A Longitudinal Evaluation of Novel Outcome Measures in Chronic Obstructive Pulmonary Disease

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A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Background
Chronic obstructive pulmonary disease (COPD) is a multimorbidity disease associated with increased risk of cardiovascular events, arterial stiffness and changes in body composition, potentially features of premature ageing and frailty. The aim of this thesis was to assess the change in aortic pulse wave velocity (PWV) over two years and its contributing factors in COPD. In addition, this thesis also aimed to examine the concept of frailty in patients with COPD and its change over a two-year follow-up.

Methods
Aortic stiffness and frailty were assessed cross-sectionally in 500 patients with COPD and 150 comparators using aortic PWV and frailty index (FI). Other assessments included spirometry, body composition, handgrip strength, Timed Up and Go test (TUG) and systemic inflammatory biomarkers. After two years, 143 consecutive patients were reassessed to examine the changes in aortic PWV and FI.

Results
In the cross-sectional data, patients with COPD had greater aortic PWV than comparators similar in age, gender and BMI, independent of traditional risk factors. After two years, the patients demonstrated a significant increase in aortic stiffness, independent of age, lung function, blood pressure and inflammation. In addition, a subset of patients was identified to have an accelerated aortic stiffness by 1.6 m/s.

At the initial visit, the patients were more frail than comparators similar in age and gender. After two years, the patients had an increase in the FI, independent of lung function and inflammation. The progression of frailty was related to loss of muscle mass and strength, and prolonged TUG time.

Conclusion
The longitudinal findings of this thesis suggest that COPD is associated with a rapid increase in aortic stiffness, independent of conventional risk factors. Frailty is a clinical feature of COPD and its progression is dependent on loss of musculoskeletal mass and strength and prolonged TUG time.

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List of Abbreviations

6MWD	Six Minute Walk Distance
AAT	α 1 Anti-Trypsin
AIx	Augmentation Index
ACCT	Anglo-Cardiff-Cambridge Trial
ACE	Angiotensin Converting Enzyme
AGEs	Advance Glycation End products
ARIC	Atherosclerosis Risk in Communities
AP	Augmentation Pressure
ARCADE	Assessment of Risk in Chronic Airways Disease Evaluation
ATS	American Thoracic Society
BIA	Bioelectrical Impedance
BLSA	Baltimore Longitudinal Study of Ageing
BMD	Bone Mineral Density
BMI	Body Mass Index
BPH	Benign Prostatic Hypertrophy
Bpm	Beats per minute
BTS	British Thoracic Society
BUA	Broadband Ultrasound Attenuation
CABL	Cardiovascular Abnormalities and Brain Lesions
CAD	Coronary Artery Disease
cf-PWV	Carotid-femoral pulse wave velocity
CAT	COPD Assessment Test
CGA	Comprehensive Geriatric Assessment
CHF	Congestive Heart Failure
CHRNA	Cholinergic Receptors Nicotinic Alpha
CHS	Cardiovascular Health Study
CI	Confidence Interval
CO	Cardiac Output
COPD	Chronic Obstructive Pulmonary Disease
CRF	Chronic Renal Failure
CRP	C-reactive protein
CT	Computed Tomography
CUBA	Calcaneal Ultrasound Bone Analyser
CVA	Cerebrovascular accident
CVD	Cardiovascular Disease
DJD	Degenerative Joint Disease
DXA	Dual X-ray Absorptiometry
DBP	Diastolic Blood Pressure
ECG	Electrocardiography
ECLIPSE	Evaluation of COPD Longitudinally to Identify Predictive
ERS	European Respiratory Society
FEV$_1$	Forced Expiratory Volume in 1 Second
FDG-PET	F18-Fluorodeoxyglucose positron emission tomography
FFM	Fat Free Mass
FFMI	Fat Free Mass Index
FI	Frailty Index
FM	Fat Mass
FM:FFM	Fat Mass to Fat Free Mass Ratio
FMI	Fat Mass Index
FMD	Flow Mediated Dilatation
FVC	Forced Vital Capacity
FEV$_1$:FVC	The ratio of FEV$_1$ to FVC
GFR	Glomerular Filtration Rate
GOLD	Global initiative for Chronic Obstructive Lung Disease
GORD	Gastro-Oesophageal Reflux Disease

GWAS	Genome-Wide Association Studies
HR	Heart Rate
HRQoL	Health Related Quality of Life
HTN	Hypertension
ICC	Intraclass Correlation Coefficient
ICS	Inhaled Corticosteroid
IL-6	Interleukin 6
IREB	Iron-Responsive Element Binding protein
kg	Kilogram
L	Litres
LABA	Long-Acting Bronchodilator Agonists
LAMA	Long-Acting Muscarinic Antagonists
LV	Left Ventricle
MAP	Mean Arterial Pressure
MESA	Multi-Ethnic Study of Atherosclerosis
mg	Miligram
MI	Myocardial Infarction
ml	Millilitre
mMol	Millimole
Min	Minute
mmHg	Millimetre(s) of mercury
mmol/L	Millimoles/litre
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
mMRC	modified Medical Research Council
NICOM	Non-Invasive Cardiac Output Measurement
NO	Nitric Oxide
OSA	Obstructive Sleep Apnoea
PI	Phase Inhibitor
PP	Pulse Pressure
PSU	Pseudouridine
PWV	Pulse Wave Velocity
ROS	Reactive Oxygen Species
SABA	Short-Acting Bronchodilator Agonists
SBP	Systolic Blood Pressure
SD	Standard Deviation
SGRQ	St George's Respiratory Questionnaire
SPSS	Statistical Package for the Social Sciences
SV	Stroke Volume
TNF-α	Tumour Necrosis Factor
TORCH	Towards a revolution in COPD health
TUG	Timed Up and Go
VSMC	Vascular Smooth Muscle Cell
WHO	World Health Organisation

Chapter One

1.1 Introduction

Chronic obstructive pulmonary disease (COPD) is a persistent low grade inflammatory disease with important extrapulmonary disorders and associated consequences (GOLD, 2014). The origin of inflammation is not fully understood, but it is likely to be multifactorial and contributes to the development of systemic manifestations (Barnes and Celli, 2009). The accepted systemic manifestations include loss of skeletal muscle mass and function, physical inactivity, osteoporosis, insulin resistance, diabetes mellitus, potential premature vascular ageing and enhanced cardiovascular risk and psychological problems including anxiety and depression (Barnes and Celli, 2009, Hanania et al., 2011).

Cardiovascular disease (CVD) is of particular importance in patients with COPD as several studies have shown increased cardiovascular morbidity and mortality in COPD independent of tobacco exposure (Hole et al., 1996, Engstrom et al., 2001). A number of studies have found a strong association between impaired lung function and CVD. A large cohort study showed reduced lung function is associated with a 3-fold increased risk of cardiovascular morbidity and mortality (Tockman et al., 1995). The mechanism(s) underlying increased cardiovascular risk is unclear, but there is growing evidence, which supports the theory of a chronic low grade inflammation in COPD could be potentially implicated the pathogenesis of the lung induced CVD, which will be discussed in the literature review (Sin and Man, 2005). Systemic inflammation is also involved in the pathogenesis of atherosclerosis and has been related to arterial stiffness in patients with COPD (Barr et al., 2012, Sabit et al., 2007). Arterial stiffness is an integrated measure of the vasculature, which reflects several pathological mechanisms including elastin degradation, endothelial dysfunction, increased oxidative stress and atherosclerosis (Maclay et al., 2012, Ives et al., 2013, Barr et al., 2012).

In the general population, increased central arterial stiffness is a major independent risk factor for CVD as well as in subjects at increased cardiovascular risk (Vlachopoulos et al., 2010). Aortic pulse wave velocity (PWV) is the accepted gold standard non-invasive assessment of arterial stiffness and is predictive of adverse cardiovascular outcomes (Laurent et al., 2006). In COPD, Sabit et al. (2007) and others demonstrated increased aortic stiffness in patients with COPD, which was related to the severity of airflow obstruction and systemic inflammation compared with controls similar in age and gender. Increased aortic stiffness was associated aortic calcification, asymptomatic left ventricle dysfunction, myocardial injury and emphysema severity (Bolton et al., 2011, Patel et al., 2013, Sabit et al., 2010a, Maclay et al., 2009). Taken together such evidence suggests that increased central arterial stiffness may reflect various risk factors that have been implicated in the pathology of CVD and could be a mediating factor in the increased cardiovascular risk associated with COPD. Therefore, it will be a valuable biomarker of monitoring and early recognition of patients with COPD who are at greater risk for cardiovascular events.

The change in arterial stiffness over time has not been evaluated in COPD so far as all the studies have only been cross-sectional. Thus, a longitudinal COPD population study is important to increase understanding of the rate at which arteries stiffen and generate information on possible cause(s) of arterial stiffening and the interaction of central arterial stiffness with other cardiovascular risk factors in COPD.

The presence of co-morbidities in COPD may resemble the accumulation of deficits with biological ageing where bodily physiological systems have become frail to resist stressors. Such deficits may represent frailty and the presence of multiple deficits in COPD be a result of premature ageing. Frailty reflects the impairment in multiple interrelated physiological systems, which range from subcellular levels to the organismic level (Fulop et al., 2010). Frailty has been established in several chronic

diseases including CVD, and the similarities between the accumulation of deficits in frailty and multiple comorbidities in COPD suggests that frailty is likely to be common in such chronic disorders (Newman et al., 2001). However, frailty is complex and not well defined, and may manifest a number of clinical changes indicating abnormal homeostasis (Rockwood et al., 2005). Therefore, frailty may quantify the risk of morbidity, mortality and healthcare and may be a useful concept to decribe heterogeneity and multimorbidity in COPD.

A number of epidemiological studies have shown that frailty is an independent predictor of adverse outcomes, disability, poor health status and death. Therefore, this study will examine the concept of frailty and its progression in COPD using the Comprehensive Geriatric Assessment short form (Rockwood et al., 2005).

Healthcare providers frequently, focus on just a single disease and overlook other comorbidities, which may have debilitating effects on health status and national resources. The management of patients with COPD is usually focused in primary care, where other comorbidities and needs are neglected. The traditional approach of treating individual components of multimorbidity in isolation is unlikely to produce optimal clinical outcomes for patients. However, an assessment of frailty provides a simple and holistic overview of multimorbidity that may identify risk factors at an early stage and enhance appropriate management decisions with a better health status for the patient and enhanced cost effective healthcare. Thus, this study would provide a comprehensive assessment of patients with COPD and potentially extend risk assessment and early recognition of CVD, which is currently underestimated, and may create an opportunity for therapeutic interventions in the future or developing preventative strategies.

1.2　COPD Definition

In the past, COPD has been defined as an irreversible disease, but now the optimistic accepted definition and most widely used is proposed by the Global Initiative for Obstructive Lung Disease (GOLD) for COPD is " a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases" (GOLD, 2014).

1.3　Diagnosis and Classification

Currently, there is no single diagnostic test for COPD; therefore, diagnosis is based on exposure to risk factors, such as cigarette smoke, domestic and occupational smoke and clinical symptoms including dyspnoea, chronic cough and sputum production. The diagnosis is usually confirmed by spirometry test that shows the presence of airflow limitation defined using the following parameters:

Forced Expired Volume in One Second (FEV_1): The volume of air expired in the first second of maximal expiration after maximal inspiration. This measures how quickly the lungs can be emptied.

Forced Vital Capacity (FVC): The maximum volume of air that can be exhaled during a forced manoeuvre.

FEV_1:FVC: The ratio of FEV_1 to FVC gives a clinically useful index of airflow obstruction.

The diagnosis of COPD is made when FEV_1:FVC is less than 0.70. Spirometry may be used to classify the severity of airflow obstruction based on the predicted value of FEV_1 as proposed by GOLD (Table 1.1). Patients with COPD may also be classified according to breathlessness (Table 1.2) or by the frequency of chest exacerbations per year (GOLD, 2014).

Recently, GOLD (2014) produced a new classification diagram to comprehensively assess patient's illness severity, incorporating patient's breathlessness or health status, alongside airflow obstruction and the number of exacerbations (Figure 1.1).

Table 1.1: Spirometric classification of patients with COPD according to (GOLD)

Stage	Severity of COPD	FEV_1:FVC	FEV_1 % Predicted
I	Mild	<0.7	>80
II	Moderate	<0.7	50-80
III	Severe	<0.7	30-50
IV	Very Severe	<0.7	<30

Abbreviations: FEV_1= forced expired volume in one second; **FVC=** forced vital capacity.

Table 1.2: Medical Research Council (MRC) dyspnoea scale (Fletcher et al. 1959)

Grade	Description of Breathlessness
0	I only get breathless with strenuous exercise.
1	I get short of breath when hurrying on level ground or walking up a slight hill.
2	On level ground, I walk slower than people of the same age because of breathlessness, or have to stop for breath when walking at my own pace.
3	I stop for breath after walking about 100 yards or after a few minutes on level ground.
4	I am too breathless to leave the house, or I am breathless when dressing.
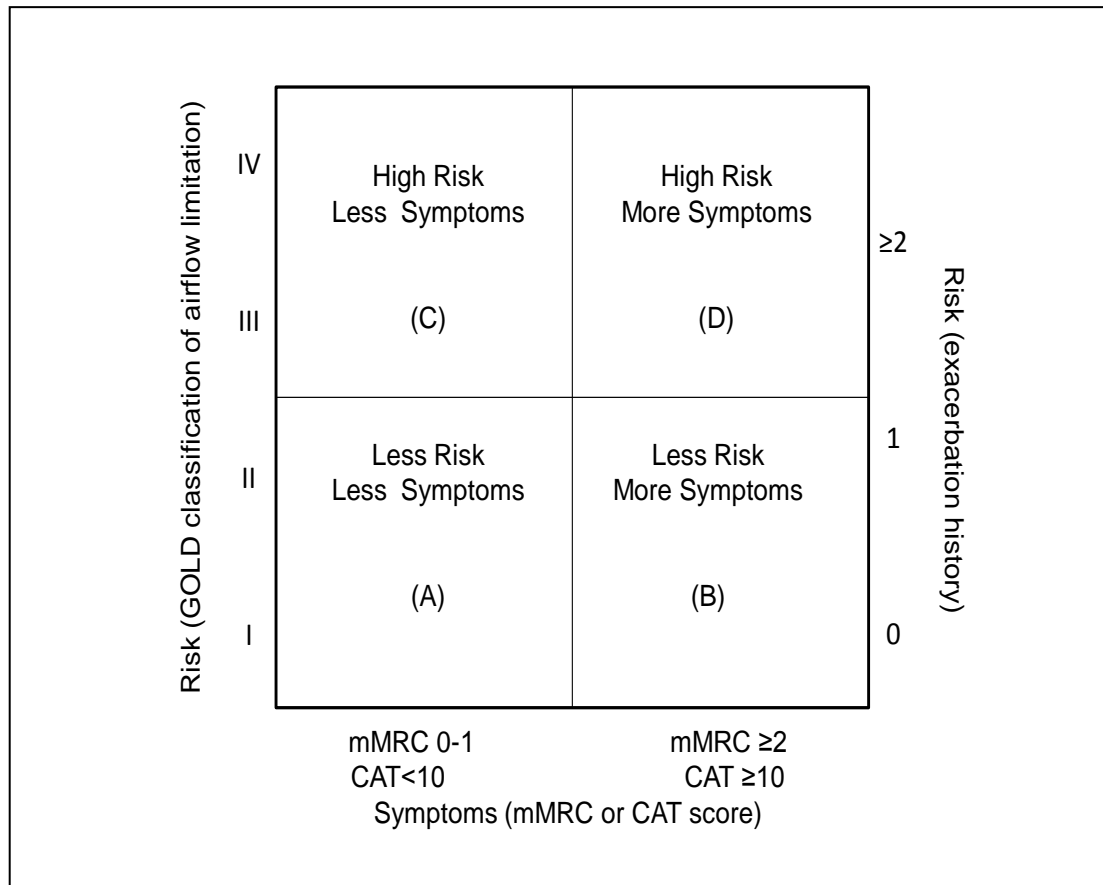
Figure 1.1: New GOLD (2014) classification

Abbreviations: mMRC= Modified Medical Research Council; **CAT=** the COPD Assessment
Test.

1.4 Aetiology

Smoking is the most widely accepted cause of COPD; it is suggested that approximately 50% of smokers will develop COPD (Lundback et al., 2003). Smoking results in a deterioration in the rate of FEV_1, by a factor of three, and an increase in mortality related to the total number of cigarettes smoked. It is accepted that reducing smoking will gradually diminish the prevalence of COPD (Doll et al., 2004, Xu et al., 1994). For this reason, the term 'preventable' has been introduced in the definition of COPD in the joint ATS/ERS definition. Tobacco smoking causes changes in the structure and function of the lungs before clinical signs of airway obstruction become apparent. These changes can be detected early in the disease process by body plethysmography and diffusing capacity measurement with routine spirometry (Perez-Padilla et al., 1996). Although, smoking is not the only predisposing factor, the contribution of environmental factors is still unknown, their effect is still minimal compared to those of smoking (Salvi and Barnes, 2009). In developing countries, a major contributor to the prevalence of COPD in non-smoking subjects is the burning of biomass fuel in poorly ventilated areas (Salvi and Barnes, 2009). Other risk factors for COPD include occupational exposure (noxious fumes, coal and silica dust, etc.), airway hyperresponsiveness, poor nutrition, deficiencies in anti-oxidant vitamins, childhood infections and socioeconomic class (Yach et al., 2004).

Airway hyperresponsiveness is considered one of the risk factors that causes an accelerated decline in lung function in individuals with non-specific airway hyperreactivity and with a smoking history. Non-specific airway hyperreactivity is inversely related to FEV_1 and may predict a decline in lung function (Lundback et al., 2003). The possible role of airway hyperresponsiveness as a risk factor for the development and progression of COPD in subjects who smoke is still unknown (Silverman et al., 1998). Moreover, airways hyperreactivity may be a consequence of airway inflammation observed with the development of smoking-related chronic

bronchitis. This may contribute to airway remodelling, leading to a more fixed obstruction, as seen in patients with COPD.

While around 50% of smokers develop COPD, this suggests that some individuals may have a genetic propensity for the development of COPD (Demeo et al., 2004, Lundback et al., 2003). Familial clustering of COPD is well recognised, and compromise in lung function in the general population has a heritability of approximately 50%. Several genes have been identified that may predispose to the development of COPD. The most extensively studied genetic abnormality is α-1 antitrypsin deficiency. This is thought to be accountable for less than 2% of cases of COPD (Tarjan et al., 1994). AAT decelerates serine proteases and is recognised to have a prominent selective action against neutrophil elastase. It is a member of a larger family of structurally unique serine protease inhibitors known as serpins, which have been implicated in other disease pathogenesis including angioedema, neurodegenerative diseases and coagulation abnormalities (Hersh et al., 2004, Stoller and Aboussouan, 2005). The AAT gene is located on the long arm of chromosome 14, and more than 90 different phenotypes have been identified according to their electrophoretic properties, giving rise to the phase inhibitor (Pi) nomenclature (Sandford and Silverman, 2002). Approximately 24 variants of the AAT molecule have been identified and the most widely known allele variant is PiM, found in 90% of the people, which regulates the activities of AAT. Conversely, the most abnormal alleles are S mutation and Z mutation. They are associated with a reduction in the plasma level of AAT and function. PiZ is the most anomalous genotype, accounted for 16% of anti-protease activity and patients with this gene have a progressive decline in FEV_1 and unrelated to smoking effect. However, symptoms may vary between individuals, ranging from asymptomatic in some, to fatal in others (Hackett et al., 2003).

Recently, genome-wide association studies (GWAS) make it possible to study people without prior knowledge of their disease, which significantly impacts on the

understanding of COPD. In GWAS pooled results, three novel genes have been identified to be associated with increased susceptibility of COPD. Pillai and colleagues (Pillai et al., 2009) found genome-wide significant associations between COPD and the cholinergic receptors nicotinic alpha (CHRNA3 and CHRNA5) and iron-responsive element binding protein 2 (IREB2) regions on chromosome 15. Although these results are interesting and envisage for identifying possible subjects prone to the disease, a disease like COPD, a multisystem disorder, could not be linked to just an abnormality in one gene, and further work need to be done to comprehensively understand pathways that link human tissue and system that are involved in COPD (Barabasi, 2007, Barabasi et al., 2011).

1.5 Epidemiology of COPD

The prevalence of COPD increases with ageing. Although this disease is preventable and treatable, a number of people who experience shortness of breath and limited physical function remain undiagnosed (Sutherland and Cherniack, 2004). This is attributed to the understanding and interpretation of these symptoms by these individuals (i.e. part of the ageing process or common symptoms of smoking). In addition, obtaining an estimate of the true prevalence of COPD largely relies on the instruments used, the population studied and the spirometric criteria for airway obstruction (Halbert et al., 2003). This heterogeneity has contributed to an underestimation of the true figure worldwide. The prevalence worldwide varies depending on the spirometric criteria for COPD. In an international study, Burden of Obstructive Lung Disease, the prevalence of COPD among the population aged over 40 was estimated at 10±4.8% (Buist et al., 2007). However, this is likely to underestimate the actual figure of people having the condition, since most people do not seek medical help until the symptoms of COPD have become quite advanced. In the UK, it is estimated that the number of people who have been diagnosed with COPD is around one million. However, this number is likely to underestimate the true prevalence (Halbert et al., 2006). In 2004, COPD was a principal cause of death in England and Wales (National Statistic, 2004). Currently, COPD is cited as the fifth leading cause of death in the UK and the fourth worldwide (National Statistic, 2004, Pauwels et al., 2001). Surprisingly, despite advances in the management of COPD, the mortality from COPD continues to increase, while other leading causes of death, such as cancer and cardiovascular disease, continue to decline. By 2020, COPD is expected to be the third leading cause of death worldwide (GOLD, 2014).

1.6 Pathogenesis of COPD

COPD is associated with an abnormal inflammatory response of the lungs to the inhalation of exogenous noxious stimuli. This involves various types of inflammatory cells and mediators. The inflammatory response extends to both the lungs and other body systems. Although smoking is considered to be the primary trigger to elicit this inflammatory response, only a proportion of smokers develop COPD (Gottlieb et al., 2001). The susceptibility to developing COPD is still unknown, however, other contributing factors, such as genetic and immune system abnormalities may play a role in the development of COPD (Feghali-Bostwick et al., 2008, Houben et al., 2009). The inhalation of toxic particles and gases activates an abnormal inflammatory response that is thought to play an integral role in mucous hypersecretion, fibrosis and proteolysis, which in consequence leads to chronic bronchitis, emphysema and airway obstruction (Gottlieb et al., 2001, He et al., 2010, Lundback et al., 2003). The extent of airway inflammation relates to the severity of COPD and exists even many years after smoking cessation (Agusti and Faner, 2012, Barnes and Celli, 2009).

Despite the existence of technology and extensive research on the pathogenesis of COPD, the exact mechanism of central and peripheral airway inflammation remains unclear. Cigarette smoke, other exogenous noxious gases and infectious organisms are believed to activate alveolar macrophages, bronchial epithelial cells and other cellular elements in the airways of genetically susceptible individuals (Marin et al., 2012). Once activated, these cells produce a variety of chemokines and cytokines including macrophage chemotactic protein-1, which lead to the pulmonary parenchyma and vasculature abnormalities and airway remodelling (Keatings et al., 1996). Compelling evidence suggests that the neutrophil is the key effector cell in COPD (Mannino et al., 2006). Increased neutrophil in the bronchial glands suggests a development of mucus hypersecretion and tissue damage (Rovina et al., 2009). In the peripheral airways, the inflammatory cell infiltrate consists predominantly of

mononuclear cells and macrophages (Tsoumakidou et al., 2009). The macrophages count increase in the airways and lung parenchyma, in parallel with the increase in severity of the disease (Tsuda et al., 2009).

Another potential mechanism involved in the pathogenesis of COPD is of proteases/antiprotease imbalance. Patients with COPD demonstrated a high prevalence of matrix melloproteinase (MMP) 9 in their skin (Maclay et al., 2012). This suggests that there is a breakdown of lung connective tissue and parenchyma.

A further mechanism that involves the pathogenesis of COPD is oxidative stress. Oxidative stress has also been attributed a central role in the pathogenesis of COPD, because, in addition to causing direct injury to the respiratory tract, oxidative stress triggers and exacerbates inflammation, protease/antiprotease imbalance and apoptosis (Barnes et al., 2003).

1.7 Comorbidity in COPD

COPD is not just a chest disease, but it is associated with extrapulmonary comorbidities. Comorbidities and systemic manifestations are features of COPD and commonly used interchangeably. However, they are really two different entities. Comorbidities are chronic diseases that associated with COPD, while the systemic manifestations are consequences of COPD. Nevertheless, both comorbidities and systemic consequences worsen the patient's condition.

A number of comorbidities can present in patients with COPD and recently Divo et al. (2012) represented them as a "Comobidome" (Figure 1.2). Comorbidities share common pathophysiological mechanisms with COPD and this accelerates the disease progression and increases hospitalisation and mortality rate (Divo et al., 2012). A number of factors may link these comorbidities to COPD including genetic predisposition, physical inactivity and inflammation (Barnes and Celli, 2009). Therefore, COPD is currently considered as a complex heterogeneous disease with various pulmonary and extrapulmonary comorbidities (Vanfleteren et al., 2013).
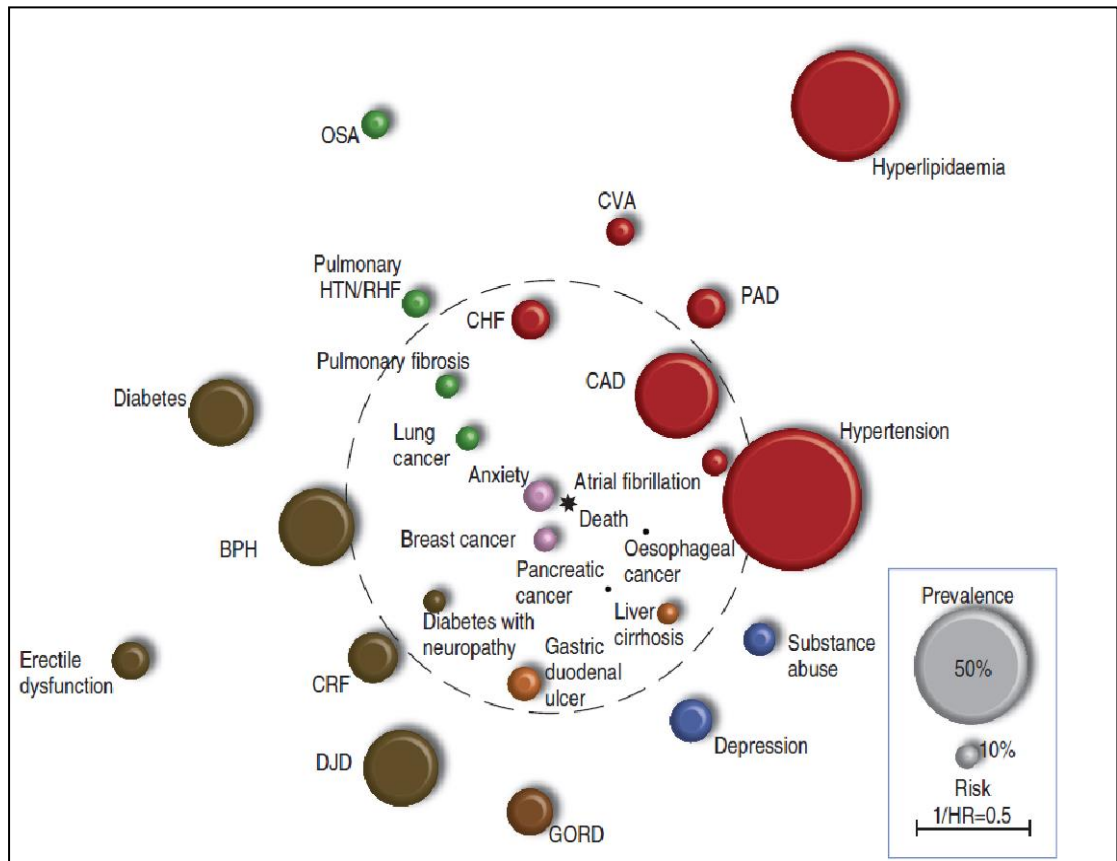
Figure 1.1: Comorbidities in COPD

The "Comorbidome" is a graphical expression of comorbidities. The area of the circle relates to the prevalence of the disease. The colours of the bubbles represent organ systems or disease clusters. Red: cardiovascular; pink: female-specific comorbidities; green: pulmonary; blue: psychiatric; brown and orange: others. OSA: obstructive sleep apnoea; CVA: cerebrovascular accident; HTN: hypertension; RHF: right heart failure; CHF: congestive heart failure; PAD: peripheral artery disease; CAD: coronary artery disease; BPH: benign prostatic hypertrophy; CRF: chronic renal failure; DJD: degenerative joint disease; GORD: gastro-oesophageal reflux disease. **Figure was taken from Divo et al. (2012) article**.

1.7.1 Systemic Inflammation in COPD

There is a growing evidence to suggest that COPD is not just a pulmonary disease, but also a systemic disease with several extrapulmonary manifestations including systemic inflammation (Thomsen et al., 2012, Vanfleteren et al., 2013). Patients with COPD have evidence of increased systemic inflammation especially at late stage of the disease or during exacerbation (Gan et al., 2004).

A meta-analysis by Gan and colleagues (2004) concluded that individuals with COPD had significantly higher levels of many circulating inflammatory mediators, such as C-reactive protein (CRP) and fibrinogen in the peripheral blood of COPD compared to individuals free from the disease. Persistent systemic inflammation continues even after smoking cessation suggesting that COPD is a systemic inflammatory disease (Agusti and Faner, 2012, Fabbri et al., 2012). Moreover, patients with a higher level of systemic inflammatory markers are more likely to have a progressive deterioration in their lung function and have an increased risk of hospitalisation (Valvi et al., 2012, Vernooy et al., 2002, Vestbo et al., 2011).

The association between COPD and systemic inflammation is not fully understood. Several hypotheses have been proposed and investigated. The hypothesis that the inflammatory process in the airways "spills over" into the systemic circulation has been questioned in the literature, since the sputum and plasma levels of important inflammatory mediators such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) have no clear relationship (He et al., 2010, Vernooy et al., 2002). Recently, the understanding of the mechanism of persistent inflammation in COPD, despite smoking cessation has considered another avenue, which is autoimmune system deficiency (Nunez et al., 2011). The hypothesis of immune system dysfunction addresses a few missing links in the pathogenesis of COPD (Agusti et al., 2003). This is evident from the presence of antinuclear antibodies in patients with COPD when compared with healthy controls, and these molecules are not related to smoking

history or lung function (Agusti et al., 2003). This suggests that the immune system adapts with a specific response to the inflammatory process that is driven by the repeated proliferation of B- and T- cells, indicating that the autoimmune system is involved in the pathogenesis of COPD and activation of systemic inflammation (Cosio et al., 2009). Another possible mechanism is that patients with COPD have a common genetic susceptibility or epigenetic changes to both systemic and pulmonary inflammation (Hackett et al., 2003, Houben et al., 2009). Having excluded sources of systemic inflammation not originating from the lung, the increased systemic inflammatory markers in patients with COPD may derive from other sources, such as circulating leukocytes, skeletal muscle or even cardiac muscle. Systemic hypoxia commonly seen in patients with severe stage of COPD, may also contribute to systemic inflammation since it has been shown to correlate with both IL-6 and TNF-α, and soluble receptors are predominantly higher than in control subjects (Sabit et al., 2010b, Takabatake et al., 1999). Moreover, local and systemic inflammations do not occur concurrently, and there may be more than one pathway involved at a given time during the progression of the disease. These mechanisms are regulated differently, locally and systemically, in response to inflammation and are dependent on disease severity (He et al., 2010). This leads to the possibility that systemic inflammation is a primary phenomenon, which itself leads to airway injury and inflammation.

Finally, due to the complexity of this disease and the involvement of other comorbidities which play a role in aggravating systemic inflammatory markers, this makes judging the source of systemic inflammation difficult. Additionally, the current evidence lacks a well-conducted study. Most studies are cross-sectional which, due to their methodological limitations, cannot determine the association between inflammatory markers and comorbidities.

1.7.2 Hypertension in COPD

Hypertension is the most common comorbidity occurring in patients with COPD and estimated to be between 40-60% (Mannino et al., 2008). This seems to rise with severity of the disease with an odds ratio of 1.4 in GOLD II and 1.6 in GOLD III & IV compared with control subjects. Hypertension is a key risk factor for atherosclerosis and the leading cause of cardiovascular events including coronary heart disease, myocardial infarction and stroke and its presence in COPD increases the risk of hospitalisation and mortality (Mannino et al., 2008). The link between increased blood pressure and COPD is not fully understood, but accelerated vascular ageing through elastin breakdown and deposition of collagen in the arterial wall, which consequently leads to arterial stiffness may be a factor (Maclay et al., 2012).

1.7.3 COPD and Type 2 Diabetes Mellitus

Several cross-sectional studies have shown a relationship between type 2 diabetes and COPD even at early stage of COPD (Carrasco-Garrido et al., 2009, Cazzola et al., 2010). The prevalence of diabetes in patients with COPD has been shown to range from 1.6% to 16% (Mannino et al., 2008, Rana et al., 2004). In the UK, patients with COPD have double the relative risk 1.8 (95%CI: 1.1 to 2.8) of developing type 2 diabetes compared with non-COPD subjects (Rana et al., 2004). COPD and diabetes mellitus share similar risk factors, including smoking. However, stopping smoking for more than 5 years can mitigate this risk in diabetes but not in COPD. Several studies have found a link between diabetes mellitus and abnormal lung function (Rana et al., 2004, Walter et al., 2003). A prospective cohort study of diabetes found an association between impaired lung function, as measured by FEV_1, and FVC, which is more pronounced in smoking subjects (Walter et al., 2003). However, in a recent large cohort study, diabetes mellitus and decreased FEV_1 were found to be related regardless of smoking status, and this relationship is more remarkable in people with central (abdominal) obesity (Lam et al., 2010). The underlying mechanism of the

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increased prevalence of type 2 diabetes in COPD remains uncertain. However, both COPD and diabetes mellitus share other common risk factors including increased obesity, physical inactivity, hypoxia, oxidative stress and inflammation.

1.7.4 Osteoporosis

Osteoporosis is common in COPD, and is characterised by loss of bone mineral density and microarchitectural changes resulting in an increased risk of fracture (Biskobing, 2002). A number of cross sectional studies have shown an increased prevalence of osteoporosis and osteopenia in COPD (Bolton et al.,2004, Sabit et al., 2007). The risk of osteoporosis has been found to increase with declining lung function as measured by FEV_1, independent of age, BMI and corticosteroid use (Graat-Verboom et al., 2009). Osteoporosis also contributes to vascular calcification and increased arterial stiffness, which may account for increased cardiovascular risk in patients with COPD (Bolton et al., 2011, Sabit et al., 2007).

The association between osteoporosis and COPD is unknown, but is likely to be multi-factorial. A number of known risk factors for osteoporosis are associated with COPD including increasing age, smoking, low levels of vitamin D, reduced physical activity, alteration in body composition and hypogonadism (Barnes and Celli, 2009). Although systemic steroid use in COPD has been linked to loss of bone mass and osteoporosis, the association with inhaled corticosteroids is still not obvious. Hip and vertebral fractures are common in COPD, with the latter reducing intra-thoracic volumes and causing compromised lung function and therefore it is prudent to put great efforts to diagnose and intervene at an early stage to minimise the complications associated with fracture.

1.7.5 Physical Activity

COPD is a heterogeneous disease associated with physical inactivity (Barnes and Celli, 2009). Patients with COPD frequently complain of dyspnea during everyday activity and subsequently tend to avoid performing normal daily tasks, which eventually leads a downward spiral symptom-induced inactivity (Pitta et al., 2006). Reduced physical activity occurs in different patients to different extents, if at all. Some patients have severely impaired lung function, yet have well preserved physical exercise capacity and maintain their functional status, whereas other patients have severely impaired physical and functional capacity, but have only mild or moderate disease airway obstruction (Spruit et al., 2010). Part of this variation may be attributable to the balance of the underlying aetiology (lung growth impairment in early life, smoking and occupational exposures), the underlying predominant pathophysiology (airway disease versus emphysema) alongside the patient's genetic makeup. This emphasises the idea that FEV1 is a poor predictor of exercise capacity and cannot reflect patient's physical performance (Kapella et al., 2011).

Physical inactivity plays a key factor in causing morbidity, hospital admission related to exacerbation, accelerate the progression of disease severity and health related quality of life (Pitta et al., 2006). Patients with low physical activity level have doubled the risk of disability (Garcia-Aymerich et al., 2006).

Although physical inactivity is a modifiable risk factor and reversible, its assessment in clinical settings is limited due to limited resources. Regular physical activity has been found to reduce the risk of CVD, but also reduced the hospitalisation rate and death related to COPD (Watz et al., 2009).

1.7.6 Body Composition in COPD

1.7.6.1 Loss of Muscle Mass

A common comorbidity of COPD, which is well established, is the loss of musculoskeletal mass, which manifests as weight loss (Bolton et al., 2004). This is a common clinical manifestation of COPD, and in particular those with emphysema (Engelen et al., 2000b). As the severity of the disease increases, the patient is more prone to loss of weight and low body mass index (BMI). Several studies have shown higher COPD-related mortality rates in underweight and normal weight patients compared to overweight and even obese patients (Landbo et al., 1999, Schols et al., 1998). Such a relationship contrasts with the U-shaped survival curve that is seen for BMI in other population studies (Bray, 1987). This discrepancy is attributed to the fact that BMI does not reliably reflect metabolic change in muscle mass (Rutten et al., 2013).

Fat Free Mass (FFM) has been shown to be a reliable indicator of mortality and prognosis in several studies (Schols et al., 2005, Van Den Borst et al., 2011, Vestbo et al., 2006). Vestbo and co-authors (2006) demonstrated that patients with COPD have some depletion of muscle mass in the presence of a normal or high BMI, and this is inversely related to the severity of the lungs' condition, especially in GOLD stages III and IV. Interestingly, this subgroup of patients, known as having muscle mass loss, are also at increased risk of death, suggesting that FFM is a predictor of mortality independent of BMI (Vestbo et al., 2006).

Loss of FFM adversely affects peripheral and respiratory muscle function, resulting in further respiratory compromise, exercise intolerance and reduced health status (Engelen et al., 1994, Hopkinson et al., 2007). In addition, loss of FFM increases the risk of exacerbation, hospital admission and mortality (Engelen et al., 2000b, Schols et al., 2005). Furthermore, Eisner and colleagues (2007) demonstrated that increased fat mass was strongly associated with activity limitation.

The underlying mechanism of both weight loss and loss of FFM seems to be complex and remains poorly understood. Nevertheless, various hypotheses have been proposed, which include systemic inflammation, protein metabolism imbalance and deregulation of muscle homeostasis. Bolton et al. (2004) demonstrated that the urinary pseudouridine (PSU), a marker of cellular protein breakdown, is significantly associated with the loss of FFM and particularly in severe and very severe COPD patients.

1.7.6.2 Fat Tissue and Adipokines

Pathophysiological mechanisms linking COPD and development of comorbidities remain largely unknown, but increased systemic inflammation has been suggested to play a role (Vanfleteren et al., 2013). Systemic inflammation has been implicated in the development of musculoskeletal dysfunction, atherosclerosis, osteoporosis and CVD (Eid et al., 2001, Bolton et al., 2004, Sin and Man, 2005). However, the source of inflammation is not fully elucidated. Patients with COPD often have altered body composition. Previously, great interest was shown in loss of FFM and the link to increased mortality. More recently attention has focused on an increased fat mass (FM), even in the presence of a normal FFM, because of it's relationship to systemic inflammation, loss of physical function and cardiovascular risk. Adipose tissue is now accepted as an active endocrine organ that produces a variety of pro-inflammatory mediators including interleukin-6, which contributes in the development of insulin resistance and the increased risk of diabetes mellitus and cardiovascular disease in COPD (Barnes and Celli, 2009, Rutten et al., 2010, Watz et a., 2008). Adipose tissue also produces a variety of adipokines, which influence a wide range of bodily physiological systems (Rutten et al., 2010). COPD is associated with intermittent or chronic hypoxemia, which is suggested to lead to adipose tissue hypoxemia and consequently adipose tissue inflammation (Agusti et al., 2010). The most studied adipokines in COPD are adiponectin and leptin. Adiponectin plays a crucial role in

inhibiting inflammation, improving vascular haemostasis and reducing cardiovascular morbidity and mortality (Sood, 2010, Breyer et al., 2012, Yoon et al., 2013). In contrast, increased levels of leptin are associated with increased local inflammation, oxidative stress and development of CVD (Breyer et al., 2011, Sattar et al., 2006).

Adiponectin has a pleiotropic effect and its regulatory role is not fully understood. In a subset of patients from the ECLIPSE study, Breyer and colleagues (2012) have found that patients with COPD demonstrated raised levels of serum adiponectin and were inversely related to BMI compared with age, gender and BMI matched control subjects. They showed the levels of adiponectin were inversely related to CRP and independent of gender. Similar findings have been reported in a small sample size of Chinese COPD population and serum adiponectin increased with the airways severity (Chan et al., 2010). However, unlike the study by Beryer et al. (2012), Chan and colleagues (2010) reported a positive relationship between CRP and serum adiponectin. Contrary to previous studies, in a fair sample sized COPD study, Breyer and co-authors (2011) reported no association between plasma adiponectin and inflammatory biomarkers (i.e. CRP or TNFα) and the levels of serum adiponectin were similar between patients with COPD and age and gender matched controls.

Recently, the longitudinal results from the Lung and Health Study showed increased levels of serum adiponectin were associated with lower hospitalisations and reduced risk of cardiovascular mortality (Yoon et al., 2012). This is supported by a number of clinical studies, which have found increased plasma levels of adiponectin has a cardioprotective role against coronary heart disease, increased risk of myocardial infarction and remodelling of cardiomyocytes after infarction (Sattar et al., 2006).

Nevertheless, this study showed other debilitating effects of circulating adiponectin on the lung, where raised levels of plasma adiponectin were associated with bronchial hyperactivity, rapid decline in lung function and doubled the risk of respiratory-related mortality (adjusted hazard ratio, 2.09; 95% CI, 1.41-3.11). Consistent with these

findings, other studies have reported that increased levels of adiponectin were associated with the severity of airway obstruction and increase the risk of exacerbations (Kirdar et al., 2009, Chan et al., 2010).

Another adipokine that has been studied in COPD populations is leptin. Patients with COPD showed elevated levels of plasma and pulmonary leptin, which were associated with increased inflammation compared with age and gender matched control subjects (Broekhuizen et al., 2005, Beryer et al., 2011). A conflicting result has also been reported in a study by Kirdar and colleagues (2009) who showed patients with COPD and age and gender matched controls had similar levels of serum leptin. Plasma leptin has been shown to be gender and BMI dependent (Breyer et al., 2011). Female patients demonstrated higher levels of leptin than their male counterparts (Breyer et al., 2011). These differences are not fully understood, but may attribute to differences in sex hormones and body fat distribution.

Increased serum and pulmonary leptin and it's relationship with other pro-inflammatory biomarkers suggest a potential role for leptin in the pathogenesis of COPD and the increased risk of CVD in this population.

Nevertheless, the role of adipokines in COPD is still controversial due to limited studies and knowledge in this field. Understanding the physiological and pathophysiological role of adipokines in local and systemic inflammation is crucial. Future studies are needed to explore the role of adipokines and their involvement in the disease process.

1.7.7 Chronic Kidney Disease

Chronic kidney disease has recently received a great attention in COPD and the GOLD guidelines recommended that patients with COPD should be screened for renal disease (GOLD, 2014). The prevalence of COPD is reported to be between 6-11% and is not associated airways severity (Patel and Hurst, 2011). The presence of chronic kidney disease in patients with COPD has been associated with a high

mortality rate compared to patients without chronic kidney disease (Incalzi et al., 1997). Association between chronic renal disease and COPD is not fully elucidated, but it may be related to inability of the kidney to clear inflammatory biomarkers and prothrombotic molecules or presence of other comorbidities such as hypertension and type 2 diabetes may amplify the risk. Screening for chronic kidney disease may help to understand the link between COPD and CVD.

1.7.8 Sarcopenia in COPD

As part of the ageing human body, body composition experiences several changes including loss of muscle mass and strength, which is known as sarcopenia. Age related sarcopenia involves loss of muscle mass and function with a subsequent decrease in physical performance and health related quality of life (Cruz-Jentoft et al., 2010). Skeletal muscles undergo several changes during the ageing process including a decrease in their mass and cross-sectional area, and a reduction in the size and number of type II fibre. However, the exact underlying pathophysiological mechanism of sarcopenia is poorly understood. It is likely to be a multifactorial involvement of neuromuscular integrity, neuroendocrine and physical inactivity (Joseph et al., 2005). The latter is thought to play an important role in the development of loss of musculoskeletal mass and strength in health and disease (Cesari et al., 2006).

Age related sarcopenia features are frequently seen in patients with COPD at younger age, suggesting that COPD has the potential to accelerate these physiological changes (Eid et al., 2001). Results from the cross-sectional studies have shown that a decline in muscle mass and function is associated with reduced fat oxidation rate and increased insulin resistance (Bolton et al., 2004, Eid et al., 2001, Gosker et al., 2007). Maintaining muscle mass and function is an integrative process and extremely complex. However, physical activity has a strong preventative influence on muscle mass loss (Pitta et al., 2006, Watz et al., 2009). Longitudinal studies are needed to examine the changes in body composition and physical performance in the natural history of COPD.

1.8 Cardiovascular Diseases

Cardiovascular disease (CVD) is the most common comorbidity associated with COPD. Over the past three decades, there has been a growing body of epidemiological evidence suggesting a strong association between COPD and CVD. The association between COPD and CVD has been previously attributed to smoking. However, several studies have suggested that smoking may partially contribute but cannot entirely explain the increased risk of cardiovascular events in COPD. Other contributing factors may explain why COPD induces CVD are likely to be reduced lung function, inflammation and increased sympathetic overactivity.

1.8.1 Lung Function Impairment

Reduced lung function is another factor that can link COPD to CVD. In the general population of Tecumseh (USA), individuals with FEV_1 less than 2.0 L had a fivefold increase in their relative risk of cardiovascular mortality than those with FEV_1 greater than or equal to 2.0L (Higgins and Keller, 1970). This relationship was subsequently confirmed in other prospective cohort studies (Hole et al., 1996, Schunemann et al., 2000). Interestingly, in these studies, the risk of cardiovascular death continuously decreases as the FEV_1 increases suggesting that CVD is lung function dependent.

In a large UK cohort study; Hole and colleagues (1996) followed 7058 men and 8353 women aged between 45 and 64 for 15 years and found that reduced FEV_1 accounted for 26% of all deaths related to ischaemic heart disease in men and 24% in women.

Although these early studies strongly suggested that reduced pulmonary function is responsible for increased cardiovascular mortality, they did not consider other comorbidities such as hypertension, hypercholesterolemia and diabetes and confounding factors such as sex, tobacco exposure and physical activity. In addition, these studies did not classify individuals according to their lung function.

In the general adult population, the cause of FEV_1 reduction is of two types, either obstructive or restrictive airway diseases. However, a reduced FEV_1/FVC ratio is a

hallmark indicator of obstructive airway disease. Engstrom and colleagues (2001) prospectively followed a cohort of 621 males for over thirteen years. During this period, all cardiovascular events and mortality were documented. They found that men in the lowest quintile of FEV_1/FVC were more than twice as likely to die of coronary events and complex ventricular arrhythmias as those in the highest quintile. This remained significant after controlling for important risk factors, such as tobacco exposure, alcohol consumption, physical activity and the presence of diabetes mellitus. Although the results of this study were interesting, the authors did not consider the role of other comorbidities or previous cardiac events on causing cardiac mortality. Additionally, the authors considered abnormal systolic blood pressure above 160 mmHg, which is 20 mmHg above the accepted limit for normal systolic blood pressure, which increases the risk factor for cardiovascular events and mortality (Lewington et al., 2002). Similarly, the lung health study reported that cardiovascular events were responsible for 25% of deaths over 5 years in a large cohort of smokers with airways obstruction (Anthonisen et al., 1994). This study also showed that cardiovascular events were responsible for 42% of first hospitalisations and 48% of second hospitalisations. In general, for every 10% decrease in FEV_1, cardiovascular mortality was increased by 28%, and non-fatal coronary events increased by 20%, after controlling for relevant risk factors (Sin and Man, 2005).

In two large cohort studies (Atherosclerosis Risk in Communities (ARIC) and the Cardiovascular Health Study (CHS)) comprising of 20,296 patients over the age of 45 years, the cardiovascular mortality in 5 years follow-up was nearly 6% and was airway severity dependent (Mannino et al., 2008). Recently, the "TOwards a revolution in COPD health" (TORCH) study recruited 6,184 patients with moderate to very severe COPD and followed them up for three years. The incidence rate of cardiovascular events during this period of time was from 20.8% to 24.3%, and the death rate was 14% (Calverley et al., 2010). However, considering the effects of bronchodilators, and

in particular ipratropium bromide and its probability to increase cardiovascular events, this may raise the question of whether these adverse events stem purely from COPD or are attributable to drug effects. In addition, the influence of other potentially confounding factors, such as diabetes mellitus, hyperlipidaemia, socioeconomic status and physical inactivity, were not controlled for in their analysis. Therefore, the percentage of cardiovascular deaths in patients with COPD reported in the TORCH study may not precisely address or link completely to the effects of COPD.

In the Baltimore Longitudinal Study of Ageing (BLAS), individuals who had a progressive decline in FEV_1 during a 16 years follow-up were three to five times more likely to die from a cardiovascular event than those who had the slowest decline in FEV_1 (Tockman et al., 1995). Although these results were adjusted for age, baseline FEV_1, smoking status, blood pressure, BMI and serum cholesterol, the effects of other confounding factors, such as gender, race, dyslipidaemia, socioeconomic status and physical inactivity, which are well-recognized risk factors for cardiac mortality, were not considered.

Similarly, a Finnish-based population study found that symptoms of chronic bronchitis were associated with a 50% increase in coronary deaths, after correcting for age, gender, serum cholesterol, systolic blood pressure and smoking history (Jousilahti et al., 1996). However, the diagnosis and severity of chronic bronchitis were based only on subjective information obtained from a simple questionnaire, which cannot quantify the severity of the disease. In addition, lack of quantification of lung function may question the relationship.

Moreover, the relationship between the decline in FEV_1 and cardiovascular disease is independent of smoking, though it has also been demonstrated in lifetime non-smokers (Hole et al., 1996; Tockman et al., 1995). In the BLSA study, the accelerated decline in FEV_1 amongst lifetime non-smokers was associated with a five- to ten-fold increase in the risk of cardiac death. This further adds to the notion that the

confounding effects of smoking cannot describe the relationship between reduced FEV_1 and CVD.

COPD is also a risk for cardiovascular events. In the UK, the prevalence of angina and myocardial infarction (MI) in newly diagnosed patients with COPD was >1% within the first year after their diagnosis (Soriano et al., 2005). Recently, from a large database of primary care comprising over one million, 29870 patients were identified with COPD and were five times more likely to have CVD compared with those without COPD (Feary et al., 2010). In the follow-up analyses and after adjustment for confounding factors including age, sex and smoking status, the incidence of MI was three times greater in patients with COPD than non-COPD individuals. Interestingly, the incidence of MI was age dependent. In the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) study, " heart trouble" and MI were found to be higher in patients with COPD (26% and 9%) than smoking controls (11% and 9%), respectively (Hurst et al., 2010). Likewise, the prevalence of CVD in the ARIC and CHS studies was between (20% and 22%) in patients with COPD compared with 9% in non-COPD group (Barr et al., 2009, Mannino et al., 2006). In a cross-sectional study involving 405 patients with COPD in primary care with unknown heart failure, 83 patients were identified to have heart failure after they underwent a thorough clinical investigation (Rutten et al., 2005a). Interestingly, none of the patients had a right side heart failure.

