the SAS system version 9.1 (SAS Institute; Cary, NC, USA). A preliminary analysis, conducted to determine if the distribution met the statistical assumptions (normality, linearity, and

homoscedasticity), indicated major violations in normality assumptions. Therefore, non-parametric statistics were utilized. For clinical parameters, missing data occurred for pulmonary function tests in 4 subjects (3 in sarcoidosis, and 1 in IPF), length of diagnosis in 5 subjects (4 in sarcoidosis, and 1 in IPF), and UCSD-SOBQ in 11 sarcoidosis subjects. Given the small sample size, no imputation technique was attempted.

Descriptive statistics (mean, median, standard deviation, percentage, and 95% confidence intervals of the mean) were used to describe all demographic and clinical variables. Exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) was measured at baseline in all groups (IPF, sarcoidosis, controls). For those newly diagnosed or active sarcoidosis patients who were not yet on treatment, data collection occurred at least twice (time 1 and time 2). In all cases, *p*-value less than 0.05 was considered statistically significant. The statistical analysis for each Specific Aim was as follows:

Specific Aim 1*. To compare FeNO levels measured at 7 flow rates (50, 100, 150, 200, 250, 300, and 400 ml/s) between patients with IPF or sarcoidosis and healthy non-smoking subjects (controls)*

Due to the violation of normality distribution that could not be solved by transformation, *Mann-Whitney U* test was used.

Specific Aim 2*. To compare calculated airway wall NO flux ($J_{AW}NO$) and alveolar NO concentrations ($C_{Alv}NO$) between patients with IPF or sarcoidosis and healthy non-smoking subjects (controls)*

Due to the violation of normality distribution that could not be solved by transformation, *Mann-Whitney U* test was used.

Specific Aim 3. *To examine the relationship between exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) and selected clinical variables (dyspnea, pulmonary function tests) in patients with IPF or sarcoidosis*

Spearman's rank correlation was used.

Exploratory Aim 1. *To compare exhaled NO (FeNO, C_{AlvNO}, $J_{AW}NO$) between patients with active sarcoidosis and patients with inactive sarcoidosis*

Mann-Whitney-U test (non-parametric) was used.

Exploratory Aim 2. *To examine changes in exhaled NO (FeNO, C_{AlvNO}, $J_{AW}NO$) over time (from initial clinic visit to follow-up visit) in patients with active sarcoidosis completed follow-up.*

Wilcoxon signed rank test was used.

4.0 RESULTS

4.1 SAMPLE DESCRIPTION

The study sample consisted of a total of 78 participants (sarcoidosis, n=43; IPF, n=15, controls n=20) recruited from May 2006 to November 2007 (Table 4).

Patients with IPF were significantly older than patients with sarcoidosis and controls, $F(2, 75) = 16.94$, $p < 0.001$. Whereas patients with IPF were all White Caucasian, approximately 40% of patients with sarcoidosis were African American. Length of diagnosis were significantly longer in patients with sarcoidosis than that of patents with IPF, $t(45.61) = 2.80$, $p = 0.007$. In patients with sarcoidosis, a majority of patients were on ICS.

Table 4. Subject Characteristics at enrollment

	Sarcoidosis (n=43)	IPF (n=15)	Controls (n=20)
Gender (% male)	37.2 %	46.6 %	50 %
Age (years)	50.63 ±10.96	66.0±10.58	45.35±10.33
Race White Caucasian African American Asian	 26 (60.5 %) 17 (39.5 %) 0	 15 (100 %) 0 0	 18 (90 %) 0 2 (10 %)
Smoking history Current Never Ex-smoker	 4 (9.3 %) 26 (60.5 %) 13 (30.2 %)	 0 4(26.7 %) 11(73.3 %)	 0 20 (100 %) 0
Length of diagnosis (months)	87.64 ± 104.6	38.0 ± 21.47	N/A
ICS use	30 (69.8 %)	3 (20%)	N/A
PFTs (% predicted) FEV_1 FVC D_LCO	 74.85 ±16.67 75.0 ±13.51 70.33 ±25.03	 68.43 ±19.26 60.71 ±21.07 46.86 ±16.75	N/A
UCSD-SOBQ^	31.16 ±24.50	N/A	N/A

**Note*: Data are missing for: PFTs 4 subjects (Sarcoidosis=3; IPF=1); Length of Diagnosis 5 subjects (Sarcoidosis=4; IPF=1); SOBQ 11 subjects (Sarcoidosis=11)
Definition of abbreviations: ICS, inhaled corticosteroids; PFTs, pulmonary function tests; UCSD-SOBQ, University of SanDiego Shortness of Breath Questionnaire.
^UCSD-SOBQ total scores range from 0 to 120 (higher score= worse shortness of breath).