1.8.2   Inflammation

Systemic inflammation is a feature of extrapulmonary manifestations of COPD, which has also been implicated in the pathogenesis of CVD. Although the origin of inflammation is poorly understood, COPD is characterised by a persistent lung and systemic inflammation, which increased during exacerbation (Van Eeden et al., 2012, Patel et al., 2013). Most importantly, inflammation that happens at level of the small airways and alveoli, may play a significant role in the lung induced CVD. The

mechanism by which inflammation induces CVD is complex and not fully understood. However, it is likely that inflammation is involved in the pathogenesis of atherosclerosis and formation of plaque, which is thought to be the first phase of CVD (Lusis, 2000). Animal studies have shown that chronic exposure of the lung to small noxious particles are responsible for increased local and systemic inflammation, accelerated atherosclerotic changes in coronary arteries, endothelial dysfunction and increased oxidative stress compared with control animals (Suwa et al., 2002, Sun et al., 2005). Additionally, although the mechanism/s behind these changes was obscure, these studies elucidated a key point, which these abnormal responses were directly related to lung inflammation. Similar findings have been demonstrated in a double-blind, randomised, crossover study of men with stable ischaemic heart disease who had been exposed to either dilute diesel exhaust (induce lung inflammation) or filtered air during one hour training (Mills et al., 2007). Men who were exposed to dilute diesel exhaust demonstrated impaired fibrinolytic activity and reduced myocardial oxygenation compared to filtered air group. Consistent with these findings, Smeeth and colleagues (2004) analysed the data of five millions patients using the UK General Practice Research Database and showed that the risk of MI was five times greater within the first three days from the onset of respiratory infection compared to upper tract of urinary infection. The role of inflammation has been recently implicated in the pathogenesis of arterial stiffness, which is an independent predictor of adverse cardiovascular events. In patients with COPD, the role of systemic inflammation in increased arterial stiffness is contentious. This controversy is due to the fact that these studies have used non-specific systemic inflammatory biomarkers such as CRP and fibrinogen. This is supported by a recent study by Coulson and colleagues (2010) who found that increased arterial stiffness in patients with COPD was related to increased vascular inflammation, which was independent of systemic inflammation.

1.8.3 Autonomic Nervous System Dysfunction

Recently, the role of increased sympathetic nervous system activity has been recognised as a responsible mechanism for increased CVD in COPD (Van Gestel et al., 2012). Patients with COPD have a higher resting heart rate and a decrease in heart rate variability (Agusti et al., 2003). An increase of 10 beats is associated with increased risk of cardiovascular and all cause of mortality in patients with COPD compared to COPD free individuals (Agusti et al., 2003). Additionally, concomitant use of cardioprotective drugs (statins and angiotensin-converting enzyme inhibitors) may increase the risk of arrhythmia, myocardial infarction and cardiovascular death (Mancini et al., 2006). Another possible factor worsening heart function in patients with COPD risk is autonomic nervous system instability, which may be mediated by the use of inhaled anti-cholinergic drugs. These drugs are believed to increase the sympathetic drive manifested by reduced heart rate variability and can increase the risk of myocardial infarction and coronary heart disease (Anthonisen et al., 2002). Physical inactivity and lower socioeconomic status have also been implicated in the increased cardiovascular risk in COPD (Watz et al., 2008).

1.9 Arterial Stiffness

There is a growing evidence linking COPD to increased risk of cardiovascular events, and the nature of this relationship is proportional to the severity of the disease. Although both diseases share common predisposing factors, such as age and smoking exposure, the nature of this association still exists after controlling for these confounding factors (Sin and Man, 2005, Van Eeden et al., 2012).

The interaction mechanism linking COPD to CVD is not fully defined. However, several proposed mechanisms have been suggested including increased arterial stiffness.

1.9.1 Definition

Arterial stiffness characterises an artery's capacity to expand and contract with cardiac pulsation and relaxation. Terms such as arterial compliance, distensibility, elastic modulus, augmentation index and pulse wave velocity are all specific measures that come under the general term of arterial stiffness (Laurent et al., 2006). Stiffening of the larger arteries is a normal part of the ageing process; as the arteries lose part of their elasticity and become stiffer (McEniery et al., 2005). This is known as arteriosclerosis and is commonly seen in elastic arteries, such as the aorta and carotid, and little change is seen in muscular arteries such as the femoral and radial (Lee and Oh, 2010).

Elastin fibres play a major part in determining the mechanical strength of the vessels at lower pressures, while collagen fibres provide most of the strength at higher pressures. Therefore, elasticity of the arteries is needed to accommodate the flow of blood with every cardiac cycle by absorbing the energy during the systole phase and releasing it during the diastole phase (Blacher and Safar, 2005).

1.9.2 Structural Component of Artery Stiffness

The integrity of the arterial wall is dependent on two prominent components, elastin and collagen. The proportion between elastin and collagen is usually regulated by a

slow dynamic process of formation and degradation. Disturbance of this balance typically results in a reduction of elastin production and accumulation of the collagen components in the arterial wall (Zieman et al., 2005). Overproduction of collagen is mainly triggered by an inflammation milieu, but also stimulated by high blood pressure. Ageing of the arterial system is manifested by a two- to threefold increase in intima-media thickness during a normal lifetime (Jani and Rajkumar, 2006). Histological examination of the intima of stiffened arteries shows abnormal and disorganised endothelial cells, overproduction of collagen content, diminished elastin molecules, infiltration of smooth muscle, inflammatory activity and increased matrix metalloproteinases (MMP) (Lakatta, 2003).

The tensile strength of the arterial wall is mainly achieved by cross-linked collagen molecules. However, collagen is particularly susceptible to non-enzymatic glycation and cross-linking due to its slow turnover rate (Zieman et al., 2005). This leads to more misalignment and a dysfunctional collagen fibre structure with less elasticity.

Elastin fibre plays a key role in providing arterial wall elasticity. However, activated metalloproteases stimulate production of frayed elastin fibre, and disruption of the cross-links predisposes protein mineralization and an increase in arterial stiffness (Zieman et al., 2005).

Advanced glycation end products (AGEs) play an important role in arterial stiffening by forming irreversible cross-links in collagen (Konova et al., 2004). AGEs also increase arterial stiffness via impairment of the endothelial function by reducing nitric oxide bioavailability and the formation of free oxygen (Zieman et al., 2005). Furthermore, AGEs stimulate inflammatory responses via activation of metalloproteinases, which are known to promote endothelial dysfunction, increase vascular smooth muscle cell (VSMC) tone and accelerate the progression of atherosclerosis (Wendt et al., 2002).

1.9.3   Assessment of Large Artery Stiffness

For many decades, the concept of arterial stiffness was only associated with ageing and the clinical significance of its waveform and its importance in clinical examination of patients was not appreciated until recently.

Arterial stiffness can be quantified non-invasively using several methods. One of these methods is a regional measurement between two arteries, commonly between the carotid-femoral arteries.

1.9.3.1   Pulse Pressure

Pulse pressure is the difference between systolic and diastolic blood pressure. Pulse pressure is a surrogate measure for arterial stiffness, as it is mainly determined by cardiac output, aortic and large artery stiffness, and pulse wave reflection (Dart and Kingwell, 2001).

Both systolic and diastolic blood pressures tend to increase with age. However, diastolic blood pressure reaches its peak around age 60 and thereafter starts to decline, which consequently results in rapidly increasing pulse pressure (Roman, 2012).

Pulse pressure is a simple and reproducible marker of arterial stiffness, and it can be easily assessed with a standard blood pressure machine (Benetos et al., 1998). Despite the simplicity and availability of pulse pressure measurement, unfortunately, it can be an inaccurate measure of arterial stiffness as it does not take changes in volume into consideration. Moreover, peripheral pulse pressure is taken from the brachial artery, which may not reflect a true reading of central pulse pressure as it has been noted there is a variation of up to 20 mmHg (Wilkinson and Cockcroft, 2000).

Pulse pressure has been shown to predict cardiovascular risk in a number of populations (Benetos et al., 1998, Cockcroft et al., 2005). Additionally, pulse pressure has been shown to be a better predictor of cardiovascular mortality and morbidity than mean arterial pressure (Millar et al., 1999). In contrast, a large study on African-

Americans found no association between pulse pressure and mortality (Pastor-Barriuso et al., 2003). This suggests that pulse pressure may not be a good measure for arterial stiffness.

1.9.3.2 Pulse wave velocity

Pulse wave velocity (PWV) is defined as a propagation of the pressure wave within the arterial system with every cardiac contraction. PWV is mainly determined by arterial wall elasticity and the size of its lumen (Laurent et al., 2006). The regional measurement of arterial stiffness using applanation tonometry involves taking recordings from two different sites and matching them with simultaneous recording of the R wave from a recorded simple electrocardiography (ECG). As the aorta is the first artery subjected to left ventricle ejection contraction, it is more likely to be prone to stiffness than other arteries. Carotid-femoral pulse wave velocity (cf-PWV), also called aortic PWV, is considered as the gold standard for aortic stiffness measurement (Laurent et al., 2006).

The assessment of pulse wave velocity using cf-PWV is a relatively simple technique and has been widely used in several populations. By recording ECG gated waveforms in the carotid and femoral arteries, wave transit time can be calculated using system software. Aortic PWV is then calculated by dividing the distance between the two recording sites by the wave transit time (Figure 1.3). A higher aortic PWV suggests increased large artery stiffness.

Pulse wave velocity can be determined throughout the vasculature, but the aorta is the main vessel of interest, for two reasons:

1) Aortic pressure reflects the left ventricular systolic load, relevant to cardiac risk;

2) The aorta plays a major role in buffering pulse pressure and wave volume.

When blood is ejected into the aorta, a pressure wave is generated and travels throughout the arterial tree. Young healthy arteries are elastic and absorb the

pulse pressure from the heart efficiently. With age and pathology, the arteries stiffen, which causes the pulse waves to increase in pressure and velocity (Lee and Oh, 2010). When a pulse wave meets greater resistance at arterioles, a reflected wave is created. In healthy arteries, this reflected wave returns to the heart during the diastolic phase, which augments myocardial oxygenation and coronary perfusion. If the arteries stiffen, the reflected wave travels at higher velocity, returning to the heart late in the systolic phase. The result is an increase in systolic blood pressure (SBP), left ventricular afterload and pulse pressure (Laurent et al., 2006). There is also a reduction in diastolic pressure, which results in reduced coronary perfusion, with increased risk of myocardial ischaemia and subsequent myocardial fibrosis and loss of ventricular diastolic distensibility (Laurent et al., 2006).

1.9.3.3 Augmentation Index

Augmentation index (AIx) is another measure of arterial stiffness obtained from the aortic waveform and defined as the difference between the systolic peak and the second reflected wave (P2 − P1) expressed as a ratio of the pulse pressure (Laurent et al., 2006). Increased AIx results from an early arrival of the reflected pulse wave from the periphery and augment systolic blood pressure. Although AIx has been used as a measure of arterial stiffness, it is influenced by a number of variables including gender and heart rate (Gatzka et al., 2001, Wilkinson et al. 2000). In the Anglo-Cardiff-Cambridge Collaborative Trial (ACCT) study, McEniery and colleagues (2005) found that AIx is not a good measure for arterial stiffness for individuals over 50 years of age.

Other assessment methods include ultrasound scans and magnetic resonance imaging (MRI). Ultrasound can be used to measure changes in arterial diameter during systole and diastole to determine arterial distensibility and compliance

(Laurent et al., 2006). However, ultrasound has a number of limitations; it can only be used on larger, easily accessible arteries and requires an operator skilled in imaging vessel walls (Laurent et al. 2006). Arterial distensibility and compliance can also be determined by MRI. However, its use is limited, given the expense and expertise required to assess arterial stiffness (Laurent et al. 2006).
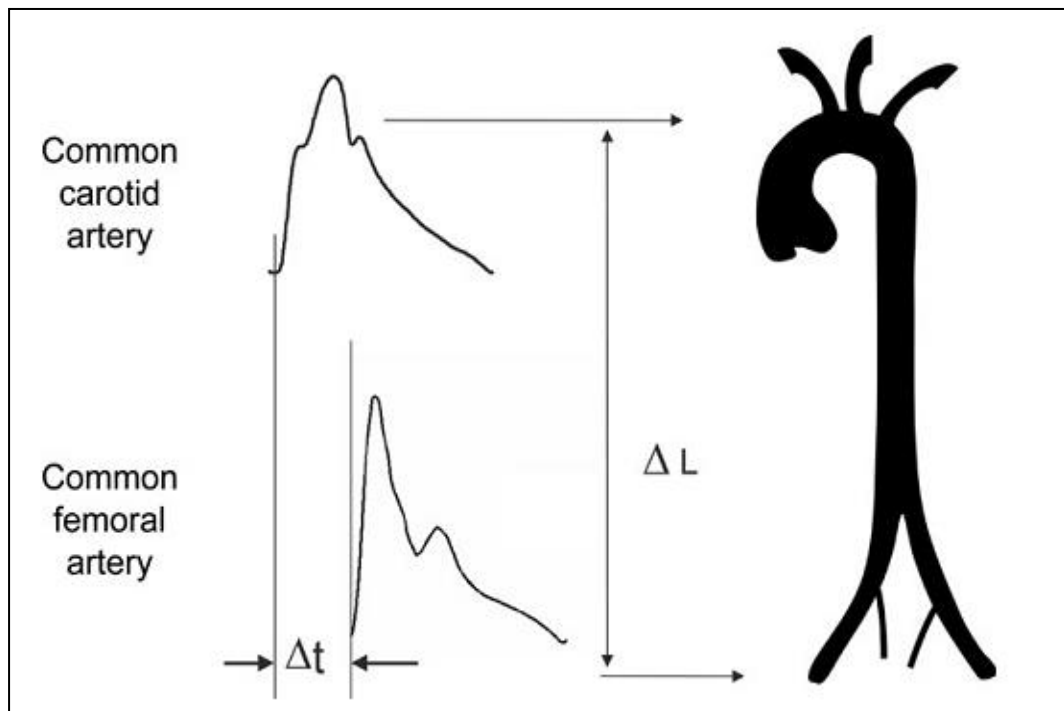
Figure 1.2: Measurement of carotid-femoral pulse wave velocity with a foot-to-foot method.

ΔL: distance between both measurement sites, Δt: transit time

Figure taken from Laurent et al. (2006)

1.9.4 Factors Affecting Artery Stiffness

1.9.4.1 Age

Inevitably, large artery stiffness occurs as part of the normal human ageing process and is consequently considered the most influential determinant of arterial stiffness (Lee and Oh, 2010). Several studies have used different techniques and found a positive association between arterial stiffness and age (McEniery et al., 2005, Mitchell et al., 2004). However, only large central arteries experience structural and mechanical changes and progressively stiffen with age, whereas the elastic properties of the peripheral arteries exhibit few changes with age (Cecelja and Chowienczyk, 2009). Data from a large cohort of healthy individuals in the ACCT study showed that aortic PWV experience changes after age of 50 (McEniery et al., 2005).

Increased aortic stiffness was thought to be as part of normal ageing process that associated with structural and functional changes in the arterial wall (Lee and Oh, 2010). However, this concept has changed, as it cannot explain why some elderly people have an aortic PWV like someone in his twenties. This largely attributed to the fact that normal or healthy ageing mechanism has not fully understood. Therefore, further mechanism may be involved and alter the properties of the arterial wall (Maclay and MacNee, 2013).

Disproportion between elastin and collagen proportion within the media layer of the arterial wall may contribute to large artery stiffness. As age increases, there is a higher collagen to elastin ratio, and this increases the risk of large artery stiffness (Lee and Oh, 2010).

1.9.4.2 Gender

Several cross-sectional studies have examined the gender related difference for PWV using different methods (Borlaug and Paulus, 2011, Duprez et al., 2009, Rossi et al., 2011b).

In the Cardiovascular Abnormalities and Brain Lesions (CABL) cohort study, women showed higher arterial stiffness than men as measured by augmentation index, independent of body size and heart rate (Russo et al., 2012). However, this relationship disappeared after adjustment for other cardiac risk factors. Likewise, the Multi-Ethnic Study of Atherosclerosis (MESA) study, women demonstrated lower large and small arteries elasticity than men, which was dependent on height and body size (Duprez et al., 2009). In contrast, in the BLSA cohort study, men showed more pronounced aortic stiffness than women independent of the effect of traditional risk factors (Alghatrif et al., 2013). This controversy is attributed to methodological variation among these studies of arterial stiffness measurements. In addition, none of these studies used the gold standard measure of arterial stiffness, which is aortic PWV.

The gender related difference for increased arterial stiffness in women may be the result of variation in sex hormones, obesity, low grade inflammation and endothelial dysfunction. Increased arterial stiffness and wave reflections, and changes in central haemodynamics increase left ventricle afterload, enhance the left ventricular remodelling process and also decrease the coronary blood flow, resulting in subendocardial ischemia and myocardial injury (Patel et al., 2013, Roman et al., 2007, Sabit et al., 2010a).

1.9.4.3 Body Composition

A number of studies have found an association between global and regional index of obesity and aortic stiffness (Mackey et al., 2002, Recio-Rodriguez et al., 2012, Sutton-Tyrrell et al., 2001). Excessive body fat generates abundant proinflammatory biomarkers and adipokines that directly or indirectly are involved in arterial pathogenesis and subsequently increase the risk of cardiovascular events (Sutton-Tyrrell et al., 2001, Recio-Rodriguez et al., 2012).

Obesity affects the level of testosterone in COPD, which is an independent risk factor for CVD (Atlantis et al., 2013, Herring et al., 2013). Testosterone deficiency is associated with increased aortic stiffness and enhances the risk of cardiovascular morbidity and mortality (Araujo et al., 2011, Dockery et al., 2003). Patients with COPD have evidence of both testosterone deficiency and increased aortic stiffness (Laghi, 2005, Sabit et al., 2007).

1.9.4.4 Hypertension

High blood pressure markedly accelerates ageing through elastin breakdown and deposition of collagen in the arterial wall, which consequently leads to arterial stiffness (Roman et al., 2007). In a large cohort study, Benetos et al. (2002) found that in poorly controlled hypertensive patients, age-induced pulse wave velocity acceleration was three times greater than in normotensive and well-controlled hypertensive patients. A recent study suggests that increased aortic stiffness in COPD may be associated with loss of elastic connective tissue (Maclay et al., 2012). Another mechanism that may link hypertension to arterial stiffness is low birth weight, which is a determinant of hypertension (Te Velde et al., 2004). This could be as a result of impaired elastin synthesis in the aorta during intra-uterine growth retardation, which is associated with the development of hypertension (Martyn and Greenwald, 1997). Interestingly, recent results demonstrate that aortic stiffness is an independent predictor of the progression to hypertension in normotensive subjects without clinical

signs of CVD (Kaess et al., 2012, Najjar et al., 2008). This suggests that measurement of aortic stiffness is a useful measure to predict and identify individuals at high risk of developing hypertension.

1.9.4.5 Diabetes and Metabolic Syndrome

COPD and diabetes mellitus share a common risk factor for increased CVD, which is increased arterial stiffness. The relationship between arterial stiffness, diabetes and metabolic syndrome has been established in several studies. In patients with metabolic syndrome, increased arterial stiffness is proportional to the degree of metabolic derangement (Teoh et al., 2013). Increased arterial stiffness is also shown in populations with impaired glucose tolerance and increased insulin resistance (Henry et al., 2003, Sengstock et al., 2005). Arterial stiffening, therefore, may precede the development of type 2 diabetes, just from the early stages of an insulin-resistant state (Sliem and Nasr, 2010). Therefore, increased insulin resistance may contribute to increased arterial stiffness in COPD.

1.9.4.6 Hypercholesterolaemia

A high level of cholesterol is a major risk factor for CVD. An elevated cholesterol level can induce premature arterial stiffness via the deposition of cholesterol in the intimal layer of the artery, which increases the risk of coronary heart disease (Wilkinson and Cockcroft, 2007). Several studies have evaluated the relationship between hypercholestolaemia and arterial stiffness. However, the results of these studies are conflicting. Lehmann et al. (1992) found increased aortic distensibility, as measured by aortic PWV, in young patients with familial hypercholesterolemia (FH), but in contrast; they reported decreases in adults with the same disorder. In a middle-aged population with metabolic syndrome, there is a direct relationship between aortic PWV and triglyceride and a negative link to high density lipoprotein (HDL), even after adjustments for age and sex (Czernichow et al., 2005).

1.9.4.7   Vascular Dysfunction

Aortic stiffness is elevated in patients with COPD in comparison to matched age and sex controls (Sabit et al., 2007). Structural components of the arterial wall including extracellular matrix, vascular smooth muscle and endothelium are the main determinant of arterial stiffness (Zieman et al., 2005). Flow mediated dilatation (FMD) is an accepted measure of endothelial function and patients with COPD showed to have abnormal endothelial function (Barr et al., 2007, Eickhoff et al., 2008, Ives et al., 2013). Recently, patients with COPD showed impaired vascular function, measured by carotid-radial PWV and FMD, compared with age matched subject controls (Ives et al., 2013). Interestingly, this study showed that vascular dysfunction could be reversed by an ingestion of antioxidant cocktail, which has an effect on altered redox balance and nitric oxide bioavailability. In addition, nitric oxide is an important antiatherogenic molecule, which can, therefore, affect large artery stiffness via its augmentation of atherosclerosis (Bäck, 2008). Furthermore, damage to small vessels is another risk factor for increased CVD (Anavekar et al., 2004, Gibson et al., 2003). Casanova et al (2010) measured urinary albumin to creatinine ratio and showed that patients with COPD without overt CVD had renal microvascular damage compared with smoking controls, independent of traditional cardiovascular risk factors. This finding was extended further by John et al. (2013) and found that aortic stiffness was related to urinary albumin/creatinine ratio, which may suggest the effect of aortic stiffness on vital organs such as the kidney.

1.9.4.8   Connective Tissue Degradation

Arterial function is not just limited to the endothelium, but the extracellular matrix is also involved in large artery compliance. An increase in vascular smooth muscle tone further increases synthesis of structural proteins, including collagen resulting in increased arterial stiffness (Maclay et al., 2009). Patients with COPD experience intra and extra pulmonary elastin breakdown that contribute to arterial stiffness (Black et

al., 2008, Maclay et al., 2012). In a study by Patel and colleagues (2006) showed that patients with COPD had more facial skin wrinkles, which is an indicator of elastin degradation, compared with non-COPD smoking subjects. Skin elastin degradation has been found in patients with COPD, which related aortic stiffness (Maclay et al., 2012). In addition, the authors also found an increased MMP-9 expression in the skin of the patients, which was associated with aortic stiffness. A similar relationship has also been reported in a healthy population (Yasmin et al., 2006).

1.9.4.9 Systemic Inflammation

A number of studies have shown a relationship between low-grade chronic inflammation and aortic stiffness in COPD. However, the exact mechanism underlying the association between systemic inflammation and arterial stiffness is still unclear. Systemic inflammation is strongly implicated in the pathogenesis of atherosclerosis, which may increase large artery stiffness (Hansson, 2005, Herrington et al., 2004). In addition, systemic inflammation has been associated with endothelial dysfunction, which may cause arterial stiffening through reduced nitric oxide bioavailability and increased activity of endothelin-1 (Eickhoff et al., 2008, Ives et al., 2013). Recently, aortic wall calcification, elastin degradation and production of MMP-9 have been found in patients with COPD, which are associated with arterial stiffness (Bolton et al., 2011, Maclay et al., 2012). Vascular inflammation is lately recognised to play a crucial role in increased aortic stiffness and developing CVD (Coulson et al., 2010, Figueroa et al., 2013).

1.9.4.10 Smoking

Smoking is a common and important risk factor for both CVD and COPD. A number of studies have investigated the effects of smoking on arterial stiffness; however, the results of these studies are controversial. Mahmud and Feely (2003) demonstrated that smoking a single cigarette produces a transient effect in aortic PWV in both smokers and non-smokers. A similar finding between smoking and arterial stiffness was reported by other researchers (Jatoi et al., 2007, Vlachopoulos et al., 2004). These results were supported by an improvement in PWV after stopping smoking, and this relationship was proportional to the duration of smoking cessation. However, this is not the case in COPD, as arterial stiffness still exists even after smoking cessation (Sabit et al., 2007). Smoking may increase arterial stiffness in some individuals, but not in others, which may suggest the involvement of a genetic component (Doonan et al., 2010).

1.10 Arterial Stiffness and COPD

Increased arterial stiffness is associated with a higher risk of cardiovascular events, due to the physiological consequences of stiffened arteries (Mitchell et al., 2010). A number of consequences of arterial stiffness are associated with increased cardiovascular risk in COPD, including increased inflammation, left ventricular dysfunction, endothelial dysfunction and connective tissue degradation (Coulson et al., 2010, Ives et al., 2013, Maclay et al., 2012, Sabit et al., 2010a). Several longitudinal epidemiological studies have found that aortic PWV is an independent predictor of cardiovascular morbidity and mortality in a variety of different diseased and healthy populations. These include patients with essential hypertension, type II diabetes, renal failure, elderly subjects and the general population (Blacher et al., 1999, Boutouyrie et al., 2002, Cruickshank et al., 2002, Sutton-Tyrrell et al., 2005, Willum-Hansen et al., 2006). Moreover, in a cross-sectional study of 194 middle-aged males free of coronary heart disease, Zureik and colleagues (2001) reported an inverse relationship between FEV_1, FVC and their ratio, and aortic PWV. Although this study did find an association between airway obstruction and arterial stiffness, these results cannot be extrapolated to patients with COPD. In addition, the severity of airway obstruction in this population was not classified, and it is unclear what proportion of their participants had restrictive or normal lung function. Additionally, this study was limited to men; thus its results cannot be generalised to women.

The first well designed study, which highlighted the relationship between arterial stiffness and COPD was by Sabit and co-authors (2007) in 74 patients with COPD and 42 controls similar in age and gender proportion. In this cross-sectional study, the authors found that patients with COPD, when compared with healthy controls, had an increase in arterial stiffness as measured by aortic PWV. The degree of arterial stiffness was proportional to the severity of airway obstruction even after adjustment for other confounding factors, such as diabetes, smoking, physical inactivity, high

cholesterol and blood pressure. Additionally, patients with COPD and osteoporosis showed greater aortic PWV than those without osteoporosis. Interestingly, this relationship remained significant even after controlling for other confounding factors, including age, sex, level of physical activity, smoking history, lung function and mean arterial pressure. However, there was no relationship between inflammatory biomarkers and osteoporosis. Nevertheless, IL-6 was found to be related to aortic PWV and was severity dependent. Although this study reported a significant relationship between arterial stiffness and COPD, the cross-sectional nature of the study cannot infer a causal relationship. Documentation of physical activity using a questionnaire may be subjected to recall bias, which may underestimate the effect of physical activity on arterial stiffness measurement. Additionally, the contribution of other confounding factors, including controls and patients with high blood pressure, who are on antihypertensive medications, which has an impact on arterial stiffness; may have affected the readings of aortic PWV.

Another study by McAllister et al. (2007) examined the relationship between emphysema and carotid-radial PWV in 157 patients. The authors found that PWV was high in patients with severe and very severe airway obstruction, and men demonstrated to have higher significant readings than women. The carotid-radial PWV did not differ between patients with ischemic heart disease more than those without ischemic heart disease. In this study, there was no association found between circulating CRP and carotid-radial PWV. A limitation of this study was the use of carotid-radial PWV as a measure of aortic stiffness, which is less robust than carotid-femoral PWV. The radial artery experiences less changes in the functional and structural features and therefore does not mirror what exactly happens in the aorta (Laurent et al., 2006). Given that, unlike the aortic artery, the radial artery is more muscular and not subjected to atherosclerotic changes and pressure variability of the cardiac cycle. In addition, the radial artery has less elastin and is barely affected by

the ageing process and other cardiovascular risk factors such as hypertension, this measure may not be suitable to predict cardiovascular risk. Furthermore, this study lacked controls and included patients with cardiac disease, which has an adverse effect on the arterial system. Given the fact that both COPD and heart disease share common risk factors, this makes it difficult to differentiate the source of origin.

In another large cross-sectional study from the same research group, the association between COPD and arterial stiffness was also confirmed. Mills and colleagues (2008) measured arterial stiffness in 102 patients and 103 controls similar in age and sex using the AIx. In this study, the authors found that patients with COPD had increased arterial stiffness, compared with the controls. This difference was maintained between patients with COPD, free from CVD, and control subjects. In addition, the authors found that arterial stiffness was associated with inflammatory biomarkers (i.e. CRP) in patients but not in controls. Although this study found a significant relationship between arterial stiffness as measured by AIx and COPD, Mills and co-authors (2008) failed to adjust for sex and heart rate in their analysis, which have a great influence on AIx (Gatzka et al., 2001, Wilkinson et al., 2000). In addition, lung function was not measured in the control subjects, which may raise the possibility of undiagnosed airway obstruction among current or past smokers. Cigarette smoking plays a crucial role in aggravating the abnormal inflammatory response inside the lungs and is a central part in the pathogenesis of COPD. Furthermore, although AIx increased in the patients compared with the controls, a number of factors may limit the ability of AIx to predict arterial stiffness and cardiovascular events in a COPD population. Firstly, unlike aortic PWV, which is the gold standard measure of arterial stiffness, AIx is a less robust and indirect measure of stiffness and is dependent on multiple components of the arterial pressure wave (Laurent et al., 2006). The AIx also exhibits different age-related changes to aortic PWV. This was highlighted in the Anglo-Cardiff-Cambridge Collaborative trial, which found that age-related changes in AIx and

aortic PWV follow different patterns (McEniery et al., 2005). Whereas changes in AIx were found to be more prominent in younger individuals (<50 years), aortic PWV changes were found to be more marked in older individuals (>50 years).

Moreover, in a prospective cross-sectional study, Janner et al. (2012) studied arterial stiffness in 494 (246 males) patients with COPD. The authors found a significant difference between patients with COPD and non-COPD controls in both sexes. However, AIx was independent of the degree of airway obstruction. Nevertheless, when the authors limited the analysis to patients less than 60 years old and adjusted the analysis for confounding factors that contribute to CVD (i.e. age, blood pressure, heart rate, smoking history, physical activity, CRP and education), they found no association between AIx and COPD. Therefore, Janner and co-workers (2012) concluded that COPD is not an independent factor of arterial stiffness after controlling for confounding factors. In the aforementioned study, the authors based the diagnosis of COPD on just pre-bronchodilator spirometry results, which is not the standard method of diagnosing COPD. The diagnosis of COPD should be based on post-bronchodilator results to eliminate the possibility of any reversibility element (GOLD, 2014). This may have led to include some patients with asthma, which has a different pathophysiology. Additionally, the characteristics of patients with COPD and non-COPD controls were not obvious, as the authors referenced demographic data that was published in 2001; however, the data collection for this study was carried out between 2001 and 2003. This raises a question about the general health of the subjects, who may, during this period, have developed some comorbidities that trigger changes in the arterial system. Furthermore, the authors limited their analysis to patients younger than 60 and with FEV_1 <80; this led to the inclusion of only 63 patients in their analysis, which may have led to underestimation of the link between COPD and arterial stiffness.

The conflicting results may be related to the outcome measure used to quantify arterial stiffness. In this study, AIx was used as a marker of arterial stiffness; however, this index is less rigorous than the gold standard of arterial stiffness for several reasons. These include the fact that an elevated heart rate causes the early return of the pulse wave to the aorta during the cardiac cycle, and this is affected by increased peripheral vascular resistance and is age-dependent (Wilkinson et al., 2000, McEniery et al., 2005).

Recently, in a prospective cross-sectional study, aortic PWV was measured in 62 patients with stable COPD, free from CVD, and 22 controls (Cinarka et al., 2013). The authors extended the previous research findings and found that even patients free from hypertension, type 2 diabetes and hypercholesterolemia had greater aortic PWV compared with control subjects, independent of age and smoking. Similar to Sabit et al. (2007) study, patients with an $FEV_1\%$ <50 had greater aortic stiffness than those with an $FEV_1\%$ >50. In this study, aortic PWV was negatively related to partial pressure of arterial oxygen. In contrast to Sabit et al. (2007) study, in this study, aortic PWV was not associated with CRP or tobacco exposure. The limitation of this study, in addition to its nature, was the majority of patients (96%) were male and therefore these results cannot be generalised to the female population.

The results of these studies have clearly demonstrated that COPD has an adverse effect on the arterial system and accelerates the features of premature vascular ageing and could be a potential factor in developing CVD. However, although some of the used aortic PWV as an outcome measure in their studies, none of them has examined the reliability of aortic PWV in COPD, which limits the generalisibility of their findings. As far these studies have been cross-sectional in nature, which limits their findings, therefore, further longitudinal studies are needed to evaluate the effect of COPD on arterial system over time and its contributing factors.

The change in aortic stiffness over time has been evaluated in different populations, but none of them was in a COPD population (Benetos et al., 2002, Briet et al., 2011, Utescu et al., 2013). In a hypertensive population, Benetos et al. (2002) found that aortic PWV increased by 0.17 m/s per year compared with 0.07 m/s in normotensive subjects, independent of blood pressure. A similar finding has been reported in 109 renal failure patients with chronic haemodialysis (Utescu et al., 2013). In this study, the adjusted progression of aortic stiffness, 0.84 m/s, was independent of age, blood pressure, systemic inflammation, diabetes and atherosclerotic CVD. In contrast, Briet et al. (2011) studied 180 patients with chronic kidney disease for four years and found no annual significant change in aortic PWV.

1.11 Frailty in COPD

COPD is a respiratory disease associated with a multisystem impairment including an increased risk of cardiovascular disease, systemic inflammation, osteoporosis, loss of skeletal muscle mass and function, hypertension and type 2 diabetes that consequently impact on health related quality of life (HRQoL) (Barnes and Celli, 2009, Divo et al., 2012, Vanfleteren et al., 2013). These co-morbidities are usually seen in natural ageing and may be regarded as deficits in physiological systems. With advance ageing, subjects are increasingly more susceptible to chronic diseases that weaken the normal physiological response to internal and external stressors (Fried et al., 2001, Rockwood, 2005). These features are mistakenly confused with disability associated with COPD. However, it is not really understood how COPD leads to disability. It is evident that COPD could lead to loss of function in the sense of failed physical function, such as a reduction in exercise tolerance. However, it seems likely that the systemic manifestations of COPD may be important factors in functional limitation and disability both of which lead to frailty. Disability, comorbidity and frailty are frequently used interchangeably because both are seen in old populations and share most common risk factors (Fried et al., 2004). Disability may lead to frailty and worsen comorbidity, and frailty could contribute to the progression of chronic diseases (Fried et al., 2004).

Frailty is a separate clinical entity and not necessarily to be associated with the elderly. The frailty concept has been around for nearly three decades, but, unfortunately, there is no operational definition for it (Morley et al., 2013). Recently, a group of experts agreed to acknowledge that frailty is not like disability and is a clinical syndrome that presents some individuals that could be reversible in some cases and progressive in others (Rodriguez-Manas et al., 2013). However, these experts failed to agree on one operational definition for frailty. Rockwood has previously defined frailty as an accumulation of deficits (Rockwood et al., 2005). This definition has been

embraced by several studies that found its utility in predicting adverse outcomes and mortality (Mitnitski et al., 2002, Song et al., 2010).

Frailty is a complex mechanism and the exact pathophysiology is still unclear. However, it was hypothesised that inability of one or multiple physiological systems to return to normal homeostasis after the perturbation, provokes a cascade of dysregulation that influence many clinical features and comorbidities (Fulop et al., 2010, Rockwood and Mitnitski, 2007). Failure to resume equilibrium status with the loss of the complexity of physiological systems increases the vulnerability to further poor prognosis that is frequently associated with frailty (Morley et al., 2006, Puts et al., 2005a, Rockwood et al., 2006, Walston et al., 2006).

There are two different clinical models to assess and quantify frailty (Fried et al., 2001, Rockwood et al., 2005). The frailty phenotype model was derived from the Cardiovascular Health Study in the United States, which defined frailty by the presence of at least three of the following five deficits in an individual: slow walking speed, weak handgrip strength, a self-reported decline in activity levels, unintentional weight loss, or exhaustion (Fried et al., 2001). Although this phenotypic approach is valid and useful in detecting frailty, it is mainly based on physical frailty and ignored other potentially important factors such as comorbidities, psychosocial and cognitive capacity, which is highly associated with functional impairment and disability (Rothman et al., 2008). Additionally, this approach is limited to identify the frailty, but not to classify or determine the degrees of frailty and heavily relies on patient recollection for much of the data (Rockwood and Mitnitski, 2011). Another approach to define frailty is by quantifying the number of deficits including cognitive, psychosocial status, comorbidities, and disabilities (Rockwood et al., 2005). The Comprehensive Geriatric Assessment is a questionnaire based tool that measures impairments, the level of frailty and predict outcomes in older individuals (Rockwood et al., 2006). The number of deficits relative to the total number of potential deficits sought can be used

to calculate a Frailty Index (FI) ranging from 0 to 1 (Rockwood and Mitnitski, 2007). This model supports the idea that FI increases with the number of deficits independent of the nature of this deficit. Consequently, this validates the concept of frailty, which is a progressive accumulation of deficits due to integral loss of homeostasis mechanism. On top of that, the FI may be used clinically to stratify risk or predict adverse outcomes and a good candidate to express biological age (Rockwood et al., 2006). Moreover, the value of 0.67 can be used as a warning sign, which beyond the incidence of mortality is highly likely (Scheffer, 2010).

Ageing and COPD share similar systemic manifestations and probably share an underlying mechanism. In the elderly, frailty predicts adverse outcomes independent of co-existing medical conditions and is associated with an increased risk of falls, hospitalisation, residential care, reduced HRQoL, progression to disability and increased mortality (Rockwood and Mitnitski, 2006, Rockwood et al., 2006, Song et al., 2010). The similarities between the accumulation of deficits in ageing and multiple co-morbidities in COPD suggest that COPD may accelerate the features of premature ageing and the patients could present with early signs of premature ageing and are likely to have high levels of frailty.

However, frailty in COPD has not been well studied, and the literature has only two studies on frailty in COPD. A study by Galizia et al. (2011) who examined the predictive role of frailty on long-term mortality in patients with and without COPD, reported that frail patients with COPD had higher rates of mortality compared to non-frail patients after 12 years follow-up. However, this study was not intended to examine the relationship between COPD and frailty. Moreover, diagnosis of COPD was based on patient's level of symptoms and report, which may have led to inclusion of patients with a wide spectrum of respiratory diseases such as asthma. A recent secondary cross-sectional study, using survey data from the National Health and Nutrition Evaluation Survey, which included only 70 patients with confirmed COPD,

suggested the prevalence of frailty was 57.8% based on a nine-item self-reported questionnaire. This study was not limited to only COPD, but included patients with known cancer and kidney diseases, which the prevalence of frailty is high (Balducci, 2013, Dalrymple et al., 2013). Additionally, the lack of a specific comparator group and validation of the frailty questionnaire limits the interpretation of this data.

COPD and frailty are both progressive with increased risk of adverse outcomes and poor health status. Therefore, emerging the assessment of frailty in routine clinical practice in patients with COPD would yield an early identification of those who are at high risk and subsequently implementing appropriate management to tackle this problem and improve the quality of life.

1.12 Summary

Several epidemiological studies have confirmed the association between COPD and CVD, however, this intimate relationship is not fully understood. Recently, increased aortic stiffness has been identified as an attributing factor. A number of cross-sectional studies have found that patients with COPD have increased aortic stiffness and associated with different risk factors including local and systemic inflammation and increased fat mass, which overall are likely to be factors in causing other co-morbidities such as increased insulin resistance and changes in body composition with loss of bone mineral density.

The presence of co-morbidities in COPD is similar to a multisystem deficit that is frequently seen in natural ageing or prematurely of what so called frailty. Frailty is considered to represent weakness or failure of physiological systems to sustain further an internal or external insult, and COPD may cause premature physiological changes or is itself an outcome of adverse outcomes.

Therefore, the aim of this thesis is to examine the progression of cardiovascular risk in COPD as measured by change in aortic stiffness and its interaction with other risk factors such as inflammation and other co-morbidities in COPD over a two-year follow-up. Additionally, this thesis aimed to explore the concept of frailty and the role of physical activity in the development and progression of frailty in COPD. Prior to that, the reliability of non-invasive outcome measures, including arterial stiffness, cardiac haemodynamics and bone density need to be established for use in patients with COPD.

1.13 Hypotheses

1. Aortic PWV will be a reliable outcome measure for assessing aortic stiffness in COPD using the SphygmoCor machine.

2. Assessment of cardiac haemodynamics in COPD (i.e. stroke volume and cardiac output) will be reliable using the Cheetah machine.

3. Calcaneal Ultrasound Bone Analyser will be a reliable and valid machine for assessment of heel bone density in COPD.

4. Timed Up and Go would be a simple test for physical performance in COPD.

5. Patients with COPD would have elevated aortic stiffness compared with similar age and sex comparator group.

6. COPD would cause an increase in aortic PWV independent of traditional risk factors after two years follow-up.

7. Patients with COPD will be more frail compared with similar age and sex comparator subjects.

8. Patients with COPD would have an increase in the frailty index and more impairment after two years follow-up.

1.14 Study Objectives

1. Assess the reliability of aortic stiffness in COPD using the SphygmoCor machine.

2. Assess the reliability of cardiac haemodynamics in COPD (i.e. stroke volume and cardiac output) using the Cheetah machine.

3. Examine the reliability and validity of Calcaneal Ultrasound Bone Analyser in COPD.

4. Examine the feasibility of using Timed Up and Go test as a rapid physical performance test in COPD.

5. Evaluate aortic stiffness in a large population with COPD and compare them with similar age and sex non-COPD subjects.

6. Evaluate the change in aortic stiffness in COPD and its contributing factors over a period of two years follow-up.

7. Assess the concept of frailty in patients with COPD and compare them with similar age and sex comparator subjects.

8. Assess the rate of frailty progression in COPD and its predicting factors after two years follow-up.

Chapter Two

Methods

2.1 Study Protocols

This chapter describes the assessment methods undertaken in this thesis.

Part I

In this part, the reliability of physiological measures including aortic stiffness, cardiac haemodynamics (i.e. cardiac output and stroke volume), broadband ultrasound attenuation and the validity of calcaneal ultrasound bone analyser (CUBA) (Chapter 3).

Part II

This part described a large cross-sectional study from an ongoing longitudinal cohort that assesses the risk factors in patients with COPD. The study evaluated the use of a simple test to measure physical performance in COPD (Chapter 4). Patients with COPD were compared with non-COPD subjects similar in age and sex. Physical performance, body composition, inflammatory markers and health-related quality of life were assessed during the initial assessment.

Part III

This part evaluated aortic stiffness cross-sectionally in patients with COPD compared with similar in age and sex non-COPD subjects. Then a group of patients with COPD was followed up for two years to examine the change in aortic PWV and its contributing factors (Chapter 5). Aortic PWV, spirometry, body composition, central and peripheral blood pressure (systolic and diastolic blood pressure, pulse pressure and mean arterial pressure), cardiac haemodynamics, heart rate, tissue oxygen saturation, kidney function, inflammatory biomarkers and health-related quality of life were assessed at the initial assessment and at the two years follow-up visit.

Part IV

This part assessed the concept of frailty cross-sectionally in COPD and similar age and sex comparators. Then a group of patients with COPD were followed up to examine the change in frailty over a period of two years (Chapter 6). The comprehensive geriatric assessment questionnaire was administered from which the frailty index was calculated. Other assessment including spirometry, body composition, cardiac haemodynamics, heart rate, tissue oxygen saturation, physical performance, inflammatory biomarkers and health-related quality of life were assessed at the initial assessment and at the two years follow-up visit.

2.2 Participants Recruitment

2.2.1 Patient Recruitment

Patients with COPD who were suitable for the study were identified through the respiratory outpatient clinics including Professor Shale's clinic and general practice databases. Inclusion and exclusion criteria for recruitment were described (Table 2.1).

2.2.2 Comparator Recruitment

Comparator subjects were recruited into the study using two methods. The first method was to invite participants of previous research projects at Wales Heart Research Institute free from respiratory disease. The second method was to recruit participant's relatives who were current smokers or ex-smokers with a minimum of 10 pack-years of smoking. All comparator participants underwent a full medical history check and spirometry to rule out any cardiorespiratory disease, airflow obstruction or other exclusion criteria (Table 2.1).

Table 2.1: Inclusion and Exclusion Criteria

	COPD	Comparators
Inclusion	Age 35-80 years Previously diagnosed with COPD Clinically stability for at least 4 weeks	Age 35-80 years Current or ex-smokers with minimum of 10 pack-years
Exclusion	Long-term oxygen therapy Malignancy in the last 5 years Hepatic or renal failure Inflammatory or metabolic disease Pregnancy Diseases affecting mobility e.g. Parkinson's disease Maintenance oral steroids	Respiratory disease and COPD Malignancy in the last 5 years Hepatic or renal failure Inflammatory or metabolic disease Pregnancy Diseases affecting mobility e.g. Parkinson's disease Maintenance oral steroids

2.3   Study Investigations

Subjects interested and eligible were provided with an information sheet to read through at their leisure or discuss with their family or friends (Appendices A & B). After one week, the patients were contacted again by a member of the research team and asked if they were happy to take part in the study. The individuals who agreed were asked about their general health, medical history and current medication (including inhaled therapy) or the presence of any other medical conditions. In particular, every attempt was made to rule out those who did not meet the inclusion criteria for the study or met any of the exclusion criteria (Table 2.1). All participants completed health questionnaires about their general health (Appendix C). On arrival, patients were welcomed and asked to sign a consent form (Appendix D). Participants were asked if they had any questions about the study; and if there were any questions, these were answered and were told to feel free to ask any further questions during the study, or even after they went back home.

Patients were examined during their clinical stability and those who suffered any exacerbation prior to their visit, their test postponed for at least four weeks until they felt their pulmonary symptoms had returned to baseline. In addition, comparators assessments were postponed if they had influenza or cold symptoms in the previous two weeks.

Definition of Clinical Stability

Clinical stability was defined according to the following criteria:

- No chest exacerbation four weeks prior to recruitment;

- No change in pulmonary symptoms four weeks prior to recruitment;

- No reduction in FEV_1>10% compared to patients' normal values within daily variation during the previous 6 months;

- No change in medication four weeks prior to assessment;

- An exacerbation was defined as:

- Increased or new respiratory symptoms (cough, sputum volume, colour, dyspnoea);

- Increased new systemic symptoms, including any of the following: fever, loss of appetite, weight loss, tiredness;

- Reduced FEV_1 >10% compared to usual clinical stability in the previous 6 months.

Smoking History

A history of exposure to cigarette smoke was calculated as pack year exposure. This can be calculated by multiplying the number of packs of cigarettes smoked per day, by the number of years a person has smoked. For example, smoking 1 pack of 20 cigarettes per day for 1 year is equal to 1 pack year.

Number of pack years = (packs smoked per day) x (years as a smoker).

2.3.1 Height and Body Composition

Subjects had their height measured barefoot, with their back straight against a wall using a stadiometer (Seca; Vogel & Halke, Hamburg, Germany). Weight and body composition were recorded with subjects wearing lightweight clothing and barefoot using a single frequency segmental bioelectrical impedance analyser (BC-418 MA, Tanita Corp., Tokyo, Japan), operating at 50 kHz, with eight-point contact electrodes (Figure 2.1). This system consists of a platform with stainless-steel footpads and two handgrips with stainless-steel contacts. The four contacts on the platform are arranged in pairs with two contacts for each foot. Each handgrip has one pair of contacts. Measurements were taken in a standing position with the arms slightly abducted. The electrode arrangement allows separate measurements for each arm and leg, the trunk and the whole body. The muscle mass and fat mass of limbs and trunk were calculated from the measured resistance values, height, body weight, sex, age and training condition.

Figure 2.1: Body Composition Analyser (TANITA, BC-418)

2.3.2   Heel Bone Density Measurement

The heel bone density of the patients was examined by the researcher using the Clinical CUBA machine (McCue Ultrasonics, Winchester) (Figure 2.2). The CUBA machine produces ultrasound waves from two distinct piezoelectric probes in the form of a sinusoid impulse. These waves are detected once they have passed through the calcaneus. The participants sat on a chair and inserted their right foot into the footwell to assess its size prior to measurement. The assessment was performed by using lines marked in the footwell and by assessment of the position of the tip of the big toe in relation to the lines, which indicates which of the inserts is required. The patient's foot was removed from the device, and the insert positioned if required. The foot insert was designed to raise the foot by a set distance from the base of the footwell, so that the patient's calcaneus is positioned between the transducers.

Ultrasound gel was applied to both the transducers and the patient's heel. The foot was repositioned between the two probes, emitting and receiving, and the leg was supported with velcro straps that pass around the calf and prevent excessive movement of the heel during measurement. The patient's details, such as age, sex and which foot was inserted into the machine were entered. The transducers were then closed so that they apply light pressure to either side of the calcaneus.

The measurement took approximately one minute, including an initial 30-second settling period to allow for any air bubbles in the gel to dissipate and for some compression of the surrounding soft tissue. The measurement was then taken, and the transducers dis-attached, releasing the heel. Calibration was checked every morning using a quality assured phantom model prior to examinations to ensure correct functioning of the transducers in terms of the ultrasound signal emitted and a determination of the speed of propagation of the impulse emitted by the probes.

Figure 2.2: Calcaneal Ultrasound Bone Analyser (CUBA) machine

(Picture taken from: http://www.medical-supplies-and-equipment.com/mccue-

cubaclinical.html)

2.3.3 Pulse Wave Analysis and Measurement of Pulse Wave Velocity

Prior to the assessment of arterial stiffness, participants were requested to abstain from caffeine, tobacco and their inhalers (short-acting β2 agonists for six hours and long-acting β2 agonists for twelve hours) before testing.

Arterial stiffness was assessed by an experienced operator using a measurement of pulse wave velocity (PWV) using a SphygmoCor device (AtCor medical) (Figures 2.3) connected to a laptop computer. Both these methods rely on the principle of applanation tonometry, where the curved surface of an artery is flattened by pencil-shaped probe, known as a tonometer (SPC-301, Millar instruments, Houston, Texas). Incorporated into the tip of the tonometer is a high-fidelity micro-manometer, consisting of a 0.5 × 1.0 mm piezoelectric crystal with a frequency response greater than 2 kHz. By flattening the curved surface of an artery, circumferential pressures are equalised and intraluminal pressures can be recorded accurately in the form of arterial pressure waveforms.

Pulse wave analysis was derived by placement of a tonometer on the radial artery of the right arm. A pulse trace was recorded for a minimum of 10 consecutive beats and an average waveform was processed by the in-built software package. A generalised transfer function, based on Fourier analysis, was then used to generate a corresponding central aortic waveform (Figure 2.4). The generalised transfer function has been prospectively validated for the assessment of ascending aortic blood pressure and showed good repeatability of measurements (Wilkinson et al., 1998). With the integral software, augmentation pressure (AP) was calculated as the difference between the second and first systolic peaks of the central waveform, and the AIx was calculated as AP expressed as a percentage of pulse pressure (PP) (Figure 2.4).

Measurement of aortic PWV was performed using the principle of applanation tonometry using a high-fidelity micro-manometer with the participant in the supine

position. Electrocardiographic-gated carotid and femoral artery waveforms were measured. Distances from the carotid pulse site to the manubrium sternum and to the femoral artery were measured as straight lines between points on the body surface using a tape measure. The time (t) between the onset of femoral and carotid waveforms was determined as the mean of 10 consecutive cardiac cycles. The PWV (m/sec) was calculated by dividing the distance between measurement points (D) to the measured time delay (t) (Figure 2.5). All measurements were duplicated, unless they differed by more than 0.5 m/sec, in which case a third reading was taken, with the mean values being used in subsequent analysis.

Calibration of the SphygmoCor device was undertaken using average brachial blood pressure in the dominant arm using a validated oscillometric method (HEM-750CP; OMRON Corporation, Japan) after 10 minutes of seated rest (O'brien et al., 1996). Three independent measurements of systolic and diastolic blood pressure were taken, and the average of the last two measurements was used. Blood pressure was classified according to the British Hypertension Society (Table 2.2) (Williams et al., 2004).
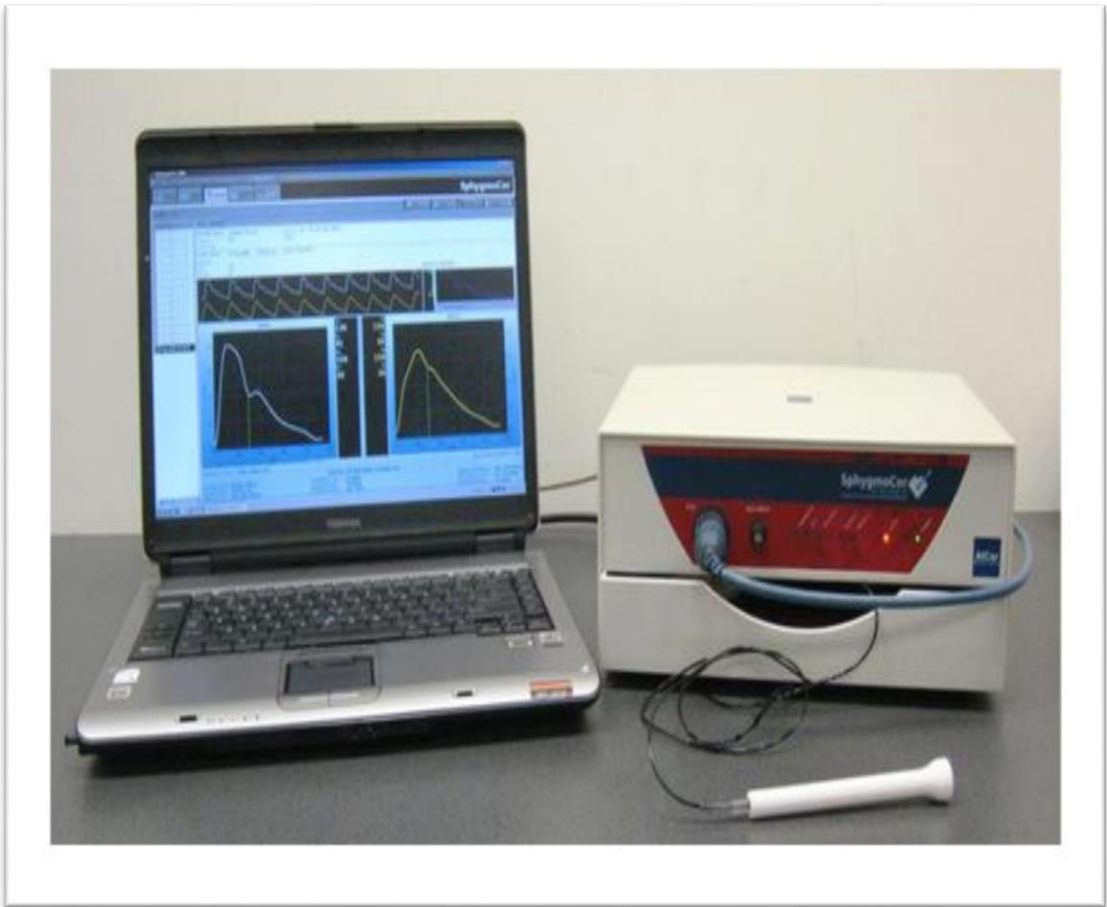
Figure 2.3: Picture of SphygmoCor Machine (AtCor, Sydney)
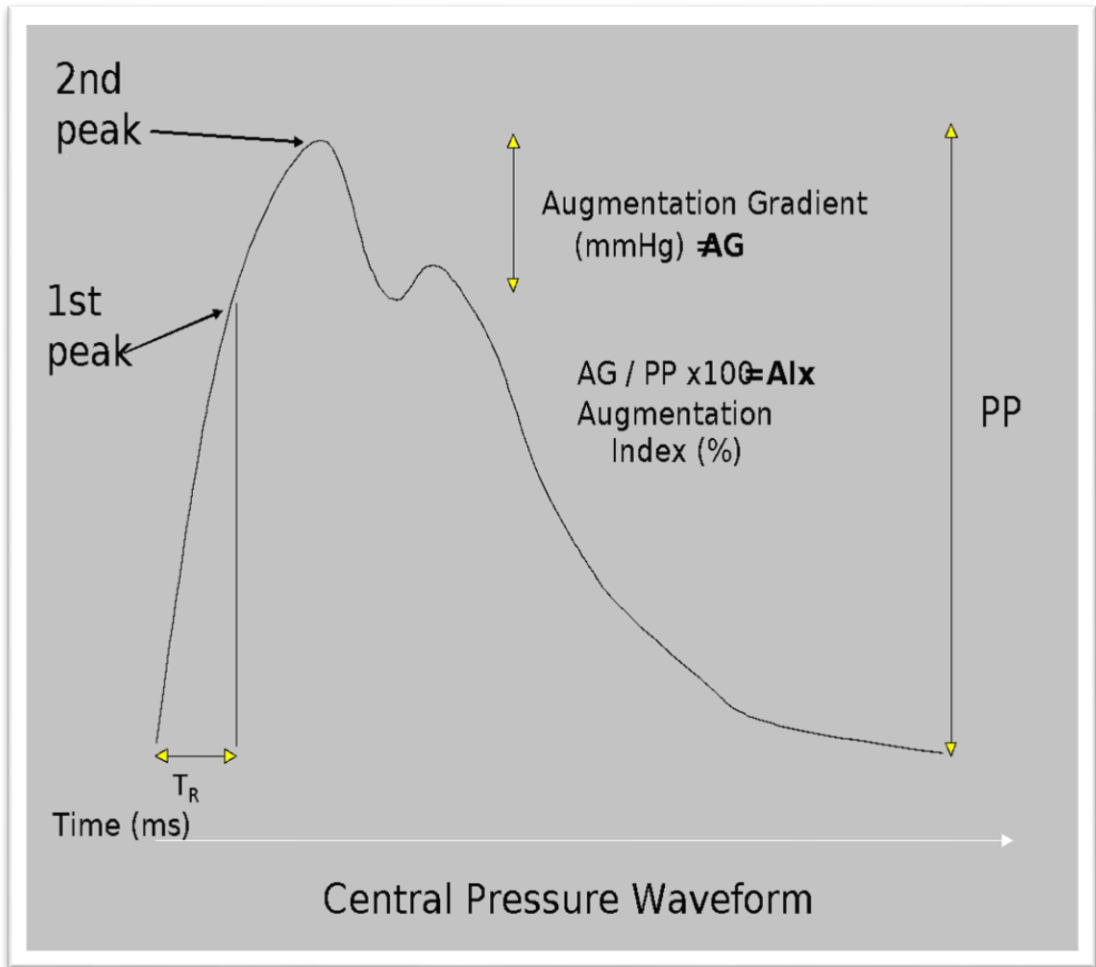
Figure 2.4: Central arterial pressure waveform and its derivatives
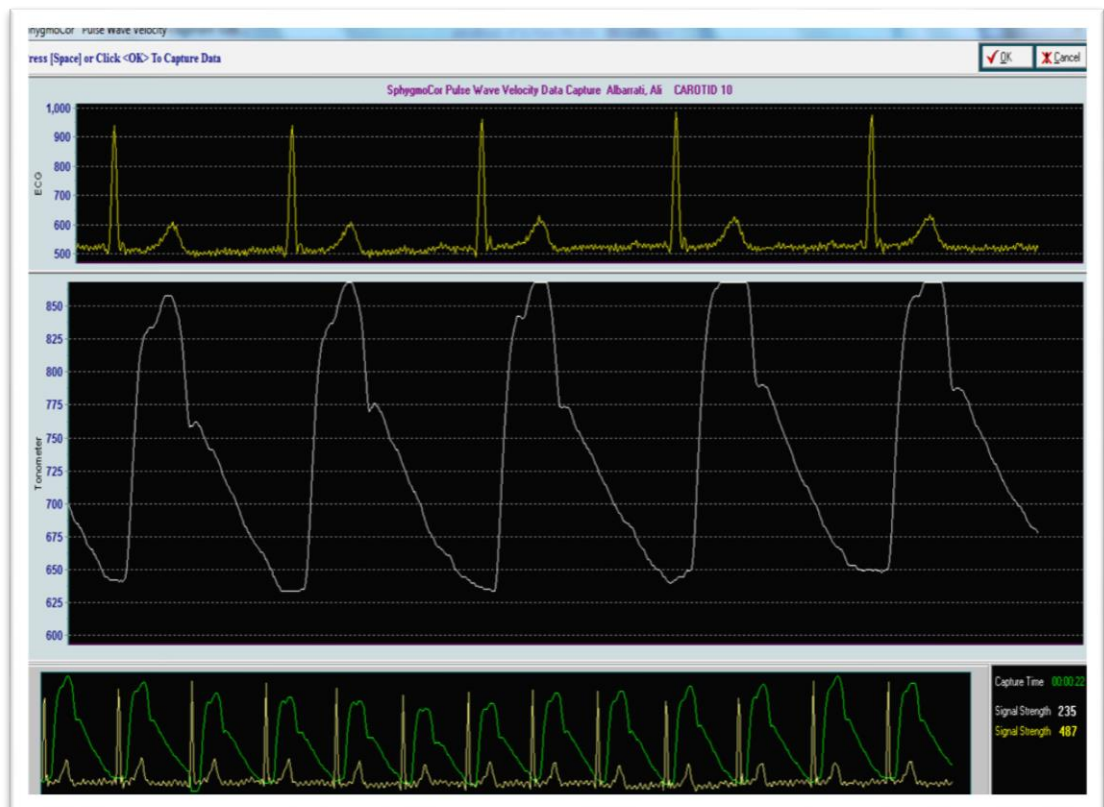
Figure 2.5: Carotid and femoral arterial waveforms and aortic PWV

Table 2.2: Classification of blood pressure levels by the British Hypertension Society (Williams et al., 2004)

	Systole (mmHg)	Diastole (mmHg)
Normotensive	<120	<80
Hypertension	≥140	≥90
Isolated systolic hypertension	140-159	<90

2.3.4 Cardiac Output Measurement

With the participant lying supine, the researcher entered participant's personal data into the machine (identity, sex, height and weight). Cardiac haemodynamics were measured non-invasively using a bioreactance technology (NICOM, Cheetah, Medical Inc., Indianapolis, IN) (Figure 2.6). This system is based on the discovery that changes in aortic blood flow induce small changes in the frequency of electrical signals propagating across the thorax. Changing the frequency across the chest cavity causes this system to be less affected by body movement, location of the electrodes, or the filtering of electrical interference. The NICOM system consists of four disposable pairs of double electrodes, which were attached to the patient's chest—two on either side of the lower thorax (positioned marginally below the ribs) and two just below the clavicle (Figure 2.7). Each pair of electrodes delivers a low alternating current, and its propagation characteristics are sensed along the thorax by the other electrode pairs. Therefore, cardiac haemodynamics are measured separately from each side; then, the average of two measurements is calculated. These measurements took about one minute to complete.

The signal-processing unit of the NICOM system determines the relative phase shift ($\Delta\Phi$,) of the input signal relative to the output signal. The relative phase shift is in turn due to changes in blood flow in the aorta with every beat. It has been shown that stroke volume can be estimated by:

$$SV = C \cdot VET \cdot d\Phi/dt_{max}$$

Where C is a constant of proportionality and VET is the ventricular ejection time, which is determined from the NICOM and electrocardiogram signal. The value of C is optimized with regard to participant age, sex and body size. The cardiac output is then calculated using the equation:

Cardiac output = Stroke volume X Heart rate

Figure 2.6: NICOM device

(Picture taken from: http://www.cheetah-medical.com)
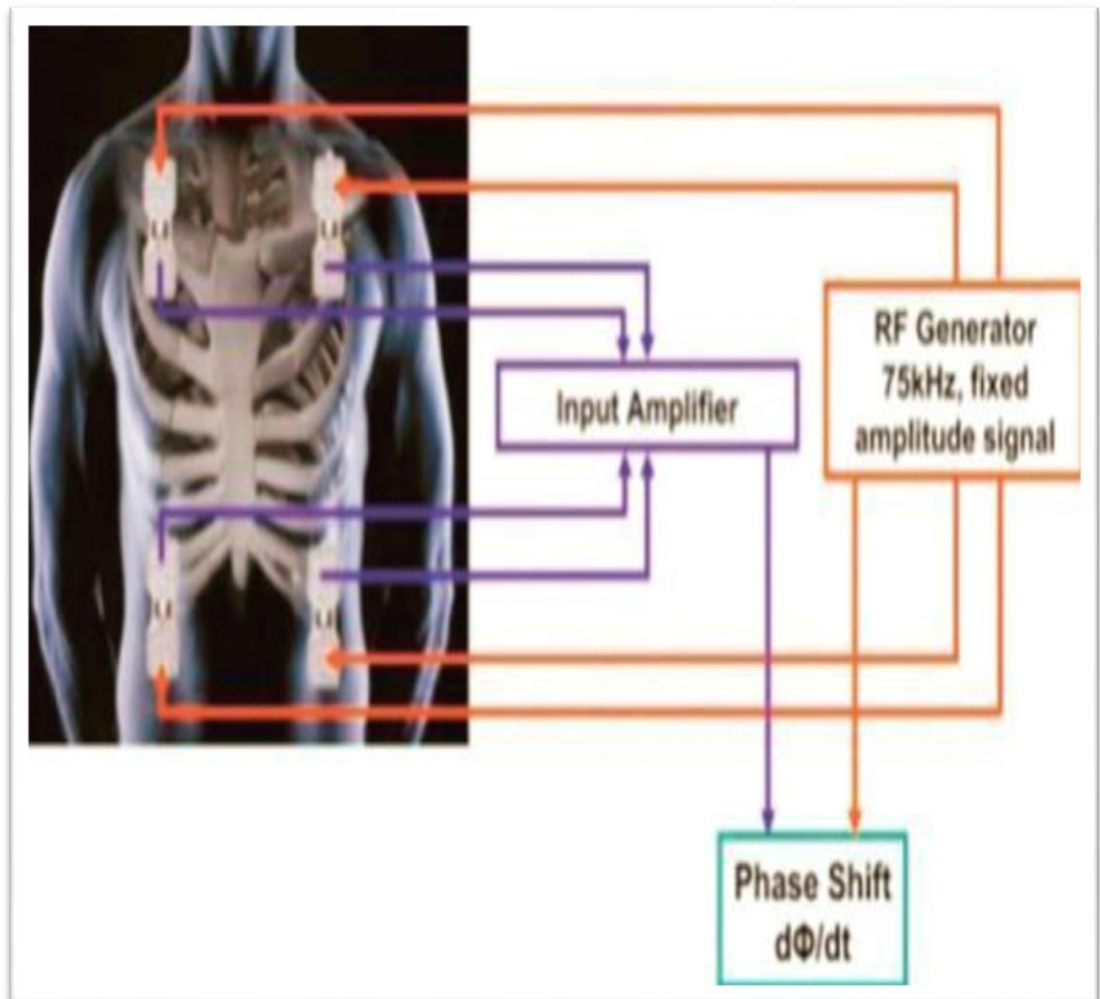
Figure 2.7: The NICOM system and its connection to the body

2.3.5 Pulmonary Function Tests

All pulmonary function tests were performed and interpreted according to the GOLD guidelines (GOLD, 2014). The subjects underwent spirometric evaluation (forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), FEV_1/FVC ratio) using a Vitalograph Alpha (Bucks, UK) spirometer. The spirometer was annually calibrated by vitalograph technician. Patients were asked to refrain from using their inhalers for 6 hours for short acting bronchodilators and 12 hours for long acting bronchodilators prior to their visit. The tests were performed with the subject in a sitting position and with nose clips in place. Participants were instructed to take the deepest possible breath, place their lips around the mouthpiece of the spirometer, and breathe out as fast as possible, as hard as possible and for as long as possible. All participants were given a demonstration of the procedure prior to the test. The FEV_1, FVC, FEV_1/FVC were recorded along with percentage predicted values based on reference values for age, sex, height, race and smoking status. Each subject performed at least three spirometric tests with at least two reproducible and acceptable manoeuvres. Reliability was considered satisfactory when the second highest values of FEV_1 and FVC were within 5% of the highest value.

Subjects with obstructive spirometry (FEV_1/FVC <70%) underwent reversibility testing, where 400µg of salbutamol is given via a spacer device using the tidal breath technique. After a ten minute wait, spirometry was repeated a further three times. Reversibility was defined as an increase in FEV_1 of 12% and 200 ml (GOLD, 2014).

2.3.6 Timed Up and Go

Timed Up and Go (TUG) measurement was obtained by the researcher using an armchair (45 cm high) and a stopwatch (accurate to 0.1 s). Subjects were seated with their back supported against the chair back. They were instructed to stand up, walk three metres (to a mark on the floor), cross the mark, turn around, walk back to the chair and sit down (Podsiadlo and Richardson, 1991). The task was to be performed at a normal comfortable pace. The stopwatch was started on the word "go" and stopped as the subject sat down. The TUG time was documented in seconds.

2.3.7 Six–Minute Walk Distance (6MWD)

Blood pressure, heart rate, oxygen saturation and breathlessness score using a Borg scale were taken by the researcher before the test, while the participant was sitting comfortably on a chair with arms. Resting heart rate and oxygen saturation were measured using a pulse oximeter (Pulsox-3iA, Konica-Minolta, Japan). The probe was placed on the index finger of each participant until the heart rate and oxygen saturation stabilised. The subjects were asked to rate their breathlessness using the Modified Borg Scale at the start and end of the walk; this is constructed as a category scale with ratio properties (Borg, 1982). A score of 0 would indicate nothing at all; 0.5 would indicate very, very slight (just noticeable) breathlessness, followed by whole numbers 1 through to 10 (Table 2.3). Particular care was taken to check for absolute contraindications prior to performing the test, as the American Thoracic Society guidelines (2002).

All 6MWD were performed in the Wales Heart Research Institute by the researcher certified in cardiopulmonary resuscitation. Oxygen, sublingual nitroglycerin, a short-acting inhaled bronchodilator and crash cart were in the immediate vicinity of the test area, and a protocol was in place to call for immediate medical assistance if required.

Subjects were instructed to wear light clothing and footwear suitable for walking. The patients were given 400μg of salbutamol via a spacer device 30 minutes prior to the test, after they had rested for at least 15 minutes.

The 6MWD was carried out in accordance with a protocol adapted from the American Thoracic Society's (ATS) guidelines (2002) using a 30-m straight level course in an enclosed corridor. The course was marked with cones at either end. Chairs were placed at each end and at the midway point to allow resting points if required. Standardised instructions were read aloud to the subjects before the test. Patients were told to stop walking and inform the researcher if they experienced any chest pain or dizziness. At the end of each minute, patients were notified of the elapsed time and given standard encouragement.

If a subject rested during the 6MWD, the following encouragement was given at 15 s intervals: 'begin walking as soon as you feel able'. In the event that profound oxygen desaturation occurred (defined as SpO_2 <88%), the researcher instructed the patient to stop walking immediately and to recommence walking if SpO_2 recovered to >88%. Patients remained in the clinical area for a minimum of 10 min. following an uncomplicated 6MWD. During this time, they were closely observed and any abnormal signs and symptoms recorded.

Table 2.3: Modified Borg Scale (Borg, 1982)

SCALE	SEVERITY
0	No breathlessness at all
0.5	Very very slight (just noticeable)
1	Very slight
2	Slight breathlessness
3	Moderate
4	Somewhat severe
5	Severe breathlessness
6	
7	Very severe breathlessness
8	
9	Very very severe (almost Maximum)
10	Maximum

2.3.8 Handgrip Strength

Maximal right and left handgrip strength (HGS) was determined twice in each hand using a hand dynamometer (T.K.K. 5401 grip-D, Takei, Japan) (Roberts et al., 2011). In a standing position, with arms by the sides, participants were asked to perform two maximum contractions alternating between left and right, each lasting 3-5 seconds and at least 15 seconds apart. The maximum handgrip was determined as the average of four measurements.

2.3.9 Health Related Quality of Life Measurement

Patients completed the St George's Respiratory Questionnaire (SGRQ), a 50-item status questionnaire designed to measure health impairment (quality of life) in patients with COPD (Jones et al., 1992). The questionnaire consists of three domains; symptoms, activity and impact (Appendix E). The first part asks about the patient's symptoms over the last twelve months, and the questions should be answered based on the patient's perception of their recent respiratory problems. The second part of the questionnaire focuses on the patient's activity in daily living and the impact of COPD on their psycho-functional activity. The questionnaire was completed to the best of the patient's knowledge. If the patient had a query regarding a question on the questionnaire, an explanation was given without answering the question. After completion of the questionnaire, the researcher went through it to check if there were any missing answers.

Each domain of the questionnaire was scored from 0 to 100, where 0 represents the best possible health status and 100 represents the worst possible. A total score was calculated which summarises the effect of the disease on health status and is expressed as a percentage.

Patients also completed a questionnaire about the impact of COPD on their health status. The COPD Assessment Test (CAT) is a simple, reliable and validated designed to assess the impact of COPD on health status (Jones et al., 2009). It is a

scoring questionnaire consists of eight domains that cover the effect of COPD on daily life and health (Appendix F).

2.3.10 Comprehensive Geriatric Assessment

Each participant was interviewed to complete a comprehensive geriatric assessment (Rockwood et al., 2005). It is a one-page form to assess the effect of ageing on general health and identify the number of deficits. This questionnaire consists of 16 standard domains (Appendix G). The patient scored 1 if he/she had a deficit and 0 if did not. The total score for this questionnaire is out of 61 points. Frailty index (FI) was then calculated by dividing the number of deficits that the patient had by the maximum, 61.

2.3.11 Dual Energy X-ray Absorptiometry (DXA)

Data from a sub-set of patients who had been diagnosed with osteoporosis and had a dual energy X-ray absorptiometry (DXA) scan as part of their normal medical care within a twelve month period from the initial visit was accessed and the results of the DXA scan were used to examine the validity of the CUBA scan. The DXA scan was performed using a Hologic Discovery bone densitometer (Hologic Inc, Waltham, MA, USA) using the whole body protocol and the fan beam array method. The scan was performed by a radiographer with the patient lying in a supine position with total effective radiation dose of 5.2 µSv (Blake and Fogelman, 1998). Calibration was performed daily using a phantom spine to check bone mineral density (BMD).

The BMD at individual lumbar spine (L1-L4) and hip (neck, trochanter and intertrochanteric regions) sites were measured, along with a mean BMD at the lumbar spine and hip. The BMD at all sites was recorded as an absolute figure (g/cm^2) and as a standardised T and Z score. The T score standardises the BMD by relating the results as a standard deviation from the mean of a young healthy population of the same gender, while the Z score relates the BMD as an SD from the mean of an age and gender matched population (WHO, 1994).

2.3.12 Blood

Peripheral blood samples were collected by a research nurse from all subjects from the ante-cubital fossa following haemodynamic measurements. Serum creatinine, estimated glomerular filtration rate, high sensitivity protein and fibrinogen were measured using standard enzymatic methods in the department of Biochemistry, University Hospital of Wales.
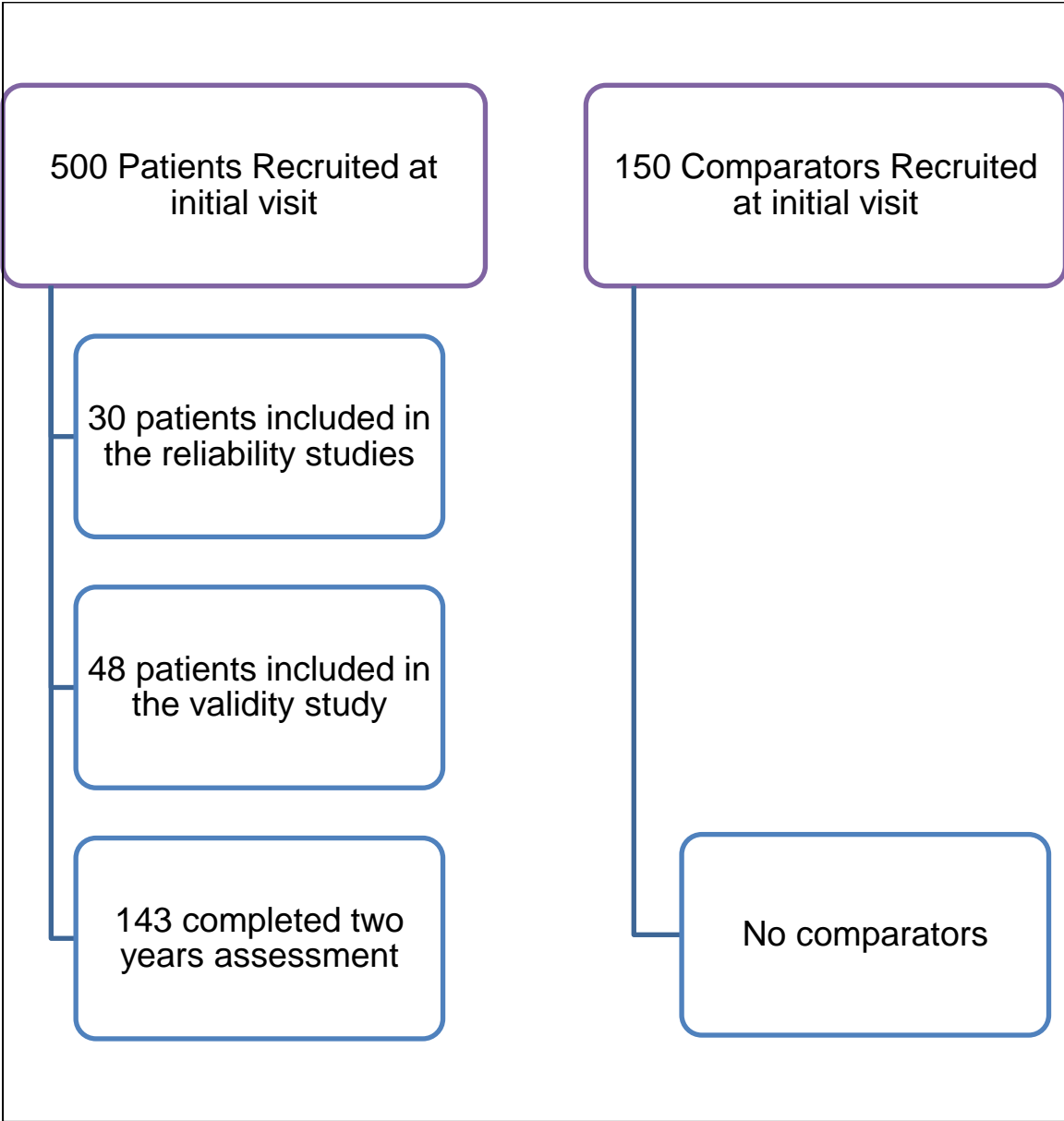
Figure 2.8: Research Flow Chart

Chapter Three

Methodological Studies

3.1 Methodological Consideration

The reliability of an outcome measure reflects how reproducible or repeatable the measurement is under a given set of circumstances. This chapter of the thesis examined the reliability of outcome measures to be of use in the longitudinal studies and its feasibility in routine clinical practice.

3.1.1 Reliability

For an outcome measurement to be useful, it must provide stable or reproducible values with small errors of measurement when no variable is influencing the attribute that the measurement is quantifying (Rankin and Stokes, 1998). Measurement errors are of two types: one is systematic errors, which may constantly under-predict or over-predict values. Systematic error does not pose problems to the reliability of the outcome measure. However, it creates problems of validity since the measured value is not the true representation of the quantity measured. The second type of the measurement error is a random error. Random error does pose a problem to reliability, as they occur due to unpredictable factors such as mechanical inaccuracy, lack of experience and fatigue. Inevitability, the unpredictability of the working environment and subjects involved cannot be avoided, even if the sources of the errors are anticipated. Random errors can be diminished by taking several measurements then calculating the average score as a good estimate of the true value (Portney and Watkins, 2009). Since measurement error is inevitable and true reliability or a true value can never be calculated, reliability is estimated based on the statistical concept of variance. Variance is 'the measure of variability or the difference among scores within a sample' (Portney and Watkins, 2009:64). It helps to find out the true difference among the values measured by yielding a ratio called the reliability coefficient that has a coefficient of 1.0 for maximum reliability. Reliability coefficients are based on a measure of correlation such as Pearson's product moment correlation or the intraclass Correlation Coefficient and range between 0.00 and 1.00. The more

reliable the measurement response, the less error variability there will be around the mean (Bruton et al., 2000). The ICC is one appropriate approach that can be used to assess relative reliability among two or more trials. It is based on measures of variance from the analysis of variance (ANOVA) (Portney and Watkins, 2009). There are different types of ICCs that are available, equation ICC (3, 1), a two-way mixed model/absolute agreement, was used to assess the reliability of a fixed rater for repeated measurements (Rankin and Stokes, 1998). A coefficient below 0.50 is considered as indicating poor reliability, between 0.50 and 0.75 is considered as moderate reliability and above 0.75 suggest good reliability (Portney and Watkins, 2009). As reliability cannot rely solely on ICC, since it measures only the strength of association between the two variables not extent of agreement between them. An assessment of agreement, as in Bland and Altman should be used in conjunction with correlation in any reliability study.

There are two types of reliability, intra-rater (test-retest) and inter-rater reliability. The intra-rater reliability also called test-retest reliability. Intra-rater reliability is the reliability of measurement taken by the same researcher on different occasions. It is mainly used to establish the consistency of the measurement tool and, for the outcome measure to be reliable, the values obtained in a multiple trial should be similar. The interval between test and retest should remain stable to avoid random error (Portney and Watkins, 2009). Additionally, proper instruction and settings should be provided to maintain the uniformity of a study. The second type of the reliability is inter-rater reliability. The inter-rater reliability is the reliability of the measurement taken by multiple researchers (Bruton et al., 2000).

3.1.2 Validity

Validity refers to the extent to which an instrument measures what it claims and intends to measure accurately (Portney and Watkins, 2009). It implies that the measurements are free from error and emphasises the ability to make inferences from the values measured in the study (Portney and Watkins, 2009). Validity can be subdivided into four categories:

I) Construct Validity

This is the degree to which a test identifies the concept or variable of that which is being measured (Brink et al., 2006). To establish the construct validity of a method, the researcher must determine the degree to which the measure accords with other measures designed to measure the same thing and whether the new test behaves as expected. In addition, the tool should demonstrate appropriateness in measurement (convergent validity) and avoid extraneous information (divergent validity).

II) Criterion Validity

This is further subcategorised into concurrent validity and predictive validity.

Concurrent validity is related to which measurement tool is being examined against a previously validated measure, often a gold standard measurement. This is considered as a common method of examining the validity of a new tool or instrument (Portney and Watkins, 2009). Concurrent validity can be performed when a new or unexamined equipment is easy and feasible to administer and potentially more efficient. Predictive validity refers to a tool's capability to predict and make inferences about the future.

III) Content Validity

This refers to what extent a tool adequately measures what it asserts to be measuring. The content validity employs a reference standard, also called a 'gold standard'. It is a measure accepted by a consensus of content experts as the best available for determining the presence or absence of a particular phenomenon (Portney and Watkins, 2009).

IV) Face Validity

This refers to a measure's appropriateness and relevance to measure what it is supposed to measure as assessed by a group of experts (Brink et al., 2006). In other words, it is to examine whether the future measure seems to provide a reasonable measure of the concept it is intended to measure.

3.2 Reliability of Arterial Stiffness Measurement in COPD

3.2.1 Background

Chronic obstructive pulmonary disease (COPD) is a systemic disorder associated with an increased risk of cardiovascular disease (CVD) (Sin and Man, 2005). A number of studies have examined the mechanism that links COPD with CVD; however, the precise pathological process is still uncertain. Recently, the role of inflammation has been recognised in the pathogenesis of COPD (Thomsen et al., 2012). One of the most commonly used indicators of inflammation is CRP. CRP is one of the modulating factors of the pathogenesis in atherosclerosis (Barr et al., 2012, Iwamoto et al., 2009). In patients with COPD, the level of CRP is increased and, therefore, may contribute or accelerate aortic stiffness and calcification, which are risk factors for atherosclerosis (Bolton et al., 2011, Man et al., 2008).

Like COPD, atherosclerosis is a chronic progressive disorder affecting the elasticity of the medium and large arteries (Lusis, 2000). With increasing age, the loss of vascular properties of the arterial wall in the presence of cardiovascular risk factors contributes to stiffness of the large arteries in healthy elderly as well as in individuals with COPD (McEniery et al., 2005, Sabit et al., 2007). Several cross-sectional studies have found a relationship between arterial stiffness and COPD, suggesting that arterial stiffness could be a driving mechanism that increases the CVD risk in patients with COPD (Sabit et al., 2007, Cinarka et al., 2013). Stiffness of the large central arteries puts a great burden on cardiac function by decreasing diastolic time, which in turn leads to insufficient blood supply to myocardial tissue and the coronary arteries, and this may increase the risk of cardiovascular events (Sabit et al., 2010a, Patel et al., 2013). Changes in vessel wall structure and function composition may be determined by measuring arterial stiffness. Aortic PWV is the gold standard for measuring arterial stiffness (Laurent et al., 2006). Several studies have reported that aortic PWV is an independent predictor of cardiovascular risk in various populations (Boutouyrie et al.,

2002, Cruickshank et al., 2002, Blacher et al., 1999, Sutton-Tyrrell et al., 2005, Willum-Hansen et al., 2006).

However, few studies have evaluated the repeatability of aortic PWV in COPD. Stone and colleagues (2013) examined the reproducibility of aortic PWV measure using a Vicorder machine in 23 stable patients with hyperinflation, with two weeks in-between the measurements. In this study, the authors found this machine had a reproducibility coefficient of 4.02% and limit of agreement between -0.68 and 0.75 m/s. Although the Vicorder machine showed to be feasible for measuring aortic PWV, use of reproducibility coefficients and small sample size limits the generalisibility. The title of the study indicates that this is an inter-rater study, but it is unclear from the methodological section whether it is a reproducibility or reliability study.

A number of studies have examined the reliability of PWV in healthy individuals and in those in diseased states. A frequently cited paper is that of Wilkinson et al. (1998), which demonstrates within-day intra and inter-rater reproducibility in healthy subjects and patients with hypertension and Hypercholesterolaemia. This paper showed no difference in aortic PWV within and between operators, ($p>0.05$). The Bland-Altman plots confirmed that there was no systematic bias and that 95% of data fell within the limits of agreement (Bland and Altman, 1986). More recently, Frimodt-Moller and colleagues (2006) confirmed the within-day reliability of aortic PWV in 23 healthy middle-aged participants, but they found that there were differences following acute cigarette smoking (within four hours after last cigarette).

Whilst within-day reliability is important, interventional research requires that outcome measures are stable from day to day (Bland and Altman, 1986). The between-day reliability of PWV has been investigated for a number of populations.

As part of a larger study, Tanaka et al. (1998) investigated between-day reliability in eight men and women. Aortic PWV was measured and the results were pooled for analysis, with no difference in mean PWV between days. However, in this study, the

time interval was not stated, so it is difficult to extrapolate the findings; and pooling of aortic PWV may not be valid as the variation may be obscured when central and peripheral velocities are combined (Laurent et al., 2006). Furthermore, a larger study showed no difference in aortic PWV when measurements were repeated over a time interval of 2.5 weeks with a coefficient of variation of 3.2% and acceptable Bland-Altman plots (Liang et al., 1998). Similarly, ter Avest et al. (2005) demonstrated good reliability between days and between afternoon and morning assessment in 19 healthy subjects using the SphygmoCor device. Using the same device, Frimodt-Moller et al. (2008) assessed the intra- and inter-observer reliability of aortic PWV in 19 patients with chronic kidney disease. They showed good reliability within a week in this population. Good reliability has also been reported in a large sample of patients with kidney disease who were assessed twice, with a time interval ranging between two and 16 weeks (Savage et al., 2002).

To evaluate aortic PWV in a long-term study, reliability is critical. In healthy individuals, intra and inter-operator reliability has been established and shown to be high, as mentioned in the above studies. Although arterial stiffness has been used as a measure of cardiovascular risk in patients with COPD in a number of studies, the reliability of this measure has not been established in this population using the SphygmoCor device. Therefore, the aim of this study was to examine the reliability of aortic PWV over one week.

3.3   Reliability of Cardiac Haemodynamics Measurement

3.3.1   Background

Increased cardiovascular risk is one feature of COPD (Sin and Man, 2005). Several studies support the relationship between COPD and CVD. Cardiovascular death related to COPD is under-diagnosed and under-recognised in this population. A recent well-conducted study TOwards a Revolution in COPD Health (TORCH) found that cardiovascular causes were involved in 27% of COPD deaths (McGarvey et al., 2007). Additionally, CVD increases the risk of hospitalisation by 42% with COPD (Anthonisen et al., 2002). The exact mechanism linking COPD to CVD remains unknown. Yet, there are several contributing factors that increase cardiovascular injury and the load on the heart function including systemic inflammation, hypoxia, aortic stiffness, autonomic nervous system dysfunction and oxidative stress (Maclay et al., 2012, Sabit et al., 2010a, Sin and Man, 2005, Skwarski et al., 1998, Van Gestel et al., 2012). The existence of one or more of these factors, beside COPD, can increase the burden on the heart, which will lead to left ventricular dysfunction or heart failure, even in the mild stages of COPD (Barnes and Celli, 2009, Rutten et al., 2005b). In a study of 405 patients with COPD, almost 21% of patients were diagnosed with hidden heart failure (Rutten et al., 2005b). Moreover, in a large multi-ethnic prospective cohort study of patients with emphysema and unknown cardiovascular disease, cardiac haemodynamics and left ventricle changes were present, even in mild stages (Barr et al., 2010).

Furthermore, hyperinflation is a feature of COPD that is associated with abnormal lung function (Ferguson, 2006). The effect of hyperinflation on the heart has not been studied extensively in patients with COPD. Jorgensen et al. (2007) found that in severe emphysema patients, there was a reduction in right and left heart size compared with non-emphysematous patients. More recently, Watz et al. (2010) found that hyperinflation was associated with reduced heart size and function across the

whole spectrum of COPD severity. Increased pulmonary vascular resistance and pulmonary arterial pressure, as consequences of hyperinflation, will lead the heart chambers to adapt to these changes by changing preload filling (Holverda et al., 2009).

There is a close relationship between heart size and cardiac function. Reduced heart chambers size leads to changes in cardiac output and stroke volume, which are measures of cardiac performance (Boussuges et al., 2000).

Cardiac output (CO) is one of the fundamental measures of heart function and the main determinant of global oxygen transport from the heart to the body. Additionally, the primary function of the cardiovascular system is to supply sufficient amounts of oxygen to meet the metabolic demands of the tissues; therefore, it appears sensible to measure CO to obtain information about the efficiency of the cardiovascular system. Given that patients with COPD are at high risk of CVD, and especially heart failure, quantifying CO in routine clinical practice may provide insights into the presence and degree of heart failure (Williams et al., 2001).

A study by Barr et al. (2010) found that CO decreases by 100 ml/minute for every 10% decline in FEV_1/FVC, independent of smoking.

Moreover, stroke volume (SV) is another determinant of cardiac performance. Boussuges et al. (2000) studied 34 stable patients with COPD, without hypoxemia, hypertension or overt cardiovascular disease, and found that the patients had reduced left ventricle stroke volume, in the absence of hypertrophy. This finding is supported by another study on a small group of 25 patients with severe COPD, and with unknown high blood pressure, CVD or heart failure. In this study, patients had lower right and left ventricle stroke volumes than healthy controls, which was concomitant with right ventricle hypertrophy (Vonk-Noordegraaf et al., 2005). In a large prospective cohort study, Barr et al. (2010) confirmed the negative impact of COPD on cardiac haemodynamics. They reported that a greater extent of emphysema and airway

obstruction was associated with a decline in SV. There was a decrement of 15 ml in SV for every 10% reduction in FEV_1/FVC.

Taking the aforementioned studies together, it appears that the assessment of cardiac haemodynamics in patients with COPD is sensible, and will provide good insights into cardiac performance. Additionally, this will allow clinicians to detect the presence of heart dysfunction at an early stage and offer accurate management. The aforementioned studies have confirmed the changes in cardiac haemodynamics in patients with COPD, even at early stages, using Doppler ultrasound, echocardiography or cardiac magnetic resonance imaging. However, the application of these techniques in routine clinical settings is difficult due to their limited availability, the time needed, and the skills and knowledge of the operator.

Recently, a new method of measuring CO and SV based on a bioreactance technique has been developed to overcome the limitations associated with previous techniques such as bioimpedance technology. This non-invasive cardiac output measurement (NICOM) system is based on phase shift in the high frequency current delivered to the thoracic cavity as a result of changes in aortic blood volume. The accuracy, precision and responsiveness of this technique have been validated in several populations (Keren et al., 2007, Kossari et al., 2009, Rich et al., 2012, Squara et al., 2007).

The ideal technology for estimating CO and SV should be non-invasive, continuous and reproducible, and have a limit of agreement as assessed by a Bland and Altman plot (Bland and Altman, 1986). Furthermore, it should be convenient for both patient and health professional, and precise, and have minimum side effects. To date, the reliability of this system has been established in several patient populations, and one study demonstrated excellent reliability in healthy adults (Elliott et al., 2010). With regard to COPD, this technique should take into consideration the pathophysiology of COPD, hyperinflation and the ventilation-perfusion mismatches that are associated with COPD. To date, none of the available techniques satisfies all these criteria;

therefore, the aim of this study was to examine the reliability of measuring CO and SV non-invasively using the Cheetah machine.

Hypotheses

Aortic PWV will be a reliable outcome measure for assessing aortic stiffness in COPD after one week using the SphygmoCor machine.

Assessment of cardiac haemodynamics in COPD (i.e. CO and SV) will be reliable after one week using the Cheetah machine.

Aims

The aim of this study was to examine the reliability of aortic PWV in COPD over one week using the SphygmoCor machine. In addition, this study aimed to assess the reliability of cardiac haemodynamics (i.e. CO and SV) using the Cheetah machine.

3.3.2 Methods

A sub-group of patients were recruited from a large cross-sectional study of the Assessment of Risk in Chronic Airways Disease Evaluation (ARCADE), which had gained ethical approval from South East Wales ethic committee (REC reference number 11/WSE02/7). Patients gave written informed consent. Inclusion criteria were: aged 40-80; clinically stable with no exacerbation in the preceding four weeks. An exacerbation is defined as "an event in the natural course of the disease characterised by a change in the patient's baseline dyspnoea, cough and/or sputum beyond day to day variability sufficient to warrant a change in management" (GOLD, 2014).

Full inclusion and exclusion criteria were detailed (Chapter 2: Table 2.1, p 65).

The sample size was based on data from a published study on COPD from South Wales: mean (SD) aortic PWV was 11.4 (2.7) (Sabit et al. 2007). This equates to an effect size of 0.24. The G power software was used to calculate the sample size, with 80% of power at $p=0.05$ and an effect size of 0.24 for repeated measures ANOVA, and found to require 30 patients.

Anthropometric Measurements

Subjects had their height measured barefoot with their back straight against a wall using a stadiometer (Seca; Vogel & Halke, Hamburg, Germany) and their weight using an electronic scale (Chapter 2:2.3.1, p 68).

Measurement of Pulse Wave Velocity

Carotid-femoral PWV was assessed non-invasively using the SphygmoCor device connected to a laptop computer (Chapter 2:2.3.3, p 72).

Cardiac Haemodynamics Measurement

Cardiac haemodynamics were measured non-invasively using a new bioreactance technology (NICOM, Cheetah, Medical Inc., Indianapolis, IN) (Chapter 2:2.3.4, p 77).

Second Measurement

The patients came back after a week to repeat the same measurements, when the same protocol was followed.

Statistical Analyses

Descriptive statistics, repeated measures analysis of variance (ANOVA), intra-correlation coefficient and Bland and Altman plots were used to assess reliability. All analyses were carried out using SPSS software (version 18.0; SPSS Inc, Chicago, IL). The p value was set at <0.05 and ICC > 0.75. Data normality was checked prior to analysis using a Kolmogorov-Smirnov test.

3.3.3   Results

Thirty patients (17 male), mean (SD) age 67 (7), height 165 (10), weight 74.8 (18.2) and $FEV_1\%$ predicted 57 (18). The time between the two measurements was mean (SD) 7 (1) days.

A normality test showed that the variable was normally distributed as the p value showed no significant difference between the patients (p>0.05). The repeated measures ANOVA showed no variability between subject measurements of cf-PWV, as demonstrated by the F=0.002 and p=0.965. Similar findings were for CO measurements, F=0.002, p=0.965 and SV measurements, F=0.001, p=0.976. This suggests that there were no significant differences between the patients (Table 3.1).

Intra-rater test-retest reliability was analysed using the ICC (3, 1) equation. Between days reliability for cf-PWV was 0.96 (95% CI: 0.93- 0.98). Similarly for CO (0.93, 95% CI: 0.89- 0.97) and similar SV, 0.94 (0.94, 95% CI: 0.89-97). The relationship between the first and second visits cf-PWV was examined using a scatter plot with an R^2 of 0.87 (Figure 3.1). The relationship between the two occasions of CO and SV was plotted using a scatter plot (Figures 3.3) and (Figure 3.5), respectively. The R^2 between CO and SV measurements was 0.91 and 0.88. This means that 90% and 88% of the variance was shared between the first and second measurements of CO and SV, respectively.

For all the outcome measures, the majority of the points were also close to the line of best fit.

The Bland and Altman plot was calculated between the first and second cf-PWV, CO and SV. The mean difference and standard deviation with 95%CI of the mean for each outcome measure is tabulated (Table 3.2). Their difference was plotted on a Y-axis against their mean on an X-axis. The cf-PWV plot showed that all but two patients fell within the 95%CI limit of agreement (Figure 3.2). The plot also revealed that there was no systematic bias in the differences between visits, as the zero value

was included in the 95%CI. The plot for CO (Figure 3.4) and SV (Figure 3.6) showed

no systematic bias, as the zero value was included in the 95%CI and the points were

distributed evenly around the mean, with minimal random error. The plot showed two

clear outliers for each outcome measure.

Table 3.1: Physiological characteristics of patients at both visits

	First Visit Mean (SD)	Second Visit Mean (SD)	p value
Aortic PWV (m/sec)	9.7 (2.2)	9.7 (2.2)	0.965
Cardiac Output (L/min)	5.7 (1.1)	5.6 (1.1)	0.965
Stroke Volume (ml)	84 (20)	82 (20)	0.976
Peripheral SBP (mmHg)	138 (18)	135 (16)	0.347
Peripheral DBP (mmHg)	76 (8)	74 (9)	0.135
Central SBP (mmHg)	127 (17)	125 (13)	0.331
Central DBP (mmHg)	77 (9)	76 (8)	0.255
Heart Rate (bpm)	70 (16)	72 (14)	0.696

Abbreviations: **PWV=** pulse wave velocity; **SBP=** systolic blood pressure;

  DBP= diastolic blood pressure.