4.2 EXHALED NO IN IPF

4.2.1 FeNO, $C_{Alv}NO$ and $J_{AW}NO$

In patients with IPF, FeNO was not significantly different from that of controls for the 7 flow rates (Table 5). $C_{Alv}NO$ was significantly higher in patients with IPF compared to controls, *Mann-Whitney* U = 86.0, p= 0.03. There was no significant difference in $J_{AW}NO$ between patients with IPF and controls, *Mann-Whitney* U = 104.0, p= 0.13.

Table 5. Comparison of FeNO at 7 flow rates, $J_{AW}NO$, and $C_{Alv}NO$: IPF and Controls

Exhaled NO	IPF (n=15)	Controls (n=20)	*p*
FeNO (ppb) at 7 flow rates			
FeNO, 50 ml/sec			
Mean ± SD	27.76 ± 16.77	37.02 ± 16.26	0.24
Median	23.10	29.15	
95% CI	18.47 – 37.05	24.41 – 39.63	
FeNO, 100 ml/sec			
Mean ± SD	17.27 ± 10.13	18.02 ± 9.61	0.63
Median	13.70	15.90	
95% CI	11.66 – 22.88	13.53 – 22.52	
FeNO, 150 ml/sec			
Mean ± SD	14.78 ± 8.35	13.13 ± 7.85	0.52
Median	13.60	11.40	
95% CI	10.16 – 19.40	9.46 – 16.80	
FeNO, 200 ml/sec			
Mean ± SD	13.57 ± 8.33	11.91 ± 6.96	0.63
Median	10.50	10.65	
95% CI	8.96 – 18.19	8.66 – 15.17	
FeNO, 250 ml/sec			
Mean ± SD	13.81 ± 9.44	10.75 ± 6.58	0.36
Median	11.8	9.40	
95% CI	8.58 – 19.03	7.67 – 13.82	
FeNO, 300 ml/sec			
Mean ± SD	11.63 ± 5.93	9.40 ± 5.47	0.20
Median	11.8	7.40	
95% CI	8.35 – 14.92	6.84 – 11.96	
FeNO, 400 ml/sec			
Mean ± SD	10.35 ± 5.90	7.88 ± 4.26	0.27
Median	8.40	6.85	
95% CI	7.08 – 13.62	5.89 – 9.87	
$C_{Alv}NO$ (ppb)			
Mean ± SD	8.27 ± 5.41	4.71 ± 2.95	**0.03** *
Median	7.00	3.91	
95% CI	5.28-11.27	3.33-6.09	
$J_{AW}NO$ (nl/sec)			
Mean ± SD	1019.9 ± 681.59	1368.73 ± 759.28	0.13
Median	749.96	1193.40	
95% CI	642.46-1397.36	1013.38-1724.09	

Note: *Mann-Whitney* U test, *$p<0.05$

4.2.2 Correlation with clinical data

Correlations between pulmonary function data and exhaled NO (FeNO, $C_{Alv}NO$, or $J_{AW}NO$) in patients with IPF are summarized in Table 6. In patients with IPF, there were significant negative correlations between $C_{Alv}NO$ and FEV1% (r=-0.58, p=0.03) and FVC% (r=-0.67, p=0.01), but not D_LCO% (r=-0.21, p=NS). With this exception, no significant correlations were seen.

Table 6. Correlation between exhaled NO (FeNO at 50ml/sec, $C_{Alv}NO$, or $J_{AW}NO$) and pulmonary function tests in IPF (n=15)

	FEV_1%	FVC%	D_LCO%
FeNO at 50ml/sec	*r* = - 0.19	*r* = - 0.33	*r* = - 0.04
$C_{Alv}NO$	***r* = - 0.58***	***r* = - 0.67***	*r* = - 0.21
$J_{AW}NO$	*r* = - 0.14	*r* = - 0.10	*r* = - 0.03

Note; Spearman's rank correlation, * p <0.05

4.3 EXHALED NO IN SARCOIDOSIS

4.3.1 FeNO, $C_{Alv}NO$ and $J_{AW}NO$

The first research questions posed by this study involved a comparison of FeNO, $C_{Alv}NO$ and $J_{AW}NO$ measured at 7 flow rates (50, 100, 150, 200, 250, 300, and 400 ml/s). FeNO levels

showed a similar pattern for each of the three groups, i.e., the highest level at the lowest flow rate (50 ml/sec) and lowest level at the highest flow rate (400 ml/sec). For FeNO, no significant differences were found between groups with the exception of a marginally significant difference (p=.05) at 50 ml/sec, with patients with sarcoidosis having a lower mean FeNO compared to controls (Table 7). In addition, patients with sarcoidosis demonstrated a significantly lower $J_{AW}NO$ compared to controls, *Mann-Whitney* U = 293.0, p=0.04. There was no significant difference in $C_{Alv}NO$ between patients with sarcoidosis and controls, *Mann-Whitney* U = 384.5, p=.50.