Table 3.2: Limit of Agreement Calculation of Outcome Measures

Outcome Measure	Mean Difference (SD)	Limit of Agreement
Aortic PWV (m/s)	0.01 (0.83)	-1.62_1.62
Stroke Volume (ml)	2.07 (7.34)	-12.32_16.46
Cardiac Output (L/min)	0.061 (0.35)	-0.75_0.62

Data presented as mean difference (SD) and 95%CI.
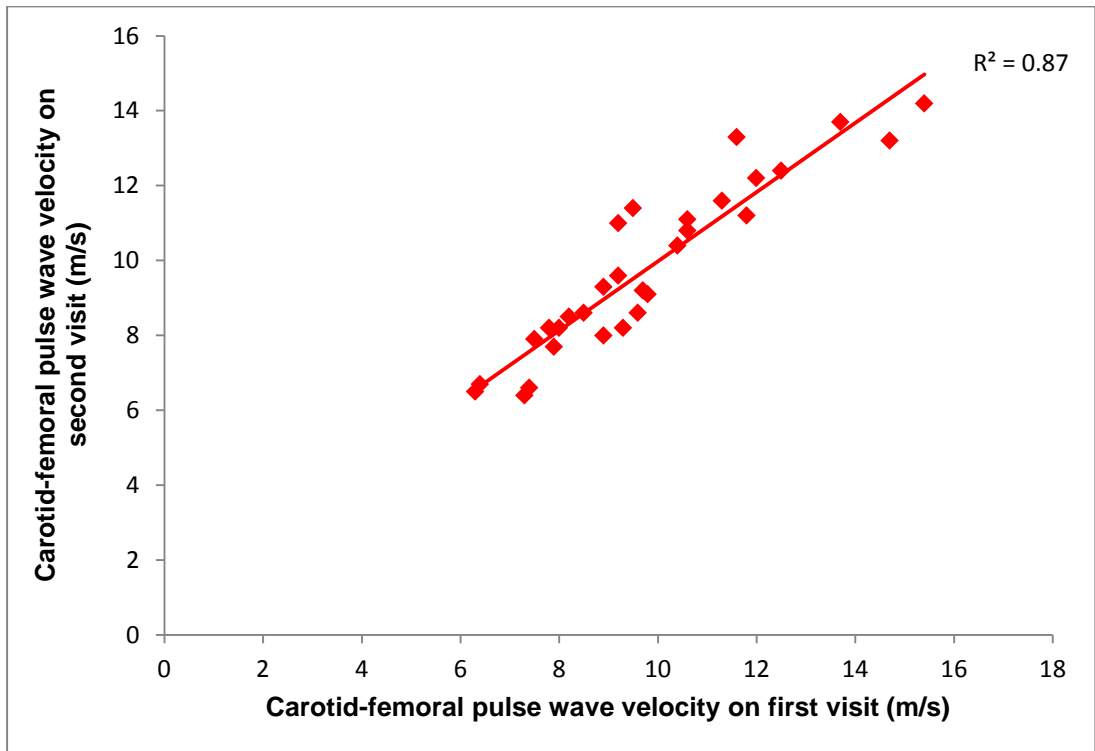
Figure 3.1: The relationship between the first and second measurement of carotid-femoral pulse wave velocity
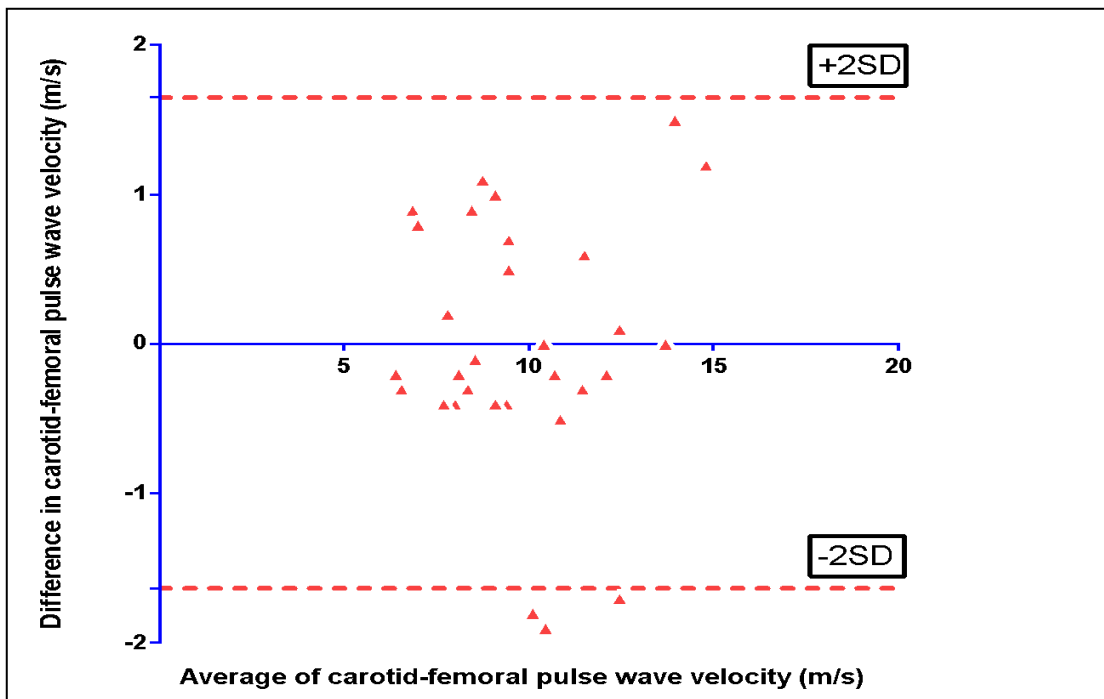
Figure 3.2: Bland and Altman plot showing between day differences in carotid-femoral pulse wave velocity
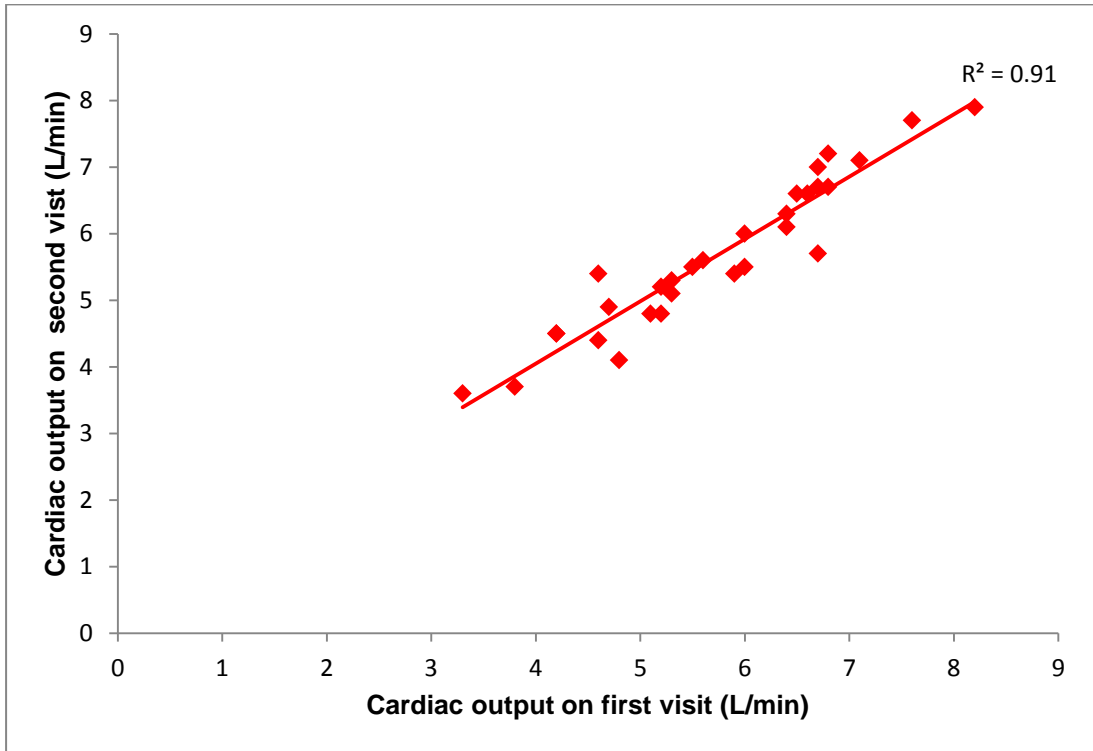
Figure 3.3: Relationship between first and second visits of Cardiac Output
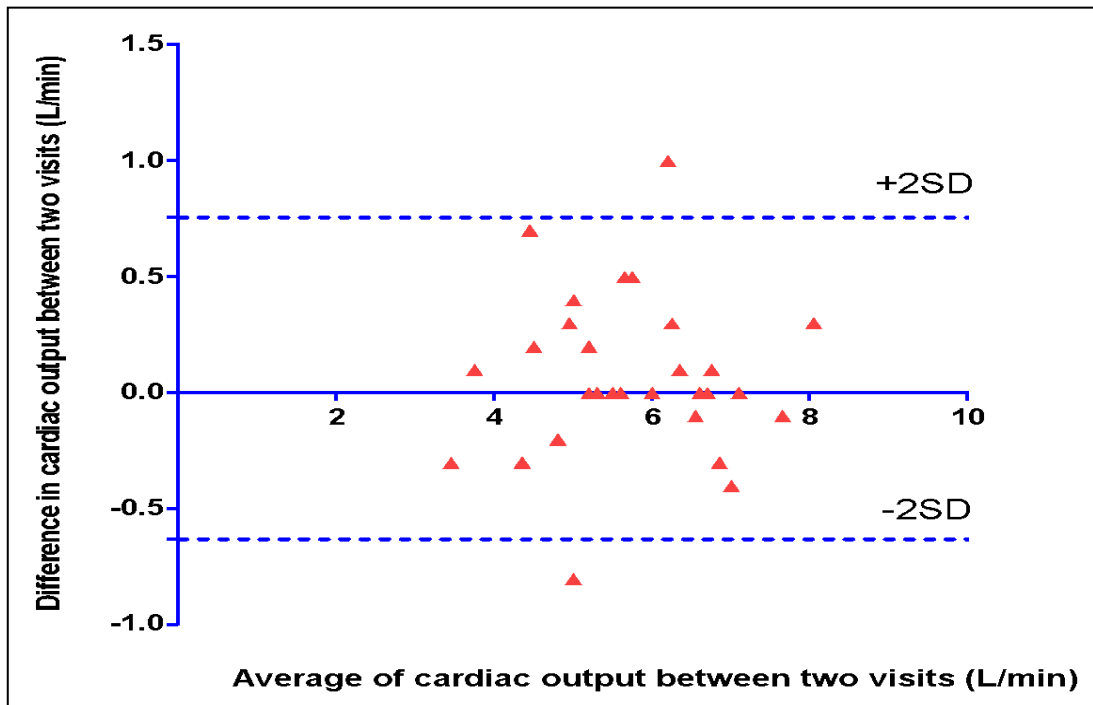
Figure 3.4: Bland and Altman plot showing between day differences in Cardiac Output
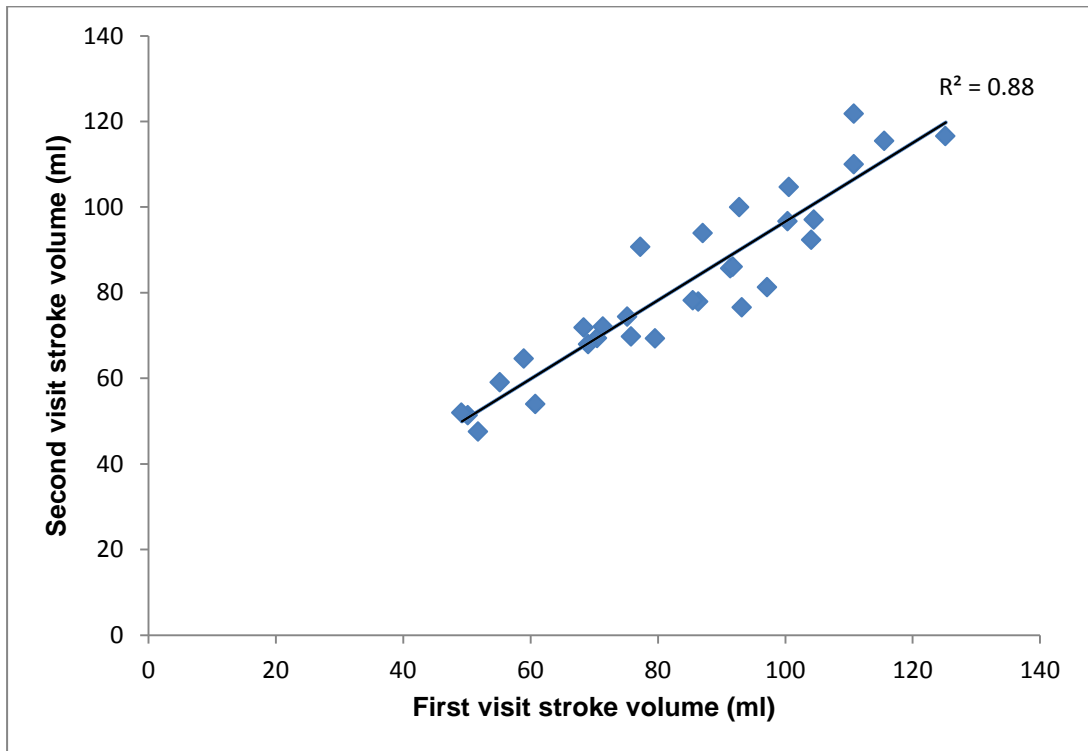
Figure 3.5: Relationship between first and second visits for Stroke Volume
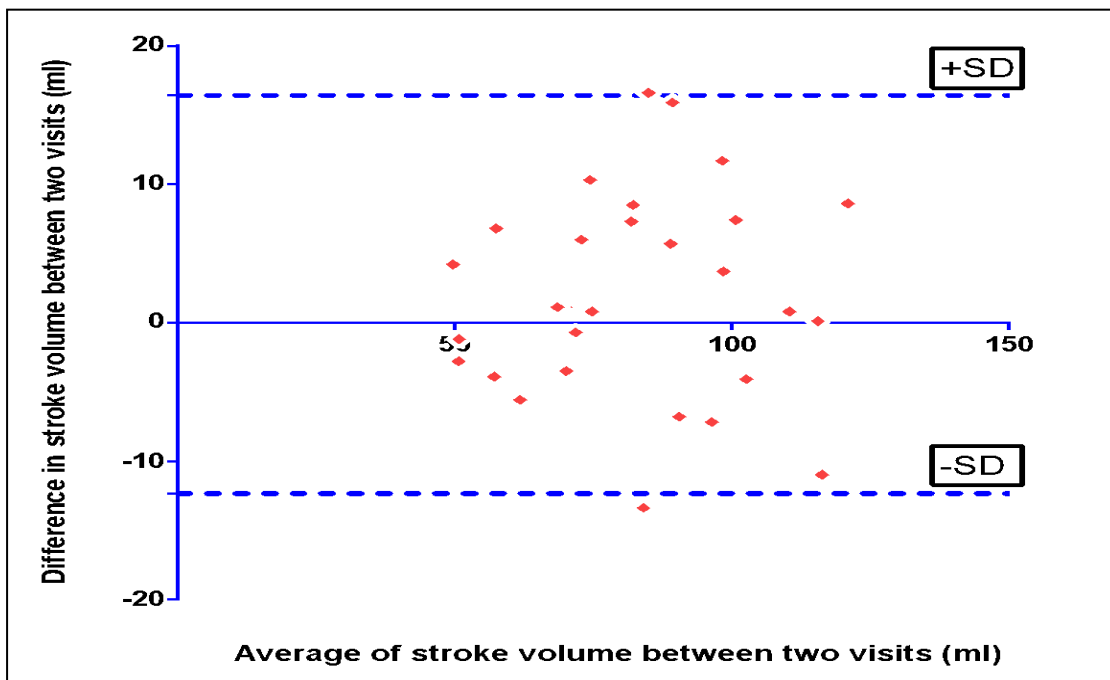
Figure 3.6: Bland and Altman plot showing between day differences in Stroke Volume

3.3.4 Discussion

Reliability of Carotid-femoral Pulse Wave Velocity

Patients with COPD are known to have elevated aortic stiffness and this may have a bearing on repeatability (Sabit et al., 2007). However, in this study, the measurement of aortic PWV showed excellent repeatability. The results obtained from the repeated measurements of ANOVA indicate that there was no significant difference in carotid-femoral PWV measurements between a week interval. This may have been as a result of the standardisation protocol used in this research.

The present study was designed to evaluate the stability (day-to-day) and reliability of carotid-femoral PWV measurements. There were two types of variability associated with this measure: systematic bias and random error. A systematic pattern in the data is characterised by data spreading in either a positive or a negative direction, and this can indicate whether a learning effect exists. Random error reflects the biological and mechanical variation inherent in the measurements and is characterised by the magnitude of the spread around the mean difference. Close examination of the Bland and Altman plot indicates no evidence of any learning effect and minimal error for carotid-femoral PWV. The mean difference between intra-operator test-retest is approximately zero (i.e. 0.01m/s). Relating this to normal clinical practice, this difference is practically non-significant in terms of making changes to clinical decision-making.

The repeatability of carotid-femoral PWV in patients with COPD using the SphygmoCor device is similar to that recently reported by Stone and co-authors (2013) using the Vicorder device. However, the present study was power calculated and used the most robust statistical test to examine the reliability. Using the same device, Wilkinson et al. (1998) showed a large variation between the two measurements taken by the same operator demonstrated by the large standard deviation of the mean difference, which is reflected in the limits of agreement. This

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variability was even maintained between operators. The inconsistency between readings could be related to an inexperienced operator, who may have had difficulty palpating and positioning the probe on the femoral artery. Similarly, in ter Avest et al. (2005) study, variability was observed between morning and afternoon measurements. Owing to a discrepancy between the measurement sides (i.e. right carotid artery and left femoral artery) and method of distance measurement, this may explain the variability. The results of this study are consistent with those obtained by Frimodt-Moller et al. (2008), who investigated the repeatability of aortic stiffness in patients with renal failure on two different occasions within a week between the measurements. Despite the bias of 0.7m/s that they found in their measurements, they showed good repeatability.

Although COPD is a heterogeneous disease with day-to-day variability in patients' physiological status, this study has demonstrated high intra-operator test-retest repeatability of carotid-femoral PWV using a well controlled standard protocol. Since carotid-femoral PWV is a reproducible measure in patients with COPD, it appears to be a useful measure to assess and monitor cardiovascular risk in this study. However, blood pressure should be taken into consideration when analysing the results. Hence, increases in distending pressure, increases carotid-femoral PWV and therefore PWV should be adjusted for changes in blood pressure (Laurent et al., 2006).

Reliability of Cardiac Haemodynamics

Cardiac haemodynamics are primary parameters for the evaluation and monitoring of cardiac performance; their measurement in clinical settings helps to quantify the presence and degree of CVD. The results of this study showed the ability of the NICOM system to measure the CO and SV accurately and non-invasively in patients with COPD over time. Adoption of any measurement technique for clinical use must show adequate between-day stability, since chronic disease management relies on repeated test paradigm analyses. Considering the heterogeneity of COPD, the NICOM system showed excellent stability over time. As no previous study has examined the repeatability of this device in patients with COPD, so no comparison could be made. However, the reliability results for the current study are consistent with the findings of a previous study of young healthy individuals (Elliott et al., 2010). In this study, Elliott and colleagues (2010) reported a high ICC of 0.94 for triple measurements of CO during the same visit. However, they did not report the amount of variability between the measurements.

Patients with COPD are at increased risk of heart failure and CVD (Rutten et al., 2005b). Increasing evidence suggests that CO and SV can be reduced in patients with COPD, even in the mild stage of airway obstruction. Similarly, patients with heart failure share the same features (Williams et al., 2001). Manifestations of cardiac damage and dysfunction may not be obvious in the early stages during routine clinical assessment in primary care settings; therefore, the progression of subclinical cardiac damage can eventually lead to heart failure (Barr et al., 2010, Sabit et al., 2010a, Watz et al., 2008). Clinically, early detection of these changes plays a vital role in providing accurate management of these patients by reducing their rate of morbidity and mortality-related CVD (Sin and Man, 2005).

Furthermore, augmentation of cardiac haemodynamics with other clinical measurements, such as aortic stiffness in routine clinical practice may provide good insights into left ventricle function and cardiac subclinical abnormalities. Patients with COPD and suspected heart failure are at high risk of left ventricle dysfunction (Rutten et al., 2005b). Therefore, the NICOM system makes it possible to quantify cardiac performance in primary care settings and to stratify patients who are at risk of developing CVD or heart failure, and thus to enhance management.

Given that the NICOM system is independent of the operator and does not need the patient's participation, and also because of its rapidity, simplicity and accuracy, this system can provide the physician with clear information about heart performance in patients with COPD.

Study Limitation

The reliability results of arterial stiffness and cardiac haemodynamics were based on a single operator measurements, therefore, these results could not extrapolated to inter-rater reproducibility.

Conclusion

Measurement of aortic PWV and cardiac haemodynamics are simple and non-invasive and provide valuable information about vascular wall properties and cardiac performance, respectively. Given the increased cardiovascular risk in COPD population, using such assessment tools in routine clinical practice could yield to early identification of patients who are high risk of developing CVD and implement appropriate management. The assessment of these outcome measures is also useful for monitoring patient's condition and treatment.

3.4 Assessment of Repeatability and Validity of Quantitative Ultrasound Scan in COPD

3.4.1 Background

Osteoporosis defined as a systemic skeletal disease characterised by diminished bone mineral density (BMD) and/or deterioration of the microarchitecture, leading to increased bone vulnerability to fractures (Biskobing, 2002). It is common in COPD estimated to be two to five times more prevalent than controls similar in age and sex (Bolton et al., 2004, Sabit et al., 2007).

The mechanistic links between osteoporosis to COPD is poorly understood. However, both diseases share similar risk factors, including use of corticosteroids, systemic inflammation, physical inactivity and weight loss (Barnes and Celli, 2009). In addition, a decline in FEV_1 is associated with an increased risk for osteoporosis and fracture (Graat-Verboom et al., 2009, Jorgensen and Schwarz, 2008). As osteoporosis develops insidiously, it often remains undiagnosed until a fracture occurs, with significant morbidity and mortality. Developing a prevention strategy that involves early detection and aggressive management to reduce the risk for osteoporotic fracture could benefit patients and reduce healthcare cost. This is of particular importance in patients with COPD, who are at greater risk of compromised lung function or adverse events with surgery (Bapoje et al., 2007, Schlaich et al., 1998).

Currently, the gold standard to assess osteoporosis non-invasively is a dual-energy x-ray absorptiometry (DXA) scan, which measures bone mineral density (BMD) at the lumbar spine and hip to monitor the efficacy of the treatment and predict the fracture (Blake and Fogelman, 1998). Nevertheless, the DXA only measures BMD, which is only one feature of bone structure, and may fail to detect deterioration of the microarchitecture of bone and the presence of osteoporosis (Siris et al., 2004). In addition, access to the DXA scan is limited and requires considerable time and expertise and therefore is not cost effective for screening. An alternative, which does

not emit radiation and has potential to identify individuals at high risk of fracture is the use of quantitative ultrasound (QUS) measurement of the calcaneus (Pluijm et al., 1999). Unlike the DXA scan which measures the BMD, the calcaneus ultrasound bone analyser (CUBA) measures the microstructure of the bone by broadband ultrasound attenuation (BUA). Osteoporosis is predominantly observed in cancellous bones, which are metabolically active tissue and affected by age and disease. The calcaneal and hip bones share similar bone structure and mechanical loading during daily activity. In addition, the calcaneal bone is relatively free from the soft tissue and easily accessible. The precision and potential diagnostic ability of the CUBA Clinical™ scanner has been demonstrated in several studies (Cook et al., 2005, Cryer et al., 2007, Greenspan et al., 1997, Stewart and Reid, 2000). Additionally, the CUBA scanner has shown the ability to predict hip and osteoporotic fracture (Khaw et al., 2004, Pluijm et al., 1999). Although the CUBA scanner has shown promising results to identify patients with osteoporosis or predict fractures, its reliability and validity has not been established in the COPD population.

Hypothesis

As patients with COPD are at high risk of developing osteoporosis and due limited resources and high cost of the DXA scan, the CUBA scanner would provide a reliable and valid measurement of heel bone density.

Aim

This study aimed to examine the repeatability of the CUBA scanner for measuring heel bone density and its sensitivity and specificity when compared to the hip DXA scan results.

3.4.2 Methods

Subjects

A sub-group of patients were recruited from the ARACDE study when they were clinically stable with no exacerbation in the preceding four weeks. An exacerbation was defined as "an event in the natural course of the disease characterised by a change in the patient's baseline dyspnoea, cough and/or sputum beyond day to day variability sufficient to warrant a change in management" (GOLD, 2014). Exclusion and inclusion criteria were detailed previously (Chapter 2, Table 2.1, p 59). Patients gave written informed consent and the study had approval from the South East Wales Research Ethics Committee.

Anthropometry Measurement

All patients had their height measured barefoot using a stadiometer (Seca; Vogel & Halke, Hamburg, Germany). Weight and body composition were recorded with patients wearing lightweight clothing and barefoot using a single frequency segmental bioelectrical impedance analyser (BC-418 MA, Tanita Corp., Tokyo, Japan) (Chapter 2:2.3.1, p 68).

Heel Bone Density Measurement

The heel bone density was assessed using the CUBA Clinical^{TM} (McCue Ultrasonics, Winchester) (Chapter 2:2.3.2, p 68).

Bone Mineral Density Measurement

Patients with a diagnosis of osteoporosis who were recruited into the ARCADE study and had the DXA scan within one year from the initial visit was taken and included in the analysis. The mean BMD at the lumbar spine and hip sites were recorded according to standard clinical practise by DXA scan at UHW Cardiff (Chapter 2:2.3.11, p 83). The BMD at all sites was recorded as an absolute figure (g/cm^2) and as a standardised T and Z score.

Pulmonary Function Tests

All patients completed post-bronchodilator spirometry and the forced expiratory volume in 1 second (FEV_1), the forced vital capacity (FVC) and FEV_1/FVC ratio were recorded (Vitalograph alpha, Bucks, UK) (Chapter 2:2.3.5, p 80).

Statistical Analyses

All analyses were carried out using SPSS software (version 18.0; SPSS Inc, Chicago, IL). Data was checked for normality using a Kolmogorov-Smirnov test.

Reliability

Normally distributed data was presented as mean (SD). Reliability was examined using repeated measures analysis of variance (ANOVA), intra-correlation coefficient and Bland-Altman plot. The p value was set at <0.05 and ICC > 0.75.

Validity

The sensitivity (proportion of patients correctly identified as having osteoporosis) and specificity (proportion of patients correctly identified without osteoporosis) of the CUBA scan was determined and compared with the gold standard DXA scan. The positive predictive value (the proportion of patients with positive CUBA scan findings who had osteoporosis) and negative predictive value (proportion of participants with negative CUBA scan findings who did not have osteoporosis) were expressed as percentages. The cut-off points were determined by 90% of sensitivity as an upper limit and 90% specificity as a lower limit by the receiver operating characteristics (ROC) curve.

3.4.3 Results

Reliability

The between-day repeatability study included 30 (17 male) patients, mean (SD) age 67 (7), height 165 (10), weight 74.8 (18.2) and FEV_1% predicted 57 (18). The period between measurements was mean (SD) of 7 (1) days.

The repeated measures ANOVA indicated no variability between patient's measurements as demonstrated by F-value (0.282) and P-value (0.599). The between-day ICC was 0.81 (95%CI: 0.63-0.90). The Bland-Altman plot mean difference between repeated measures was 1.36 (14) dB/MHz and the 95%CI was between -28.92 and +26.32 dB/MHz. All except two patients fell between the 95%CI limits of agreement and there was no systematic bias as the points distributed evenly around the mean (Figure 3.7).
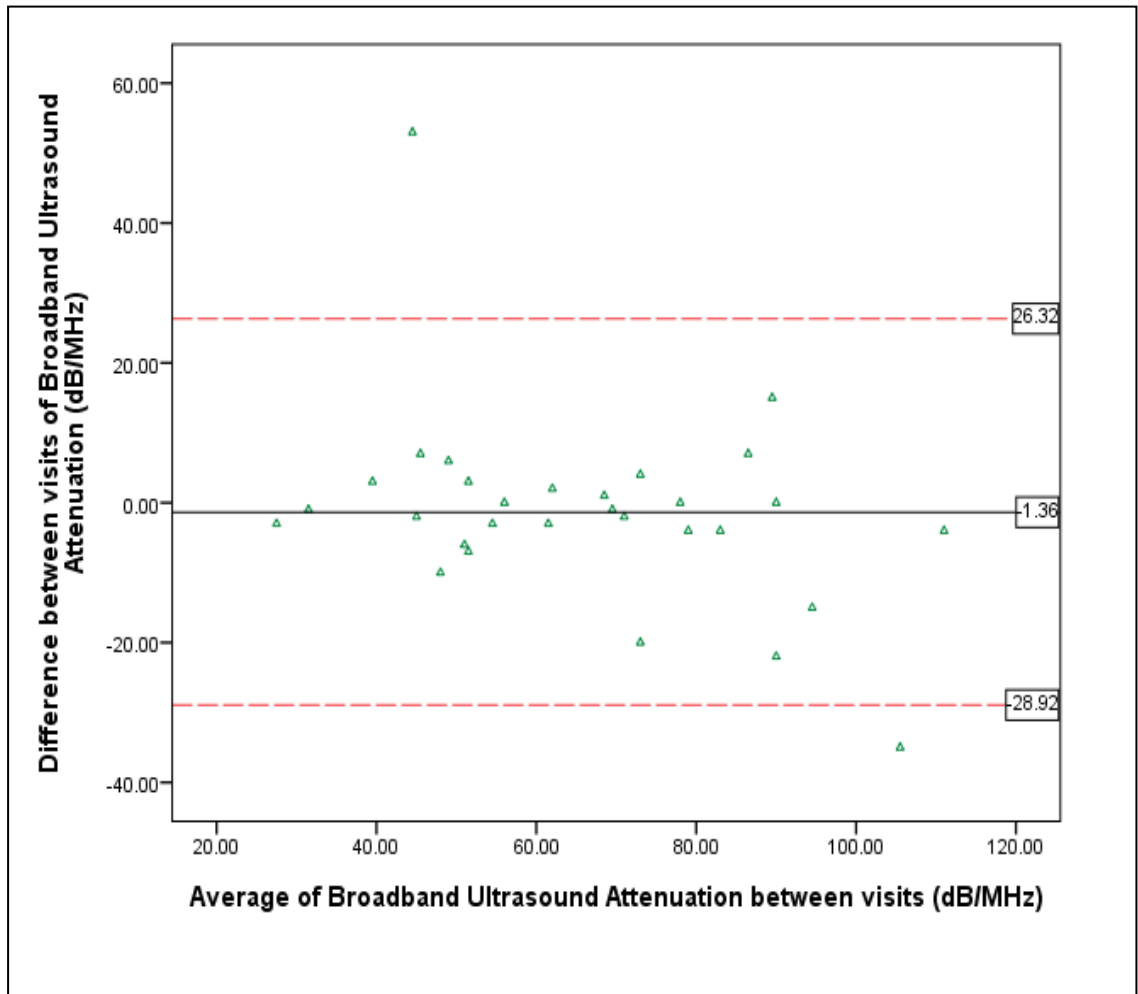
Figure 3.7: Bland and Altman plot showing between day differences in Broadband

Ultrasound Attenuation Measurement

Validity

Of the 48 patients who underwent the CUBA and DXA scans, 8 had a normal scan, 28 had osteopenia and 12 had osteoporosis according to DXA scan. Mean age was 68 (7), FEV_1% 57 (18), BUA 49 (17) dB/MHz, heel T-score -2.44 (1.02), hip BMD 0.72 (0.11) g/cm^2 and hip T-score -1.84 (0.80). The BUA correlated with hip BMD (r=0.38, p=0.01) and hip T score (r=0.35 p= 0.02), and CUBA T score related to hip BMD (r= 0.39, p= 0.01) and hip T score (r=0.35, p= 0.01).

In the first analysis, those patients who had a left hip DXA T- score of <-1 were classified as positive bone mass loss, while those who had a normal bone densiometry were considered as negative cases. Using the manufacture cut- off point of T score between -2 and -1 as a positive indication of loss of bone density, the CUBA scan identified 38 out of 40 patients as having bone mineral density loss with 95% sensitivity and 13 % specificity (Table 3.3).

Using the receiver-operating characteristic curve to determine the best sensitivity and specificity values for the CUBA clinical machine to identify bone mass loss in this population, the cut-off value was -2.66 and the area under the curve was 0.752 (95%CI: 0.580 to 0.923) (Figure 3.8). According to this value, out of 40 patients with bone mass loss, only 15 patients were identified, with 38% sensitivity and 100% specificity (Table 3.4).The accuracy of this machine based on the sensitivity and specificity analysis was 48%.

The second analysis, the ROC curve was used to determine the best sensitivity and specificity for the CUBA scanner to identify patients with osteoporosis (Figure 3.9). Patients who were classified as "osteopenic" were excluded from the analysis. From this analysis, the cut-off T-score for the CUBA scanner based on 90% sensitivity and 90% specificity was -2.74. The area under the curve was 0.750 (95%CI: 0.522 to 0.968). Based on this cut-off point, the CUBA detected 7 out of 12 patients with osteoporosis; however, it did not misclassify any patient as osteoporotic (Table 3.5).

In comparison to the ROC curve cut-off, the recommended cut-off value set by the manufacture to diagnose osteoporosis revealed that this machine had a sensitivity and specificity of 75% and 63%, respectively. The positive predictive value was 75% where the negative predictive value was 63%. The percentages of incorrect diagnosis (i.e. false negative and false positive values) were equal 15%.

Combining the two analyses, a T-score between -2.73 to -1 indicated loss of bone mass in patients with COPD where a T-score of -2.74 or below suggested osteoporosis.

Table 3.3: Sensitivity and Specificity calculation for bone mass loss based on the manufacture recommendation

CUBA scanner	DXA scanner				
	Normal	Bone Mass loss	Total	PPV	NPV
Normal	1	2	3		33%
Bone Mass Loss	7	38	45	84%	
Total	8	40	48		
	Specificity=13%	Sensitivity =95%		FP=15%	FN=4%

Abbreviations: CUBA= Calcaneal Ultrasound Bone Analyser**; DXA=** Dual-energy X-ray Absorptiometry**; PPV=** positive predictive value**; NPPB=** negative predictive value.

Table 3.4: Sensitivity and Specificity calculation for bone loss based on the ROC curve

CUBA scanner	DXA scanner				
	Normal	Bone Mass loss	Total	PPV	NPV
Normal	8	25	33		24%
Bone Mass Loss	0	15	15	100%	
Total	8	40	48		
	Specificity=100%	Sensitivity =38%		FP=0%	FN=52

Abbreviations: CUBA= Calcaneal Ultrasound Bone Analyser**; DXA=** Dual-energy X-ray Absorptiometry; **PPV=** positive predictive value**; NPP=** negative predictive value.

Table 3.5: Sensitivity and Specificity calculation for osteoporosis based on the ROC curve

CUBA scanner	DXA scanner				
	Normal	Osteoporosis	Total	PPV	NPV
Normal	8	5	13		62%
Bone Mass Loss	0	7	7	100%	
Total	8	12	20		
	Specificity =100	Sensitivity =58%		FP=0%	FN=25

Abbreviations: CUBA= Calcaneal Ultrasound Bone Analyser**; DXA=** Dual-energy X-ray Absorptiometry; **PPV=** positive predictive value**; NPP=** negative predictive value.
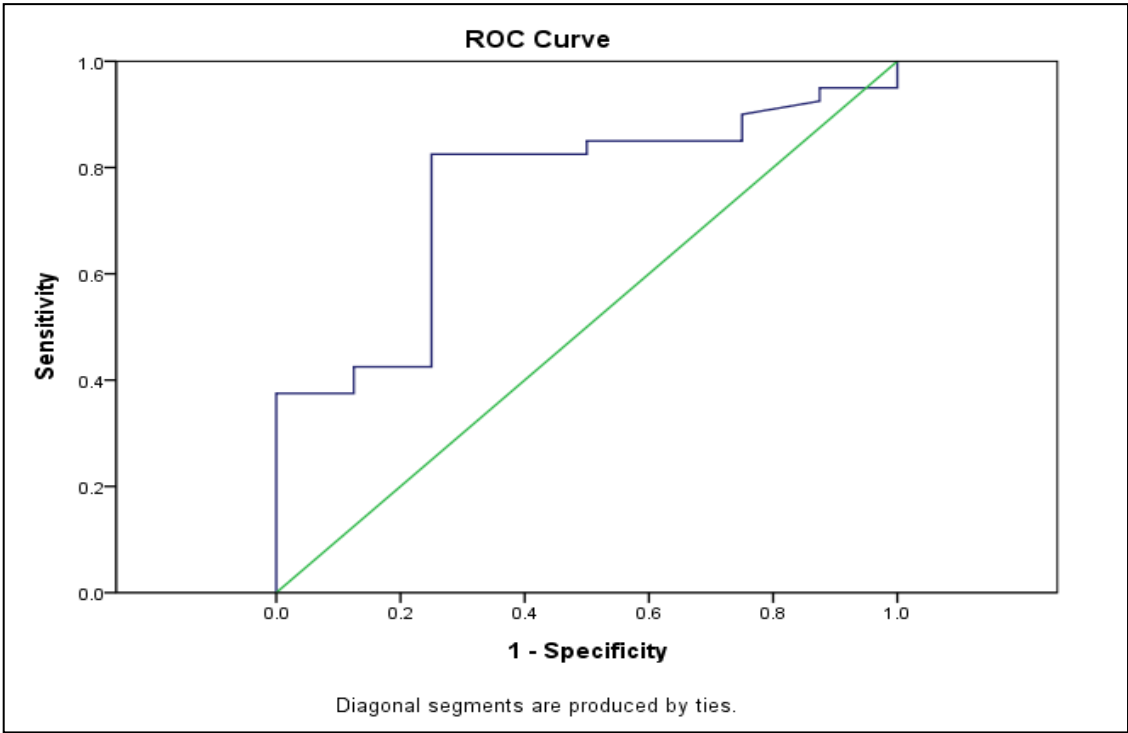
Figure 3.8: Receiver Operating Characteristics Curve for determining cut-off values for bone mass loss
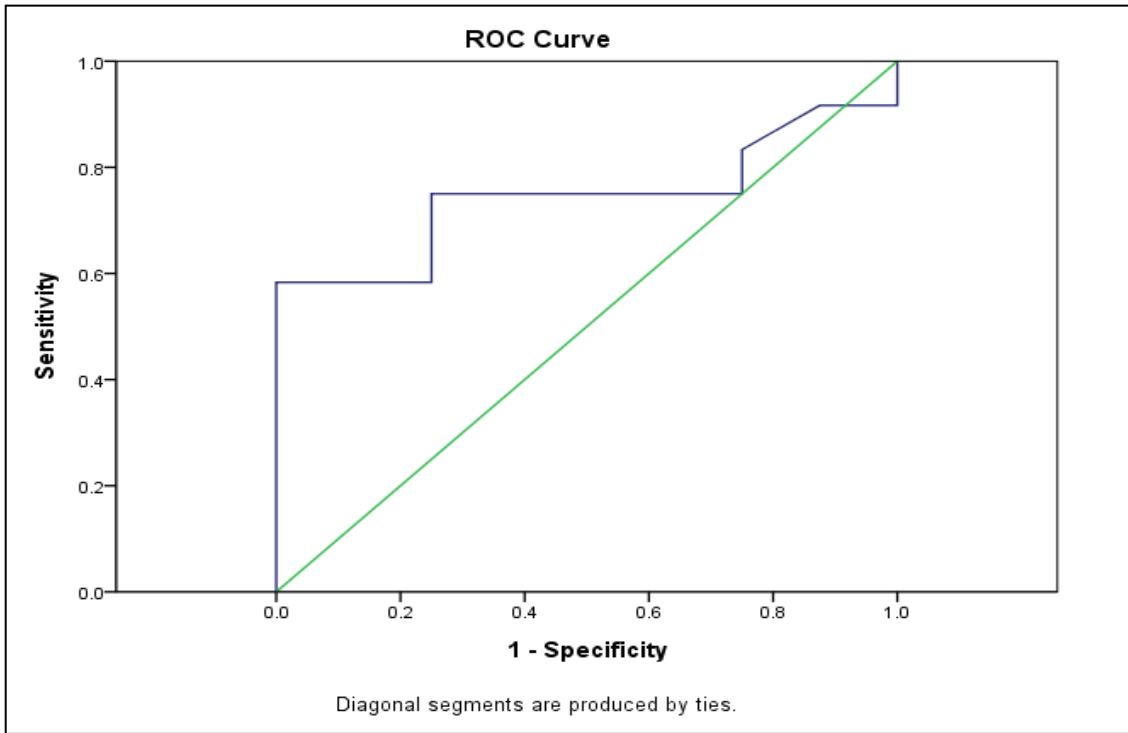
Figure 3.9: Receiver Operating Characteristics Curve for determining cut-off values for osteoporosis

3.4.4 Discussion

The CUBA Clinical^{TM} scanner demonstrated an excellent between-day repeatability of heel bone density in patients with COPD, with a slight variability between the measurements. It also showed the ability to identify patients with loss of bone mass and excluded those who do not have osteoporosis.

Adoption of any measurement tool for use in clinical practice must show adequate between days repeatability to identify changes in chronic disease. The Bland-Altman plot showed some variability between the measurements of the patients, though consistency was optimised, as it should be according to the manufacture recommendations. However, previous studies have suggested that results may be influenced by the size and width of the foot as well as the position of the ankle (Cheng et al., 2002, Greenspan et al., 1997). As no previous study has examined the repeatability of this device in patients with COPD, no comparison could be made. Two previous studies examined inter-operator measurements precision of the CUBA scanner and found this scanner has less variability between the measurements. Although, these studies used the coefficient of variation, which can underestimate repeatability, the results of the current study are still comparable with their findings (Greenspan et al., 1997, Stewart and Reid, 2000).

The correlation between the CUBA clinical scan and DXA scan is similar to other previous studies with consideration of different anatomical sites (Naganathan et al., 1999, Stewart and Reid, 2000, Cook et al., 2005, Cryer et al., 2007). The discriminatory ability of the CUBA scanner to identify patients with bone loss showed a good correlation between the CUBA clinical scan and DXA scan. For a useful clinical diagnostic test in case of a common comorbidity in COPD such as osteoporosis, obtaining high sensitivity is usually recommended. Thus, the results of this study showed that this machine had a sensitivity of 95% and specificity of 13%. This means if bone mass loss is diagnosed by the CUBA scanner, it is likely to be true

(84%). However, only 33% will be classified as a disease free if the results are negative. This has changed notably when the cut-off values were set by the ROC curve values. The ability to diagnose loss of bone mass decreased to an unacceptable value of 38%, but the specificity increased markedly to 100%. Therefore, the 8 patients with normal bone density were identified by both the DXA and the CUBA scanner.

For clinical use, a screening test should demonstrate a good sensitivity to rule out any patient who does not have the disease. Balancing between the false positive and false negative obtained from both cut-off levels, the identification of patients with early stage of bone mass loss using the CUBA scan could be based on a T score of -1 or below as proposed by manufacture and international osteoporosis foundation (IOF) recommendations (Vasikaran et al., 2011). Using this value, the clinician can be 84% confident that the patient has some degree of bone weakness and worthy of further investigation. The data produced by the ROC curve analysis, to determine the ability of the CUBA clinical scanner to identify osteoporosis, showed that the CUBA scanner can exclude patients without osteoporosis. Nevertheless, to keep the incorrect diagnosis of osteoporosis to a minimum, both cut-off values set by the ROC analysis and manufacture maintain the false negative results to its minimum (25%). Even a false positive value, should not be ignored, as the CUBA scanner may give different information about trabecular bone mass and its density and early changes in bone microstructures could be demonstrated in the heel bone before it can be detected by the DXA scan at the hip region. Several studies have found that low BUA is an independent risk factor for hip and osteoporotic fracture.

The current study findings are in agreement with Stewart and Reid (2000) study, who found that the CUBA scanner has the ability to discriminate between those with loss of bone mass and who have apparently healthy bone. In contrast, Naganathan et al. (1999) found the CUBA scanner was unable to identify people with bone mass loss.

Measuring the BUA at the heel area appears to predict the loss of bone mass at the hip region and this is because these bones share similar anatomical and biomechanical characteristics. The sensitivity and specificity of the CUBA scanner has been examined by several studies to identify subjects with osteoporosis, but conflicting results have been reported. The results of this study are consistent with Stewart and Reid (2000) and Cryer et al. (2007) studies who found a high sensitivity (94% and 90%, respectively) of the CUBA scanner to identify subjects with osteoporosis. In contrast to the present findings and previous two studies, Naganathan et al. (1999) found this machine had a limited sensitivity to diagnose osteoporosis (33%) in postmenopausal women. However, the authors based the diagnosis of osteoporosis upon multiple sites and did not specify what area was most related to the CUBA clinical scanner; and recent guidelines suggest the hip region as the best estimate of osteoporosis (Qaseem et al., 2008). Nevertheless, the certainty, i.e. true positive, of this machine to diagnosis osteoporosis is controversial. The results of this research are consistent with Stewart and Reid (2000) findings but not with Naganathan et al. (1999) and Cryer et al. (2007) studies. Although Naganathan et al. (1999) and Stewart and Reid (2000) used the same method as the present study, Naganathan and colleagues (1999) did not specify which region was osteoporotic. However, Cryer and co-authors (2007) based the diagnosis of osteoporosis upon the ulnar BMD results, which is not the recommended site for diagnosing osteoporosis.

The threshold of the CUBA scanner for identifying osteoporosis in patients with COPD is similar to Cook et al. (2005) and that recommended by the manufacturer, whereas Langton et al. (1997) recommended a slightly lower cut-off value of -1.58 to -1.64. In contrast, Naganathan et al. (1999) used a lower cut-off between -2.5 to -1 to diagnose osteoporosis. This discrepancy in the threshold values between the studies is partly explained by measuring different sites to diagnose osteoporosis.

The CUBA scanner is a useful device to measure the changes in bone mass and microstructure of patients with COPD. Although it had moderate ability to identify patients with osteoporosis, it was precise to exclude those who did not have osteoporosis. Putting this in a clinical context, this machine could be used as a screening tool to identify patients who have some degree of bone mass weakness when the T-score falls between -2.73 and -1. However, if the T-score is below -2.74, the patient is more likely to have osteoporosis and worth further investigation. The CUBA scanner could be used for a case finding approach to identify patients at low or high risk and need further investigation. This will move the strategy from identification to prevention of osteoporosis and its complications, as osteoporosis is common in patients with COPD, yet often not diagnosed until a fracture occurs. Furthermore, due to limited resources, the DXA scan is not accessible in primary care settings, making this simple and portable device feasible to screen patients for changes in bone architecture and excludes the possibility of osteoporosis.

Study Limitations

Although the DXA and CUBA scans were not undertaken on the same day, they were within one year, where appreciable change of BMD is unlikely. The measurement of the BUA was obtained using the ankle whereas the BMD was measured over the top part of the hip, which may differ in the presence of osteoporosis. However, as an easily accessible weight bearing bone, the heel may be a useful surrogate of BMD.

Conclusion

The BUA is a reproducible measure of calcaneal bone density and structure in COPD. Given the prevalence of osteoporosis in COPD is high and often undiagnosed, a cheap and simple device such as the CUBA Clinical™ could be useful in primary care for screening patients with increased risk of loss of bone density and reducing the number of referral to the hospital for the DXA scan. Such a device could be feasible in promoting prevention strategy, treatment and management of osteoporosis in COPD.

Chapter Four

A Simple and Rapid Test of Physical

Performance in Chronic Obstructive

Pulmonary Disease.

4.1 Background

A major impact of chronic obstructive airway disease (COPD) is the progressive loss of physical performance, which may lead to disability with loss of the ability to perform routine activities of daily living (Eisner et al., 2008). Reduced physical activity occurs even in mild severity airflow obstruction and continuous activity monitoring has demonstrated that patients spend less time walking and standing than healthy controls (Gouzi et al., 2011, Watz et al., 2009). In addition to the impact on functional status, physical inactivity is associated with a reduced health related quality of life (HR-QoL), and contributes to loss of muscle mass, osteoporosis and cardiovascular disease, all co-morbidities of COPD (Barnes and Celli, 2009). Consequently, it becomes part of a vicious cycle of physical inactivity and changes in body composition that affect physical performance (Barnes and Celli, 2009).

The assessment of physical performance in patients with COPD in routine clinical practice is challenging and often poorly quantified, and the progressive loss of airways function, particularly the forced expiratory volume in one second (FEV_1) is a poor predictor of physical impairment (Kapella et al., 2011). Breaking the cycle of physical inactivity and detrimental changes in body composition could have major impacts on HR-QoL and earlier life-style interventions may maintain physical activity and normal body composition.

Physical performance in COPD has been assessed by various methods including questionnaires, which may have limited reproducibility and validity due to their dependence on patient recall. Quantitative measures of physical performance include the six minute walking distance (6MWD), the incremental shuttle walk test and cycle ergometry (Palange et al., 2007, Revill et al., 1999). The 6MWD is commonly used in clinical research and provides a measure of sub-maximal physical performance. Although, the 6MWD is a validated measure and reflects daily activity, it is not widely used in clinical practice owing to the requirement for substantial time, space and

expertise (Glaab et al., 2010). The Timed Up and Go (TUG) test has been used to assess physical function in the elderly (Savva et al., 2013). It is a relatively simple and reproducible test that assesses balance, gait speed and physical performance and can predict the risk of falls, functional activity and frailty in the older population (Podsiadlo and Richardson, 1991, Viccaro et al., 2011). The TUG test is also responsive to a rehabilitation intervention (Brooks et al., 2006). It has not been applied widely in COPD, but it has demonstrated to identify patients with COPD with reduced balance, co-ordination and an increased risk of falls similar to findings in the elderly (Beauchamp et al., 2009). Thus, the TUG may reflect routine daily activities, which require the integration of strength and balance.

Hypothesis

The TUG test would be prolonged in patients with COPD, compared with a non-COPD comparator population. Additionally, the TUG would be a predictor of the 6MWD.

Aim

This study aimed to assess the feasibility of using TUG test as an integrated measure of physical mobility in patients with COPD when compared to 6MWD.

4.2 Methods

Subjects

Five-hundred patients with COPD confirmed with spirometry and 150 comparators either current or ex-smokers free from respiratory disease were recruited from the Assessment of Risk in Chronic Airways Disease Evaluation (ARCADE) study. Patients and comparators were recruited following the inclusion and exclusion criteria detailed previously (Chapter 2: Table 2.1, p 65). All subjects gave written informed consent and the study had approval from the South East Wales Research Ethics Committee.

Anthropometry and Body Composition Measurement

All subjects had their height measured barefoot using a stadiometer (Seca; Vogel & Halke, Hamburg, Germany). Weight and body composition were recorded with subjects wearing lightweight clothing and barefoot using a single frequency segmental bioelectrical impedance analyser (BC-418 MA, Tanita Corp., Tokyo, Japan) (Chapter 2:2.3.1, p 68). Body mass index (BMI), fat free mass (FFM) and fat mass (FM) were also determined. Waist and Hip circumferences were measured with a stretch resistance tape.

Pulmonary Function Tests

All subjects completed spirometry and the forced expiratory volume in 1 second (FEV_1), the forced vital capacity (FVC) and FEV_1/FVC ratio were recorded (Vitalograph alpha, Bucks, UK) (Chapter 2:2.3.5, p 80).

The level of breathlessness was scored using the modified Medical Research Council (MRC) dyspnoea scale.

In the patients, the number of exacerbations, defined as an acute worsening of respiratory symptoms characterised by the increase of any combination of three key symptoms, which necessitate a change in regular medication, in the last year was recorded (Rabe et al., 2007).

Six Minute Walk Distance

The 6MWD was carried out in accordance with a protocol adapted from the American Thoracic Society (ATS) guideline (2002) using a 30-m level, straight indoor track (Chapter 2:2.3.7, p 81).

Timed Up and Go

All subjects undertook the TUG test using a standard chair and standardised instructions (Chapter 2:2.3.6, p 79). The task was to be performed at their normal comfortable pace and the time recorded in seconds.

Handgrip Strength Measurement

Maximal right and left handgrip strength (HGS) was determined twice in each hand using a hand dynamometer (T.K.K. 5401 grip-D, Takei, Japan) (Chapter 2:2.3.8, p 84).

Health Related Questionnaire

Patients completed the St George's Respiratory Questionnaire (SGRQ) and the COPD assessment test (CAT), both validated questionnaires to assess the impact of COPD on their health status (Chapter 2:2.3.9, p 82).

Inflammatory Biomarkers

A blood sample was obtained for determination of C-reactive protein (CRP, high sensitivity) and fibrinogen by standard assays (Dept. Biochemistry, University Hospital of Wales).

Statistical Analysis

The statistical software package SPSS 18.0 (Chicago, Illinois, U.S.A.) was used for all analysis. Data were checked for normality prior to analysis using a Kolmogorov-Smirnov test. Results are presented as arithmetic or geometric (for non-normally distributed) and standard deviation (SD). Comparisons between patients and comparators were performed using analysis of variance (ANOVA). Categorical data was analysed using the Chi-square test. Relationships between variables were explored using Pearson's and Spearman correlation coefficients. Multivariate analysis was performed using a stepwise multiple regression model. For all analysis $p < 0.05$ was considered significant. Receiver operating characteristics (ROC) curve was performed to determine the diagnostic ability of the TUG test for discrimination between stable patients with COPD and community population.

4.3   Results

The patients and comparators were similar in age, gender ratio and BMI. The patients had a greater tobacco exposure, lower mean FEV_1, FVC and resting oxygen saturation than the comparator group, all $p<0.001$ (Table 4.1). The severity of airflow obstruction by GOLD stratification was: GOLD 1 n=73, GOLD 2 n=251, GOLD 3 n=144 and GOLD 4 n=32 and the distribution of the modified MRC breathlessness score was: mMRC 0 n=65, mMRC 1 n= 172, mMRC 2= 97, mMRC 3 n=121 and mMRC 4 n= 44.

Measures of Physical Performance

The patients had a greater TUG, 11.1 (3.7) s than comparators 8.2 (1.2) s, $p<0.001$, however, there was no difference in the TUG for males and females in either group, $p>0.05$. The TUG in the patients was weakly related to the FEV_1% predicted (r= -0.14, $p<0.01$), oxygen saturation (r= -0.12, $p<0.03$) and mMRC dyspnoea score (r= 0.28, $p< 0.001$).

The TUG was related to age in patients (r=0.13, p=0.003) and comparators (r=0.21, p=0.01). The patients had greater TUG than the comparators across all the age decades <50-80, all $p<0.001$ (Figure 4.1). Using the upper 95%CI (8.5) s for the comparator as a cut-off value for the non-COPD range demonstrated that only 101 of the 500 patients had a TUG within the comparator reference range.

The 6MWD and HGS were less in patients than comparators, both $p<0.001$ (Table 4.1). In the patients, the TUG was inversely related to the 6MWD (r=-0.71, $p<0.001$) and HGS (r=-0.24, $p<0.001$), but only to the 6MWD in comparator subjects (r=-0.38, $p<0.001$). The 6MWD, HGS and TUG were all related to each other in the patients, all $p<0.001$.

Table 4.1: The characteristics of patients with COPD and comparator subjects

	COPD (n=500)	Comparator (n=150)	p value
Gender Male:Female	255:245	75:75	0.451
Age (years)	66 (7)	65 (8)	0.125
Smoking (pack-years)	40.6 (25.5)	21.4 (17.9)	**0.001**
FEV_1 (L)	1.64 (0.84)	2.73 (0.67)	**0.001**
FVC (L)	2.50 (0.98)	3.50 (0.90)	**0.001**
FEV_1/FVC (L)	0.53 (0.11)	0.78 (0.05)	**0.001**
FEV_1 (%)	59 (21)	105 (13)	**0.001**
FVC (%)	87 (21)	110 (15)	**0.001**
BMI (kg/m^2)	28.1 (5.5)	28.3 (4.2)	0.664
Waist circumference (cm)	100 (15.0)	95 (13)	**0.001**
Waist:Hip ratio	1.0 (0.1)	0.9 (0.1)	**0.001**
Handgrip (kg)	27.1 (9.9)	31.0 (10.2)	**0.001**
FFM (kg)	49.6 (11.0)	52.2 (9.5)	**0.011**
FFMI (kg/m^2)	18.1 (2.6)	18.5 (2.3)	0.088
Fat mass (kg)	26.9 (11.1)	26.9 (8.6)	0.985
6MWD (m)	324 (124)	502 (86)	**0.001**
TUG (s)	11.1 (3.7)	8.2 (1.2)	**0.001**
Resting O_2 saturation (%)	97 (2)	98 (1)	**0.001**
Fibrinogen (g/L)#	3.51 (1.31)	3.08 (1.25)	**0.001**
CRP (mg/L)#	3.49 (2.89)	1.76 (3.18)	**0.001**

All data mean (SD) unless otherwise indicated, # Geometric mean

Abbreviations: **FEV_1=** forced expiratory volume in 1 second; **FVC=** forced vital capacity;

BMI = body mass index; **HGS=** handgrip strength; **FFM=** fat free mass;   **FFMI =** fat free mass

index; **6MWD=** six minute walk distance; **TUG=** Timed Up and Go; **CRP=** C - reactive protein.

Figure 4.1: TUG test across age categories in COPD and comparator subjects

* p value <0.001

Body Composition and TUG Test

The TUG was related to BMI (r=0.24, p<0.001), FM (r=0.22, p< 0.001), FMI (r=0.21, p<0.001) and the waist to hip ratio (r= 0.13, p=0.01), in the patients group, but there was no relationships between these variables and TUG in the comparator group. Both TUG and 6MWD were related to FFM:FM ratio, r=-0.13, p=0.01, r=0.11, p=0.04, respectively, in the patients, but only with 6MWD in comparators (r=0.34, p<0.001).

Health Related Questionnaires and TUG Test

The TUG and the SGRQ total score were related (r=0.35, p<0.001), as were the domains of activity (r=0.35, p<0.001), symptoms (r=0.24, p<0.001) and impact (r=0.32, p<0.001). The CAT score was also related to the TUG (r=0.34, p=0.001). Both the 6MWD and HGS were also related to the total SGRQ score, r=-0.59, p<0.001 and r=-0.26, p=0.001, respectively, and similarly to the CAT score r=-0.53, p<0.001 and r=-0.27, p<0.001, respectively.

Systemic Inflammation

Circulating CRP and fibrinogen were greater in patients than comparators, p<0.001, and both were related to the TUG, CRP (r_s=0.19, p=0.001) and fibrinogen, (r_s=0.17, p<0.001), but were unrelated to TUG in the comparator group, p>0.05. In the patients, 6MWD was also related to both CRP (r_s=0.19, p=0.001) and fibrinogen (r_s=-0.24, p=0.001), while neither biomarker was related to HGS.

Frequency of Exacerbation

Of the patients, 207 reported 0-1 exacerbations/ year and 293 reported two or more exacerbations/ year. The TUG was related to the frequency of exacerbations (r=0.24, p<0.001), and frequent exacerbators had greater TUG, 11.5 (3.5) s, than infrequent exacerbators, 10.5 (4.1) s, and both were greater than the comparator group TUG, p<0.01 (Figure 4.2).
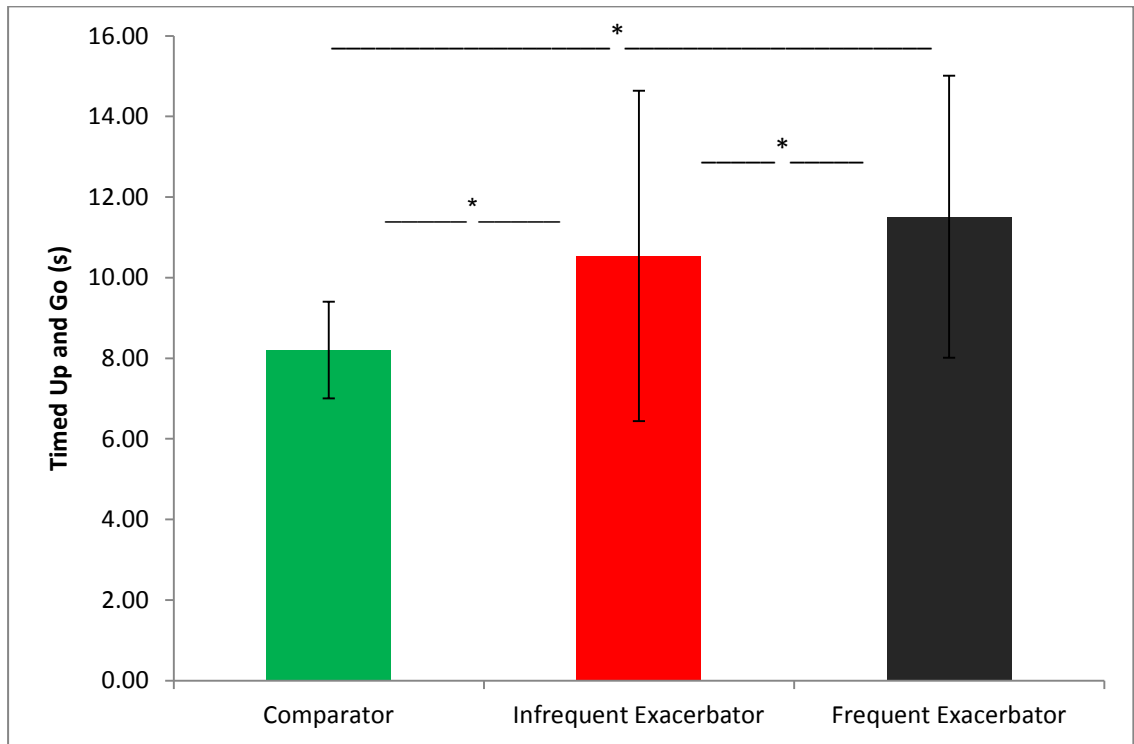
Figure 4.2: The TUG difference between infrequent and frequent exacerbators and comparator subjects at initial visit

Data presented as mean with SD
ANOVA, Bonferonni post-hoc, p<0.01

Predictive Factors for the 6MWD

In the patients, stepwise multivariate regression analysis after controlling for age and BMI showed that TUG, mMRC and CAT score explained 55% of the variability in 6MWD with $FEV_1\%$ predicted excluded from the analysis (Adjusted $R^2=0.54$,F= 182.80,p<0.001). The TUG explained 41% of the reduction in 6MWD, and mMRC and total CAT score explained 27% and 25% of the variability, respectively, all p<0.001.

Predictive Factors for the TUG

In the patients, stepwise multivariate regression analysis after correcting for age and BMI showed that 6MWD, waist:Hip ratio and HGS explained 45% of the variability in TUG with $FEV_1\%$ predicted, FM and inflammatory biomarkers excluded from the analysis (Adjusted $R^2=0.45$,F= 61.39,p<0.001). The 6MWD explained 59% of prolonged TUG, p<0.001 and waist:Hip ratio and HGS explained 14% and 12% of the variability in the TUG, respectively, all p<0.05.

Diagnostic Ability of TUG Test against 6MWD

Using the upper 95%CI (8.5 s) of the TUG for the comparator as a cut-off value for normal mobility, the TUG test showed an excellent diagnostic ability to predict the 6MWD as demonstrated by the area under the curve of 0.826 (95%CI: 0.783 to 0.870) (Figure 4.3). With 90% sensitivity and 80% specificity, this cut-off value corresponds to 360 m on the 6MWD.
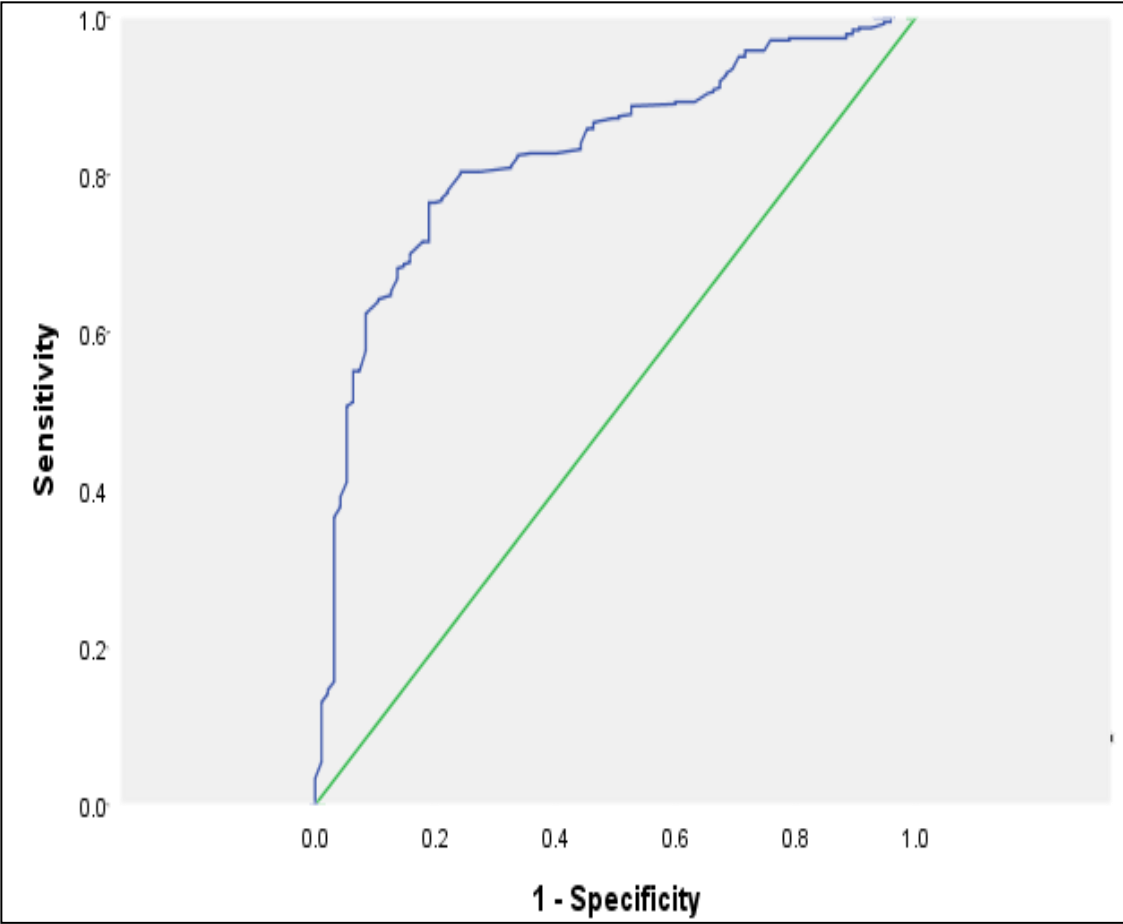
Figure 4.3: The ROC curve for the TUG test. Diagnostic ability of TUG test in patients with COPD

4.4 Discussion

The TUG test is an integrated assessment of physical performance which incorporates balance, gait speed and functional capacity (Podsiadlo and Richardson, 1991). In the present study, the TUG was greater in patients with COPD than a non-COPD comparator group and age stratified ranges reported in a meta-analysis of 21 studies in the elderly (Bohannon, 2006). Therefore, by comparing 95%CI of both groups, a cut-off point at 8.5 seconds or less is suggested for normal TUG test performance in this population. This threshold showed discriminative ability to identify patients with less physical performance with an area under the curve of 0.826. Similarly, the TUG test was found to predict mobility status and reflect physical performance with an area under the curve of 0.969 in community-dwelling elderly women (Bischoff et al., 2003). Therefore, in clinical practice, patients who perform the TUG test in > 8.5 seconds should receive a further evaluation of their physical status and early intervention to avoid subsequent complications related to physical inactivity.

A prolonged TUG identifies patients with COPD at risk of falls, a status likely to be dependent on loss of physical performance, and in the elderly at risk of disability and increased mortality (Beauchamp et al., 2009, Idland et al., 2012). The TUG test reflects various movements frequently used in everyday activities and its prolongation in COPD is a consequence of physical inactivity, skeletal muscle weakness and de-conditioning in a similar manner to that seen in the elderly. Thus in COPD, the TUG test is likely to reflect the presence of co-morbidities affecting body composition and similar to other measures of physical performance, independent of the severity of airflow obstruction (Watz et al., 2009). This is consistent with a previous study in elderly women which found that the TUG test is a measure of the interaction of body composition, muscle strength and comorbidities on the physical performance (Bischoff et al., 2003). In this study, the TUG was inversely related to the 6MWD, a measure of sub-maximal exercise capacity, and HGS as a surrogate marker for muscle strength

(Ling et al., 2010). Hence both measures were related to TUG, particularly the 6MWD, suggesting that the TUG in COPD similarly assesses impaired functional mobility. This interpretation of the longer TUG in the patients is supported by the lower HGS, which was associated with reduced physical activity in COPD and in healthy elderly populations where it is associated with reduced lower extremity strength, less daily activity and predicts future disability (Rittweger et al., 2000). The relationship between the increased TUG and SGRQ and CAT score indicates that the TUG reflects the impact of physical impairment and inactivity on patients' perceptions of their health and quality of life (Jones et al., 2009, Jones et al., 1992).

Altered body composition is an accepted co-morbidity in COPD and interest has centred on the importance the loss of skeletal muscle mass and function as a cause of impaired physical performance, similar to the sarcopenia and loss of physical performance that occurs in healthy ageing (Walston et al., 2002). However, in the studied populations, FFM was not related to deficits in physical performance, while changes in FM and its distribution were linked in both the patients and comparators. The TUG was related to abdominal obesity and FM, which supports the finding that an increased FM was the best predictor of functional limitation in COPD (Eisner et al., 2007, Kapella et al., 2011). Similarly, there is an association between functional limitation and FM in the elderly in the UK, in particular abdominal obesity determined as waist circumference was a major predictor of disability in similar aged subjects to our patients and comparators (Angleman et al., 2006).

In the present study, the TUG was related to FM and abdominal obesity only in the patients. The mechanisms linking FM and abdominal obesity to functional limitation may relate to the increased circulating levels of CRP and fibrinogen, which have been associated with reduced exercise capacity and impaired left heart function in patients with COPD (Watz et al., 2008). The association of the TUG with regional obesity in the patients suggests that the components of physical performance measured by the

TUG test have a similar relationship to systemic inflammation as other assessments used in COPD. Circulating biomarkers, including CRP and fibrinogen, have been associated with physical performance decline and cardiovascular risk in older subjects (Geffken et al., 2001). Fat produces a variety of pro-inflammatory mediators including interleukin-6, a regulator of CRP production and secretion, and is overall likely to be a factor in the development of insulin resistance and the increased risk of diabetes mellitus and cardiovascular disease in COPD (Barnes and Celli, 2009, Rutten et al., 2010). Systemic inflammatory biomarkers may also be a factor linking the TUG to the frequency of exacerbation when they are likely to increase.

The finding that the patients' TUG was independent of age and was greater than in the comparator group when stratified by decades, and published age ranges could be interpreted as the loss of physical performance in COPD being further evidence of premature ageing and is in keeping with evidence of premature vascular ageing (Sabit et al., 2007). Such an interpretation is further supported by the finding that 80% of the patient group had a greater TUG than the upper 95%CI of the comparator group. The development of co-morbidities in COPD resembles the pattern of accumulating deficits, which interrupt normal physiological homeostasis in various systems, a feature of ageing (Walston et al., 2002). In the elderly, over 85 years, a TUG in the upper 10% of a study population was associated with an increased risk of disability, odds ratio 9.02, and mortality confirming that the TUG is a valid indicator of disability in natural ageing (Martin-Ruiz et al., 2011).

The development of co-morbidities is a factor in impaired physical function and consequent disability and is clinically important in the management of COPD. Ideally, an assessment of physical performance should be part of the assessment of the disorder, but most measures of physical performance are difficult to apply in routine clinical practice. They often require substantial space, equipment, staff time and some patients may not be able to maintain activity long enough to complete an assessment.

The rapidity and simplicity of the TUG test suggest that it could be used to assess physical performance in the routine clinical settings. Furthermore, in elderly, the TUG test has been found to be a responsive measure to a rehabilitation exercise program (Brooks et al., 2006, Lihavainen et al., 2012). Combining the TUG test with an easily completed validated questionnaire, such as the CAT, as demonstrated here, could provide important information about a patient's physical performance and functional status that is not currently collected.

Study Limitations

A key limitation of this study is its cross-sectional nature, which limits understanding of the potential of this test in clinical practice. However, a limitation of physical function measures is that not all patients are able to complete them, but the short duration of TUG removes the endurance issue that limits application of some assessments in COPD and experience in the elderly suggests it is a widely applicable assessment.

Conclusions

The TUG test is a useful measure to identify limitations of physical performance and early signs of disability in the elderly and in COPD. It clearly discriminates between patients with COPD and comparators. Implementing the TUG test and simple questionnaires in clinical practice may enable clinicians to identify a patient's functional deficit and facilitate early intervention to improve the patient's health status. In addition, the TUG test could useful in monitoring adverse effects of pharmacological treatment on the musculoskeletal system.

Chapter Five

Cross-sectional and Longitudinal

Evaluation of Aortic Stiffness in COPD

Introduction

This chapter of the thesis evaluated the aortic stiffness in patients with COPD and was divided into two parts:

The first part was a cross-sectional study to examine aortic stiffness in COPD and included all the participants recruited into the ARCADE study at initial visit.

The second part of this study was a longitudinal study of a group of patients who returned for a repeated assessment after they had completed two years from their initial visit.

5.1 Background

COPD is a multicomorbidity disease associated with cardiovascular disease (Barnes and Celli, 2009). Compelling evidence from large epidemiological studies has found that COPD is an independent risk factor for CVD. However, the exact mechanism linking COPD to CVD remains poorly understood. Recently, several studies have demonstrated that COPD is associated with increased arterial stiffness, which is an independent predictor of fatal and non-fatal cardiovascular events (Laurent et al., 2006, Sabit et al., 2007).

Although arterial stiffness is increased in COPD, it is unclear how COPD is involved in the developing arterial stiffness. Previous mechanistic research in COPD has shown inconsistent results and this is not surprising due to methodological variations (Barr et al., 2007, Ives et al., 2013, Maclay et al., 2009, Maclay et al., 2012). Nevertheless, all these studies support the idea of vascular dysfunction in COPD. Alteration of vasculature that is normally associated with ageing has been suggested to happen prematurely in patients with COPD, independent of age (Maclay et al., 2009, Maclay et al., 2012). Therefore, patients with COPD may demonstrate signs of premature ageing either driven genetically or acquired through several mechanisms including elastin degradation, oxidative stress or inflammation, which subsequently leads premature cardiovascular events, independent of smoking exposure and severity of airway obstruction (Barr et al., 2012, Ives et al., 2013).

Identification of early vascular ageing in COPD may be clinically useful as it reflects subclinical features of arterial wall disorder (i.e. sub-atherosclerotic change or pre-hypertension) that has not been clinically manifested (Alghatrif et al., 2013, Bolton et al., 2011, Patel et al., 2013, Sabit et al., 2010a). Aortic pulse wave velocity is an accepted measure for vascular function and useful to identify patients with COPD who are at increased of cardiovascular risk. Previous cross-sectional studies have linked elevated aortic stiffness in COPD to lung function impairment, abnormal body

composition and inflammation. However, due to the limitations of these studies, these relationships are still questionable. Additionally, as the studies so far have been cross-sectional in nature, a longitudinal study is needed to confirm these relationships.

Hypothesis

Patients with COPD would have greater aortic stiffness than comparators similar in age and gender proportion at initial visit. Additionally, the patients would have a rapid increase in aortic stiffness after two years from the initial assessment.

Aim

This study aimed to prospectively evaluate the effect of COPD on the arterial wall over a period of two years, and the rate of change and its contributing factors.

5.2 Method

Part I:

This was a cross-sectional study of patients with stable COPD confirmed, with spirometry, and comparators from the Assessment of Risk in Chronic Airways Disease Evaluation (ARCADE) study. Comparators were current or ex-smokers free from respiratory disease. All subjects were clinically stable and free of inflammatory disease such as rheumatoid arthritis, oral maintenance corticosteroids and long-term oxygen therapy (full inclusion and exclusion criteria in Chapter 2: Table 2.1, p 65). All subjects gave written informed consent and the study had approval from the South East Wales Research Ethics Committee.

Lung Function

All subjects performed spirometry (Vitalograph), to measure forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the FEV_1/FVC ratio (Chapter 2: 2.3.5, p 78). A diagnosis of COPD was confirmed as post bronchodilator spirometry FEV_1:FVC <0.70, and the number of exacerbations per year were recorded.

Body Composition

Weight and body composition were measured barefoot in lightweight indoor clothing, fat free mass (FFM), fat mass (FM) and the percentage fat were determined using a Bioelectrical impedance device (Tanita BC-418). The FFM was expressed as a height-squared ratio, FFMI, and the body mass index (BMI kg/m^2) was calculated (see Chapter 2: 2.3.1, p 66). A stretch resistant tape measure was used to measure waist and hip circumference.

Measurement of Pulse Wave Velocity

Aortic PWV was assessed non-invasively using the SphygmoCor device connected to a laptop computer (Chapter 2: 2.3.3, p 70).

Cardiac Haemodynamics Measurement

Cardiac haemodynamics were measured non-invasively using a new bioreactance technology (NICOM, Cheetah, Medical Inc., Indianapolis, IN) (Chapter 2: 2.3.4, p 75).

Inflammatory Biomarkers

High sensitivity C-reactive protein (CRP) and fibrinogen were determined from a blood sample according to standard procedures (University Hospital of Wales, Biochemistry).

Data Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL), version 18.0. Results are presented as arithmetic or geometric for non-normally distributed data and standard deviation (SD). Analyses included One-way Analysis of Variance (ANOVA), Pearson's correlations and hierarchical multiple regression analysis. Difference between visits was performed using paired t-test. Categorical data was analysed using the Chi-square test. A $p < 0.05$ was considered significant. Subjects aged less than 50 years were added to age category of 50-59.

5.3 Result

Subject Characteristics

The baseline characteristics of patients and comparators are presented in table 5.1. Patients with COPD and comparators were similar in age, gender and BMI, but the patients had greater pack-year smoking history (Table 5.1). The severity of airflow obstruction in the patients was GOLD 1 n=73, GOLD 2 n=251, GOLD 3 n=144 and GOLD 4 n=32. Furthermore, patients were categorised into an $FEV_1 > 50\%$ predicted (n=318) (GOLD I&II) and an $FEV_1 < 50\%$ predicted (n=182) (GOLD III & IV). Of the 500 patients, 207 reported infrequent exacerbations, less than two per year, while 293 patients reported two or more per year.

Following the classification used by Landbo et al. (1999), of the 500 patients, (26) 5% had a low BMI (<19.9 kg/m^2), (196) 39% had an elevated BMI (>25 kg/m^2), (163) 33% were classified as obese (BMI>30 kg/m^2), while (115) 23% were within the normal limit for BMI (20-25 kg/m^2). Among comparator subjects, (34) 23% had an optimal BMI and (116) 77% had an elevated BMI, 28% of these subjects were obese (BMI >30 kg/m^2). Patients with COPD had greater waist circumference and waist: hip ratio, and lower fat free mass and oxygen saturation, all $p<0.001$. However, both groups had similar fat mass (Table 5.1).

Patients with COPD and comparator subjects were similar in kidney function indices, $p>0.05$ (Table 5.1). Circulating CRP and fibrinogen were greater in patients with COPD compared to non-COPD subjects, all $p<0.001$ (Table 5.1).

The proportion of subjects with a prior diagnosis of comorbidities was greater in the patients (Table 5.2). Details of medications in both groups are also given (Table 5.2). Most of the patients (64%) and comparators (82%) were ex-smokers and had a different pack year exposure.

Table 5.1: Baseline characteristics of patients with COPD and comparator subjects at initial visit

	COPD (n= 500)	Comparator (n= 150)	p value
Gender Male:Female	255:245	75:75	0.451
Age (years)	66 (7)	65 (8)	0.125
Smoking (pack-years)	40.6 (25.5)	21.4 (17.9)	**0.001**
FEV_1 (L)	1.64 (0.84)	2.73 (0.67)	**0.001**
FVC (L)	2.50 (0.98)	3.50 (0.90)	**0.001**
FEV_1/FVC (L)	0.53 (0.11)	0.78 (0.05)	**0.001**
FEV_1 (%)	59 (21)	105 (13)	**0.001**
FVC (%)	87 (21)	110 (15)	**0.001**
BMI (kg/m^2)	28.1 (5.5)	28.3 (4.2)	0.664
Waist circumference (cm)	100 (15)	95 (13)	**0.001**
Waist:Hip ratio	1.0 (0.1)	0.9 (0.1)	**0.001**
FFM (kg)	49.6 (11.0)	52.2 (9.5)	**0.011**
FFMI (kg/m^2)	18.1 (2.6)	18.5 (2.3)	0.110
Fat mass (kg)	26.9 (11.1)	26.9 (8.6)	0.985
Resting O_2 saturation (%)	97 (2)	98 (1)	**0.001**
Fibrinogen (g/L)#	3.5 (1.3)	3.1 (1.3)	**0.001**
CRP (mg/L)#	3.5 (2.9)	1.76 (3.2)	**0.001**
Serum Creatinine (µmol/l)	70 (15)	70 (12)	0.389
eGFR (mls/min/1.73m^2)	94 (20)	93 (14)	0.786

Data presented as mean (SD) or mean (%) unless otherwise stated. #Geometric mean
Abbreviations: **BMI=** body mass index; **FVC=** forced expiratory volume; **FEV_1=** forced expiratory volume in 1 second; **FFM=** fat free mass; **FFMI=** fat free mass index; **CRP=** C-reactive protein; **eGFR=** estimated glomerular filtration rate.

Table 5.2: Reported comorbidities and medications in patients with COPD and comparator subjects at initial visit

	COPD (n=500)	Comparator (n=150)	p value
Hypertension	229 (46%)	33 (22%)	**0.001**
High Cholesterol	222 (44%)	40 (27%)	**0.001**
Peripheral vascular disease	17 (3%)	3 (3%)	0.304
Angina	54 (11%)	-	**0.001**
Heart Attack	44 (8%)	-	**0.001**
Transit Ischemic Attack	35 (7%)	-	**0.001**
Atrial Fibrillation	39 (8%)	-	**0.001**
Other heart problem	46 (9%)	-	**0.005**
Diabetes Mellitus	63 (13%)	-	**0.001**
Osteoporosis	87 (%)	8 (5%)	**0.001**
Osteoarthritis	174 (35)	40 (26)	**0.001**
ACE inhibitors	113 (23%)	8 (5%)	**0.001**
Beta Blocker	39 (%8)	5 (%3)	**0.420**
Calcium Channel Blocker	109 (22%)	8 (%5)	**0.001**
Diuretics	103 (21%)	10 (7%)	**0.001**
Statins	185 (37%)	25 (17%)	**0.001**
ICS	295 (59%)	-	-
LAMA	293 (59%)	-	-
SABA	305 (61%)	-	-
LABA	31 (6%)	-	-

Abbreviations: **ICS=** Inhaled Corticosteroid; **LAMA=** Long-Acting Muscarinic Antagonists **SABA=** Short Acting Bronchodilator Agonists; **LABA=** Long Acting Bronchodilator Agonists; **-** = not reported.

Initial Haemodynamics Data

The 500 patients with COPD had a greater mean peripheral and central systolic BP than comparators, both p<0.01, but both groups had similar peripheral and central diastolic blood pressure (Table 5.3). Peripheral and central MAP were greater in patients with COPD than their individual counterparts, p<0.05 (Table 5.3). Mean peripheral and central pulse pressure were greater in the patients than the comparators, p<0.05. Heart rate of patients with COPD was 74 (11) and greater than comparator subjects 67 (10), p<0.001. Mean aortic PWV was greater in the patients 9.9 (2.4) compared with non-COPD individuals 8.3 (1.7), p<0.001. The difference remained after adjustment for age, sex, waist circumference, peripheral MAP, smoking status and $\log_{10}$ CRP (Adjusted R^2= 0.38, F=21.8, p<0.001). Aortic PWV was associated with age in both groups, p<0.001. Aortic stiffness differed across age categories between patients with COPD and comparator subjects, all p<0.001 (Figure 5.1).

Table 5.3: Differences in haemodynamics between patients with COPD and comparators at initial visit

	COPD (n=500)	Comparator (n=150)	p value
Peripheral SBP (mmHg)	146 (18)	140 (18)	**0.001**
Peripheral DBP (mmHg)	82 (10)	81 (8)	0.283
Peripheral PP (mmHg)	64 (15)	59 (15)	**0.001**
Peripheral MAP (mmHg)	103 (11)	100 (10)	**0.011**
Central SBP (mmHg)	133 (18)	128 (19)	**0.003**
Central DBP (mmHg)	83 (10)	83 (11)	0.886
Central PP (mmHg)	50 (14)	45 (22)	**0.001**
Central MAP (mmHg)	106 (42)	102 (11)	**0.039**
Heart Rate (bpm)	74 (11)	67 (10)	**0.001**
Aortic PWV (m/s)	9.9 (2.40)	8.3 (1.66)	**0.001**
CO (L/min)	5.84 (1.21)	5.95 (1.03)	0.324
Stroke Volume (ml)	86 (20)	96 (20)	**0.001**

Data presented as mean (SD).

Abbreviations: **SBP=** systolic blood pressure; **DBP=** diastolic blood pressure; **PP=** pulse pressure; **MAP=** mean arterial pressure; **PWV=** pulse wave velocity; **CO=** cardiac output.
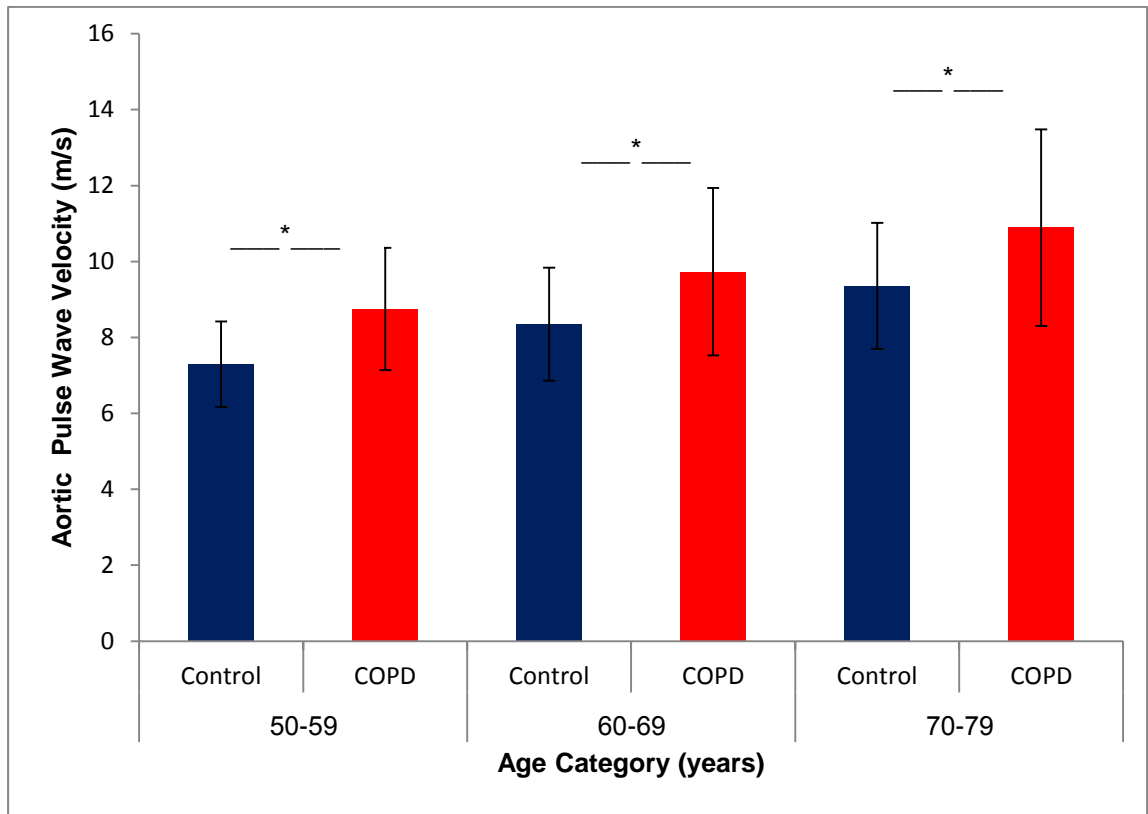
Figure 5.1: Bar chart of aortic stiffness in patients with COPD and comparator subjects by age categories at initial visit

Data presented as mean with SD.

ANOVA, Bonferonni post-hoc, p<0.001.

Relationship between Aortic Stiffness and Blood Pressure

In the patients, aortic stiffness was related to peripheral MAP (r=0.41), peripheral PP (r=0.41), central MAP (0.27) and central PP (r=0.30), all p<0.01 (Table 5.4). Similarly, aortic stiffness of the comparators was associated with peripheral and central blood pressure indices (Table 5.4). Increased heart rate was associated with increased aortic PWV in patients with COPD (r=0.24, p<0.001) and comparator subjects (r=0.21, p=0.012). Greater aortic PWV was associated with lower peripheral (r=0.12, p=0.006) and central (r=0.13, p=0.005) diastolic blood pressure. However, neither of these relationships was evident in the comparator group (Table 5.4).

Aortic stiffness did not relate to cardiac output in either group (Table 5.4). Aortic stiffness was related to stroke volume in the comparators (r=-0.25, p=0.003), but was not in the patients (Table 5.4).

To examine the relationship of kidney function with aortic stiffness, the serum creatinine level and estimated glomerular filtration rate (eGFR) were measured in patients with COPD and comparator individuals. Aortic stiffness was associated eGFR in patients with COPD (r=-0.13, p=0.005) and comparator subjects (r=-0.17, p=0.044), but was only related to serum creatinine (r=0.20, p<0.001) in the patients (Table 5.4).

Table 5.4: Relationships between aortic PWV and physiological characteristics in patients with COPD and comparators at initial visit

Pearson's correlation	COPD (n=500)	p value	Comparator (n=150)	p value
Peripheral SBP (mmHg)	0.411	**0.001**	0.517	**0.001**
Peripheral DBP (mmHg)	0.124	**0.006**	0.136	0.099
Peripheral MAP (mmHg)	0.294	**0.001**	0.370	**0.001**
Peripheral PP (mmHg)	0.407	**0.001**	0.555	**0.001**
Central SBP (mmHg)	0.313	**0.001**	.438	**0.001**
Central DBP (mmHg)	0.127	**0.005**	0.056	0.496
Central MAP (mmHg)	0.269	**0.001**	0.335	**0.001**
Central PP (mmHg)	0.296	**0.001**	0.366	**0.001**
Heart rate (bpm)	0.240	**0.001**	0.209	**0.012**
Cardiac output (L/min)	0.080	0.083	0.046	0.584
Stroke Volume (ml)	-0.068	0.143	0.248	**0.003**
Resting O_2 saturation (%)	-0.146	**0.002**	-0.160	0.149
Serum Creatinine (µmol/l)	0.195	**0.001**	0.077	0.363
eGFR (mls/min/1.73m^2)	-0.128	**0.005**	-0.169	**0.044**
log_{10} Fibrinogen (g/L)	0.033	0.476	-0.033	0.697
log_{10} CRP (mg/L)	0.131	**0.005**	0.193	**0.022**
Number of Comorbidities	0.199	**0.001**	0.421	**0.001**

Abbreviations: SBP= systolic blood pressure; **DBP=** diastolic blood pressure; **PP=** pulse pressure**; MAP=** mean arterial pressure; **eGFR=** estimated glomerular filtration rate; **CRP=** C-reactive protein.

Relationship between Aortic Stiffness and Gender

Within the patients, aortic PWV was greater in males with COPD 10.2 (2.7) than their female counterparts 9.5 (2.0), p=0.003. The difference remained after controlling for age and peripheral MAP (Adjusted R^2=26%, F=6.15, p=0.014). However, the difference was not significant after adding waist circumference to the model (Adjusted R^2=30%, F=0.16, p=0.693). In contrast, gender had no effect on aortic stiffness in comparator group.

Across gender comparison, after adjustment for waist circumference, peripheral MAP, smoking exposure and $\log_{10}$ CRP, male patients 9.9 m/s (95%CI: 9.6 to 10.2) had greater aortic PWV than male comparators 9.1 m/s (95%CI: 8.5 to 9.6), (adjusted R^2=22%, F=6.3 and p=0.012). Similarly, the aortic PWV of females with COPD 9.4 m/s (95%CI: 9.1 to 9.6) was greater than females without COPD 8.7 m/s (95%CI: 8.3 to 9.2), adjusted R^2=25%, F=5.13 and p=0.024 (Figure 5.2).
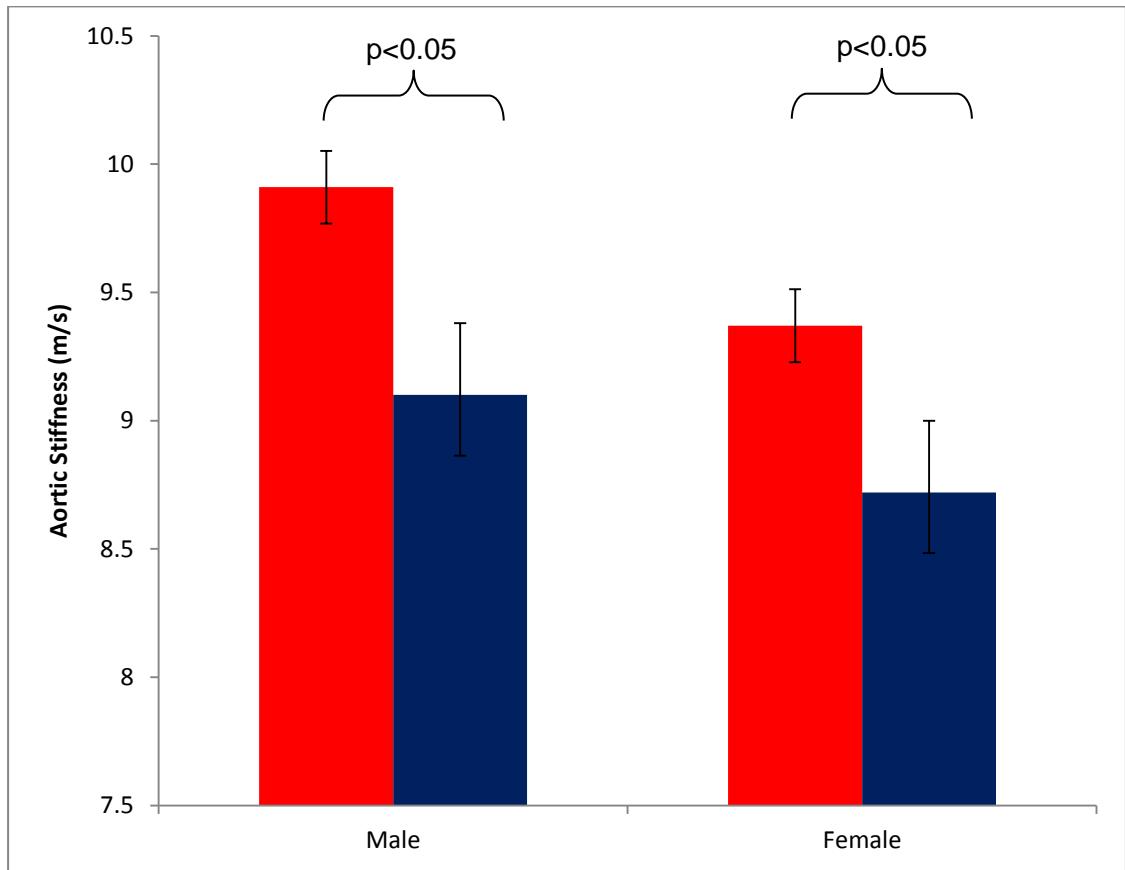
Figure 5.2: Bar chart comparing aortic stiffness by gender in patients with COPD and comparators at initial visit

 COPD   Comparator

Data presented as mean with SD.

Relationship between Aortic Stiffness and Lung Function

In the patients, aortic stiffness was related to the severity of airway obstruction (r=-0.10, p=0.037), however, in the comparators, aortic stiffness was not related to FEV_1, p>0.05 (Table 5.5). Aortic stiffness did not differ across the GOLD categories. Patients with COPD in all GOLD categories had greater aortic PWV compared with non-COPD subjects, all p<0.001 (Figure 5.3).

Patients with an FEV_1>50% predicted had lower aortic PWV 9.6 m/s (2.2) than patients with an FEV_1<50% predicted 10.70 m/s (2.6), p<0.001, and patients in both categories had greater aortic PWV compared with non-COPD subjects 8.3 m/s (1.7), all p<0.001 (Figure 5.4).

In the patients, aortic stiffness was associated with oxygen saturation (r=-0.15, p=0.002), but was not in the comparators (Table 5.5).
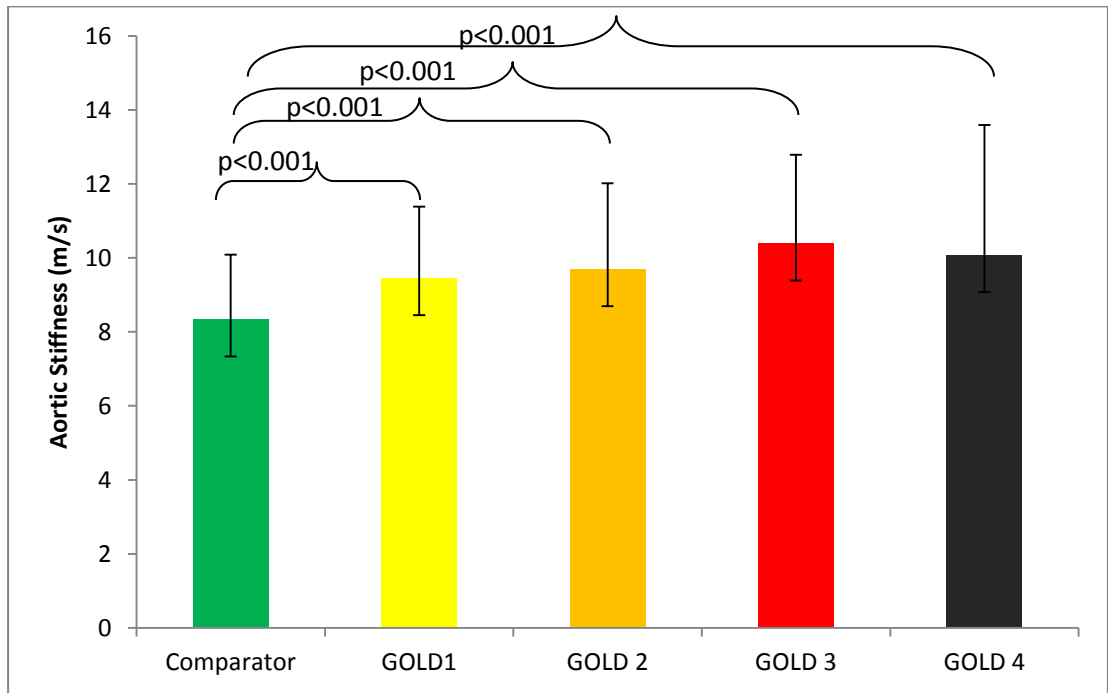
Figure 5.3: Bar chart of aortic PWV in comparators and patients with COPD across GOLD category at initial visit

Data presented as mean with SD.

ANOVA, Bonferonni post-hoc, p<0.001.
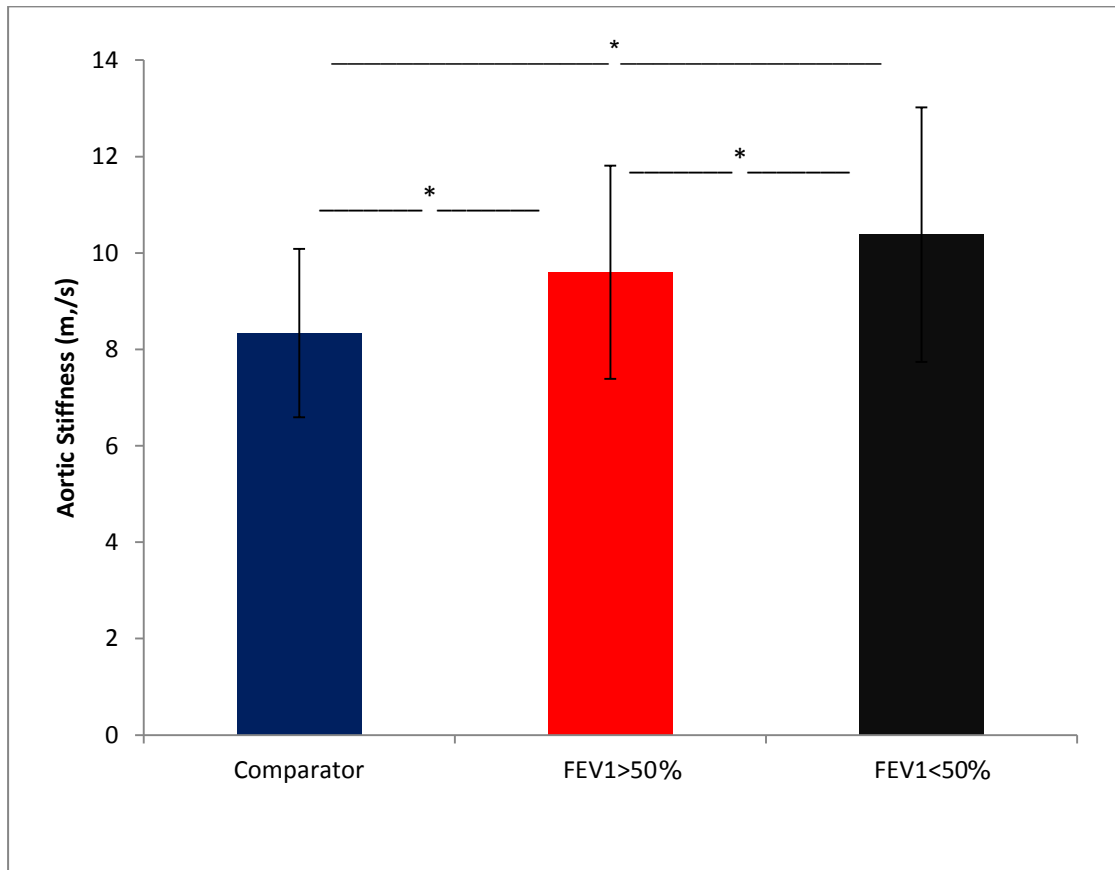
Figure 5.4: Bar chart of aortic PWV in comparators, patients with an FEV1>50% predicted and patients with an FEV1<50% at initial visit

Data presented as mean with SD.

ANOVA, Bonferonni post-hoc, p<0.001.

Relationship between Aortic Stiffness and Body Composition

In the patients, aortic PWV was related to BMI (r=0.18) waist circumference (r=0.26), waist: hip ratio (r=0.24), fat mass (r=0.17) and FFMI (r=0.14), all p<0.01. However, these relationships were not evident in comparator group (Table 5.5). Aortic stiffness did not differ across BMI categories in patients with COPD, p>0.05.

Relationship between Aortic Stiffness and Smoking

In patients with COPD, increased aortic PWV was associated with the number of smoking pack-years (r=0.13, p=0.005), but this relationship was not found in comparator subjects (Table 5.4). Within the patients, there was no difference in pack-years between previous smokers mean (SD) 35 (2) and current smokers 35 (2), but current smokers were younger 63 (8) than previous smokers 67 (6), p<0.001. The aortic PWV was similar between current smokers and previous smokers after correcting for age (F=1.26, p>0.05). Within the comparators, there was a difference in the number of pack-years smoked between current smokers mean (SD) 22 (2) and previous smokers 15 (2), but they had a similar aortic PWV, 7.8 (1.4) and 8.4 (1.7), p>0.05, respectively.

Table 5.5: Relationships between aortic PWV and physical characteristics in patients with COPD and comparator subjects at initial visit

Pearson's correlation	COPD (n=500)	p value	Comparator (n=150)	p value
Age (years)	**0.405**	**0.001**	**0.518**	**0.001**
FEV_1 (%)	**-0.095**	**0.037**	-0.079	0.339
FEV_1/FVC (L)	**-0.110**	**0.016**	-0.071	0.393
Smoking (pack-years)	**0.129**	**0.005**	-0.002	0.984
No. Exacerbations/year	-0.023	0.620	-	-
BMI (kg/m^2)	**0.177**	**0.001**	0.037	0.653
Waist circumference (cm)	**0.263**	**0.001**	0.008	0.923
Waist:Hip ratio	**0.239**	**0.001**	0.155	0.062
FFM (kg)	**0.143**	**0.002**	0.037	0.655
FFMI (kg/m^2)	**0.141**	**0.002**	0.002	0.984
Fat Mass (kg)	**0.172**	**0.001**	0.083	0.312

Abbreviations: **FEV_1=** forced expiratory volume in 1 second; **FEV_1/FVC=** forced expiratory volume in 1 second to forced expiratory volume ratio**; BMI=** body mass index; **FFM=** fat free mass; **FFMI=** fat free mass index**.**

Relationship between Aortic Stiffness and Circulating Inflammatory Biomarkers

Circulating $\log_{10}$CRP was related to aortic stiffness in patients with COPD (r=0.13, p=0.005) and comparator subjects (r=0.19, p=0.022). However, the fibrinogen level was not associated with aortic stiffness in either group, p>0.05 (Table 5.5).

To determine the effect of exacerbations on aortic stiffness, frequent exacerbators and infrequent exacerbators were compared. There was no difference in aortic stiffness between frequent exacerbators 9.9 (2.6) and infrequent exacerbators 9.9 (2.3), p>0.05. However, both groups had greater aortic PWV than comparator subjects 8.3 (1.7), p<0.001 (Figure 5.5).
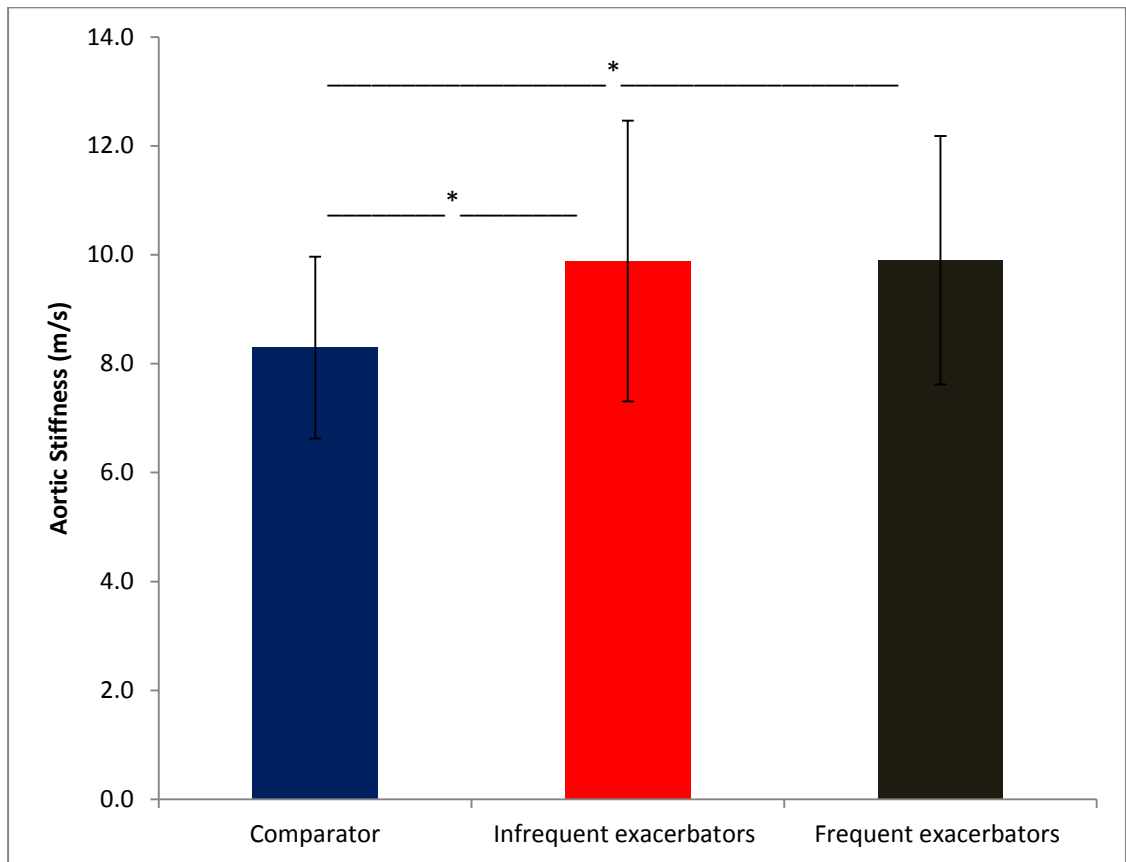
Figure 5.5: Bar chart of aortic stiffness in comparators and patients with frequent and

infrequent exacerbation

Data presented as mean with SD.

ANOVA, Bonferonni post-hoc, p<0.001.

Determinants of initial aortic PWV

A hierarchical regression analysis was used to determine predictors of aortic PWV at the initial visit. The first model included gender to examine the behaviour of aortic PWV across gender. The second model was based on the most known influential factors on aortic PWV including age and peripheral MAP. In the third model, the potential factors were included: $FEV_1\%$ predicted, waist circumference, heart rate, smoking history, eGFR, $log_{10}CRP$. For body composition, waist circumference was chosen because abdominal obesity exerts an adverse effect on the arterial system and increases aortic stiffness. Estimated GFR rather than serum creatinine was chosen to delineate kidney function because it is more robust and takes into account age and gender as well as the level of serum creatinine. In the patients, in the first model, gender explained 2% of variation in aortic stiffness (F=7.31, p<0.01). Adding age and peripheral MAP to the regression model explained another 26% with a significant change in R (R=28%, F=73.20, p<0.001). However, including potential factors in the model revealed that gender was no longer a predictor and among the potential factors only waist circumference and heart rate which could explain an additional 12% of variation in aortic PWV (R=38%, F=10.01, p<0.001). The predictors of aortic PWV in COPD were age, peripheral MAP, waist circumference and heart rate, and explained 38% of variation in aortic PWV (Table 5.6). In the comparator group, similarly to COPD group, age and peripheral MAP explained 36% of the variability in aortic stiffness (R^2= 0.33, F=22.89, p<0.001) (Table 5.7). However, none of the potential factors added any additional information to the model and the change in R was non-significant (R=0.05, F=1.49, p=0.177).

Table 5.6: Predictors of aortic stiffness in patients at initial visit

	B	SE	Beta	p value	Adjusted R^2
Model 1					**0.02**
Gender	-.594	.220	-.131	0.007	
Model 2					**0.28**
Gender	-.396	.190	-.087	0.038	
Age (years)	.137	.013	.435	0.001	
Peripheral MAP (mmHg)	.057	.008	.282	0.001	
Model 3					**0.38**
Age (years)	.135	.013	.428	0.001	
Peripheral MAP (mmHg)	.043	.008	.214	0.001	
Waist circumference (cm)	.063	.016	.409	0.001	
Heart rate (bpm)	.045	.008	.226	0.001	

Abbreviations: MAP= mean arterial pressure.

Table 5.7: Predictors of aortic stiffness in comparator subjects at their initial visit

	B	SE	Beta	p value	Adjusted R^2
Model 3					0.33
Age (years)	.090	.015	.434	0.001	
Peripheral MAP (mmHg)	.045	.012	.276	0.001	

Abbreviation: MAP= mean arterial pressure.

Part II:

Characteristics of Returning Patients

Of the 500 patients who were assessed at the initial visit, 143 consecutive patients completed a two-year follow-up visit by end of December 2013 and were included in the analysis. In this longitudinal study, there was no comparator group due to time constraint of the three-year PhD study period, which it did not allow for follow-up the comparators.

To ensure that the 143 consecutive patients included in the analysis represented the whole patient group who will have been reassessed, a comparison analysis was carried out between returning patients (n=143) and the rest of the patients (n=357). The returning patients were similar to the rest of 500 patients in body composition, peripheral and central blood pressure indices, aortic stiffness, cardiac hemodynamic indices and inflammatory biomarkers, all p>0.05. However, the returning patients were older, and had lower FEV_1 and eGFR, p<0.05 (Table 5.8).

Of the returning patients, 9 patients had developed hypertension, 4 had developed high cholesterol, 3 had angina and 3 had developed osteoporosis (Figure 5.7). Among the returning patients, 16 out of 30 patients were still smokers.

The severity of airflow obstruction by GOLD stratification was: GOLD I n=12, GOLD II n=72, GOLD III n=42 and GOLD IV n=17. Patients were further stratified into above and below 50% predicted of an FEV_1: patients with an FEV_1>50% predicted, n=94, and patients with an FEV_1<50% predicted, n=59. On the follow-up visit, the patients had a reduction in FEV_1% predicted and FVC%. The change in FEV_1 across the GOLD categories was significant only in GOLD I&II (Table 5.9).