Table 7. Comparison of FeNO at 7 flow rates, $J_{AW}NO$, and $C_{Alv}NO$: Sarcoidosis and Controls

Exhaled NO	Sarcoidosis (n=43)	Controls (n=20)	*p*
FeNO (ppb) at 7 flow rates			
FeNO, 50 ml/sec			
Mean ± SD	24.22 ± 14.25	37.02 ± 16.26	**0.05**
Median	19.90	29.15	
95% CI	19.84 – 28.61	24.41 – 39.63	
FeNO, 100 ml/sec			
Mean ± SD	15.01 ± 9.09	18.02 ± 9.61	0.15
Median	12.30	15.90	
95% CI	12.21 -17.81	13.53 – 22.52	
FeNO, 150 ml/sec			
Mean ± SD	11.90 ± 6.65	13.13 ± 7.85	0.66
Median	11.0	11.40	
95% CI	9.85 – 13.94	9.46 – 16.80	
FeNO, 200 ml/sec			
Mean ± SD	10.85 ± 6.79	11.91 ± 6.96	0.60
Median	9.40	10.65	
95% CI	8.76 – 12.94	8.66 – 15.17	
FeNO, 250 ml/sec	9.21 ± 5.13	10.75 ± 6.58	0.56
Mean ± SD	7.80	9.40	
Median	7.63 - 10.79	7.67 – 13.82	
95% CI			
FeNO, 300 ml/sec			
Mean ± SD	8.82 ± 4.93	9.40 ± 5.47	0.92
Median	8.10	7.40	
95% CI	7.31 - 10.34	6.84 – 11.96	
FeNO, 400 ml/sec			
Mean ± SD	7.71 ± 4.57	7.88 ± 4.26	0.89
Median	7.50	6.85	
95% CI	6.30 - 9.11	5.89 – 9.87	
$C_{Alv}NO$ (ppb)			
Mean ± SD	5.38 ± 4.06	4.71 ± 2.95	0.50
Median	4.56	3.91	
95% CI	4.14-6.63	3.33-6.09	
$J_{AW}NO$ (nl/sec)			
Mean ± SD	985.95 ± 709.13	1368.73 ± 759.28	**0.04** *
Median	872.46	1193.40	
95% CI	767.71 -1204.18	1013.38-1724.09	

Note: *Mann-Whitney* U test, *$p<0.05$

4.3.2 Correlation with clinical data

Dyspnea measured by the UCSD-SOBQ was available in 32 patients diagnosed with sarcoidosis. There was no significant correlation between UCSD-SOBQ scores and FeNO at 50ml/sec, $C_{Alv}NO$, or $J_{AW}NO$ (r= -0.29, p= 0.11; r= 0.16, p=0.39, and r=-0.25, p=0.17, respectively). Pulmonary function data were available 40 patients diagnosed with sarcoidosis. Correlations between pulmonary function data and exhaled NO (FeNO at 50ml/sec, $C_{Alv}NO$, or $J_{AW}NO$) are summarized in Table 8. In patients with sarcoidosis, there were significant negative correlations between $C_{Alv}NO$ and FVC% (r=-0.51, p=0.001) and D_LCO% (r=-0.41, p=0.008). With this exception, no significant correlations were seen.

Table 8. Correlation between exhaled NO (FeNO at 50ml/sec, $C_{Alv}NO$, or $J_{AW}NO$) and pulmonary function tests in sarcoidosis (n=40)

	FEV_1%	FVC%	D_LCO%
FeNO at 50ml/sec	r= 0.23	r= 0.16	r = 0.16
$C_{Alv}NO$	r = - 0.27	***r* = - 0.51***	***r* = - 0.41***
$J_{AW}NO$	r = - 0.24	r = 0.24	r = 0.18

Note; Spearman's rank correlation, * p <0.05

4.4 EXPLORATORY AIMS

4.4.1 Exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) depending on disease activity

Of 43 patients with sarcoidosis, 8 were diagnosed with active sarcoidosis. In all subjects, multiple flow-rates exhaled NO measurement was obtained prior to treatment. Table 9 summarizes characteristics of patients with active (n=8) and inactive (n=35) disease.