Following the classification used by Landbo et al. (1999), of the 143 patients, 7% had a low BMI (<19.9 kg/m^2), 37% had an elevated BMI (25-29.9 kg/m^2) and 34% were classified as obese (BMI>30 kg/m^2), while 21% were within the normal limit for BMI (20-24.9 kg/m^2).

Table 5.8: Comparison between initial visit data in patients assessed at 2 years and

patients yet to be assessed

	To be assessed (n=357)	Reassessed (n=143)	p value
Age (years)	65 (8)	68 (7)	**0.001**
FEV1 (L)	1.7 (0.8)	1.3 (0.5)	**0.001**
FVC (L)	2.4 (0.9)	2.4 (0.8)	0.753
FEV_1/FVC	53 (10)	51 (12)	**0.033**
FVC%	86 (21)	83 (20)	0.226
FEV_1%	58 (18)	54 (20)	**0.031**
BMI (kg/m^2)	27.9 (6)	28.0 (6)	0.800
Waist circumference (cm)	99 (15)	99 (15)	0.755
Waist:Hip ratio	0.96 (0.10)	0.94 (0.12)	0.303
FM (kg)	26.8 (11.4)	26.7 (10.5)	0.949
FFM (kg)	49.5 (10.9)	48.6 (10.8)	0.446
FFM:FM ratio	2.2 (1)	2.2 (1.3)	0.845
FFMI (kg/m^2)	18 (2.7)	18 (2.6)	0.669
Peripheral SBP (mmHg)	145 (19)	148 (17)	0.287
Peripheral DBP (mmHg)	82 (10)	82 (11)	0.921
Peripheral MAP (mmHg)	103 (12)	103 (11)	0.358
Peripheral PP (mmHg)	64 (16)	65 (15)	0.232
Central SBP (mmHg)	133 (19)	135 (17)	0.461
Central DBP (mmHg)	83 (10)	83 (11)	0.879
Central MAP (mmHg)	104 (12)	104 (12)	0.529
Central PP (mmHg)	50 (14)	52 (15)	0.728
Heart rate (bpm)	74 (11)	76 (11)	0.068
Aortic PWV (m/s)	9.7 (2.3)	10.2 (2.7)	0.102
CO (L/min)	5.9 (1.3)	5.7 (1.1)	0.051
SV (ml)	87 (20)	83 (19)	0.072
O_2 saturation	96 (2)	96 (2)	0.658
Smoking (packs/year)	40 (23)	42 (30)	0.384
No. Exacerbations	2.2 (2)	2.5 (1.9)	0.072
Fibrinogen (g/L)	3.4 (1.3)	3.7 (1.1)	0.179
CRP (mg/L)#	3.6 (2.9)	3.0 (2.8)	0.164
eGFR (mls/min/1.73m^2)	95 (20)	89 (20)	**0.001**

Data presented as mean (SD) unless otherwise indicated, # Geometric mean.
Abbreviations: FEV_1= forced expiratory volume in one second**; FVC=** forced vital capacity**; BMI=** body mass index**; SBP=** systolic blood pressure**; DBP=** diastolic blood pressure**; PP=** pulse pressure**; PWV=** pulse wave velocity**; CO=** cardiac output**; SV=** stroke volume**; 6MWD=** six minute walking distance**; CRP=** C- reactive protein**; eGFR=** estimated glomerular filtration rate.

Table 5.9: Change in FEV$_1$ (ml) across GOLD categories in patients with COPD at follow-up visit

	Mean	95% Confidence Interval for Mean		P value
GOLD I	-166	278	54	**0.006**
GOLD II	-178	-256	-100	**0.001**
GOLD III	-65	-120	-10	0.192
GOLD IV	47	-102	196	0.300
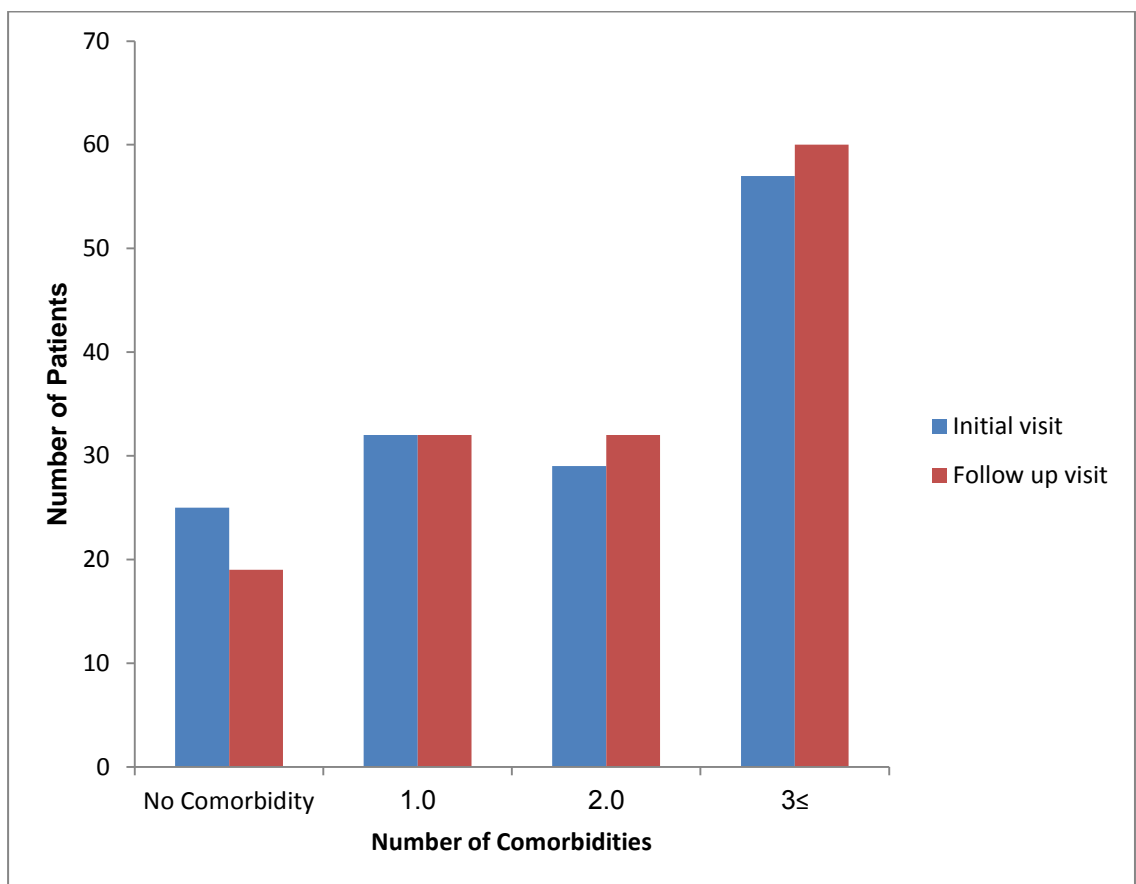
Figure 5.6: Distribution of comorbidities in patients at initial visit and two years follow-up

Changes in Cardiovascular Parameters at Two Years Follow-up

Peripheral and central blood pressure indices had decreased significantly over two years in the patients, $p<0.05$ (Table 5.10). The aortic PWV of the patients had increased after two years from the initial assessment by mean (95%CI) 0.7 m/s (0.35 to 0.95), $p<0.001$ (Table 5.10). Males and females patients showed a similar change in aortic PWV. The heart rate remained stable on both visits, but there was a reduction in stroke volume and cardiac output after two years from the initial assessment, $p<0.05$ (Table 5.10). However, the change in aortic PWV was not associated with cardiac output or stroke volume, $p>0.05$.

Relationship between Blood Pressure and Change in Aortic Stiffness

Change in aortic PWV did not relate to the changes in systolic, diastolic or mean arterial pressures.

Relationship between Lung Function and Aortic Stiffness

There was no difference in aortic stiffness across the GOLD categories. This analysis was further expanded and compared the change in aortic PWV between patients with an $FEV_1>50\%$ predicted (n=84) and patients with an $FEV_1 <50\%$ predicted (n=59). The results also showed no difference between patients with mild to moderate airway obstruction compared with severe and very severe airflow obstruction, $p>0.05$.

Table 5.10: Changes in patients with COPD after two years from the initial assessment

	Initial visit (n=143)	Follow-up visit (n=143)	Mean Change (95%CI)			p value
Gender (Male:Female)	65:78	65:78				
Age (years)	68 (7)	70 (7)	2	1.86	2.04	**0.001**
FEV_1 (L)	1.3 (0.5)	1.1 (0.5)	-0.12	-0.16	-0.07	**0.001**
FVC (L)	2.4 (0.08	2.3 (0.8)	-0.16	-0.23	-0.08	**0.001**
FVC% predicted	83 (19)	79 (22)	-4	-7	-2	**0.001**
FEV_1%predicted	54 (19)	51 (16	-3	-4	-1	**0.001**
Smoking (pack-years)	42 (30)	43 (28)	1.25	-3	5	0.519
BMI (kg/m^2)	28 (6)	27.8 (6)	-0.20	-0.59	0.21	0.356
Waist (cm)	99 (15)	99 (15)	-0.16	-1.30	0.99	0.786
Waist:Hip ratio	0.94 (0.10)	095 (0.12	0.01	0.01	0.02	0.120
Fat Mass (kg)	26.7 (11)	26.2 (10.5)	-0.55	-1.44	0.34	0.224
FFM (kg)	48.6 (10.8)	48.1 (10.8)	-0.53	-1.05	-0.01	**0.048**
Peripheral SBP (mmHg)	148 (17)	144 (17)	-4	-7	-1	**0.008**
Peripheral DBP (mmHg)	82 (11)	79 (11)	-3	-4	-1	**0.001**
Peripheral PP (mmHg)	66 (15)	65 (15)	-1	-4	1	0.207
Peripheral MAP	103 (11)	100 (11)	-3	-5	-1	**0.001**
Central SBP (mmHg)	135 (17)	130 (16)	-5	-7	-1	**0.006**
Central DBP (mmHg)	83 (11)	81 (11)	-2	-4	-1	**0.003**
Central PP (mmHg)	52 (15)	50 (14)	-2	-4	1	0.138
Central MAP (mmHg)	104 (12)	102 (12)	-2	-4	-1	**0.012**
Heart Rate (bpm)	76 (11)	76 (12)	0	-1	2	0.700
Aortic PWV (m/s)	10.2 (2.7)	10.9 (2.6)	0.70	0.37	0.95	**0.001**
CO (L/min)	5.7 (1.1)	5.5 (1.2)	-0.20	-0.30	-0.02	**0.024**
Stroke Volume (ml)	83 (19)	79 (20)	-4	-7	-2	**0.001**
Resting O_2 (%)	96 (2)	96 (2)	0	-0.08	0.63	0.128
CRP (mg/L)#	3.0 (2.8)	3.1 (2.6)	0.1	0.9	1.2	0.953
Fibrinogen (g/L)	3.7 (1.1)	3.3 (0.9)	-0.40	-0.56	-0.20	**0.001**
Serum creatinine(µmol/L)	72 (16)	76 (16)	3.43	1.76	5.11	**0.001**
eGFR (mls/min/1.73m^2)	89 (20)	81 (17)	-8	-2	-7	**0.001**

Data presented as mean (SD) unless otherwise indicated, #=Geometric mean, mean (95%CI).

Abbreviations: FEV$_1$= forced expiratory volume in one second**; FVC=** forced vital capacity**; BMI=** body mass index**; SBP=** systolic blood pressure**; DBP=** diastolic blood pressure**; PP=** pulse pressure**; MAP=** mean arterial pressure; **PWV=** pulse wave velocity; **CO=** cardiac output**; SV=** stroke volume**; CRP=** C- reactive protein; **eGFR=** estimated glomerular filtration rate

Relationship between Body Composition and Change in Aortic Stiffness

Previously in the cross-sectional analysis, aortic PWV was related to global and regional obesity indices. Therefore, in this section, the effect of body composition was examined. Body composition did not change significantly in the patients apart from a significant loss of FFM (Table 5.10). The change in aortic PWV did not differ across BMI categories, $p > 0.05$. This analysis was further extended by dividing patients into two groups: underweight and normal weight in one group, and overweight and obese in another group. Similar to whole group analysis, there was no statistical difference between the groups, $p > 0.05$.

Relationship between Change in Aortic Stiffness and Changes in Inflammatory Biomarkers

After two years from the initial visit, the fibrinogen level decreased significantly, while CRP level remained statistically unchanged and a third of the patients remained inflammation free during the study period, <3 mg/L (Figure 5.8). In contrast, the number of exacerbations of the patients had increased (Table 5.10).

Although the level of fibrinogen decreased, aortic PWV had increased. There was no association between change in aortic PWV and the reduction in plasma fibrinogen or increase in the number of exacerbations.

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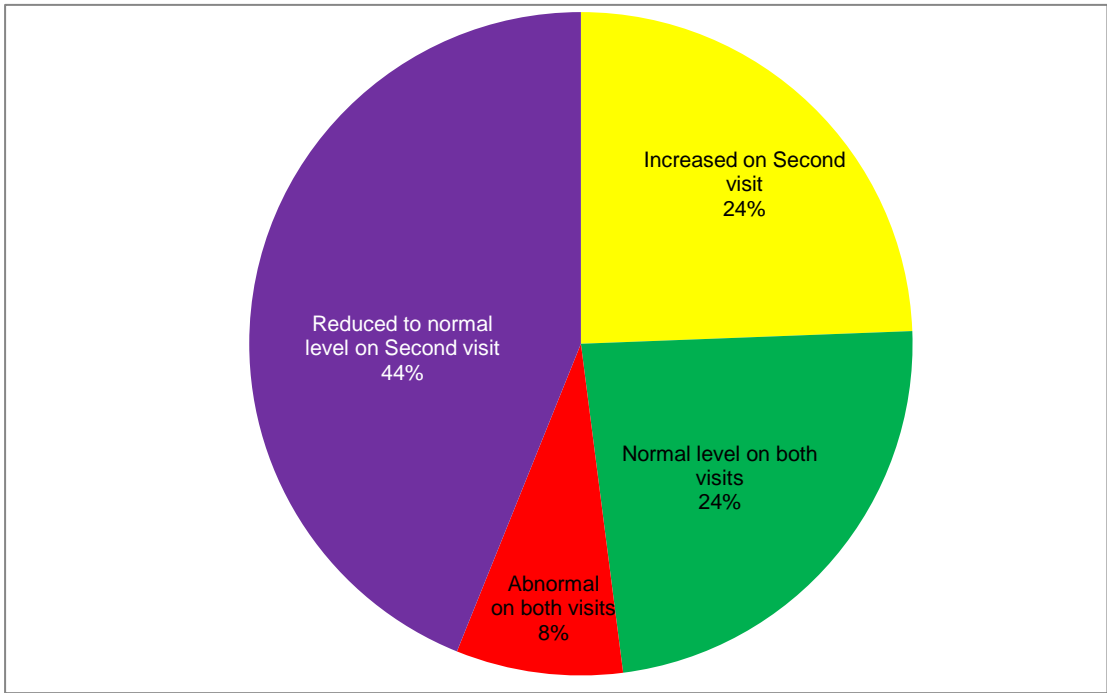
Figure 5.7A: The pie chart showing the distribution of patients according to the change in the fibrinogen level during the follow-up period
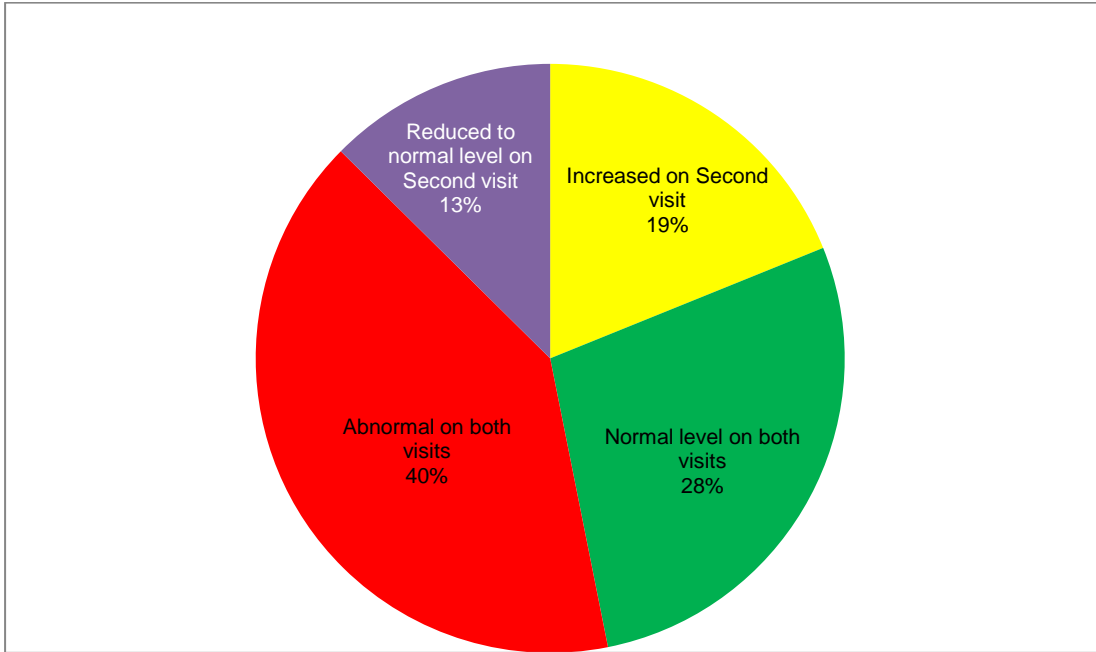
Figure 5.7B: The pie chart showing the distribution of patients according to the change in the CRP level during the follow-up period

Subgroup Analysis

After two years from the initial assessment, a subset of patients was identified to have greater progression in aortic stiffness. This group (progressor) was defined as any patients who demonstrated an increase in aortic PWV by more than 0.20 m/s from the baseline measurement (Cecelja and Chowienczyk, 2012). The progressors showed a rapid increase in aortic PWV than the rest of the group (non-progressor). Therefore, a further analysis was performed to explore the characteristics of this group and the factors associated with accelerated aortic stiffness.

Out of 143 patients with COPD, 93 patients (progressors) had greater increase in aortic PWV, 1.6 (95%CI: 1.3 to 1.9) m/s, $p < 0.001$ whereas 50 patients (non-progressor) had a significant reduction in aortic PWV -1.0 (95%CI:-1.3 to -0.7) m/s, $p < 0.001$. Out of 50 non-progressor patients, 32 (64%) were previously diagnosed with isolated systolic hypertension and on the second visit three patients had developed hypertension. Non-progressors also experienced a significant reduction in peripheral and central blood pressure indices, $p < 0.001$ (Table 5.11). However, there were no changes in peripheral and central pulse pressure. Cardiac hemodynamic indices of the non-progressor did not change during the follow-up period, $p > 0.05$, but eGFR significantly decreased by mean (95%CI) -11 (-18 to -8), $p < 0.001$ (Table 5.11). In the non-progressors, lung function as measured by FVC was reduced significantly over a period of the two years follow-up, $p < 0.001$ (Table 5.11). Non-progressors also demonstrated a reduction in the fibrinogen level while CRP remained unchanged (Table 5.11). In the non-progressor patients, improvement of aortic stiffness was not associated with the reduction in blood pressure indices.

Table 5.11: Characteristics of non-progressor patients at initial visit and 2 years follow-up

	Initial visit (n=50)	2 year-visit (n=50)	Mean change 95%CI			p value
Age (years)	68 (7)	70 (7)				
FEV$_1$ (L)	1.3 (0.5)	1.2 (0.5)	-0.08	-0.15	-0.01	**0.035**
FVC (L)	2.5 (0.7)	2.3 (0.7)	-0.13	-0.25	-0.02	**0.026**
FVC% predicted	84 (20)	79 (22)	-5	-7	-2	**0.001**
FEV$_1$%predicted	56 (24)	54 (22)	-2	-5	1	0.170
Smoking (pack-years)	44 (4)	42 (4)	-2	-9	4	0.463
BMI (kg/m^2)	28.8 (5.9)	28.8 (5.8)	-0.07	-0.68	0.53	0.810
Waist (cm)	103 (14)	102 (14)	-1	-3	1	0.244
Waist:Hip ratio	0.95 (0.10)	096 (0.10)	-0.34	-1.44	0.76	0.536
Fat Mass (kg)	28.7 (10.2)	28.3 (10.5)2	-0.55	-1.44	0.34	0.224
FFM (kg)	49.6 (1.3)	48.8 (1.4)	-0.69	-1.53	0.15	0.105
Peripheral SBP (mmHg)	152 (19)	143 (18)	-9	-15	-3	**0.002**
Peripheral DBP (mmHg)	84 (10)	78 (9)	-6	-9	-4	**0.001**
Peripheral PP (mmHg)	68 (19)	65 (16)	-23	-7	1	0.164
Peripheral MAP	106 (11)	99 (10)	-7	-11	-4	**0.001**
Central SBP (mmHg)	138 (17)	129 (16)	-9	-15	-4	**0.001**
Central DBP (mmHg)	86 (10)	79 (9)	-7	-9	-4	**0.001**
Central PP (mmHg)	53 (15)	50 (14)	-3	-7	1	0.103
Central MAP (mmHg)	107 (11)	100 (11)	-7	-11	-3	**0.001**
Heart Rate (bpm)	78 (12)	78 (13)	0	-2	3	0.736
Aortic PWV (m/s)	11.0 (2.2)	10.0 (2.1)	-1.00	-1.3	-0.7	**0.001**
CO (L/min)	5.8 (1.2)	5.6 (1.2)	-0.20	-0.45	0.05	0.115
Stroke Volume (ml)	84 (20)	80 (20)	-4	-8	1	0.108
Resting O$_2$ (%)	96 (2)	96 (2)	0	-1	1	0.128
CRP (mg/L)#	3.0 (2.9)	3.1 (2.8)	0.1	0.8	1.4	0.711
Fibrinogen (g/L)	3.7 (0.9)	3.3 (0.7)	-0.40	-0.67	-0.14	**0.004**
Serum creatinine (μmol/L)	72 (16)	79 (18)	7	4	9	**0.001**
eGFR (mls/min/1.73m^2)	90 (20)	79 (22)	-11	-18	-8	**0.001**

Data presented as mean (SD) unless otherwise indicated, #=geometric mean, mean (95%CI).

Abbreviations: FEV$_1$= forced expiratory volume in one second**; FVC=** forced vital capacity**; BMI=** body mass index**; SBP=** systolic blood pressure**; DBP=** diastolic blood pressure**; PP=** pulse pressure**; MAP=** mean arterial pressure; **PWV=** pulse wave velocity; **CO=** cardiac output; **SV=** stroke volume; **CRP=** C- reactive protein; **eGFR=** estimated glomerular filtration rate.

In the progressors, 43 (64%) had a previous diagnosis of isolated systolic hypertension on the initial visit and six new patients were diagnosed with hypertension on the second visit. Unlike non-progressors, peripheral and central blood pressure indices remained unchanged, $p > 0.05$, while stroke volume significantly decreased, $p < 0.001$ (Table 5.12). In the progressors, lung function indices had declined during the two years follow-up period, $p < 0.001$ (Table 5.12). Similar to non-progressors, progressors' kidney function index as measured by eGFR demonstrated a significant reduction in its level, $p < 0.001$ (Table 5.12). The level of inflammatory biomarkers behaved similarly as in the non-progressors, and plasma fibrinogen returned to its normal level, $p < 0.001$, while circulating CRP maintained its level as it was at the initial visit, $p > 0.05$ (Table 5.12). The progression of aortic PWV was only related to baseline age of the progressors ($r = 0.23$, $p = 0.029$). The impact of comorbidities on aortic stiffness progression was further explored and showed no association between the number of comorbidities and the progression of aortic PWV. A quarter of the progressors had no previous history of diagnosed comorbidities and nearly a third of them had just one comorbidity (Figure 5.9).

Table 5.12: Clinical characteristics of progressors at initial visit and 2 years follow-up

	Initial visit (n=93)	2 year-visit (n=93)	Mean change 95%CI			p value
Age (years)	67 (7)	69 (7)				
FEV$_1$ (L)	1.25 (0.5)	1.10 (0.5)	-0.14	-0.20	-0.08	**0.001**
FVC (L)	2.4 (0.8)	2.2 (0.9)	-0.17	-0.27	-0.07	**0.001**
FVC% predicted	83 (19)	77 (20)	-6	-9	-3	**0.001**
FEV$_1$%predicted	52 (16)	49 (17)	-3	-5	-1	**0.001**
Smoking (pack/year)	41 (29)	44 (30)	3	-1	8	0.176
BMI (kg/m^2)	27.5 (5.4)	27.3 (5.7)	-0.25	-0.78	0.28	0.353
Waist (cm)	98 (15)	99 (16)	0.37	-1.07	1.82	0.610
Waist:Hip ratio	0.94 (0.10)	0.95 (0.10)	0.01	-0.01	0.02	0.212
Fat Mass (kg)	25.5 (11.3)	24.9 (10.6)	-0.67	-1.92	0.59	0.295
FFM (kg)	48.0 (10.6)	47.6 (10.6)	-0.44	-1.12	0.24	0.200
Peripheral SBP (mmHg)	145 (17)	145 (17)	-1	-4	2	0.505
Peripheral DBP (mmHg)	80 (11)	80 (12)	0	-2	1	0.600
Peripheral PP (mmHg)	65 (16)	64 (15)	-1	-3	2	0.658
Peripheral MAP	101 (11)	100 (11)	-1	-3	1	0.505
Central SBP (mmHg)	133 (17)	132 (16)	-1	-4	2	0.481
Central DBP (mmHg)	82 (12)	81 (12)	-1	-2	2	0.679
Central PP (mmHg)	51 (15)	51 (13)	0	-3	2	0.574
Central MAP (mmHg)	102 (11)	102 (11)	0	-2	2	0.938
Heart Rate (bpm)	74 (11)	75 (12)	1	-2	2	0.816
Aortic PWV (m/s)	9.8 (2.3)	11.4 (2.7)	1.6	1.3	1.9	**0.001**
CO (L/min)	5.6 (1.1)	5.4 (1.1)	-0.20	-0.31	0.03	0.107
Stroke Volume (ml)	83 (18)	78 (19)	-5	-8	-2	**0.001**
Resting O$_2$ saturation (%)	96 (2)	96 (2)	0	-1	1	0.425
CRP (mg/L)#	3.0 (2.5)	3.1 (2.8)	0.1	0.8	1.2	0.706
Fibrinogen (g/L)	3.7 (1.1)	3.3 (0.9)	-0.40	-0.61	-0.12	**0.004**
Serum creatinine (µmol/l)	72 (16)	74 (13)	2	1	4	0.122
eGFR (mls/min/1.73m^2)	89 (18)	81 (16)	-8	-11	-4	**0.001**

Data presented as mean (SD) unless otherwise indicated, #=geometric mean, mean (95%CI).

Abbreviations: FEV$_1$= forced expiratory volume in one second**; FVC=** forced vital capacity**; BMI=** body mass index**; SBP=** systolic blood pressure**; DBP=** diastolic blood pressure**; PP=** pulse pressure**; MAP=** mean arterial pressure; **PWV=** pulse wave velocity; **CO=** cardiac output**; SV=** stroke volume**; CRP=** C- reactive protein.
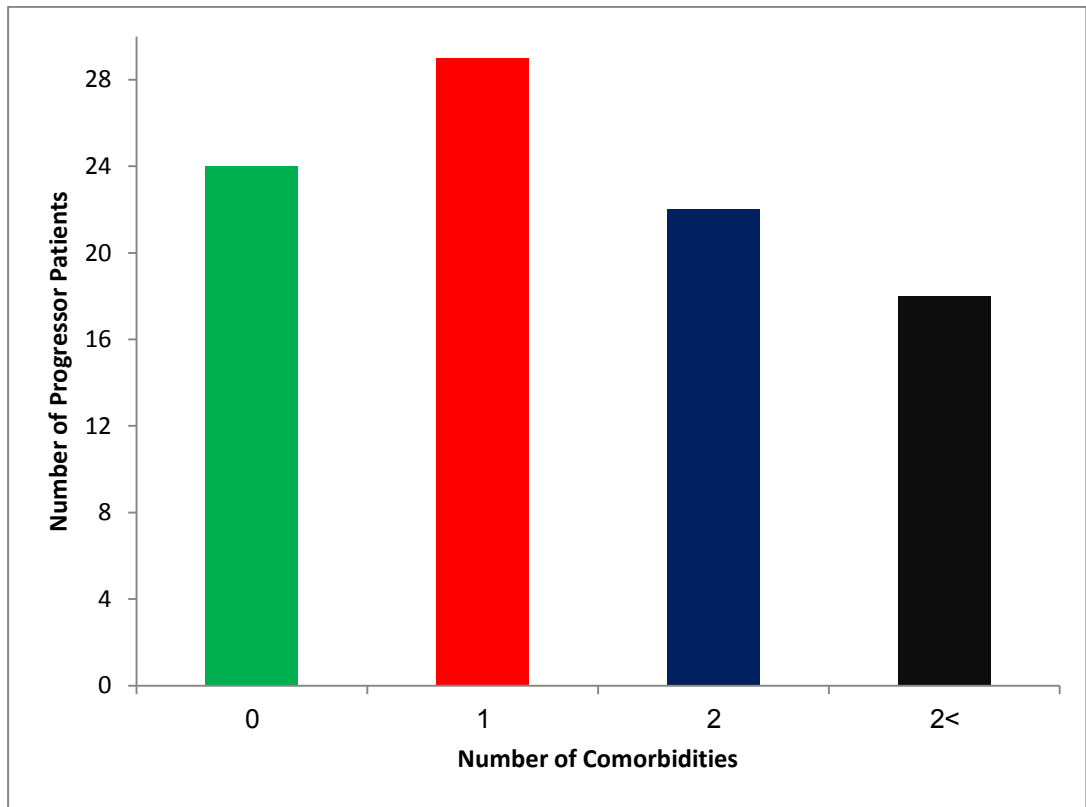
Figure 5.8: Distribution of comorbidities in progressor patients at two years visit

In contrast, the non-progressors had improvement in peripheral and central blood pressure indices, p<0.01 (Table 5.13). Although, there was a significant difference in the change of aortic stiffness between the progressors and non-progressors, peripheral and central pulse pressure remained similar between both groups, p>0.05 (Table 5.13). Comparing progressor patients with non-progressor patients revealed that the progressors experienced more rapid decline in lung function indices than the non-progressors, p<0.001 (Table 5.13). Both groups had similar reduction in plasma fibrinogen on the follow-up visit, p<0.01, while circulating CRP remained unchanged in both groups during the two years follow-up period (Table 5.13).

The progressors demonstrated a substantial increase in aortic PWV than the non-progressors after correcting for the difference in peripheral MAP (Adjusted R^2=51%, F=121, p<0.001). The same finding was found when the change in peripheral MAP was replaced by the change in central MAP. The model was further expanded and included baseline age, sex, the difference in FEV_1% predicted, difference in peripheral MAP, smoking status and showed that increased aortic PWV remained significant in the progressors (adjusted R^2=51%, F=119.8, p<0.001).

Table 5.13: Differences between progressor and non-progressor patients after two years

	Non-progressors (n=50)	Progressors (n=93)	p value
Change in FEV_1 (ml)	-80 (-150 to -100)	-140 (-200 to -80)	**0.001**
Change in FEV_1% predicted	-2 (-5 to 1)	-3 (-5 to -1)	0.594
Change in FVC % predicted	-5 (-7 to -2)	-6 (-9 to -3)	**0.001**
Smoking history (packs/year)	-2 (-9 to 4)	3 (-1 to 8)	0.334
Change in peripheral SBP (mmHg)	-9 (-15 to -3)	-1 (-4 to 2)	**0.007**
Change in peripheral DBP (mmHg)	-6 (-9 to –4)	0 (-2 to 1)	**0.001**
Change in peripheral PP (mmHg)	-2 (-7 to -1)	-1(-3 to 2)	0.304
Change in peripheral MAP (mmHg)	-7 (-11 to -4)	-1 (-3 to 1)	**0.001**
Change in central SBP (mmHg)	-9 (-15 to -4)	-1 (-4 to 2)	**0.006**
Change in central DBP (mmHg)	-7 (-9 to -4)	-1 (-2 to 2)	**0.001**
Change in central PP (mmHg)	-3 (-11 to -3)	0 (-3 to 2)	0.267
Change in central MAP (mmHg)	-7 (-11 to -3)	0 (-2 to 2)	**0.001**
Change in heart rate (bpm)	0 (-2 to 3)	1 (-2 to 2)	0.902
Change in aortic PWV (m/s)	-1 (-1.3 to 0.7)	1.6 (1.3 to 1.9)	**0.001**
Change in fibrinogen (g/l)	-0.40 (-.67 to -0.14)	-0.40 (-0.61 to -0.12)	0.936
Change in CRP (mg/l)#	0.10 (0.8 to 1.4)	0.1 (0.8 to 1.2)	0.941

Data presented as mean (95%CI) unless otherwise indicated, #=geometric mean.

Abbreviations: FEV$_1$= forced expiratory volume in one second**; FVC=** forced vital capacity**; SBP=** systolic blood pressure**; DBP=** diastolic blood pressure**; PP=** pulse pressure**; MAP=** mean arterial pressure**; PWV=** pulse wave velocity**; CRP=** C- reactive protein.

5.4 Discussion

Cross-sectional study: Baseline Findings

The cross-sectional results of this study showed that patients with COPD have greater aortic stiffness than comparators similar in age and gender proportion. The greater aortic PWV in the patients than the comparators and the association with central pulse pressure support the theory of that increased aortic stiffness is a systemic manifestation associated with COPD.

Increased aortic stiffness in patients with COPD has also been confirmed in other studies that used the same index and different indices (Maclay et al., 2009, Maclay et al., 2012, Mills et al., 2008, Patel et al., 2013). In the study by Sabit and colleagues (2007), they failed to adjust for other confounding factors in patients with COPD that may have a bearing on aortic stiffness including blood pressure and smoking, which are believed to play a role in increased aortic stiffness and may have inflated the difference. In the present study, to avoid the residual confounding factor from smoking and blood pressure, the association between aortic stiffness and COPD remained after correcting for peripheral mean arterial pressure and smoking. Other studies have used augmentation index or radial PWV as measures of arterial stiffness. Mills and co-worker (2007) used AIx as a measure of arterial stiffness. However, AIx is not the optimal measure for arterial stiffness, and is gender and heart rate dependent. A further limitation of this study was the failure to control for blood pressure, which they thought, was a determinant of arterial stiffness. The other study by McAllister and co-authors (2008) used radial PWV, which is less robust than aortic PWV as the brachial artery contains more collagen and less elastin than the aorta and, therefore, it does not reflect physiological changes associated with blood pressure and the work imposed on the heart. In contrast, Janner et al. (2012) have recently reported a conflicting result to this study and found no significant association between COPD and arterial stiffness after adjustment for cardiovascular risk factors including blood

pressure, CRP, socioeconomic and physical activity. However, the researchers in this study used the AIx as an outcome measure for arterial stiffness, which is not the gold standard for arterial stiffness measurement, in addition to other disadvantages mentioned above. Additionally, the diagnosis of COPD was based on pre-bronchodilator spirometry, which is not the gold standard for confirming COPD and may have led to inclusion of patients with other obstructive airway diseases including asthma (GOLD, 2014). Although, the authors stated that the difference was corrected for physical activity, assessment of physical activity was subjectively recorded which depends upon patients recall.

Subendocardial manifestations present in patients with COPD at an early stage of the disease even in those without overt cardiovascular problems (Sabit et al., 2010a). Aortic stiffness improves the Framingham cardiovascular risk prediction, independent of conventional risk factors (Mitchell et al., 2010). The increase in both central systolic and pulse pressure in parallel with increased aortic stiffness implicate the amount of load being exerted on the heart as well as alteration of aortic vasculature properties (Mitchell et al., 2004). Central haemodynamics may be more relevant to reflect the pathogenesis of cardiovascular disease and damage to the sensitive organs such as the kidney (Roman et al., 2007). The earlier return of the reflected arterial wave during late systole increases left ventricle afterload and consequently reduces coronary perfusion in diastole leading to sub-endocardial ischaemia (Laurent et al., 2006). In COPD, aortic stiffness is the best predictor of left ventricle dysfunction in patients without overt CVD and associated with myocardial injury (Patel et al., 2013, Sabit et al., 2010a). These findings may partly explain the underlying mechanism linking CVD in COPD and possibly the relationship between aortic stiffness and lung function in healthy individuals.

The association of aortic stiffness with heart rate may indicate a predominant role of sympathetic overactivity in aortic stiffness and subsequently increased risk of CVD.

Similar to the current study, in treated hypertensive patients, Benetos and colleagues (2002) found the increased heart rate was a significant predictor of aortic stiffness. Elevated resting heart rate, a marker of sympathetic overactivity, is associated with CVD and all cause of mortality in COPD (Jensen et al., 2013). Autonomic nervous system dysfunction has been recognised in patients with COPD and could be a potential mechanism linking COPD to CVD (Van Gestel et al., 2012). Chronic elevated heart rate may alter mechanical wall properties of the aorta through decreased compliance and increased the aortic wall stress. A cyclic increased heart rate exposes aortic wall to greater strain, and subsequently leading to elastin degradation, which is a marker of aortic stiffness and a systemic feature of COPD (Maclay et al., 2012, Mitchell et al., 2004, Whelton et al., 2013). Elevated aortic stiffness in parallel with a greater heart rate may implicate a poor cardiac function as a consequence of myocardial damage (Sabit et al., 2010a).

The association between aortic stiffness and renal function biomarkers (i.e. serum creatinine and eGFR) supports the notion that increased aortic stiffness could be related to the glomerular damage happening at the microvascular level. Recently, the GOLD guideline has emphasised the need for early detection and management of comorbidities in COPD (GOLD, 2014). One of the most common comorbidities associated with COPD is CVD. Cardiovascular morbidity and mortality is inversely and independently associated with kidney function (Anavekar et al., 2004, Gibson et al., 2003). Alteration of small vessels function of a vital organ such as the kidney and its haemodynamics could be another horizon to explore the link between cardiovascular disease and COPD. Both patients with COPD and renal dysfunction share a common risk factor for CVD, which is increased arterial stiffness. The interplay of microvascular changes and the macrovascular function in COPD could be a triggering factor for causing arterial stiffness. Casanova et al. (2010) measured urinary albumin/creatinine ratio in 129 stable patients with COPD and 51 smoking

controls, and showed higher level of microalbuminuria in the patients compared with smoking controls, independent of traditional cardiovascular risk factors. This suggests that patients with COPD have some degree of kidney vascular damage, which is a risk factor for cardiovascular events and may precipitate the development of aortic stiffness. A study by John et al. (2013) revealed that aortic stiffness in patients with COPD is related to glomerular damage as measured by urine albumin/creatinine ratio. However, John and colleagues (2013) included patients with known renal impairment and therefore, glomerular damage cannot be exclusively related to COPD and increased aortic stiffness. In contrast, in the current study, patients with COPD were free of known kidney dysfunction, which may, indicate that elevated aortic stiffness could cause subclinical damage to the kidney before its clinical manifestations.

Age is the major determinant of aortic stiffness (McEniery et al., 2005). This is not surprising, as with age, there are structural and functional changes to the vascular system, which could be accelerated by the presence of a systemic disease such as COPD. A similar finding has been recently reported in healthy and diseased populations (Alghatrif et al., 2013, Cecelja and Chowienczyk, 2009, McEniery et al., 2005, McEniery et al., 2010, Teoh et al., 2013). Interestingly, in the review by Cecelja and Chowienczyk (2009) found that age and blood pressure are the only predictors of the variability in aortic stiffness, and accounted for approximately 50% of that change. Hypertension is common in patients with COPD and is estimated between 40% and 60% (Curkendall et al., 2006, Mannino et al., 2008). The relationship between hypertension and COPD is unclear; however, increased arterial stiffness may play a role. Elastin degradation, a systemic feature of COPD, is also involved in the development of hypertension (Maclay et al., 2012, Te Velde et al., 2004). Therefore, increased aortic stiffness in COPD could be predisposing risk factor for hypertension development in the COPD population. Indeed, a recent longitudinal study from the

Framingham Offspring cohort showed that aortic stiffness is an independent predictor of the incident of hypertension in non-cardiovascular participants (Kaess et al., 2012). In the present study, aortic stiffness was more pronounced in males with COPD than the females and was independent of conventional cardiovascular risk factors including age, blood pressure and airway severity. This is recently supported in the London COPD Cohort study and the Baltimore Longitudinal Study of Ageing (Alghatrif et al., 2013, Patel et al., 2013). Conversely, a conflicting result has been reported by other studies (Duprez et al., 2009, Russo et al., 2012). In the Multi-Ethnic Study of Atherosclerosis study, women demonstrated lower large and small arteries elasticity than men, which was dependent on height and body size (Duprez et al., 2009). A similar finding has also been reported in the Cardiovascular Abnormalities and Brain Lesions cohort study, which demonstrated that women had higher arterial stiffness than men measured by AIx, independent of body size and heart rate (Russo et al., 2012). However, this relationship disappeared after adjustment for other cardiac risk factors. This controversy is attributed to the fact that these studies have used AIx as a measure of arterial stiffness, which is not the gold standard and is age and female dependent (Laurent et al., 2006). The supposition that women have greater arterial stiffness than men, because of their body size and shorter aortic path length would assume or lead to the conclusion that children would have greater aortic stiffness than adults. However, this is not the case in reality as arterial stiffness increases with age.

In the present study, males with COPD had greater aortic PWV than the females, independent of age and blood pressure, which clearly shows the high risk of premature cardiovascular events in the males is independent of the effect of age and blood pressure. Although males and females with COPD were similar in physiological measures, males had more visceral obesity than the females, which increases the risk of CVD, and may partially explain the relationship. Obesity increases aortic stiffness and both are independent risk factors for cardiovascular events (Recio-Rodriguez et

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al., 2012, Sutton-Tyrrell et al., 2005, Sutton-Tyrrell et al., 2001). The combined effect of obesity and aortic stiffness in men in conjunction with COPD culminate in worsening cardiovascular morbidity. Recently, aortic stiffness was found to be associated with subclinical left ventricle dysfunction in a group of patients with COPD and free of overt cardiovascular disease when compared with age and sex matched controls (Sabit et al., 2010a). Another potential explanation of why males with COPD can have a stiffened aorta is probably testosterone deficiency (Atlantis et al., 2013). Males with COPD have lower testosterone level than disease free males (Laghi et al., 2005). Low testosterone level is associated with increased aortic stiffness in the elderly and enhances the risk for cardiovascular morbidity and mortality (Araujo et al., 2011, Dockery et al., 2003, Jones, 2010). The association between increased aortic stiffness and low levels of testosterone has been found in several populations and yet is to be explored in COPD (Fukui et al., 2007, Kyriazis et al., 2011). Nevertheless, increased aortic stiffness is well established in COPD, which is an independent predictor for cardiovascular morbidity and mortality in several populations including the healthy elderly. As males age, the level of testosterone progressively decreases and increases the incidence of fatal and non-fatal cardiac events (Herring et al., 2013, Jones, 2010).

In the current study, patients with mild to moderate airflow obstruction had increased aortic stiffness compared with non-COPD subjects. There was also a significant difference in aortic stiffness between patients with an $FEV_1 > 50\%$ and patients with an $FEV_1 < 50\%$. These findings could be interpreted as showing that aortic stiffening occurs at early stage of the disease and progresses as the function of airway declines. The relationship between the degree of aortic stiffness and severity of airway obstruction in patients with COPD may explain the increased risk for coronary heart disease and stroke in COPD. The result of this study is supported by findings from Sabit et al. (2007) study and other research (Cinarka et al., 2013, Janner et al.,

2012, McAllister et al., 2007, Mills et al., 2008). Although, these studies used different indices to assess aortic stiffness, they agreed that there is a relationship between aortic stiffness and pulmonary function.

The relationship between pulmonary function and aortic stiffness in the general population is contentious in the literature. In this study and others, there was a negative association between lung function and aortic stiffness in non-COPD subjects (Bolton et al., 2009, Janner et al., 2012, Sabit et al., 2007). In contrast, a study in 678 Japanese American adult subjects found no significant relationship (Taneda et al., 2004). This could be due to the type of the statistical analysis they used to identify the relationship. They treated their variables as dichotomous, which may have underestimated the association.

The association between inflammatory biomarkers and arterial stiffness in COPD is contradictory. In the current study, increased aortic stiffness was associated with high levels of circulating CRP. A similar relationship between increased CRP and aortic PWV was found in a population from the same community and in others (Patel et al., 2013, Sabit et al., 2007). In contrast, Cinarka et al. (2013) reported a conflicting result. This could be related to the fact that their study was underpowered and 96% of their population were male, therefore, the relationship was underestimated. Association between aortic stiffness and frequency of exacerbation in parallel with elevated CRP may suggest the possibility of a systemic elastin degradation and acceleration of atherosclerotic features (Maclay et al., 2012, Patel et al., 2013).

COPD is a persistent inflammatory disease with elevated CRP level, which is a predictor of adverse outcomes in this population (Thomsen et al., 2012). Increased circulating CRP may be responsible for accelerating atherosclerosis leading to aortic stiffening. CRP is also involved in inducing endothelial dysfunction, which will consequently increase vascular fibrosis and subsequently lead to elevated aortic stiffness (Barr et al., 2007, Ives et al., 2013).

Another mechanism may explain the association between aortic stiffness and COPD is hypoxia. In the current research, aortic PWV was inversely related to resting oxygen saturation. A similar finding has been found in two cross-sectional studies (Cinarka et al., 2013, Sabit et al., 2007). In contrast, although McAllister and colleagues (2007) used the same method as in the current study (i.e. pulse oximeter) to measure oxygen saturation, they found no association between oxygen saturation and radial PWV. This may be due to the method they used to measure arterial stiffness (radial PWV) which is not the gold standard and unlikely to reflect physiological changes in the aorta (Laurent et al., 2006). The effect of hypoxia on arterial stiffness is still unknown. Nevertheless, hypoxia may alter arterial smooth muscle structure and function and increase its predisposition to rigidity (Lattimore et al., 2005). To support this hypothesis, a study by Bartels and co-authors (2004) demonstrated that supplemental oxygen resulted in reduced arterial stiffness in a population of patients with airflow obstruction. Hypoxia may also influence arterial stiffness by its effect on systemic inflammation (Takabatake et al., 2000). Repetitive hypoxia that is usually seen in patients with COPD either sustained as in the late stage of the disease or intermittent as during an exacerbation or exercise, which induces cardiac stress (i.e. an increase in heart rate) and renal stress (i.e. a decrease in renal blood flow) (Skwarski et al., 1998).

Tobacco exposure has a causal effect in COPD and CVD, therefore, it was considered in the analysis. In this study, although the smoking history between the patients and comparators was different, this is unlikely to account for the increased aortic stiffness observed in patients with COPD, as increased aortic stiffness was independent of pack-years of smoking. Within the comparator group, current smokers with a higher pack-years history did not have greater aortic PWV. Within the patients, there was a difference in aortic PWV between patients in GOLD I&II and patients in GOLD III&IV, who were similar in terms of current smokers and the number of pack

years smoked. A similar finding was found by Sabit et al. (2007) study, who reported that increased aortic stiffness in patients with COPD was independent of the number of pack-years smoked. Consistent with previous findings, in 157 emphysematous patients, McAllister and colleagues (2007) reported no difference in PWV between active smokers and ex-smokers. In the general population, Zureik and co-authors (2001) found similar aortic PWV in never smokers, current smokers and ex-smokers.

To the best knowledge of the researcher, this is the largest cross-sectional study to date, which has confirmed previous findings of the association between COPD and elevated aortic stiffness. In this study, aortic stiffness was greater in the patients than the comparators similar in age and gender, and it was independent of traditional cardiovascular risk factors. The present study added to previous studies that male patients had greater aortic stiffness than their female counterparts and this difference remained after controlling for age and blood pressure. Additionally, the present study found waist circumference and heart rate as well as age and blood pressure are predictors of increased aortic stiffness in COPD.

Longitudinal study: Two-year Findings

This is the first longitudinal study evaluating the change in aortic stiffness over time in COPD and showed that there was a significant increase in aortic stiffness, independent of age, airway severity, body composition, mean blood pressure and inflammation. In this study, a subset of patients was also identified to have an accelerated progression in aortic stiffness. The accelerated progression of aortic stiffness seen in patients with COPD was three-fold greater than expected with normal ageing, independent of the traditional risk factors, which suggests that COPD is an independent risk factor for increased aortic stiffness and premature vascular ageing, which subsequently enhance the risk of CVD.

A few studies have examined the progression of aortic stiffness over time. Similar to the current study, in treated hypertensives, Benetos et al. (2002) reported an annual increase in aortic PWV by 0.17 m/s, and was independent of mean arterial pressure. In a longitudinal prospective study in patients with renal failure and on chronic haemodialysis, Utescu and colleagues (2013) showed that the progression of 0.84 m/s in aortic stiffness over fourteen months was independent of age, atherosclerosis, CVD, diabetes and inflammation. In contrast, Briet et al. (2011) evaluated annual progression of aortic stiffness in 180 patients with chronic kidney disease for four years. The researchers failed to find a significant change in aortic stiffness. In this study, the researchers lost 30% of their population during the follow-up and this may explain why the rate of aortic stiffness progression was underestimated. In addition, majority of the patients in this study had been prescribed renin-angiotensin system blocker, which may have anti-progression effect on aortic vascular properties.

The link between aortic stiffness and elevated blood pressure is complex and not well understood. In this study, the rate of aortic progression was not associated with mean arterial pressure, indeed, there was a significant reduction in peripheral and central MAP after two years, while aortic stiffness had substantially increased. Similar to the

current study, Benetos et al. (2002) reported that the adjusted annual rate of aortic stiffness progression was independent of MAP. In another population, Utescu and colleagues (2013) found an increase in blood pressure after a period of over one year in chronic haemodialysis patients, however, the change in aortic stiffness was not dependent on blood pressure. This proposes that the accelerated increase in aortic stiffness is independent of blood pressure. Recently, aortic stiffness was found to predict the incidence of hypertension (Kaess et al., 2012). Indeed, this is interesting as it is in contrast to current understanding and may infer that aortic stiffness develops before increases in blood pressure takes place, which implies that there are other contributing factors involved in initiating aortic stiffness and its progression.

Depletion of FFM is a common problem in patients with COPD and increases with low BMI and the disease severity (Bolton et al., 2004, Vestbo et al., 2006). However, it is not limited to cachectic patients or those at late stage disease, as it exists in normal BMI patients with mild to moderate airway obstruction (Vestbo et al., 2006). Studies have shown that in patients with COPD, FFMI is inversely related to survival independent of BMI and other covariates such as sex, smoking and lung function (Schols et al., 2005, Slinde et al., 2005, Vestbo et al., 2006). In patients with COPD, free of overt heart disease, Sabit et al. (2010) found that left ventricle mass, FFM and cellular protein breakdown were all interrelated suggesting that alterations in cardiac and skeletal muscle may occur as part of a global protein catabolic process. Reduction of cardiac haemodynamics indices in parallel with loss of FFM may reflect the left ventricle dysfunction and its inability to deliver adequate amounts of blood to perfuse skeletal muscle leading to tissue deprivation and consequently increased protein turnover. Impaired oxygen delivery to skeletal and cardiac muscle as a result of reduced blood supply is a further explanation for loss of normal haemodynamics and reduced reserve capacity of the cardiovascular system in COPD.

Alteration of vascular wall properties may occur via endothelial dysfunction that alter collagen and elastin structure and result in arterial stiffness (Zieman et al., 2005). The role of endothelial dysfunction in promoting arterial stiffness in COPD is controversial and this is largely related to variations of methodological approaches to study endothelial function (Barr et al., 2007, Ives et al., 2013, Maclay et al., 2009). However, this does not preclude the interaction between endothelium and increased elastin degradation, which promotes calcification and atherosclerosis (Zieman et al., 2005). Aortic stiffness reflects the pathological state of the central arteries and accumulated atherosclerotic changes. Hence, aortic stiffness is elevated in COPD and has previously been shown to be associated with aortic calcification, a feature of atherosclerosis, left ventricle dysfunction and myocardial injury; suggesting that rapid progression of aortic stiffness mirrors the atherosclerotic changes in the aortic wall (Bolton et al., 2011, Patel et al., 2013, Sabit et al., 2010a). A recent exploratory study in patients with COPD, free from CVD, type 2 DM and inflammatory disease showed that deposition of aortic calcification was influenced by vascular inflammation measured by F18-Fluorodeoxyglucose positron emission tomography (FDG-PET) compared with ex-smoker control subjects (Coulson et al., 2010). Vascular inflammation may be implicated in cardiovascular events (Hansson, 2005). This has recently been supported from a large retrospective study on 513 non-cancer patients who underwent FDG-PET for suspected malignancy (Figueroa et al., 2013). In this study, the researchers found vascular inflammation, measured by FDG-PET, is an independent predictor of cardiovascular events during a four-year follow-up. Aortic atherosclerotic change is usually preceded by activation of endothelium where there is elastin breakdown caused by high sheer stress, which subsequently may lead to initiation of local inflammatory response (Hansson, 2005).

Patients with COPD have evidence of both pulmonary and extrapulmonary elastin breakdown (Black et al., 2008, Maclay et al., 2012). In the lung, degradation of elastin

results in loss of lung compliance and airway patency, while an altered ratio of elastin to collagen in the vasculature results in arterial stiffening (Maclay et al., 2012). Patients with emphysema experienced greater skin elastin degradation and increased expression of MMP-9 compared with non-COPD smoking controls, which were associated with increased aortic stiffness. Increased elastin breakdown is involved in vascular calcification, which promotes atherosclerotic features and stiffness (Bolton et al., 2011, Maclay et al., 2012, Zieman et al., 2005). Such alterations may be genetically predetermined or secondary to increased elastolytic compounds such as MMP– 9 (Maclay et al., 2012). In healthy subjects as well as patients with COPD, MMP-9 is associated with increased aortic stiffness (Maclay et al., 2012, Yasmin et al., 2006). This infers that patients with COPD may be genetically predisposed to have enhanced aortic stiffness, which could be mediated via several stimuli including connective tissue degradation. This increases the susceptibility of autoimmune involvement and may explain the failure of the immune system to suppress persistent inflammation associated with COPD (Agusti et al., 2003).

Persistent systemic inflammation in COPD may play a role in the development of aortic stiffness and increase the risk for cardiovascular disease. Several cross-sectional studies have found an association between arterial stiffness and inflammation while others did not (Cinarka et al., 2013, Maclay et al., 2009, Mills et al., 2008). Yet, the nature of these studies limits their findings as COPD is a heterogeneous disease and drawing a conclusion based on a single visit measurement is inconclusive. Controlling for other confounding factors such as tobacco exposure and blood pressure is also lacking in some studies. Therefore, the relevance of this relationship cannot be established based on this design. The current longitudinal study overcomes these limitations and found no association between progression of aortic stiffness and inflammatory biomarkers. Interestingly, in this study, although aortic stiffness increased over two years, the CRP level remained

unchanged over the period of follow-up while a third of the patients remained inflammation free (i.e. CRP within the normal limit). Similar findings have also been reported in another group of patients with chronic kidney disease and on long term haemodialysis (Utescu et al., 2013). The investigators in this study observed that the level of CRP remained stable during the follow-up period of fourteen months, and was not related to the progression of aortic stiffness.

The role of systemic inflammation in the pathogenesis of aortic stiffness cannot be excluded, but it may not be responsible for the initiation of aortic stiffness in COPD or the inflammatory biomarkers used in the current study may not reflect local inflammation in the aorta. Indeed, the recent investigative work by Coulson and colleagues (2010) who supported this assumption and showed that aortic inflammation and calcification, measured by FDG-PET, was independent of systemic inflammation. This is not extraordinary in COPD as the systemic inflammation is not a constant feature in all patients with COPD (Agusti et al., 2012). Recently, in the ECLIPSE cohort study, Agusti and colleagues (2012) found that systemic inflammation behaved differently in COPD, as about a third of their patients were free from abnormal systemic inflammation, while another third expressed no changes in their inflammatory biomarkers after a year of follow-up (Agusti et al., 2012).

Moreover, a subset of patients was identified to have a rapid increase in aortic stiffness progression, independent of blood pressure, lung severity and inflammation.

The association of aortic stiffness progression in COPD with age suggests that age related vascular changes occur prematurely in COPD. Similarly, Benetos and colleagues (2002) reported that progression of aortic stiffness in patients with hypertension was age dependent. The increase of 1.6 m/s means that the estimated biological aortic age for this group of patients is 16 years greater than their chronological age, which increases the cardiovascular risk by more than 22% (Vlachopoulos et al., 2010). The theory of premature ageing has recently been

appreciated in COPD and numbers of studies have been increasing to understand cellular and molecular mechanisms of biological ageing. From the same community population, Sabit and colleagues (2007) were the first to suggest that COPD causes premature vascular ageing characterised by increased aortic stiffness. COPD provokes elastin degradation either inside the lung by destruction of the lung parenchyma or externally via vascular elastin and collagen deposition (Black et al., 2008, Maclay et al., 2012). In the current study, the mean rate of decline in FEV_1 in the progressors was 140 ml. Rapid decline in the lung function could be responsible directly or indirectly for elastin breakdown, which subsequently increases aortic ageing. A similar finding was reported in a cross-sectional study by Zureik et al. (2001) in 194 middle-age men free from overt cardiovascular disease. They found that for every 93 ml reduction in FEV_1, aortic stiffness increased by 2.5 m/s. Patel et al. (2006) found an association between COPD and skin wrinkling, indicative of elastin degradation, and was age dependent. This finding was also extended by Maclay and co-authors (2012) who showed a relationship between skin elastin breakdown and MMP-9 and both were associated with increased aortic stiffness. Circulating MMP-9 has been implicated in the pathogenesis of atherosclerosis and predisposing factor to aortic stiffness (Yasmin et al., 2006).

Atherosclerosis and COPD are both chronic inflammatory states that may cause premature vascular ageing and certainly share common risk factors such as smoking, inflammation, endothelial dysfunction and protease/antiprotease imbalance such as circulating MMP-9. In COPD, the severity of airways disease is associated with increased carotid intima-media thickness, and this atherosclerotic change can occur during the early stage of the disease, independent of smoking. Subclinical atherosclerosis occurs early in COPD because of endothelial dysfunction and microvascular alteration and progresses with the decline in lung function (Barr et al., 2012). Endothelial dysfunction has recently been implicated in the pathogenesis of

arterial stiffness in COPD, which interestingly could be reversed by ingestion of an antioxidant cocktail (Ives et al., 2013). A study by Bolton et al. (2011) in patients with COPD showed that aortic stiffness is associated with aortic calcification and it was independent of MAP. In the light of current results and previous findings mentioned above, COPD may cause premature vascular ageing, which is characterised by increased aortic stiffness independent of conventional cardiovascular risk factors. Aortic stiffness could reflect a vascular insult occurring in its early stage and underlying hidden or sub-clinical cardiovascular disease.

Study limitations

The major limitation of this longitudinal study is lack of a non-COPD group to compare the extent of aortic stiffness progression and factors influencing it. A second limitation was no cardiac scan had been done to confirm subclinical damage to the heart as well as there was no measure performed to ascertain the presence of atherosclerosis.

Conclusion

COPD is an independent risk factor for progression of aortic stiffness. Accelerated increase in aortic stiffness occurs in a sub-group of patients with COPD, which is independent of traditional risk factors. This may enhance the risk of cardiovascular events and promote more research on the causes and mechanisms. Accelerated aortic stiffness progression may reflect cumulative, subclinical target organ damage with decline in functional reserve that is manifested as frailty. Given the simplicity and rapidity of aortic stiffness measurement, its use in primary care practice could be beneficial to identify people with COPD who are at increased risk of cardiovascular disease and administer appropriate preventive strategy. Future interventional studies are required to slow the progression of aortic stiffness and reduce the risk of CVD in COPD.

Chapter Six

Cross-sectional and Longitudinal

Evaluation of Frailty in COPD

Introduction

This chapter of the thesis evaluated frailty in patients with COPD and was subdivided into two parts:

The first part was a cross-sectional study including all the participants recruited into the ARCADE study at initial visit to establish the concept of frailty.

The second part of this chapter was a longitudinal study of patients who had been reviewed after their completion of two years from their participation at the initial visit.

6.1 Background

Frailty is a progressive clinical syndrome in parallel with COPD and ageing. Frailty and COPD are both associated with increased comorbidities and reduced functional activity and physical capacity (Fried et al., 2004, Rockwood, 2005, Savva et al., 2013, Thomsen et al., 2012, Waschki et al., 2011). The latter is a risk factor for many comorbid conditions including cardiovascular disease, osteoporosis, metabolic disorders and sarcopenia (Barnes and Celli, 2009, Watz et al., 2008). Frailty is a complex phenomenon and its relation with ageing makes the distinction between the two difficult. However, in COPD, these signs appear early and may represent premature ageing and consequently lead to frailty, which are gender dependent (Agusti et al., 2010, Aryal et al., 2013, Cesari et al., 2004, Gale et al., 2013). In parallel with the proposed theory of frailty, which implies that failure of physiological systems to respond to further stressors, leading to an interruption of homeostasis status and appearance of frailty (Walston et al., 2006). Several underlying physiological mechanisms have been proposed to cause frailty including inflammation, immunosenescence, obesity and lack of activity (Abbatecola and Paolisso, 2008, Clegg et al., 2013, Fulop et al., 2010). COPD is accompanied by various extra-pulmonary comorbidities, which increases morbidity and risk of death (Divo et al., 2012, Vanfleteren et al., 2013). Most assessments of disease status, particularly in routine clinical settings, do not include comorbidities, though recently a scoring system based on the BODE index and comorbidities predictive of non-survival was described, and was predictive of the mortality risk over a four year period (Divo et al., 2012).

Comorbidities represent deficits in individual physiological systems and are often multiply present in a single patient (Vanfleteren et al., 2013). The presence of multiple comorbidities in COPD resembles the accumulation of deficits in physiological systems seen in natural ageing (Fried et al., 2001, Rockwood and Mitnitski, 2011).

Frailty better predicts adverse outcomes than age independent of co-existing medical conditions and is associated with an increased risk of falls, hospitalisation, residential care and reduced HRQoL, and progression to disability and increased mortality (Fried et al., 2001, Jones et al., 2005, Rockwood and Mitnitski, 2007, Fried et al., 2009, Rockwood and Mitnitski, 2011, Rockwood et al., 2005, Romero-Ortuno and Kenny, 2012). The similarities between the accumulation of deficits in ageing and multiple comorbidities in COPD, suggests that frailty is likely to be common in COPD, and be related to comorbidities and increased morbidity.

However, the frailty concept in COPD needs to be established using a validated measure of frailty and subsequently confirm this finding in a longitudinal study to target future understanding and management of frailty in COPD, as there is no study so far which has examined the rate of frailty progression in either health or disease. Only a few longitudinal studies are available on frailty and these studies looked at the incidence of frailty (Barzilay et al., 2007, Baylis et al., 2013, Gale et al., 2013, Puts et al., 2005b).

Hypothesis

Patients with COPD would be more frail and have more impairments compared with similar age and sex comparator subjects. Additionally, the patients would have an increase in the frailty index after two years from the initial assessment.

Aim

The aim of this study was to examine the frailty concept in patients with COPD compared with similar age and gender non-COPD subjects. Secondly, this study aimed to evaluate the change in frailty in patients with COPD over time.

6.2 Method

This was a cross-sectional study of patients with stable COPD confirmed, with spirometry and comparators from the Assessment of Risk in Chronic Airways Disease Evaluation (ARCADE) study. Comparators were current or ex-smokers free from respiratory disease. All subjects were clinically stable and free of inflammatory disease such as rheumatoid arthritis, oral maintenance corticosteroids and long-term oxygen therapy (full inclusion and exclusion criteria in Chapter 2: Table 2.1, p 65). All subjects gave written informed consent and the study had approval from the South East Wales Research Ethics Committee.

Lung Function

All subjects performed spirometry (Vitalograph), to measure forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the FEV_1/FVC ratio (Chapter 2:2.3.5, p 80). The number of exacerbations per year was recorded.

Body Composition

Weight and body composition were measured barefoot in lightweight indoor clothing, fat free mass (FFM), fat mass (FM) and the percentage fat were determined using a Bioelectrical impedance device (Tanita BC-418). The FFMI was expressed as a height-squared ratio and the body mass index (BMI kg/m^2) was calculated (Chapter 2:2.3.1, p 68). A stretch resistant tape measure was used to measure waist and hip circumference.

Physical Performance

All subjects performed the Timed Up and Go test (Chapter 2:2.3.6, p 79). The subjects also undertook the six minute walking distance (6MWD) test. Prior to the test, resting heart rate and oxygen saturation was recorded by pulse oximetry with the subjects breathing air (Chapter 2:2.3.7, p 81).

Handgrip Measurement

Maximal right and left handgrip strength (HGS) was determined twice in each hand using a hand dynamometer (T.K.K. 5401 grip-D, Takei, Japan) (Chapter 2:2.3.8, p 84).

Measurement of Pulse Wave Velocity

Aortic PWV was assessed non-invasively using the SphygmoCor device connected to a laptop computer (Chapter 2:2.3.3, p 72).

Cardiac Haemodynamics Measurement

Cardiac haemodynamics were measured non-invasively using a new bioreactance technology (NICOM, Cheetah, Medical Inc., Indianapolis, IN) (Chapter 2:2.3.4, p 77).

Inflammatory Biomarkers

High sensitivity C-reactive protein (CRP) and fibrinogen were determined from a blood sample according to standard procedures (University Hospital of Wales, Biochemistry).

Frailty

A modified version of the comprehensive geriatric assessment specific to community dwelling individuals was administered to all participants (Chapter 2:2.3.10, p 85). A Frailty Index was calculated by dividing the total number of CGA deficits by the maximum, 61.

Health Related Quality of Life

The patients completed the St. George's Respiratory Questionnaire (SGRQ) and the COPD Assessment Test (CAT) that were previously detailed (Chapter 2:2.3.9, p 85). The number of reported diagnosed comorbidities in patients and comparators were also recorded.

Data Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL), version 18.0. Results are presented as arithmetic or median (range) (for non-normally distributed), $\log_{10}$ transformed mean and standard deviation (SD). Analyses included One-way or Two-way Analysis of Variance (ANOVA) as appropriate, Pearson's correlations and hierarchical multiple regression analysis. Difference between visits was performed using paired t- test. Categorical data was analysed using the Chi-square test. The $p<0.05$ was considered significant. Subjects aged less than 50 years were added to the 50-59 age category.

6.3 Result

Subject Characteristics

The patients (n=500) and comparators (n=150) were similar in age, gender and BMI, but the patients had a greater tobacco exposure and a lower FEV_1 and FVC compared with comparators, all p<0.001 (Table 6.1). The severity of airflow obstruction in the patients was GOLD 1 n=73, GOLD 2 n=251, GOLD 3 n=144 and GOLD 4 n=32. Of the 500 patients, 207 reported infrequent exacerbations, less than two per year, while 293 patients reported two or more per year.

Body composition was classified according to Landbo et al. (1999), of the 500 patients, (26) 5% had a low BMI (<19.9 kg/m^2), (196) 39% had an elevated BMI (>25 kg/m^2), (163) 33% were classified as obese (BMI>30 kg/m^2), while (115) 23% were within the normal limit for BMI (20-25 kg/m^2). Among comparator subjects, (34) 23% had an optimal BMI and (116) 77% had an elevated BMI, 28% of these subjects were obese (BMI >30 kg/m^2). Patients had greater waist circumference and waist: hip ratio, and lower fat free mass and oxygen saturation, all p<0.001. However, both groups had similar fat mass (Table 6.1). The proportion of subjects with a prior diagnosis of comorbidities was greater in the patients (Table 6.2). Details of reported comorbidities and medications in both groups were previously mentioned (Chapter 5, Table 5.2). Most of the patients (64%) and comparators (82%) were ex-smokers and had a different pack year exposure.

The TUG test of patients with COPD 11.1 (3.7) s was greater than the comparators, 8.2 (1.2) s, while the 6MWD of the patients 320 (126) m was less compared with non-COPD subjects 501 (86) m, all p<0.001. Likewise, the HGS of the patients, 25.6 (9.8) was weaker than the comparators, 31.1 (10.4) kg, p<0.001.

Circulating CRP and fibrinogen were greater in patients with COPD compared to non-COPD subjects, all p<0.001 (Table 6.1).

Table 6.1: Physical and clinical characteristics of patients with COPD and comparators at initial visit

	COPD (n=500)	Comparator (150)	p value
Gender Male:Female	255:245	75:75	0.451
Age (years)	66 (7.3)	65 (8.0)	0.125
Smoking (pack-years)	41 (25.5)	21 (17.9)	**0.001**
FEV_1 (L)	1.64 (0.84)	2.73 (0.67)	**0.001**
FVC (L)	2.50 (0.98)	3.50 (0.90)	**0.001**
FEV_1/FVC (L)	0.53 (0.11)	0.78 (0.05)	**0.001**
FEV_1 (%)	59 (21)	105 (13)	**0.001**
FVC (%)	87 (21)	110 (15)	**0.001**
BMI (kg/m^2)	28.1 (5.5)	28.3 (4.2)	0.664
Waist circumference (cm)	100 (15)	95 (13)	**0.001**
Hip circumference (cm)	104 (11)	105 (12)	0.891
Waist:Hip ratio	1.0 (0.1)	0.9 (0.1)	**0.001**
HGS (kg)	27.1 (9.9)	31.0 (10.2)	**0.001**
FFM (kg)	49.6 (11.0)	52.2 (9.5)	**0.011**
FFMI (kg/m^2)	18.1 (2.6)	18.5 (2.3)	0.088
Fat mass (kg)	26.9 (11.1)	26.9 (8.6)	0.985
Aortic PWV (m/s)	9.9 (2.40)	8.3 (1.66)	**0.001**
CO (L/min)	5.84 (1.21)	5.95 (1.03)	0.324
Stroke Volume (ml)	86 (20)	96 (20)	**0.001**
6MWD (m)	324 (124)	502 (86)	0.001
TUG (s)	11.1 (3.7)	8.2 (1.2)	**0.001**
Resting O_2 saturation (%)	97 (2)	98 (1)	**0.001**
Fibrinogen (g/L)#	3.51 (1.31)	3.08 (1.25)	**0.001**
CRP (mg/L)#	3.49 (2.89)	1.76 (3.18)	**0.001**

All data mean (SD) unless otherwise indicated, # Geometric mean

Abbreviations: **FEV_1=** forced expiratory volume in 1 second; **FVC=** forced vital capacity; **BMI=** body mass index; **HGS=** handgrip strength; **FFM=** fat free Mass; **FFMI=** fat free mass index; **6MWD=** six minute walk distance; **TUG=** Timed Up and Go; **CRP=** C-reactive protein.

Frailty

The total CGA score and the FI were greater in patients than comparators (Table 6.2). The possibility that comorbidities might unduly weigh the FI in COPD was addressed by comparing the scores after exclusion of the medical comorbidity scores, the CGA score remained greater in the patients than in non-COPD subjects, p<0.001 (Table 6.2). The FI was not related to age in either group and did not differ across age categories, p>0.05. However, patients with COPD had a greater FI than comparator subjects across age categories (Figure 6.1). The total FI was related to the number of previously diagnosed comorbidities, which included hypertension, angina, atrial fibrillation, hypercholesterolaemia, diabetes and osteoporosis, in patients (r =0.456) and comparators (r =0.421), all p<0.001 (Table 6.3).

Using the upper 95%CI of the FI for the comparator group (0.05), as an upper limit for non-frail status in non-COPD subjects, 446 (92%) of the 500 patients were frail, and 56 (37%) of the comparators were frail.

Table 6.2: Questionnaire scores in patients with COPD and comparators

	COPD (n= 500)	Comparator (n= 150)	p value
CGA Total	8.3 (0.25-28.5)	2.25 (0.0-11.25)	**0.001**
CGA without medical	2.8 (0.0-14.0)	0.25 (0.0-5.25)	**0.001**
Frailty Index	0.15 (0.08)	0.05 (0.04)	**0.001**
SGRQ Symptom	66 (20)	-	-
SGRQ Activity	66 (26)	-	-
SGRQ Impact	38 (21)	-	-
SGRQ Total	51 (20)	-	-
Total CAT Score	21 (9)	-	-

Data are Median (range) or Mean (SD), - = not assessed

Abbreviations: **CGA=** Comprehensive Geriatric Assessment; **SGRQ=** St George's Respiratory Questionnaire; **CAT=** COPD Assessment Test.

Table 6.3: Relationships of FI with physical and clinical characteristics in patients with COPD and comparator subjects at initial visit

Pearson's correlation	COPD (n=500)	p value	Comparator (n=150)	p value
Age (years)	-0.004	0.937	0.122	0.138
FEV_1 (%)	-0.193	**0.001**	-0.201	**0.014**
FEV_1/FVC	-0.035	0.439	0.097	0.238
Smoking (pack-years)	0.037	0.409	-0.002	0.977
No. Exacerbations/year	0.262	**0.001**	-	-
BMI (kg/m^2)	0.173	**0.001**	0.281	**0.001**
Waist circumference (cm)	0.157	**0.001**	0.177	**0.035**
Hip circumference (cm)	0.130	**0.005**	0.202	**0.014**
Waist:Hip ratio	0.089	0.050	0.014	0.869
FFM (kg)	-0.057	0.206	-0.084	0.307
FFMI (kg/m^2)	0.043	0.341	-0.005	0.947
Fat Mass (kg)	0.148	**0.001**	0.325	**0.001**
HGS (kg)	-0.353	**0.001**	-0.182	**0.026**
Aortic PWV	0.088	0.052	0.192	**0.019**
CO (L/min)	0.004	0.930	-0.015	0.857
SV (ml)	-0.140	**0.003**	-0.112	0.184
6MWD (m)	-0.581	**0.001**	-0.371	**0.001**
TUG (s)	0.516	**0.001**	0.082	0.324
Resting O_2 saturation (%)	-0.155	**0.001**	-0.206	**0.012**
log_{10}Fibrinogen (g/L)	0.162	**0.001**	0.191	**0.023**
log_{10} CRP (mg/L)	0.156	**0.001**	0.271	**0.001**
Number of Comorbidities	0.456	**0.001**	0.421	**0.001**

All data mean (SD) unless otherwise indicated.

Abbreviations: **FEV_1=** forced expiratory volume in 1 second; **BMI=** body mass index; **FFM=** fat free Mass; **FFMI=** fat free mass Index; **HGS=** handgrip strength; **CO=** cardiac output; **SV=** stroke volume; **6MWD=** six minute walk distance; **TUG=** Timed Up and Go; **CRP=** C-reactive protein.
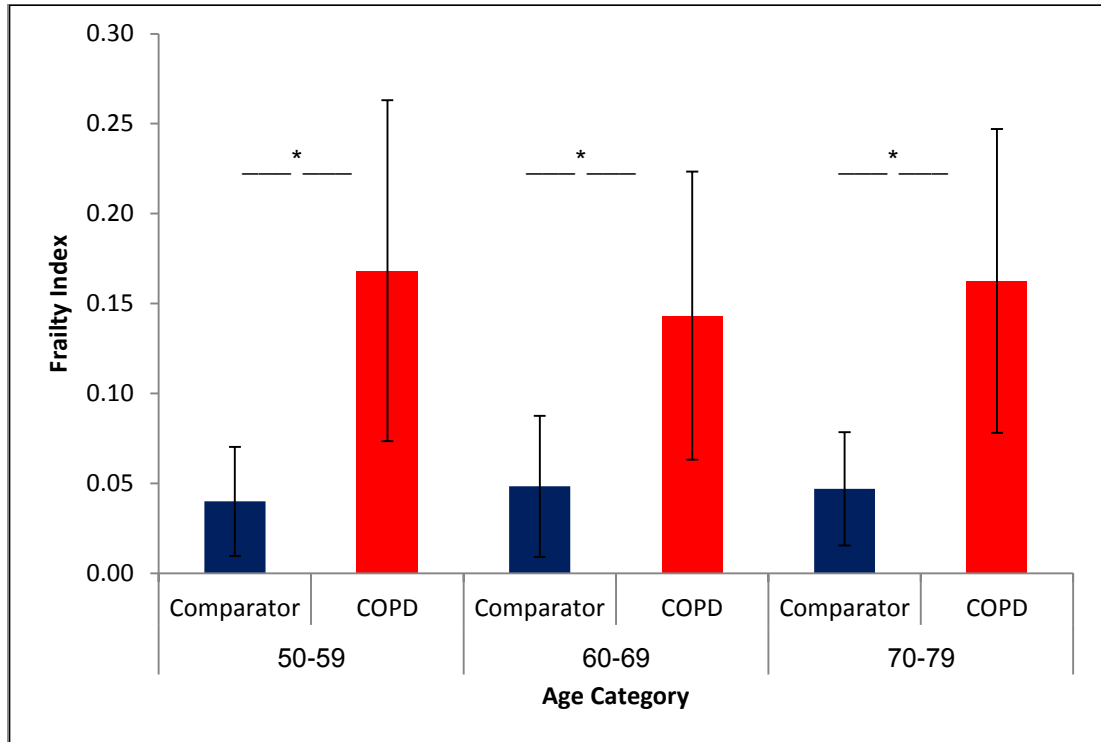
Figure 6.1: Bar chart illustrating the difference in FI between patients with COPD and

comparators across age categories at initial visit

Data presented as mean with SD.
ANOVA, p<0.001.

Relationship between Frailty and Physical Performance

The FI was related to the 6MWD (r=-0.58) and TUG (r=0.52) in patients with COPD, all p<0.001, but was only related to the 6MWD (r=-0.37) in the comparators, p<0.001 (Table 6.3).

Relationship between Frailty and Body Composition

The patients and comparators had similar BMI, p>0.05 (Table 6.1), but patients with COPD had greater FI than the comparator group across the BMI category, p<0.001 (Figure 6.2). In the patients, the FI was greater at the extremes of BMI (Figure 6.2). However, in the comparator group, the FI increased with BMI, p<0.01. The FI was related to the FM and waist circumference in both patients and comparators, all p<0.01, but not the FFM (Table 6.3).

Relationship between Frailty and Gender

The FI of female patients 0.17 (0.08) was greater than their male counterparts 0.14 (0.09), p<0.001. Similarly for the comparators, females 0.05 (0.04) were more frail than males 0.04 (0.03), p<0.001. The difference remained after controlling for age, waist: hip ratio and $\log_{10}$ CRP in the patients (Adjusted R^2= 7%, F=23.80, p<0.001) and the comparators (Adjusted R^2= 12%, F=9.27, p=0.003). A Two-way ANOVA showed that female patients with COPD had a greater FI after the age of 60 than the male patients, F= 2.51, p=0.040.
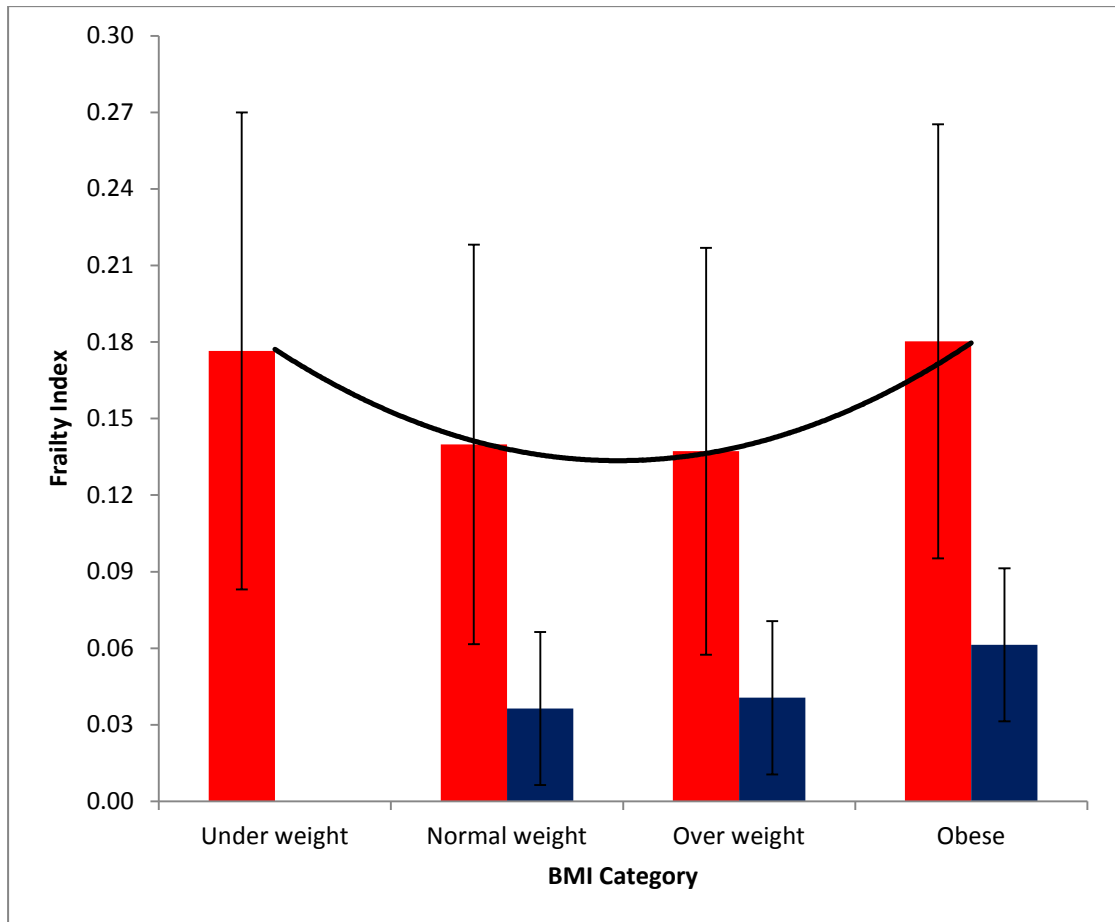
Figure 6.2: Bar chart illustrating the difference in frailty index between patients with COPD and comparator subjects across BMI categories at initial visit

Data presented as mean with SD.

ANOVA, Bonferonni post-hoc, p<0.001.

COPD   Comparator

Relationship between Frailty Index and Lung Function

The FI was related to FEV_1% predicted in the patients (r=-0.19, p<0.001) and the comparators (r=-0.20, p=0.019). In the patients, there was a significant difference in FI across GOLD categories, p<0.01. Post hoc analysis showed the only difference existed across the GOLD categories was between GOLD1 and GOLD3, p<0.001. However, the FI of the comparators was lower than the patients across all GOLD categories, p<0.001 (Figure 6.3).

Relationship between Frailty and Cardiac Function

In the patients, the FI was related to stroke volume (r=-0.14, p=0.003), but was not in the comparators. The stroke volume of the patients was also associated with FFM (r=0.58, p<0.001) and FFMI (r=0.46, p<0.001). The FI was not associated with cardiac output in either group, p>0.05 (Table 6.3). There was no relationship between FI and aortic stiffness in the patients, but it was evident in the comparators (r=0.19, p=0.019).
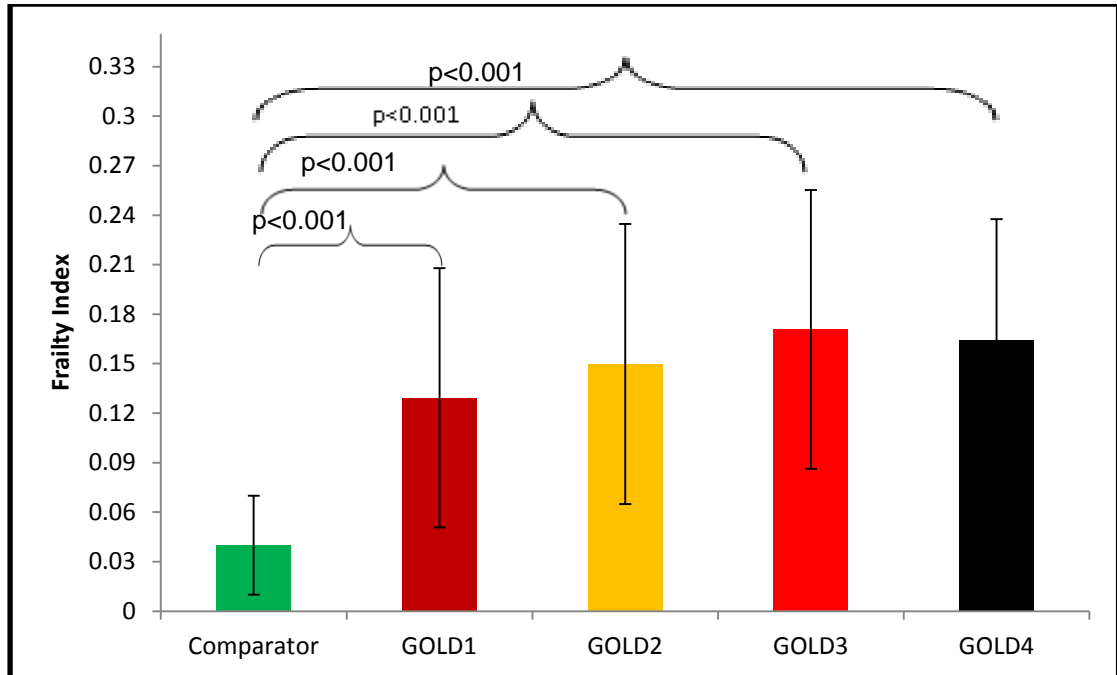
Figure 6.3: Bar chart representing FI in patients with COPD across GOLD categories and comparator subjects at initial visit

Data presented as mean with SD.
ANOVA, Bonferonni post-hoc.

Relationship between Frailty and Inflammatory Biomarkers

Both circulating CRP and fibrinogen were related to the FI in the patients and comparators, all p<0.05 (Table 6.4). The frailty score in the patients was also related to the number of exacerbations per year, (r=0.262, p<0.001). The FI of frequent exacerbators, 0.17 (0.08), was greater than infrequent exacerbators, 0.13 (0.08), and both were greater than that of the comparators, 0.05 (0.04), p<0.001 (Figure 6.4). Based on the FI upper 95%CI of the comparators, 165 of 204 (81%) infrequent exacerbators and 277 of 296 (94%) frequent exacerbators were frail.

Relationship between Frailty and Health Related Quality of Life

The FI score of the patients was related to SGRQ (r=0.60) and its sub-domains; activity (r=0.57), symptom (r=0.42) and impact (r=0.57), all p<0.001. Similarly, the health status as measured by the total score of the CAT was related to the FI score of the patients (r=0.54, p<0.001).
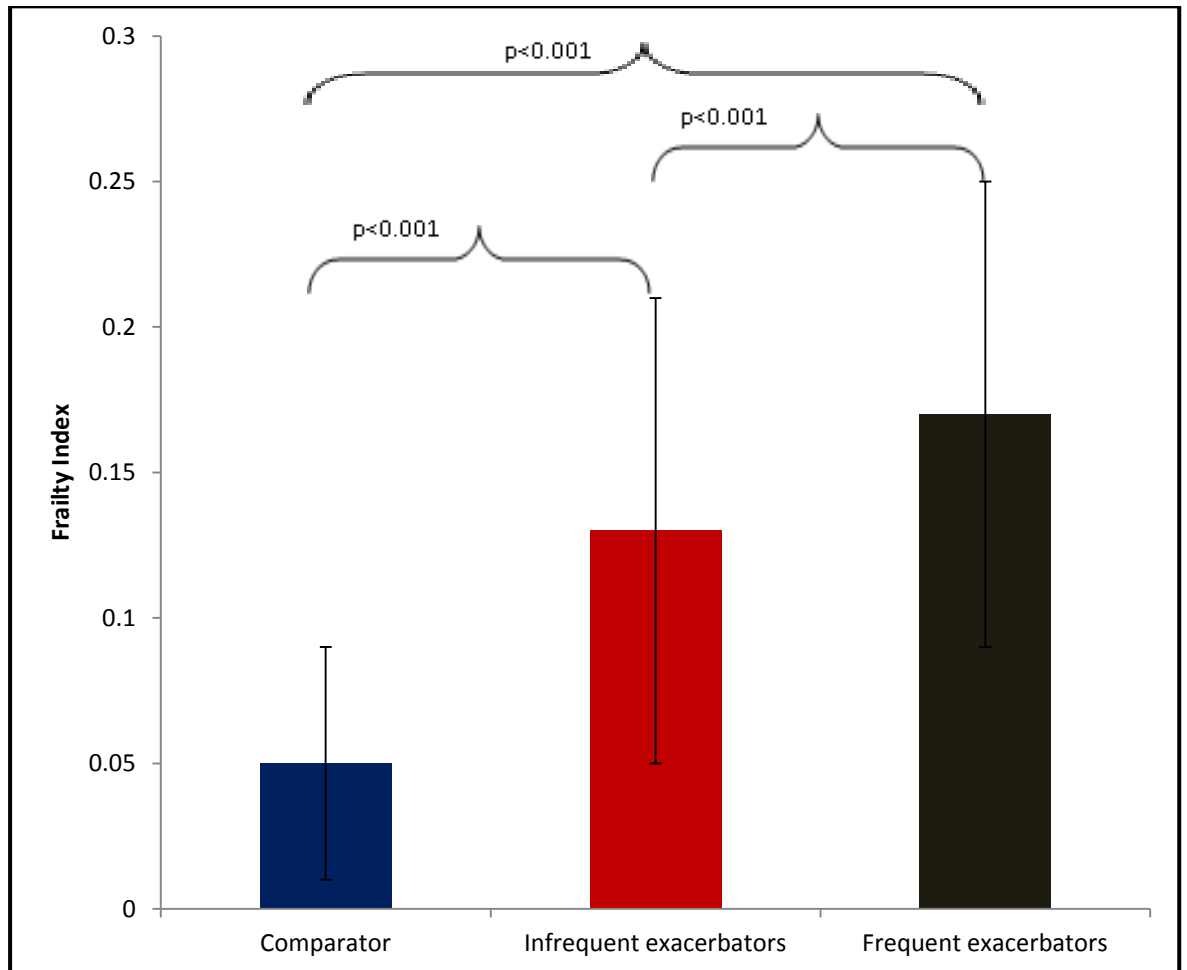
Figure 6.4: Comparison between comparators and patients with infrequent and frequent

exacerbation at initial visit

Data presented as mean with SD.
ANOVA, Bonferonni post-hoc.

Disease status and frailty

Based on the upper 95%CI of the FI for the comparator group (0.05), to define an upper limit for non-frail status in all subjects, patients and comparators were classified as frail or non-frail. The differences between non-frail and frail comparators were a shorter 6MWD, p=0.003, and CRP, p=0.029. Similarly, comparison between non-frail comparators and non-frail patients revealed a shorter 6MWD in the patients, p=0.002, a greater level of CRP, p=0.023, apart from spirometric indices. Comparison between frail comparators and non-frail patients only showed differences for spirometry measures, all p<0.001. Comparisons between frail comparators and frail patients demonstrated a lesser 6MWD, with greater number of co-morbidities and exacerbations per year in the patients, all p<0.001. Comparing the non-frail and frail patients revealed that frail patients had a lesser stroke volume, 6MWD and HGS with a greater aortic PWV, TUG test, waist circumference, number of diagnosed co-morbidities and exacerbations than non-frail, all p<0.001 (Table 6.4). The COPD specific measures of the total SGRQ and CAT score were greater in the frail patients, p<0.01.

Table 6.4: Comparison between frail and non-frail patients with COPD at initial visit

	Frail (n=446)	Non-Frail (n=54)	p value
Age (years)	66 (7)	65 (7)	0.380
FEV$_1$% predicted	58 (20)	66 (21)	**0.011**
SGRQ	53.1 (19.1)	21.6 (13.6)	**0.001**
BMI (kg/m^2)	28.2 (5.6)	26.4 (3.2)	**0.049**
Waist circumference (cm)	100 (15)	94 (10)	**0.025**
Waist:Hip ratio	0.96 (0.10)	0.93 (0.06)	0.054
Fat Mass (kg)	27.2 (11.4)	23.5 (7.2)	0.227
FFM (kg)	49.6 (11)	50.3 (11.2)	0.704
HGS (kg)	26.6 (9.7)	32.5 (10.1)	**0.001**
Aortic PWV (m/s)	10.0 (2.4)	9.0 (1.9)	**0.012**
CO (L/min)	5.8 (1.2)	5.8 (1.0)	0.482
SV (ml)	85 (20)	92 (23)	**0.042**
6MWD (m)	313 (121)	450 (72)	**0.001**
TUG (s)	11.3 (3.8)	8.7 (1.4)	**0.001**
Smoking (packs/year)	41 (25)	36 (23)	0.229
No. Exacerbations/ year	2.3 (1)	1.0 (1)	**0.001**
CAT	21.4 (8.3)	11.3 (6.9)	**0.001**
CRP (mg/L)#	3.6 (2.9)	2.80 (2.2)	0.174
Fibrinogen (g/L)#	3.6 (1.3)	3.1 (1.2)	**0.006**

All data mean (SD) unless otherwise indicated, # Geometric mean

Abbreviations: FEV$_1$= forced expiratory volume in 1 second; **BMI=** body mass index; **FFM=** fat free mass; **HGS=** handgrip strength; **PWV=** pulse wave velocity; **CO=** cardiac output; **SV=** stroke volume; **6MWD=** six minute walk distance; **TUG=** Timed Up and Go; **SGRQ** = St George's Respiratory Questionnaire; **CAT=** COPD Assessment Test; **CRP** = C-reactive protein.

Predictive Factors for Frailty

A hierarchical multiple regression was conducted with the FI as a dependent variable. Gender was entered at stage one to control for gender. The TUG, HGS, waist circumference, 6MWD, $FEV_1\%$ predicted, MRC and SGRQ Activity score were entered at stage two because these variables are commonly associated with frailty. Third stage included potential variable including stroke volume, CRP, fibrinogen and number of exacerbations.

In the patients, the hierarchical multiple regression revealed that gender contributed 2% to variation in frailty (F=10.59, p=0.001). Introducing HGS, 6MWD, TUG and SGRQ Activity score to the model explained an additional 43% of variation in frailty and this change in R^2 was significant (R^2=45, F=48.6, p<0.001). However, adding potential variables to the regression model demonstrated no additional variation in frailty (R^2=0.001, F=0.438.6, p=0.646). The most important predictors of frailty were SGRQ Activity score, TUG, HGS and 6MWD, and these four predictors explained 45% of variation in frailty (Table 6.5).

Similar to the patients, gender explained 4% of variation in frailty in comparator group (F=6.75, p<0.001). Introducing 6MWD to the model added an additional 13% of variation in frailty with significant change in R^2 (R^2=17%, F=5.05, p<0.001). However, adding potential variables to the regression model revealed no significant change in the model (R^2=0.005, F=0.437, p=0.647). The only predictor of frailty in the comparators was 6MWD, which explained 17% of variation in frailty (Table 6.6).

Table 6.5: Hierarchical multiple regression for variables predicting frailty in patients with COPD at initial visit

	B	SE	Beta	p value	Adjusted R^2
Stage 1					.02
Gender	.024	.007	.144	.001	
Stage 2					.45
HGS (kg)	-.002	.000	-.201	.000	
6MWD (m)	.000	.000	-.148	.004	
TUG (s)	.006	.001	.251	.000	
SGRQ Activity score	.001	.000	.303	.000	
Stage 3					.45
HGS (kg)	-.002	.000	-.200	.000	
6MWD (m)	.000	.000	-.144	.005	
TUG (s)	.006	.001	.252	.000	
SGRQ Activity score	.001	.000	.303	.000	

Abbreviations: HGS= handgrip strength; **6MWD=** six minute walk distance; **TUG=** Timed Up and Go; **SGRQ=** St George's Respiratory Questionnaire.

Table 6.6: Hierarchical multiple regression for variables predicting frailty in comparator subjects at initial visit

	B	Std. Error	Beta	p value	Adjusted R^2
Stage 1					0.04
Gender	.015	.006	.210	0.010	
Stage 2					0.17
6MWD (m)	.001	.001	-.288	0.001	
Stage 3					0.17
6MWD (m)	.001	.001	-.274	0.003	

Abbreviation: 6MWD= six minute walk distance

Frailty Longitudinal Results

The frailty index of the 143 patients who completed the two years follow-up had increased (Table 6.7). Using the predetermined frailty index of 0.05 as an acceptable upper limit for non-frail patients with COPD (page. 197), 133/143 patients were frail at the baseline and continued to be frail at the follow-up visit, five patients had progressed from non-frail status at baseline to be frail at two years follow-up, while two patients remained non-frail during the study period. The frailty status of three patients reduced and patients became non-frail at the two years follow-up assessment. The severity of airflow obstruction by GOLD stratification was mentioned previously (Chapter 5, page. 158). The distribution of the modified MRC breathlessness score was: mMRC 0 n= 14, mMRC 1 n= 42, mMRC 2 n=34, mMRC 3 n=48 and mMRC 4 n=5.

After two years, 10% had lost some weight while 8% had gained weight. Following the classification used by Landbo et al. (1999), of the 143 patients, 7% had a low BMI (<19.9 kg/m^2), 37% had an elevated BMI (25-29.9 kg/m^2) and 34% were classified as obese (BMI>30 kg/m^2), while 21% were within the normal limit for BMI (20-24.9 kg/m^2). Based on previous published data on patients with COPD from this geographical region (South Wales), Bolton et al. (2004) classified a low FFMI for men as <16.89 kg/m^2 and <13.28 kg/m^2 for women. Using these cut-off values, 9 patients had a low FFMI. Four of these had a low BMI, therefore 5 had a hidden loss of FFM.

The patients demonstrated significant loss of FFM and HGS after two years from their initial visit, while there was no change in BMI and other body composition indices, p>0.05 (Table 6.7).

Table 6.7: Physical and physiological changes in the patients after two years from the initial assessment

	Initial visit (n=143)	Follow-up visit (n=143)	Mean change 95%CI			p value
Age (years)	68 (7)	70 (7)				
Gender (Male:Female)	65:78	65:78				-
FI	0.16 (0.08)	0.18 (0.09)	0.02	0.01	0.03	**0.001**
FEV$_1$ (L)	1.3 (0.5)	1.1 (0.5)	-0.12	-0.16	-0.07	**0.001**
FVC (L)	2.4 (0.08)	2.3 (0.8)	-0.16	-0.23	-0.08	**0.001**
FVC% predicted	83 (19)	79 (22)	-4	-7	-2	**0.001**
FEV$_1$%predicted	54 (19)	51 (16	-3	-4	-1	**0.001**
Smoking (pack-years)	42 (30)	43 (28)	1.25	-3	5	0.519
BMI (kg/m^2)	28 (6)	27.8 (6)	-0.20	-0.59	0.21	0.356
Waist (cm)	99 (15)	99 (15)	-0.16	-1.30	0.99	0.786
Waist:Hip ratio	0.94 (0.10)	095 (0.12)	0.01	0.01	0.02	0.120
Fat Mass (kg)	26.7 (11)	26.2 (10.5)	-0.55	-1.44	0.34	0.224
FFM (kg)	48.6 (10.8)	48.1 (10.8)	-0.53	-1.05	-0.01	**0.048**
FFF:FM	2.2 (1.3)	2.2 (1.2)	0.01	-0.15	0.18	0.932
HGS (kg)	26.2 (9.8)	24.6 (9.9)	-1.6	-3.63	-0.87	**0.002**
CO (L/min)	5.7 (1.1)	5.5 (1.2)	-0.20	-0.30	-0.02	**0.024**
Stroke Volume (ml)	83 (19)	79 (20)	-4	-7	-2	**0.001**
6MWD (m)	307 (83)	328 (145)	21	3	39	**0.003**
TUG (s)	11.4 (3.9)	13.2 (8.3)	1.8	0.7	2.8	**0.001**
No. Exacerbation/ year	2.5 (1.95)	2.9 (2.2)	0.4	0.03	0.70	**0.034**
mMRC	1.95 (1.1)	2.24 (1.22)	0.31	0.08	0.50	**0.008**
SGRQ Symptom	67 (19)	68 (20)	-1	-2	-4	0.486
SGRQ Impact	42 (19)	40 (18)	-2	-3	-5	0.087
SGRQ Activity	72 (22)	73 (23)	-1	-2	-4	0.499
Resting O$_2$ saturation (%)	96 (2)	96 (2)	0	-0.08	0.63	0.128
CRP (mg/L)#	3.0 (2.8)	3.1 (2.6)	0.1	0.9	1.2	0.953
Fibrinogen (g/L)	3.7 (1.1)	3.3 (0.9)	-0.40	-0.56	-0.20	**0.001**

Data presented as mean (SD), # Geometric mean and mean (95%CI).

Abbreviations: FI= frailty index**; FEV$_1$=** forced expiratory volume in one second**; FVC=** forced vital capacity**; BMI=** body mass index; **FM=** fat mass; **FFM** = fat free mass; **FFM:FM=** fat free mass to fat mass; **HGS=** handgrip strength; **CO=** cardiac output; **SV=** stroke volume; **6MWD=** six minute walk distance; **TUG=** Timed Up and Go; mMRC**=** modified Medical Research Council; **SGRQ=** St George's Respiratory Questionnaire; **CRP=** C-Reactive protein.

The Relationship between Physical Performance and Increased Frailty

Increased frailty was associated with reduced physical performance as assessed by TUG test (r=0.39, p<0.001) and 6MWD (r=-0.19, p=0.034). The change in the TUG score was associated with the change in the 6MWD (r=-0.25, p=0.006).

The relationship between Body Composition and Increased Frailty

In the patients, increased frailty index was associated with loss of fat free mass (r=-0.19, p=0.023) and handgrip strength (r=-0.25, p=0.004). Patients continued to follow a U-shaped pattern that was seen in the cross-sectional analysis. Frailty was higher on the extreme than who were considered to be normal or overweight (p<0.05).

The Relationship between Gender and Increased Frailty

In the cross-sectional analysis, female patients were more frail than the males. The gender variation persisted at the follow-up assessment with female patients demonstrating a greater change in the frailty index compared to their male counterparts (Figure 6.5). The difference remained after correcting for age (R^2= 2%, F=5.16, p=0.025). The TUG time of the female patients had increased by mean (95%CI) 2.7 (0.9 to 4.5) s while the males had no change in the TUG test. In contrast, the 6MWD of the female patients remained as it was at the initial assessment, 2 (-20 to 24) m, p>0.05, while the males had increased by mean (95%CI) 43 (15 to 72) m, p<0.01. The change in the 6MWD did not relate to the change in FI in the male patients.
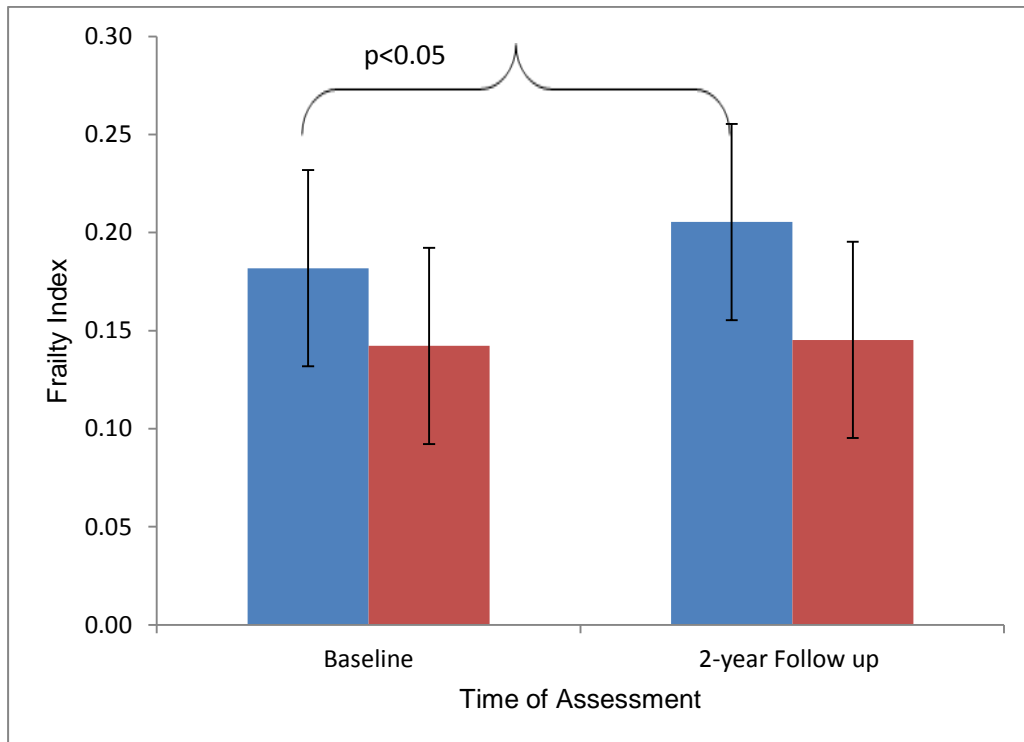
Figure 6.5: Gender difference of frailty index in the patients at 2 years

■ **Female**　　■ **Male**

Data presented as mean with SD.

The Relationship between Cardiac Function and Increased Frailty

The increase in frailty index was associated with a decline of cardiac hemodynamic indices as measured by stroke volume (r=-0.22, p=0.009) and cardiac output (r=-0.19, p=0.029). The decline in stroke volume and cardiac output in the patients was also related to the loss of fat free mass (r=0.23, p=0.008), (r=0.19, p=0.030), respectively. There was no association between the change in frailty index and the change in aortic PWV and its subcategories (progressors and non-progressors).