Table 9. Baseline Demographic and Clinical Characteristics: Active & Inactive sarcoidosis

	Sarcoidosis (n=43)		*p*
	Active (n=8)	Inactive (n=35)	
Gender (M/F)	2/6	14/21	0.43
Age (years)	46.00 ± 12.15	51.69 ± 10.57	0.19
Race White Caucasian African American Asian	 3 (37.5%) 5 (62.5%) 0	 23 (70.6%) 12 (34.3%) 0	0.14
Smoking history Current Never Ex-smoker	 1 (12.5%) 6 (75.0%) 1 (12.5%)	 3 (8.6%) 20 (57.1%) 12 (34.3%)	0.48
Length of diagnosis (months)	48.63 ± 73.70	97.71 ± 109.91	0.13
ICS use	7 (87.5 %)	23 (65.7 %)	0.23
PFTs (% predicted) FEV_1 FVC D_LCO	 68.63 ± 24.01 74.38 ± 19.51 74.38 ± 30.36	 76.41 ± 14.31 75.16 ± 11.99 69.31 ± 23.98	 0.41 0.92 0.67
UCSD-SOBQ	38.17 ± 29.77	37.0 ± 23.6	0.53

Note: χ^2-test was used for gender, race, smoking history, and ICS use. Independent sample t-test was used for age, and PFTs. Mann Whitney U test was used for length of diagnosis and UCSD- SOBQ. UCSD-SOBQ total scores range from 0 to 120 (higher score= worse shortness of breath).
Definition of abbreviations: ICS, inhaled corticosteroids; PFTs, pulmonary function tests; UCSD-SOBQ, University of SanDiego Shortness of Breath Questionnaire.

Patients with active and inactive sarcoidosis did not differ significantly in regard to baseline demographics and clinical characteristics. No significant differences was found in FeNO or $J_{AW}NO$ depending on disease activity, except significantly lower $C_{Alv}NO$ in patients with active sarcoidosis (*Mann-Whitney* U=73.0, p=0.04) (Table 10).

Table 10. Comparison of FeNO at 7 flow rates, $J_{AW}NO$, and $C_{Alv}NO$ at baseline: Active & Inactive sarcoidosis

Exhaled NO	Sarcoidosis (n=43)		p
	Active (n=8)	Inactive (n=35)	
FeNO (ppb)			
FeNO, 50 ml/sec	20.48±13.03	25.07±14.55	0.35
FeNO, 100 ml/sec	12.94±6.73	15.49±9.56	0.59
FeNO, 150 ml/sec	10.05±4.33	12.32±7.05	0.49
FeNO, 200 ml/sec	8.34±4.30	11.43±7.17	0.36
FeNO, 250 ml/sec	8.10±3.38	9.47±5.47	0.61
FeNO, 300 ml/sec	7.35±3.58	9.16±5.17	0.43
FeNO, 400 ml/sec	5.65±3.19	8.17±4.74	0.10
$C_{Alv}NO$ (ppb)	3.78±3.34	5.75±4.16	**0.04** *
$J_{AW}NO$ (nl/sec)	927.42±729.65	993.42±714.55	0.75

Note: Mann-Whitney *U* test, * p<0.05

4.4.2 Difference in exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) over time

In 6 patients with active sarcoidosis, exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) was measured before and after initiating treatment (Table 11). With one exception, all were on ICS at enrollment. Follow-up measurements were obtained at intervals ranging from 3 to 23 weeks. During this time interval, each subject was treated with prednisone and methotrexate using various doses.

Table 11. Characteristics of subjects with Active sarcoidosis (n=6)

Gender (Age)	Race	Length of diagnosis (month)	Smoking	ICS	UCSD-SOBQ	PFTs (% predicted)			Follow-up interval (weeks)
						FEV_1	FVC	D_LCO	
# 1 Female (37 years)	African American	17	Never	Yes	42	93	98	64	23
# 2 Male (57 years)	White Caucasian	22	Never	No	3	106	97	118	3,14
# 3 Female (31 years)	African American	6	Current	Yes	88	42	42	48	6
# 4 Female (63 years)	African American	20	Never	Yes	37	58	65	39	7
# 5 Male (51 years)	White Caucasian	36	Never	Yes	13	90	83	109	6, 8
# 6 Female (47 years)	African American	228	Quit (~ 25 years ago)	Yes	46	58	67	63	6, 8

Note: UCSD-SOBQ total scores range from 0 to 120 (higher score= worse shortness of breath).
Definition of abbreviations: ICS, inhaled corticosteroids; PFTs, pulmonary function tests; UCSD-SOBQ, University of SanDiego Shortness of Breath Questionnaire.

When exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$) was compared between before (Time 1) and after treatment (Time 2), there was no significant change in FeNO, $C_{Alv}NO$, and $J_{AW}NO$ (*Wilcoxon Signed Rank Test*, Z = -.676, p=NS, Z = - 1.342, p=NS, Z = -.314, p=NS, respectively).

5.0 DISCUSSION

The major findings in patients with IPF were: 1) FeNO was not significantly different from that of controls for the 7 flow rates; 2) while there was no significant difference in $J_{AW}NO$ compared with controls, $C_{Alv}NO$ was significantly higher, and 3) $C_{Alv}NO$ showed significant negative correlations with FEV_1% and FVC%. In patients with sarcoidosis, major findings were: 1) FeNO at a flow rate of 50 ml/sec was lower than that of controls but the level of significance was marginal (p=.05); 2) in addition, $J_{AW}NO$ was significantly lower in patients with sarcoidosis compared to controls, a similar directional change; however $C_{Alv}NO$ did not differ significantly from controls; 3) $C_{Alv}NO$ showed significant negative correlations with FVC% and D_LCO%.

In addition, the subset of patients with active sarcoidosis (n=8) had significantly lower $C_{Alv}NO$ compared with those with inactive sarcoidosis (n=35), but no significant difference in FeNO and $J_{AW}NO$. In patients with active sarcoidosis who completed follow-up at various intervals (n=6), exhaled NO (FeNO, $C_{Alv}NO$ and $J_{AW}NO$) did not change significantly as a result of treatment.

5.1 IPF

In the present study, FeNO in IPF was not significantly different from controls for all 7 flow rates. Contradictory findings were reported from the one study that exclusively recruited patients

with IPF (Paredi et al., 1999). In 11 patients with IPF, Paredi et al. (1999) found that FeNO was significantly higher than that of healthy non-smokers. They also reported that, although FeNO in patients on oral steroids was significantly lower compared to those not on steroids, FeNO levels in steroid users were still higher than healthy non-smokers (Paredi et al., 1999). When patients enrolled in our study were compared with those enrolled by Paredi et al (1999), there appeared to be a difference in time since the diagnosis of IPF based on the extent of decrement in pulmonary function tests. In the present study, the mean length of time since diagnosis in IPF subjects was 38 months. Although the duration of time since patients were diagnosed with IPF was not mentioned by Paredi et al. (1999), a comparison of the mean FVC (73%) and D_LCO levels (61%) of subjects enrolled in their study with those in the present study (68.4%, 46.8%, respectively), suggests that patients in the present study had more advanced disease. Based on findings from the study by Saleh, et al. (1997), that evaluated inflammation in tissue samples, differences in disease progression in IPF might cause differences in production of FeNO (Saleh, Ernst, Lim, Barnes, & Giaid, 1998). In early stages of IPF, there was increased activation of lung inflammatory cells which stimulated the production of iNOS and ultimately increased the production of NO. Whereas iNOS activity is increased in inflammatory cells, little or no iNOS activity was reported in fibrotic lesions (Saleh, Barnes, & Giaid, 1997). Based on these reports, it can be speculated that FeNO might be increased in early inflammatory stage of IPF. However once fibrosis becomes more predominant, FeNO can be lower than normal. In future studies, it would be important to consider disease stage when comparing findings in patients with IPF.

In patients with IPF, whereas $J_{AW}NO$ did not show any significant difference compared with that of healthy controls, $C_{Alv}NO$ was significantly higher. Our finding suggests there may be inflammations at the alveolar level in patients with IPF. Our finding is consistent with the finding

from the study by Brindicci et al. (2005) that reported significantly higher $C_{Alv}NO$ in IPF compared to healthy non-smoking controls. In our study, a significant negative correlation was found between $C_{Alv}NO$ and FVC% and FEV_1%. This finding supports the potential of inflammatory changes at the alveolar level in patients with IPF that is associated with worsening of restrictive and obstructive changes in their lungs.

5.2 SARCOIDOSIS

In the present study, FeNO at a flow rate of 50 ml/sec was lower than that of controls with a marginal statistical significance. For the remaining flow rates (from 100 ml/sec to 400 ml/sec), there was no significant difference between patients with sarcoidosis and controls. This finding is contradictory to prior studies which reported either no difference (O'Donnell et al., 1997; Ziora et al., 2004; Wilsher et al., 2005) or a significantly higher FeNO (Moodley et al., 1999) in patients with sarcoidosis compared with healthy non-smoking controls. There are several possible reasons for these contradictory findings. First, approximately 70% of patients with sarcoidosis recruited for this study were prescribed ICS, whereas none of the patients in prior studies were on oral or inhaled corticosteroids (Moodley et al., 1999; O'Donnell et al., 1997; Ziora et al., 2004; Wilsher et al., 2005). The potential of ICS to reduce airway inflammation and, hence, exhaled NO levels is well established (S. Kharitonov et al., 2000; S. Kharitonov et al., 1996). However, it is unclear whether use of ICS could cause FeNO levels to decrease to a level lower than that of healthy non-smokers. No literature was identified that examined the change in FeNO in response to the use of ICS in patients with sarcoidosis.

In the study by Moodley et al. (1999), the effect of 6 weeks of oral steroids (30mg/day) was measured in a subgroup of patients with active sarcoidosis (n=8). In this study, FeNO was significantly decreased after the treatment (from 9.7 ± 0.5 ppb to 5.9 ± 0.7 ppb, $p<0.05$), but FeNO values remained higher than those of healthy controls (4.1 ± 0.2 ppb). Yates et al. (1995) reported no change in FeNO in response to oral steroids (30mg/day for 3 days) in healthy children (n=6) whereas FeNO was significantly decreased after asthmatic children were treated with the same regimen (n=7) (D. H. Yates, Kharitonov, Robbins, Thomas, & Barnes, 1995). Based upon the findings from Yates et al. (1995), it appears that FeNO in healthy individuals is not affected by the use of steroids. Given this evidence, it is difficult to explain the mechanisms responsible for the lower FeNO seen in patients with sarcoidosis.

Compared with FeNO levels reported in 4 prior studies (range of mean 6.7- 9.8 ppb; Moodley et al., 1999; O'Donnell et al., 1997; Ziora et al., 2004; Wilsher et al., 2005), mean FeNO in our study was much higher for both patients and controls. It is possible that extraneous factors influenced FeNO e.g. ambient NO, room temperature, etc. To explore this possibility, the relationship between FeNO and ambient NO at the time of measurement was examined. No significant correlation was found between FeNO and ambient NO (*Spearman's rho* = -0.16, p=0.93). Howeever, the ambient NO that each subject was exposed to prior to obtaining study data could also influence FeNO. Influence of this potentially confounding variable was not measured or controlled in this study. It is possible that control subjects had undiagnosed conditions that might have led to an elevated FeNO level. However, controls were carefully screened during recruitment.

Prior studies have attributed changes in FeNO to changes in airway caliber. Because pulmonary function tests were not performed for controls, it is unknown if these parameters were

within normal limits. Mean FEV_1% in patients with sarcoidosis was below normal. Bohadana et al. (2008) reported a significant prediction of FeNO by FEV1%/FVC ratio in 39 healthy non-smokers (t=7.4, p<0.001) (Bohadana, Michaely, Teculescu, & Wild, 2008). Based upon findings from Bohadana et al. (2008), the lower FeNO in sarcoidosis might be due to a mechanical change in airways. Ho et al. (2000) reported a significant positive correlation between FEV_1% and FeNO in steroid naïve patients with asthma (n=12; r=0.9, p<0.001), but no correlation in healthy controls (n=17; r=-0.13, p=0.06) (Ho, Wood, Robson, Innes, & Greening, 2000). Ho et al. (2000) reasoned that decreased airway caliber during bronchoconstriction may increase airflow velocity when patients exhale with a constant flow rate, with a resultant change in FeNO. This could be a possible explanation for patients with sarcoidosis in our study.

$J_{AW}NO$ in patients with sarcoidosis was significantly lower than that of controls. FeNO level measured at lower flow rates reflects more contribution from airway compartment. Therefore, FeNO, which is measured at a flow rate of 50 ml/sec, would also reflect contribution from the airway compartment. The finding of a significantly lower $J_{AW}NO$ in our sample is consistent with the finding of a significantly lower FeNO and can be attributed to the same rationale discussed in the prior paragraph.

In patients with sarcoidosis, whereas FeNO and $J_{AW}NO$ did not show a significant correlation with any pulmonary function test result, $C_{Alv}NO$ showed significant correlations with FVC% and D_LCO%. These findings indicate worsening of restrictive pattern and decreased diffusion capacity that could alter the alveolar concentration of NO. Subtle increase in $C_{Alv}NO$ in patients with sarcoidosis reflects increased alveolar inflammation.

5.3 EXPLORATORY AIM: INACTIVE VS. ACTIVE SARCOIDOSIS

In 43 patients with sarcoidosis, 8 patients diagnosed with active sarcoidosis were recruited before being on treatment. Compared with patients with inactive sarcoidosis (n=35), $C_{Alv}NO$ was significantly lower in active sarcoidosis, whereas other values (FeNO and $J_{AW}NO$) did not show any significant difference. Two possible reasons can be suggested. First, unequal sample size between two groups (8 vs. 25) is problematic in this comparison. Second, a majority of patients with active sarcoidosis (7 of 8, 87.5%) were on ICS at enrollment. Therefore, the influence of ICS might be an explanation for the lower $C_{Alv}NO$ in active sarcoidosis.

To further examine possible differences in our sample, an effort was made to compare the difference in $C_{Alv}NO$ after controlling for the effect of ICS. However, due to limitations in conducting the analysis (e.g., normality violation and unequal sample size), this analysis could only be done for patients with inactive sarcoidosis (23 on ICS, 12 not on ICS) as only 8 patients were enrolled with active sarcoidosis. Contradictory to our assumption, $C_{Alv}NO$ was significantly higher in patients with inactive sarcoidosis who were on ICS compared to those not on ICS (5.94±2.88 ppb vs. 5.38 ± 6.55 ppb, respectively; *Mann-Whitney* U = 71, p=0.02). Due to limitations in this post-hoc analysis resulting from the sample size and large standard deviation, it remains unclear whether the effect of ICS on $C_{Alv}NO$ is similar in patients with active sarcoidosis. The effect of ICS on $C_{Alv}NO$ in sarcoidosis needs further exploration.

For patients with active sarcoidosis who completed follow-up, in all 6 cases, exhaled NO (FeNO, $C_{Alv}NO$ and $J_{AW}NO$) did not change significantly as a result of treatment with prednisone and methotrexate. Our finding is not consistent with the report by Moodley et al. (1999) that showed a significant decrease in FeNO after 6 weeks of treatment with oral prednisone (40 mg) in 8 newly diagnosed patients with sarcoidosis. Potential effects of ICS,

heterogenous patient characteristics, various treatment regimens and follow-up intervals might be possible explanations for such inconsistency between two studies.

Although our sample was composed of patients who met ATS criteria for the diagnosis of sarcoidosis, our findings must be interpreted cautiously for several reasons including an unequal sample size (8 active vs. 35 inactive), the variety in follow-up intervals and large individual variance in exhaled NO (FeNO, $C_{Alv}NO$, $J_{AW}NO$). Heterogeneous demographic and clinical characteristics in patients with active sarcoidosis may also contribute to our findings.

5.4 LIMITATIONS

This study had a number of limitations. First, the sample size was small despite a relatively long recruitment interval (18 months) in an ILD specialty clinic. Second, exhaled NO levels are known to vary due to multiple reasons, e.g. individual difference, different analyzer, etc (American Thoracic Society, 2005; Borrill et al., 2006). In our study, large individual variances in exhaled NO were consistent across three subject groups. Although attempts have been made to standardize measurement, there are still many factors that potentially influence exhaled NO measurement that have not been clarified. The goal of recruiting a larger sample would require a multi-center study. Despite the benefit in terms of available subjects, using multiple sites would introduce new challenges. Exhaled NO measurement could vary depending on equipment made by different manufacturers, slightly different calibration and measurement ranges. Therefore, for feasibility and consistency of measurement, obtaining exhaled NO measurement with the same equipment in the same laboratory environment is the safest choice. In the present study, all measurements were obtained in the same laboratory using a single analyzer. To maintain

consistency, all measures of exhaled NO were obtained in the same space during the daytime (between 9am to 5pm). In spite of this effort, there was variability in room temperature and distribution of ambient air due to failure of the air conditioning system in the examination area for 3 weeks of data collection.

In this study, we did not exclude subjects who were on ICS. Exhaled NO levels decrease in response to the use of inhaled corticosteroids (S. A. Kharitonov, Yates, Chung, & Barnes, 1996; Smith et al., 2005). Almost 70% of patients with sarcoidosis and 20% with IPF were on ICS at enrollment, which makes it difficult to compare findings of the present study with previous studies. In prior studies, none of the subjects were on inhaled or oral corticosteroids at enrollment (Moodley et al., 1999; O'Donnell et al., 1997; Ziora et al., 2004; Wilsher et al., 2005). We did not apply the use of ICS as exclusion criteria because it would have limited enrollment as inactive patients managed in our clinic are commonly prescribed ICS for symptom management of reactive airways disease. It would be preferable to exclude patients on ICS to improve understanding on mechanisms of exhaled NO production in these population. However, clinically, airway hyperrreactivity is common in many patients with sarcoidosis (Aggarwal, Gupta, Chandrasekhar, & Jindal, 2004; Manresa Presas, Romero Colomer, & Rodriguez Sanchon, 1986; Marcias, Ledda, Perra, Severino, & Rosetti, 1994; Shorr, Torrington, & Hnatiuk, 2001) and ICS are commonly prescribed to manage this problem. Therefore, accomplishing this goal would be difficult.

In healthy non-smokers, subject screening was conducted based upon self-reported health information. Therefore, there is always possibility to miss important information which potentially influence exhaled NO levels, e.g. smoking, alcohol use, and unknown allergy. No

pulmonary function tests data was obtained in healthy non-smokers, which might be helpful to understand the relationship between exhaled NO levels and pulmonary function tests results.

For exploratory aims, a limited numbers of subjects were available. Because many individuals with active sarcoidosis were already on treatment, it was difficult to recruit subjects with active sarcoidosis prior to initiating treatment regimen. Obtaining timely follow-up data was a challenge due to frequent cancellation of regular follow-up visit by patients. Given the small sample and variable follow-up intervals, it is premature to hypothesize the direction of change in exhaled NO in sarcoidosis depending on disease activity or response to treatment regimen.

5.5 IMPLICATIONS FOR FUTURE RESEARCH

Findings from the present study are premature to determine the usefulness of exhaled NO monitoring in patients with sarcoidosis or IPF. Evaluation of the usefulness of exhaled NO in IPF and sarcoidosis would require a substantially larger sample in order to examine subgroup differences. In these disease conditions, the pattern of monitoring disease progression relies heavily rely on patients' individual symptoms, pulmonary function tests, and radiographic images. Therefore, in order to obtain measurements at uniform intervals it would be necessary to provide incentives for study participation. Additional well-controlled studies are needed to determine the benefits of this monitoring technique in this group of patients.

This study used 7 flow rates that ranged between 50 ml/sec and 400 ml/sec to estimate $C_{Alv}NO$ and $J_{AW}NO$. The range of flow rates is set differently for different analyzers and there is no standard regarding the number of breaths and range of the flow rates. Multiple flow rates NO measurement involves more time than a single breath measurement. Some low or high flow

rates, e.g. 50 ml/sec or 400 ml/sec, required more effort from patients to maintain a constant flow rate. If similar $C_{Alv}NO$ and $J_{AW}NO$ values can be estimated with using fewer breaths and less challenging flow rates, it will decrease time spent in measurement and subject burden.

APPENDIX

UCSD Medical Center Pulmonary Rehabilitation Program
Shortness of Breath Questionnaire

Instructions: For each activity listed below, please rate your breathlessness on a scale between zero and five where 0 is not at all breathless and 5 is maximally breathless or too breathless to do the activity. If the activity is one which you do not perform, please give your best estimate of breathlessness. Your response should be for an "average" day during the past week. Please respond to all items. Read the two examples below then turn the page to begin the questionnaire.

0 Not at all
1
2
3
4 Severely
5 Maximally or unable to do because of breathlessness

Example 1:

How short of breath do you get while:
Brushing teeth ... 0 1 2 3 4

Harry has felt moderately short of breath during the past week while brushing his teeth and so circles a three for this activity

---

Example 2:

How short of breath do you get while:
Mowing the lawn ... 0 1 2 3 4 5

Anne had never mowed the lawn before but estimates that she would have been too breathless to do this activity during the past week. She circled a five for this activity.

ID :_____ Date:_____

0 Not at all
1
2
3
4 Severely
5 Maximally or unable to do because of breathlessness

How short of breath do you get:

1. At rest…	0	1	2	3	4	5
2. Walking on a level at your own space…	0	1	2	3	4	5
3. Walking on a level with others your age…	0	1	2	3	4	5
4. Walking up a hill…	0	1	2	3	4	5
5. Walking up stairs…	0	1	2	3	4	5
6. While eating…	0	1	2	3	4	5
7. Standing up from a chair…	0	1	2	3	4	5
8. Brushing teeth…	0	1	2	3	4	5
9. Shaving and/or brushing hair…	0	1	2	3	4	5
10. Showering/ bathing…	0	1	2	3	4	5
11. Dressing…	0	1	2	3	4	5
12. Picking up and straightening…	0	1	2	3	4	5
13. Doing dishes…	0	1	2	3	4	5
14. Sweeping/ vacuuming…	0	1	2	3	4	5
15. Making bed…	0	1	2	3	4	5
16. Shopping…	0	1	2	3	4	5
17. Doing laundry…	0	1	2	3	4	5
18. Washing car…	0	1	2	3	4	5
19. Mowing lawn…	0	1	2	3	4	5
20. Watering lawn…	0	1	2	3	4	5
21. Sexual activities…	0	1	2	3	4	5

How much do these limit you in your daily life?

22. Shortness of breath…	0	1	2	3	4	5
23. Fear of "hurting myself" by overexerting…	0	1	2	3	4	5
24. Fear of shortness of breath…	0	1	2	3	4	5

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