The Relationship between Lung Function and Increased Frailty

There was no association between the change in frailty index and the change in $FEV_1\%$ predicted. The analysis was further expanded and no change in frailty across the GOLD categories was found.

Relationship between Inflammatory Biomarkers and Increased Frailty

Neither circulating CRP nor fibrinogen level was related to the change in frailty index of the patients, though the fibrinogen level had significantly decreased over the period of follow-up.

There was no association between the change in frailty index and the change in the number of exacerbations, p>0.05.

Relationship between the Number of Comorbidities and Increased Frailty

In this longitudinal study, the change in frailty index did not relate the number of comorbidities, p>0.05. Interestingly, based on the upper limit of 95%CI of the frailty index for the comparator group (0.05), a group of patients without reported comorbidities was identified to be frail.

Health Related Questionnaires.

Although, in this study, there was an increase in the frailty index over the two years follow-up, the health-related quality of life measured by the SGRQ and CAT questionnaires did not change.

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Predictive factors for Increased Frailty Index

A hierarchical multiple regression was conducted with the FI change as a dependent variable. Gender was entered at stage one to control for gender. The change in FFM and HGS were entered at stage two to examine the effect of loss of musculoskeletal mass and strength on increased frailty. The third stage included the change in physical performance as measured by the TUG and 6MWD. Finally, the level of dyspnoea was entered at stage four.

The hierarchical multiple regression revealed that gender could explain 2% of variation in the change of frailty (F=4.49, p=0.036). Introducing loss of fat free mass and HGS to the model added an additional 6% to the variation in the change of frailty information and the change in R^2 was significant (R^2=8%, F=5.50, p=0.021). Adding the change in the TUG time to the regression model explained an additional 11% of variation in the change of frailty and this change in R^2 was significant (R^2=19%, F=19.66, p<0.001). However, gender was no longer a predictor of the change in frailty as was the 6MWD. Adding breathlessness score to the regression model demonstrated no additional variation in the change of frailty (R^2=0.013, F=2.26, p=0.136). The most important predictors of the change in frailty index were loss of fat free mass, loss of HGS and increased TUG time and these predictors explained 19% of variation in the change of frailty (Table 6.8).

Table 6.8: Hierarchical multiple regression for variables predicting the change in frailty in the patients at 2-year follow-up visit

	B	SE	Beta	p value	Adjusted R^2
Stage 1					0.02
Gender	-0.02	0.01	-0.18	0.036	
Stage 2					0.08
Gender	-0.019	0.009	-0.16	0.047	
Loss of FFM	-0.003	0.001	-0.17	0.036	
HGS	-0.003	0.001	-0.19	0.022	
Stage 3					0.19
Loss of FFM	-0.003	0.001	-0.16	0.039	
HGS	-0.002	0.001	-0.16	0.036	
Increased TUG time	0.002	0.001	0.35	0.001	

Abbreviations: **FFM=** fat free mass; **HGS=** handgrip strength; **TUG=**Timed Up and Go.

6.4 Discussion

Cross-sectional study: Baseline Findings

The results of this chapter demonstrated that patients with COPD were more frail than the comparator group, free from respiratory disease, as demonstrated by their greater CGA deficits and higher frailty index score. More patients, 92% were frail compared with the 57.8% previously reported by Park et al. (2013). Comparisons between this study and that of Park et al. (2013) and Galizia et al. (2011) are difficult due to different assessments of frailty and smaller study populations. Park and colleagues (2013) included 70 patients with physician diagnosed COPD without an objective measure of airflow limitation, lacked a specific comparator group, or previous validation of the frailty questionnaire used. However, the current research used the widely validated CGA assessment tool, which allowed to compare subject's FI with other populations where the FI has been widely used. The FI is a better predictor of outcome than chronological age or disability and the need for healthcare support (Rockwood et al., 2005, Romero-Ortuno and Kenny, 2012). In the current study, female patients and comparators had greater FI than males. A similar relationship was reported in the general population, and was similar to the mean FI of 0.15 reported for women over 65 years of age (Hubbard et al., 2010, Hubbard and Rockwood, 2011, Rossi et al., 2011a).

In the general population, frailty is associated non-linearly with increasing age and with female gender, functional dependence, systemic inflammation and chronic disease (Fried et al., 2009, Hubbard et al., 2010, Jones et al., 2005, Rockwood et al., 2005). Strong associations have been reported between frailty and cardiovascular and respiratory disorders, while there are few specific studies in individual chronic diseases, such as reported here in patients with COPD (Vaz Fragoso et al., 2012, Woods et al., 2005). In the Women's Health and Ageing Studies, the risk of frailty increased with inflammatory comorbidities, e.g., the combination of pulmonary

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disease with anaemia carried a risk ratio of 5.57 compared with control subjects who had fewer comorbidities (Chang et al., 2010). A further study in this female cohort demonstrated that frailty was related non-linearly to the number of abnormal physiological systems, which was more predictive than the degree of abnormality in any one system and resembles the impact of multiple comorbidities in COPD (Fried et al., 2009). Physiological deficits in COPD include loss of skeletal muscle mass (sarcopenia) and function with progressive decline in physical activity, loss of bone mineral density, systemic inflammation, reduction in HRQoL and increasing cardiovascular morbidity and mortality (Bolton et al., 2004, Rossi et al., 2011a, Van Eeden et al., 2012). In the present study, the number of comorbidities was related to frailty in the COPD group. Hence, there are clear parallels with COPD where the number of comorbidities was related to frailty in the patients. Frailty was largely independent of age in the patients, indicating that an assessment of frailty was an additional measure of impairments over and above chronological age in COPD and may represent premature ageing.

Increased numbers of comorbidities associated with COPD clearly could add to the degree of frailty, however, after removing these elements from the total score of CGA, frailty remained ten-fold different from the comparators, indicating that other features of COPD contributed to the presence of frailty. In the patients, the best predictors of frailty were the SGRQ activity score, TUG, HGS and 6MWD, which suggests loss of physical function and capacity, and muscle strength are key factors in frailty in COPD. The predictive association between reduced muscle strength and physical activity and frailty could explain why chronic lung inflammation and comorbidities could lead to premature ageing and frailty in COPD, and is supported by the association between frailty and exacerbation frequency. Hence, frailty in COPD is a multifactorial function of reduced quality of life, muscle strength and physical function. However, comorbidities and multisystem deficits in COPD are an important factor in the overall

morbidity and mortality, and their association with frailty suggest the latter may be used to predict a poor outcome (Divo et al., 2012). Loss of muscle strength and impairment of physical activity are an important factor of COPD and in the aetiology frailty pathway. The TUG test is a simple measure of physical performance and has been widely used as a measure of frailty. Although frailty has not been well studied in the COPD population, the presence of a multisystem disease such as COPD could accelerate ageing process by developing several manifestations including loss of muscle mass, weakness, decreased walking speed and comorbidities. Comparing patients with COPD to their comparators using this integrated test showed that patients with COPD were slower to perform this task which may be due to the contribution of several factors including loss of lower extremity strength as part of the nature of the disease that involve musculoskeletal system. Finding a relationship between TUG and frailty measure in patients with COPD but not in the comparators may suggest that these patients are experiencing accelerated ageing than comparators of the same age. This supports previous research that suggests that TUG is a useful measure for screening and identifying people with frailty (Viccaro et al., 2011). Using the TUG test in this population appears to come in parallel with what has been published in the literature in a frail elderly population and may allow clinicians to capture different aspects of frailty, which include weakness of the lower extremity, slow walking speed and impaired balance.

This study also showed that frailty was associated with components of body composition, including BMI, FM, FFM, their ratio and hip and waist circumference, all changes that occur in both COPD and ageing (Angleman et al., 2006, Cesari et al., 2006, Eisner et al., 2007, Rossi et al., 2011a, Walston et al., 2002). The U-shaped relationship between frailty and accepted categories of the BMI seen in the patients is similar to the U-shaped distribution in both males and females reported in the English Longitudinal Study of Ageing (Rossi et al., 2011). In the comparator group, although

frailty increased with BMI, the lack of individuals with a low BMI in this group limited finding a U shaped distribution. In the patients, the FFM was less than in the comparator group, but when expressed as the FFMI, there was no difference. Loss of muscle mass as in sarcopenia in the elderly has been considered to be a major factor leading to frailty, particularly in the phenotypic characterisation (Fried et al., 2001). However, in both COPD and natural ageing, increases in the FM and abdominal obesity have been closely linked to impaired physical function (Eisner et al., 2007, Cesari et al., 2006). In both groups, FM was directly related to the FI, as were the waist and hip circumferences in the patients and hip circumference in the comparators, which parallels the importance of the FM as a factor in frailty in the general population (Cesari et al., 2006). Increased FM and frailty in the elderly has also been linked to increased levels of circulating biomarkers of inflammation (Hubbard and Woodhouse, 2010, Walston et al., 2002). Circulating CRP an indicator of IL-6 activity increases with age and has been linked to frailty and abdominal obesity, independent of BMI, which may reflect the negative impact of chronic low grade systemic inflammation on physical function and wellbeing (Angleman et al., 2005). These reported associations with frailty and ageing, in many ways parallels findings in COPD, where systemic inflammation may be causal, compensatory or an epi-phenomenon, but is associated with an increased risk of major comorbidities and is a predictor of mortality (Celli et al., 2012, Hubbard and Woodhouse, 2010, Thomsen et al., 2012).

Frailty is a risk factor for the incident and development of CVD (Fried et al., 2001, Newman et al., 2001, Khan et al., 2013). The relationship between frailty and stroke volume in the patients suggests reduced normal physiological reserve of the heart or a subclinical abnormality such as myocardial ischaemia and left ventricle dysfunction. In COPD, asymptomatic left ventricle systolic and diastolic dysfunction, a feature of impaired cardiac capacity, was associated with loss of FFM (Sabit et al., 2010a). In

agreement with the results from Sabit et al. (2010), Barr and colleagues (2010) provided an evidence of decreased left ventricle volume and SV in a large population with COPD without a history of CVD and was related to the degree of airway severity. Although, the interaction between COPD and left-sided heart disease is complex and is not fully understood, it is known that lower SV will lead to impaired oxygen delivery to the tissue, which subsequently results in loss of left ventricle muscle mass and left ventricle dysfunction (Collis et al., 2001). This is supported by the data from the Cardiovascular Health Study, which showed that frail patients had reduced left ventricle function and poor myocardial function (Newman et al., 2001).

In the elderly, frailty independently predicts the risk of falls, worsening mobility, loss of activities of daily living, increased risk of hospitalisation and death over short term periods (Fried et al., 2001). The CGA quantifies the number of deficits, and as a generic tool, it is useful to compare frailty in different populations, and as a prognostic indicator of their health outcomes. The comparison of frail and non-frail status within the patients based on a reference range derived from the same population source clearly identified patients with major disease related deficits giving a potential global assessment tool that might predict adverse outcomes, including the risk of death or the need for institutional care, based on the impact of accumulating deficits, and be useful for planning interventions.

Discussion

Longitudinal study: Two-year Findings

This is the first longitudinal study exploring the progression of frailty in COPD. Patients with COPD had an increase in their frailty index over the two years follow-up period with progressive decline in physical performance and loss of muscle mass and strength. This supports the findings of the cross-sectional analysis that COPD is an independent risk factor for frailty. As there is no previous study that has assessed the frailty longitudinally in COPD, comparisons are difficult.

In the patients, loss of FFM and handgrip strength was associated with the progression of frailty. Concomitant loss of FFM and skeletal muscle mass and strength (sarcopenia) is one of the comorbidities associated with COPD and similarly a hallmark of frailty. An increased FI in parallel with sarcopenia in patients with COPD suggests that COPD precipitates features of premature ageing. The cause of sarcopenia in COPD is not fully understood and likely to be multifactorial. In this cohort study, though, the level of plasma fibrinogen decreased while circulating CRP remained stable; there was no association between systemic inflammation and sarcopenia in the patients. The association between loss of FFM and systemic inflammation is still debated, as the studies in this topic are cross-sectional in design, which limits their findings. Loss of muscle mass in COPD does not just reduce patient's mobility, but it also impairs glucose metabolism and increases insulin resistance (Barnes and Celli, 2009). Insulin plays a potent role in maintaining muscle mass by balancing between muscle protein synthesis and breakdown, up-regulating glucose uptake and inhibiting fat tissue degradation (Magkos et al., 2010). In COPD, abdominal obesity acts as an active endocrine gland producing abundant of inflammatory cytokines that induce insulin resistance in skeletal muscle (Rutten et al., 2010, Gosker et al., 2007). Increased insulin resistance predisposes to intramuscular fat gain, which enhances fatty acid delivery and subsequently reduces fat oxidative

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capacity of skeletal muscle. In type 2 diabetes, the patients experience a high rate of muscle mass loss compared with non-diabetic individuals due to increased insulin resistance (Park et al., 2009). Given the high prevalence of type 2 diabetes in patients with COPD, this enhances insulin resistance and may accelerate musculoskeletal mass wasting. A similar relationship has been evident in natural ageing and in the elderly with sarcopenia and frailty (Abbatecola and Paolisso, 2008).

The association between loss of handgrip strength and frailty in patients with COPD independent of chronological age is more likely to reflect the accelerated ageing process that COPD imposes on the musculoskeletal system as manifested by sarcopenia and loss of muscle strength. Loss of muscle mass and strength have been linked in COPD to serious consequences including exercise intolerance, poor health related quality of life and increased mortality (Bolton et al., 2004, Schols et al., 2005, Rutten et al., 2013). In COPD, reduced handgrip strength is associated with reduced muscle mass and physical activity (Engelen et al., 2000b, Pitta et al., 2006). Likewise, in healthy elderly, handgrip is associated with reduced lower extremity strength and daily activity, and predicts future disability and mortality (Al Snih et al., 2004, Ling et al., 2010). Furthermore, handgrip strength, a measure of frailty, was found to predict future frailty in 717 elderly men and women, independent of age (Syddall et al., 2003). The cause of sarcopenia in COPD and frailty is multifactorial, including inflammation, endocrine changes and most importantly physical inactivity (Watz et al., 2009, Cesari et al., 2004). Patients with COPD often avoid involvement in moderate to high intensity activities due to their breathlessness and subsequently and unconsciously adopt sedentary lifestyles. Compelling evidence supports the role of physical inactivity in skeletal muscle loss and sarcopenia, which are cardinal features of frailty.

Following the patients for two years allowed for further exploration of the features of frailty in COPD and the interaction between them and defined the best predictor of the progression of frailty in COPD. The only predictor of the progression of frailty in

patients with COPD was the change in TUG test. Reduced physical performance over a short period of time (i.e. two years) reflects the impact of COPD on frailty and strengthens the notion of an accelerated ageing process, which is supported by loss of muscle mass, handgrip weakness and physical inactivity and parallels the same factors associated with natural ageing.

The TUG is a strong predictor of frailty and mortality in the elderly and has been used to assess the risk of falls in patients with COPD (Savva et al., 2013, Beauchamp et al., 2009). The predictive relationship of the TUG test of the progression of frailty in COPD parallels what is published in the literature on frailty in the elderly population. In a cohort study of 417 elderly subjects living in the community, the TUG test was a good predictor of one year decline in functional and physical activities, which are the hallmark of physical frailty (Viccaro et al., 2011). Furthermore, in the Irish longitudinal study of ageing, the TUG test was discretely able to discriminate between frail and nonfrail individuals (Savva et al., 2013). Poor physical performance is an accepted criterion for frailty and a feature of COPD. Frailty is a dynamic clinical syndrome and its transition from one stage to another can be missed. Similarly, in COPD, a patient's level of activity is not routinely assessed in clinical practice. Therefore, the TUG test could be a useful screening test to reflect the level of impairment in patients with COPD. The TUG test incorporates muscle function, strength, balance and gait speed, which capture age-related changes and physiological deterioration (Podsiadlo and Richardson, 1991). These symptoms are commonly associated with COPD and features of the frailty phenotype (Fried et al., 2001). Physical inactivity could be the first step in the development of musculoskeletal dysfunction and trigger the frailty down spiral phase. Therefore, clinicians should be alert to different aspects of frailty in patients with COPD especially physical inactivity.

In this study, there was no relationship between the 6MWD and the progression of frailty. Although the 6MWD is a measure of exercise capacity, its improvement did not

reflect on the frailty status of the patients as frailty progressed. The concept of exercise capacity is totally different from physical activity. It has been suggested that an improvement in exercise capacity will subsequently lead to an improvement in the level of physical activity. Daily physical activity is a life style habit that requires continuous motivation and repetitive activity while exercise capacity is a maximum tolerability the patients could sustain. Unlike the TUG test, the 6MWD did not reflect patient's physical activity and musculoskeletal strength. Although, the 6MWD is a good outcome measure of submaximal exercise capacity than lung function, it does not reflect the level of daily activity that involves repetitive movement and subsequently stimulating the musculoskeletal system. In addition to that, the 6MWD cannot capture associated impairments in COPD including loss of muscle mass and strength (Spruit et al., 2010).

Frailty and COPD are both progressive and multifactorial based on the interplay of genetic, physiological and physical factors. The response and behaviour of the human body to these changes is gender dependent (Hubbard and Rockwood, 2011, Aryal et al., 2013). The number of females with COPD has recently been increasing especially among non-smokers or those with less than 20 pack-years of smoking (Salvi and Barnes, 2009). Increased level of frailty in female patients with COPD is not surprisingly limited to this group of patients and in agreement with previous research from the general population (Fried et al., 2001, Hubbard et al., 2010). Similarly, in the Cardiovascular Health Study, females were more frail and had more physiological impairments than males during the three years of follow-up (Fried et al., 2001). Contrary to this, in a cohort study of 2962 elderly subjects, followed up for nearly five years, Klein and colleagues (2005) reported that frailty was higher in males than females. This controversy is due to different frailty assessment and classification. The gender difference has not been studied extensively in COPD and likewise in frailty is still not known why females are more prone to frailty than males. In this study,

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although females with COPD had better lung function than males, they were more symptomatic and had reduced handgrip strength and prolonged that impacted on their health related quality of life, which resemble the physiological characteristics of frailty. Therefore, reduced physical performance and loss of muscle strength could induce inflammation that may be possibly causally related to the frailty in COPD. Thus, physical inactivity and inflammation could be a unifying physio-biological process leading to clinical frailty that is more prevalent in females than males with COPD.

Another explanation of why females with COPD are more frail than their male counterparts is the type of comorbidity. Males with COPD are at a higher risk of fatal diseases without a prior event of impaired health such as cardiovascular disease and rapidly progressive cancer while women with COPD present with a different pattern of comorbidities such as osteoporosis and depression (Agusti et al., 2010). Females with COPD in this study presented with better lung function than males, but they had worse symptoms and poor quality of life, which are frequently associated with anxiety disorder. Recently, in the ECLIPSE cohort study, depression and anxiety were most frequently seen in females with COPD, which were mainly determined by disease symptoms and health status (Hanania et al., 2011).

This is the first longitudinal study in COPD, which links the progression of frailty to reduced cardiac haemodynamics. The progression of frailty and its association with reduced cardiac haemodynamics over a short period suggests a subclinical damage to the heart prior to the appearance of its clinical manifestations. Cardiovascular disease is often underdiagnosed in patients with COPD and, therefore, the presence of frailty in parallel with a reduction in the heart reserve may implicate reduced physiological reserve of the heart to respond to further challenges, such as chest infection. Disintegration of normal harmony between body organs leads to a decline in their functional reserve and subsequently intolerance to further insult that is defined as frailty (Rockwood and Mitnitski, 2007). Since COPD is a multisystem disease, patients

with COPD may have accumulated subclinical organ damage related to vasculature abnormality including left ventricle dysfunction, aortic calcification and kidney impairment (Sabit et al., 2010, Bolton et al., 2007, John et al., 2013). Sabit and colleagues (2010) have shown that patients with COPD, free of CVD, had a symptomatic left ventricle dysfunction, which was associated with loss of muscle mass, independent of airway severity. Decreased blood supply to the skeletal muscle causes muscle mass loss and in a consequence produces abundant amino acids that subsequently cause further damage to other muscles including cardiac muscle, leading to left ventricle dysfunction (Rasmussen et al., 2006, Sabit et al., 2010a). Skeletal muscle is highly metabolic and consequently requires an enhanced metabolic demand, FFM may govern the required adequate blood supply for a given heart rate (Collis et al., 2001). Impaired oxygen delivery to skeletal and cardiac muscle as a result of reduced blood supply may be a further explanation for this relationship between frailty and reduced cardiac haemodynamics in COPD. In the Cardiovascular Health Study, frail patients with CVD had reduced left ventricle function and poor myocardium performance (Newman et al., 2001). In line with Newman et al. (2001) findings, the Health Ageing and Body Composition study showed that frailty was an independent risk factor for the incidence of heart failure after the adjustment for other confounding factors (Khan et al., 2013). In COPD, depletion of FFM, a feature of frailty, is believed to be secondary, in part, to a protein catabolic state as consequent of an imbalance in protein synthesis and breakdown (Eid et al., 2001, Engelen et al., 2000a). It has been previously shown in patients with COPD that cellular protein breakdown as measured by urinary pseudouridine (PSU) was inversely related to fat free mass (Bolton et al., 2004). Recently, Sabit et al. (2010a) took this a step further and showed that cellular protein breakdown and FFM were related to left ventricle mass. In the present study, reduced cardiac haemodynamics may further suggest another global feature of frailty in COPD, which may occur in parallel with loss of

musculoskeletal mass as part of the global catabolic process in COPD. Decreased cardiac haemodynamics may explain the cause of muscle mass loss and therefore cardiac dysfunction.

The role of inflammation in COPD is well established and may play a role in the frailty (Thomsen et al., 2012, Walston et al., 2002). The reason for not finding any association between inflammatory biomarkers in COPD and frailty progression could be related to various reasons. Firstly, most of the inflammatory biomarkers studied in frailty are not just specific to frailty syndrome as no study has examined the relationship between inflammatory biomarkers and frailty in absence of other comorbidities. Therefore, the presence of multimorbidity in these studies could inflate the association between frailty and inflammation. The majority of the studies investigating the relationship between frailty and inflammation are cross-sectional in design, which their results cannot be used to draw a definite conclusion on the predictive role of inflammation on frailty and its development. There has been no longitudinal study so far which examined the relationship between the progression of frailty and the change in inflammatory biomarkers. The cohort studies on frailty coming from the Cardiovascular Health Study, Longitudinal Ageing Study Amsterdam, Hertfordshire Ageing Study and the English longitudinal Study of Ageing have examined the incidence of frailty-related inflammation, and the results are sparse and inconsistent (Barzilay et al., 2007, Baylis et al., 2013, Gale et al., 2013, Puts et al., 2005b).

Secondly, before proceeding to the second explanation, it is still unknown if frailty is a cause or consequence of a specific disease or combination of multimorbid conditions. COPD is a multisystem disease including neuroendocrine, cardiovascular and musculoskeletal disorders (Barnes and Celli, 2009). The most frequently cited inflammatory biomarkers are CRP, IL-6 and fibrinogen. However, these biomarkers lack reproducibility and specificity (Cazzola et al., 2008). Furthermore, none of the

biomarkers has been found viable to predict or monitor COPD progression (Tzortzaki et al., 2006). Recently, a one-year follow-up from the ECLIPSE cohort study showed that 16% of the patients did not have high inflammatory biomarkers while 30% of the patients had no change in the inflammatory biomarkers (Agusti et al., 2012). A three-year cohort study in 218 stable patients with moderate to very severe COPD failed to show that CRP can discriminate between survivors and deceased patients (De Torres et al., 2008). An interventional study to determine the effect of fluticasone and fluticasone plus salmeterol on CRP or IL-6 in 289 patients with COPD showed neither fluticasone nor the combination therapy had a significant effect on serum CRP or IL-6 (Sin et al., 2008). Absence of the relationship between the progression of frailty and inflammation in COPD does not preclude the possible role of inflammation, but probably the chosen biomarkers in this study may not have been specific and sensitive enough to explain this kind of relationship.

This is first cohort study to explore the relation between COPD and frailty, which stimulates several questions. The debate on frailty has been ongoing for two decades and recently frailty is accepted as a clinical syndrome (Morley et al., 2013). Frailty is a multisystem deficit in parallel with COPD and requires a trigger to develop and exhaust the reserve of the body system. COPD is a systemic disease that precipitates the physiological reserve of other systems. Frailty does not exist until the body system is unable to respond to further stressors (Fried et al., 2001). COPD causes multiple impairment and functional limitations, which are fundamental features of frailty. At this stage, the reserve function of the body system has already begun to decline and eventually become unresponsive to further stressors. Thus, frailty could be a clinical manifestation of COPD. For example, in this cohort study, patients had loss of muscle mass and strength, which influenced their physical performance and increased their level of frailty.

Frailty is an important aspect in COPD and patients with COPD should be examined for frailty in routine clinical practice to implement early identification and management. Applying a simple questionnaire can capture different aspects of comorbidities and avoid possible consequences associated with COPD such as physical inactivity, loss of FFM, musculoskeletal mass and strength and reduced cardiac haemodynamics, healthcare utilization and health related quality of life.

Study Limitations

It is acknowledged that this cohort study lacked a comparator group as with many published studies. However, due to the time constraint of the PhD, it was not possible to follow-up the comparator group. Another limitation was this study was not powered to identify the progression of frailty in COPD. However, this is a community study, which represented patients with stable COPD. In this study, there was no measure to ascertain left ventricle dysfunction or the protein turnover.

Conclusion

Patients with COPD were more frail than the comparator group of current or ex-smokers, independent of age. Frailty in COPD was related to reduced physical performance and health status. In each age category, FI was greater in patients than comparators, which supports the possibility that COPD is associated with premature ageing.

In the follow-up study, frailty progressed in the patients and predominantly in the females. The progression of frailty in COPD was dependent on the decreased physical performance in conjunction with loss of muscle mass and strength. The CGA is a potential quick, simple and inexpensive questionnaire that provides a global assessment of COPD. It is an assessment that highlights multisystem deficits, which could guide interventions and monitor disease progress.

Chapter Seven

Conclusions and Future Research

7.1 Conclusions

7.1.1 A Simple and Rapid Measure of Physical Performance in COPD

Physical inactivity is one of the common comorbidities associated with COPD and increased cardiovascular risk, loss of muscle mass and mortality. However, the assessment of physical performance is not routinely examined in clinical practice due to limited resources. Timed Up and Go is a rapid and simple test that has been widely used in the elderly to assess physical performance and frailty.

The cross-sectional results showed that patients with COPD had longer TUG time compared with non-COPD subjects. The TUG test was related and a predictor of the most common test of submaximal exercise capacity, the 6MWD. The TUG test had shown the potential to be a useful clinical test in primary care to capture different aspects of physical performance.

7.1.2 Aortic Stiffness in Patients with COPD

Cardiovascular disease is the most common comorbidity associated with COPD. Aortic stiffness is an independent predictor of CVD morbidity and mortality is several populations including healthy elderly and undoubtedly plays a pivotal role in the increased risk of CVD in COPD.

The cross-sectional results revealed that patients with COPD had greater aortic stiffness than similar age and gender comparators, independent of blood pressure, heart rate, airway severity, tobacco exposure and inflammation. Aortic stiffness was greater in males with COPD than their female counterparts and was dependent on waist circumference.

The results of this longitudinal study clearly showed that COPD is an independent risk factor for aortic stiffness progression and premature vascular ageing. Patients with COPD showed a rapid aortic stiffness progression by six-fold compared with estimated normal aortic ageing. A subset of patients demonstrated an accelerated aortic biological ageing by 16 years over a two-year follow-up, independent of

conventional risk factors and consequently their CVD morbidity and mortality increased by more than 22%. Aortic PWV is a simple, reliable and non-invasive measure that can be used in clinical practice to identify patients who are at high risk of cardiovascular events and provide proper management.

7.1.3 Frailty in COPD

Presence of the multimorbidity in patients with COPD resembles the multideficit associated with frailty.

The results of the cross-sectional study demonstrated that patients with COPD were more frail compared with similar age and gender non-COPD individuals, independent of airway severity and inflammation. Females were more frail than males in both groups. Frailty in COPD was dependent on physical performance and function and musculoskeletal strength.

The longitudinal results showed that the progression of frailty in COPD was related to the reduced physical performance and loss of muscle mass and strength. The progression of frailty in COPD clearly supports the hypothesis of premature ageing in COPD and reduced homeostasis capacity of patient's physiological system to sustain further internal or external insult. Since frailty is an independent predictor of adverse outcomes and mortality in non-COPD populations, implementing frailty assessment in parallel with a simple test such as the TUG test would optimise management for patients with COPD.

7.2 Future Research

7.2.1 A Simple and Rapid Measure of Physical Performance in COPD

- Since TUG test is related to the 6MWD, a measure of exercise capacity, a field study is needed to examine the applicability of the TUG test in primary and secondary care as a routine assessment of physical performance.

- As the TUG test is a predictor of physical performance, it is useful to examine body composition and bone mineral density using the DEXA scan and this will give insight about the impact of fat mass and fat free mass and their ratio on functional status.

- Since the TUG test is related to handgrip strength, body composition and exercise capacity, future studies are needed to incorporate lower extremity function, body composition, exercise capacity and daily physical activity, and this will give more insight about the physical impairment.

7.2.2 Aortic Stiffness in Patients with COPD

- Future longitudinal studies investigating progression of arterial stiffness in patients with COPD should include comparator subjects with similar smoking history to provide a realistic comparison between these groups.

- Future longitudinal studies of arterial stiffness in COPD should quantify anatomical emphysema using high resolution computed tomography. This may determine whether an emphysematous phenotype or a chronic bronchitis phenotype patients are at particular risk of aortic stiffness progression.

- Since loss of FFM correlates with cardiac haemodynamics, future longitudinal studies investigating the progression of aortic stiffness should include measurement of left and right ventricular function and their relationship with aortic stiffness progression and changes in body composition. Such longitudinal studies may also be able to indicate whether sub-clinical left and right

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ventricular dysfunction exists in parallel with loss of FFM and progression of aortic stiffness.

- Since this longitudinal study showed a subset of patients who had a rapid progression of aortic stiffness independent of blood pressure and inflammation, future longitudinal studies are needed to investigate vascular properties and include both endothelia and oxidative mechanism.

- Osteoprotegerin (OPG) is a marker of bone attenuation as well as vascular remodelling and aortic stiffness has been previously shown to be elevated in patients with COPD and osteoporosis, therefore, including OPG measurement if future longitudinal studies would provide another horizon of increased CVD in COPD.

- Future longitudinal studies should include newly diagnosed patients with COPD at different stages and assess the effects on inhaled bronchodilators on aortic stiffness progression and incident of CVD. This should include measurement of aortic stiffness, airways obstruction, cardiac haemodynamics and systemic inflammation at baseline and repeated annually. This will give an insight of which treatment has a substantial effect on aortic stiffness progression and increased risk for CVD.

7.2.3 Frailty in COPD

- Since this research has shown that frailty is another manifestation of COPD, frailty assessment should be included as part of patient's general assessment to capture other comorbidities beyond lung function.

- Future longitudinal studies should include comparator subjects to determine the rate of frailty progression compared with a COPD population.

- Future studies should include patients with COPD without comorbidities and monitor the development of physiological deficits in comparison with a comparator group.

- Since this study has shown that frailty progression was related to loss of muscle mass and strength independent of inflammation, future longitudinal studies are needed to investigate the cause of sarcopenia.

- Future longitudinal studies of frailty in COPD should include lower extremity strength and function, and daily physical activity in parallel with simple tests such TUG, HGS and mMRC and body composition to determine if physical inactivity is the main trigger of frailty phenotype.

- Since reduced functional reserve of the heart was related to frailty progression, future studies of frailty in COPD should assess cardiac function to provide early detection of cardiovascular abnormalities.

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Appendix A: Participant Information Sheet for Patients

Dennis J Shale MD

Professor of Respiratory Medicine

J R Cockroft

Professor of Cardiology

Nichola S Gale PhD

Research Associate

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Cardiff University School of Medicine
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Heath park
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"Assessment of Risk in Chronic Airways Disease Evaluation - ARCADE**"**

You are invited to take part in a research study. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. One of our research team members will go through this sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with relatives, friends and your GP if you wish. We feel that this would take about 15 minutes to go through. Please ask us if there is anything that is not clear or if you would like more information. This study is funded for the first three years by a research grant from GlaxoSmithKline.

Why are we doing this study?

As we get older, our arteries generally stiffen over a period of time. This leads to an increase in blood pressure and may place us at a greater risk of strokes and heart attacks. A similar process occurs in patients with chronic obstructive pulmonary disease (COPD) causing premature arterial stiffening. However, we are not sure how quickly their arteries stiffen, and whether this is related to the severity of COPD. Also, the reasons for arterial stiffening in COPD are not well understood. We now have the means to assess arterial stiffness and the aim of this study is to measure how quickly arteries stiffen over time in COPD, and what factors are associated with this.

Why have I been asked to participate?

You have been asked to participate because you fall into the following group:

- Between 35-80 years
- Have a diagnosis of Chronic Obstructive Pulmonary Disease

Individuals who fall in this categories have been similarly approached by a letter and we are looking to recruit 1500 patients with COPD.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

Visit 0:

If you agree to take part, you will be asked to attend an initial visit lasting approximately 1 hour at a clinic at the University Hospital of Wales, Cardiff or your General Practitioner's surgery if it is one of the participating research sites. Here we will go through the information sheet, consent form and obtain a detailed lifestyle and medical history from you in addition to a medical examination.

You will then be requested to make 3 visits to the Wales Heart Research Institute at University Hospital of Wales, Cardiff, spaced over the next 5 years. All measurements made at these visits will be the same and each visit will last approximately 2 hours. Visit 1 will be baseline, visit 2 will be 2 years and visit 3 will be 5 years following your baseline visit.

Visit 1:

It would be preferable if you could refrain from smoking for at least 6 hours prior to attending. The following measurements will be made:

- We will check your height, weight and hip and waist circumference (using a tape).
- Blood pressure: After lying down for 15 minutes, you will have your blood pressure taken, just like at your General Practitioner's surgery.
- Arterial stiffness measurements: A small, pencil-like probe will be placed on the artery (blood vessel) at your wrist, neck and upper leg to calculate how stiff your arteries are. We will also record an electrocardiogram (ECG) with stickers on your chest to track small changes in your heart rate.

- Cardiac output: We will place some stickers on your chest and neck and measure how much blood is being pumped around with each heart beat.
- Lung function tests: You will then be asked to blow out as hard as you can into a tube after inhaling salbutamol (a safe drug using an inhaler with a spacer device) unless contraindicated to measure your lung function (the size of your lungs and how quickly you can empty them).
- Measurement of body composition: At this point we will check the proportion of muscle and fat tissue in your body by asking you to stand barefoot on a machine with hand grips. A tiny electric current which is not painful or harmful in any way is then passed and the measurements recorded. None of these measurements are painful.
- Hand-grip strength: This will be measured using a spring device.
- Bone density scan: We will then measure the bone density in your heels by using an ultrasound device which has no radiation.
- Blood sample: We will then ask you to give a blood sample of approximately 50 mls (5 tablespoons of blood) from your arm. This sample is used to measure various levels of naturally occurring substances in the blood, (ex: cholesterol, glucose and inflammatory molecules). This blood sample will be disposed after the study is completed in 5 years.
- Timed up and go (TUG) test: You will be requested to get up from a chair, walk to a distance of 3 metres, turn around, walk back and sit down again while we measure the time taken to do so.
- 6-minute walking test: You will then be requested to walk for 6 minutes on a flat surface at your own pace with breaks if necessary while we also check your oxygen levels in the blood using a non-invasive probe on your finger.
- Urine sample: Finally we will ask you for a urine sample (20ml or equivalent of 4 teaspoons) in a container to test for certain molecules.

A small proportion of participants will be invited to attend repeat assessments within 2 weeks of the assessment visit 1. They will be given activity monitors to get an idea of their exercise levels in real life.

Visits 2 and 3:

The data collected on subsequent visits (2 years & 5 years later) will be compared with the baseline to demonstrate any changes in blood vessel stiffening, inflammatory molecules in blood and evolution of cardiovascular risk factors. Data generated from the study will be stored at the WHRI for a period of 10 years in the safe custody of Professor D J Shale.

Will there be any side-effects?

Taking blood may lead to minor discomfort, but only experienced people will take blood samples.

Are there any risks involved in taking part?

Apart from the blood sample, all of the measurements are non-invasive and we don't foresee any risks involved.

Are there any possible benefits to taking part?

You will have a detailed assessment of your blood pressure and other cardiovascular risk factors (eg glucose, cholesterol levels) and we will be happy to make your results available to your General Practitioner if you agree.

Will my taking part in this study be confidential?

By consenting to take part in this research, you will consent to the collection, processing, disclosure and transfer of your personal data for medical research purposes only. Named information will be sent to the NHS Central Register and you will be flagged by the NHS Information Centre (NHS IC) for the purpose of follow-ups. Any data that is sent will be encrypted and we will follow ethical and legal practice to ensure that all information about you is handled in confidence. This is done to identify any new health problems that you may develop in relation to the heart while you are enrolled in our study. Information held by the NHS and records maintained by the General Register Office may be used to keep in touch with you and follow your health status. Your data will not be available to GSK as they are financially sponsoring this as an independent study.

Will my GP be informed?

The doctor in charge of the study will inform your GP of your participation in the study with your consent. In addition with your permission, they will be informed of any clinically relevant results that may become available during the study.

What will happen to the study results?

The results of the study may be published in scientific journals or presented at medical conferences, a few months after completing the study. You will not be identified in any report or publication. An overall summary report (fully anonymised) will be sent to your

General practitioner at the end of the study. You will be able to access this through your GP upon the completion of the study.

What happens if something goes wrong?

The doctors involved in the study, and the Wales Heart Research Institute have suitable indemnity insurance if you are harmed due to someone's negligence. However, there are no special compensation arrangements for non-negligent harm. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

What will happen if I withdraw from the study?

If you withdraw from the study, this will in no way affect your future medical care. Data or tissue already collected up to the point of withdrawal will be anonymised and utilised for study purposes.

The same would apply if capacity to consent is lost during the study. You will be withdrawn from the study and data or tissue already collected under consent will be anonymised and utilised for study purposes.

Will I be reimbursed for any travel expenses?

Travel expenses of up to £30 will be given as necessary for trips to hospital to cover bus, mileage and parking. Taxis can also be ordered from our department to pick you up or drop you off.

Who is organising this study?

This study is being organised by the Wales Heart Research Institute of the Cardiff University. The investigators in charge are Professor D J Shale and Professor J R Cockcroft.

Who has reviewed this study?

The South East Wales Research Ethics Committee has reviewed this study and given it a favourable opinion.

Contacts for research-related enquiries:

If you require more information about the study, then please contact any of the research team members namely Dr M Irfan (Clinical Research Fellow), Dr Nichola Gale (Research Associate) or Mrs Margaret Munnery (Research Nurse) on Tel 02920742352. Alternatively, you may wish to speak to the Principal Investigator: -

Professor J R Cockcroft	OR	**Professor D J Shale**
Consultant Cardiologist		Professor in Respiratory Medicine
Level 1,		Level 1
Wales Heart Research Institute		Wales Heart Research Institute
University Hospital of Wales		University Hospital of Wales
Heath Park		Heath park
Cardiff		Cardiff
C14 4XN		CF14 4 XN
0292074 3489		0292074 3489

Thank you for reading this information sheet.

Remember - You are under no obligation to participate in this study. If you wish to leave the study at any point you may do so for any reason. Please take as much time to read this leaflet as require; do not feel that you have to make a decision quickly. Researchers will be available to answer any questions you may have. All the data collected will be confidential and is only for the purposes of research.

Dennis J Shale MD
Professor of Respiratory Medicine
J R Cockroft
Professor of Cardiology
 Nichola S Gale PhD
Research Associate

Department of Respiratory Medicine
Cardiff University School of Medicine
Level 1, Wales Heart Research Institute
Heath park
Cardiff
CF14 4XN

PARTICIPANT INFORMATION SHEET FOR COMPARATOR SUBJECTS

"Assessment of Risk in Chronic Airways Disease Evaluation - ARCADE"

You are invited to take part in a research study. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. One of our research team members will go through this sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with relatives, friends and your GP if you wish. We feel that this would take about 15 minutes to go through. Please ask us if there is anything that is not clear or if you would like more information. This study is funded for the first three years by a research grant from GlaxoSmithKline.

Why are we doing this study?

As we get older, our arteries generally stiffen over a period of time. This leads to an increase in blood pressure and may place us at a greater risk of strokes and heart attacks. A similar process occurs in patients with chronic obstructive pulmonary disease (COPD) causing premature arterial stiffening. However, we are not sure how quickly their arteries stiffen, and whether this is related to the severity of COPD. Also, the reasons for arterial stiffening in COPD are not well understood. We now have the means to assess arterial stiffness and the aim of this study is to measure how quickly arteries stiffen over time in COPD, and what factors are associated with this.

We are comparing these measurements in patients with COPD against those in subjects free of COPD who have a history of smoking.

Why have I been asked to participate?

You have been asked to participate because you fall into the following group:

Between 35-80 years

Have a history of smoking

Other individuals in this group have been similarly approached by a letter and we are looking to recruit 300 such subjects in this study to act as comparators for the 1500 patients with COPD.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

Visit 0:

If you agree to take part, you will be asked to attend an initial visit lasting approximately 1 hour at a clinic at the University Hospital of Wales, Cardiff or your General Practitioner's surgery if it is one of the participating research sites. Here we will go through the information sheet, consent form and obtain a detailed lifestyle and medical history from you in addition to a medical examination.

You will then be requested to make 3 visits to the Wales Heart Research Institute at University Hospital of Wales, Cardiff, spaced over the next 5 years. All measurements made at these visits will be the same and each visit will last approximately 2 hours (please see figure 1 on page 5). Visit 1 will be baseline, visit 2 will be 2 years and visit 3 will be 5 years following your baseline visit.

Visit 1:

It would be preferable if you could refrain from smoking for at least 6 hours prior to attending. The following measurements will be made:

- We will check your height, weight and hip and waist circumference (using a tape).

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- Lung function tests: You will then be asked to blow out as hard as you can into a tube after inhaling salbutamol (a safe drug using an inhaler with a spacer device) unless contraindicated to measure your lung function (the size of your lungs and how quickly you can empty them).

- Measurement of body composition: At this point we will check the proportion of muscle and fat tissue in your body by asking you to stand barefoot on a machine with hand grips. A tiny electric current which is not painful or harmful in any way is then passed and the measurements recorded. None of these measurements are painful.

- Hand-grip strength: This will be measured using a spring device.

- Bone density scan: We will then measure the bone density in your heels by using an ultrasound device which has no radiation.

- Blood sample: We will then ask you to give a blood sample of approximately 50 mls (5 tablespoons of blood) from your arm. This sample is used to measure various levels of naturally occurring substances in the blood, (ex: cholesterol, glucose and inflammatory molecules). This blood sample will be disposed after the study is completed in 5 years.

With your permission some blood will also be taken and stored for future analyses, which will examine the genes which are known or likely to be important in determining how stiff or elastic your arteries are. This sample taken for genetic analysis will be stored for 10 years in the custody of Professor D J Shale and Professor J R Cockcroft at the Wales Heart Research Institute (WHRI), Cardiff.

Further ethical approval will be sought if any genetic testing is required beyond 5 years. As these genetic markers are mainly to establish a genetic link for the natural evolution of COPD it would not have any implications on inherited risk or insurance status. GlaxoSmithKline, the sponsors of the first three years of the study will have access to the genetic data, but you will not be identified individually to the sponsors or anyone else in respect of this data.

- Timed up and go (TUG) test: You will be requested to get up from a chair, walk to a distance of 3 metres, turn around, walk back and sit down again while we measure the time taken to do so.

- 6-minute walking test: You will then be requested to walk for 6 minutes on a flat surface at your own pace with breaks if necessary while we also check your oxygen levels in the blood using a non-invasive probe on your finger.
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Professor J R Cockcroft	OR	**Professor D J Shale**
Consultant Cardiologist		Professor in Respiratory medicine
Level 1,		Level 1
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Thank you for reading this information sheet.

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Participant's Questionnaire

'Assessment of Risk in Chronic Airways Disease Evaluation'

'ARCADE'

Thank you very much for your participation in the 'ARCADE' study. We appreciate you taking time to complete this questionnaire.

Please answer the questions to the best of your ability and feel free to leave blank any questions you do not wish to answer.

Please use **black** ink.

Yours Sincerely,

Prof. J. Cockcroft   Prof. D. Shale

SECTION 1 - PERSONAL DETAILS

1   Name ....

Today's Date

☐☐ ☐☐ ☐☐☐☐

2   **a)** Date of Birth   **b)** Age   **c)** Birth Weight

☐☐ ☐☐ ☐☐☐☐   ☐   ☐

3   Address ....

....

Postcode ....   Telephone # ....

4   GP's Name ....

GP's Address ....

.... Postcode ....

5   **a)** Are you employed?   Yes ☐   No ☐

b) Have you had any jobs which involved working with any of the following?

Coal ☐   Asbestos ☐   Chemicals ☐   Dust ☐   No ☐

If **Yes**, please specify approximately how long for, in years: ☐

6   **a)** Are you married/cohabiting?   Yes ☐   No ☐

b) If not married/cohabiting, are you

Single, ☐   Widowed ☐   Divorced ☐   Separated ☐
Never married

c) If widowed/divorced or separated what year did this happen? ☐☐☐☐

1

7

a) Do you get short of breath?

All the time ☐   Worse at ☐   On ☐   At ☐   No ☐
certain times   exercise   night
of the day

b) Do you notice a wheeze?   Yes ☐   No ☐

c) Do you cough?   Yes ☐   No ☐

If **Yes**, do you produce sputum?

Yes, most mornings ☐   Occasionally ☐   No ☐

d) Does anything make your breathing worse (e.g. Cats, Dogs, Pollen, Aerosol, Perfume, Cold, Heat)?

Yes ☐   No ☐

8

When were you diagnosed with COPD?

....

9

a) How far can you normally walk on a flat surface without having to stop for a breath·on a good day? (Please give a number in yards, feet or miles).

....

b) If not limited by breathlessness what limits your mobility?

....

10

a) How often do you get chest infections in a year?

....

b) How many courses of antibiotics or steroids (tablets) did you need for your chest over the last year?

....

11

a) Are you on home oxygen?   Yes ☐   No ☐

If **Yes**, how many hours a day?   ☐

b) Are you on home nebulisers?   Yes ☐   No ☐

12

a) Did you have asthma as a child?   Yes ☐   No ☐

b) Were you admitted to a hospital as a child for chest problems?   Yes ☐   No ☐

c) Do you suffer from eczema or hay fever?   Yes ☐   No ☐

2

303

13

a) Have you had the flu vaccine?   Yes ☐   No ☐

b) Have you had the pneumonia vaccine?   Yes ☐   No ☐

c) Have you had a chest x-ray in the last 12 months?

Yes ☐   No ☐

14

Have you been admitted to hospital (including as a day case) in the last **3 years**?

Yes ☐   No ☐

If **Yes**, please specify the number of times: ☐

Please specify the reason for hospitalisation(s) and the dates:

Cause 1 ....   ☐☐ ☐☐☐☐

Cause 2 ....   ☐☐ ☐☐☐☐

Cause 3 ....   ☐☐ ☐☐☐☐

Cause 4 ....   ☐☐ ☐☐☐☐

15

a) Which statement best describes your breathlessness?
Please tick the appropriate box

0 – "I only get breathless with strenuous exercise"   ☐

1 – "I get short of breath when hurrying on the level or walking up a slight hill"   ☐

2 – "I walk slower than people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level"   ☐

3 – "I stop for breath after walking about 100 yards or after a few minutes on the level"   ☐

4 – "I am too breathless to leave the house" or "I am breathless when dressing"   ☐

b) Can you climb a flight of stairs without stopping?

Yes ☐   No ☐

3

16 How likely are you to doze off or fall asleep in the following situations in contrast to just feeling tired? This refers to your usual way in recent times. Even if you have not done some of these things recently, try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation.

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing   Chance of dozing (0-3)

Sitting and reading ☐

Watching TV ☐

Sitting inactive in a public place (eg. a theatre or meeting) ☐

As passenger in a car for an hour without break ☐

Lying down to rest during the day when circumstances permit ☐

Sitting and talking to someone ☐

Sitting quietly after lunch without alcohol ☐

In a car, while stopped for a few minutes in traffic ☐

17 a) Do you snore?   Yes ☐   No ☐

b) Do you have Sleep Apnoea?   Yes ☐   No ☐

18 Have you done Pulmonary Rehabilitation?   Yes ☐   No ☐

19 Have you lost or gained weight in the last 12 months?

Yes ☐   No ☐

4

20 Have you ever been told by your doctor that you have any of the following?

a) High blood pressure   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

If **Yes**, are you on therapy for it?   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

b) High cholesterol   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

If **Yes**, are you on therapy for it?   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

c) Peripheral vascular disease
(narrowing of the arteries in the leg)   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

If **Yes**, are you on therapy for it?   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

d) Atrial fibrillation
(irregular pulse)   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

If **Yes**, are you on therapy for it?   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

e) Diabetes   Yes ☐   No ☐   If Yes, in what year? ☐☐☐☐

If **Yes**, which type?   Type I ☐   Type II ☐   Don't Know ☐

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21   Has a doctor told you that you have had any of the following?

a) Angina   Yes ☐   No ☐

b) Heart attack   Yes ☐   No ☐   If Yes, ☐☐☐☐
(myocardial infarct/   in what
coronary thrombosis)   year?

c) Stroke or Transient attack   Yes ☐   No ☐   If Yes, ☐☐☐☐
(mini-stroke/TIA?)   in what
                                            year?

If Yes: Stroke ☐   Transient ☐   Other ☐
                                Ischemic   (please specify)
                                Attack
                                (TIA)   . . . .

d) Any other heart trouble   Yes ☐   No ☐
suspected or confirmed?
(e.g. valve disease, congenital
heart disease or irregular heart beat)

If Yes, please specify: . . . .

---

22   Have you ever been told that you have increased risk of heart disease?

No ☐   Yes, by ☐   Yes, by a ☐   Yes, other ☐
         my GP   consultant   (please specify)

                                    . . . .

---

23   Have you ever been told by a doctor
that you have, or have had, any of   If Yes, what
the following?   year did the
                                       doctor first

	Yes	No	tell you?
Cancer	☐	☐	☐☐☐☐
Osteoarthritis ('wear & tear' arthritis)	☐	☐	☐☐☐☐
Rheumatoid arthritis	☐	☐	☐☐☐☐
Gout	☐	☐	☐☐☐☐
Osteoporosis	☐	☐	☐☐☐☐

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24 Apart from these, do you have any other longstanding illness, diseases or medical conditions for which you have sought treatment in the last **12 months**? (Longstanding means anything that has troubled you over a period of time or that is likely to affect you over a period of time).

Yes ☐   No ☐

If **Yes**, please list below:

. . . .

. . . .

25 Have you taken any inhalers, medicines or tablets in the last **14 days**?

Yes ☐   No ☐

If **Yes**, please list any medicines below:

Inhalers	Dose	Puffs	Amount /day
. . . .	. . . .	. . . .	. . . .
. . . .	. . . .	. . . .	. . . .
. . . .	. . . .	. . . .	. . . .

Nebuliser	Dose	Amount /day
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .

Other Medication	Dose	Amount /day
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .
. . . .	. . . .	. . . .

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26 Was your father ever diagnosed with any of the following?

Please tick the appropriate box(es)	Yes	No	Don't Know	Younger than 60 when diagnosed?
High blood pressure	☐	☐	☐	☐
Angina	☐	☐	☐	☐
Heart attack	☐	☐	☐	☐
Stroke	☐	☐	☐	☐
Peripheral vascular disease	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐
Asthma	☐	☐	☐	☐
COPD	☐	☐	☐	☐

27 Was your mother ever diagnosed with any of the following?

Please tick the appropriate box(es)	Yes	No	Don't Know	Younger than 60 when diagnosed?
High blood pressure	☐	☐	☐	☐
Angina	☐	☐	☐	☐
Heart attack	☐	☐	☐	☐
Stroke	☐	☐	☐	☐
Peripheral vascular disease	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐
Asthma	☐	☐	☐	☐
COPD	☐	☐	☐	☐

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28 Were your brother(s)/sister(s) ever diagnosed with any of the following?

Please tick the appropriate box(es)	Yes	No	Don't Know	Younger than 60 when diagnosed?
High blood pressure	☐	☐	☐	☐
Angina	☐	☐	☐	☐
Heart attack	☐	☐	☐	☐
Stroke	☐	☐	☐	☐
Peripheral vascular disease	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐
Asthma	☐	☐	☐	☐
COPD	☐	☐	☐	☐

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SECTION 5 - ABOUT YOUR LIFESTYLE

29 How often do you take part in sport or activities that are mildly energetic, moderately energetic or vigorous?

	3 times a week	Once or twice a week	About once to 3 times a month	Never/ hardly ever
a) Mildly energetic (e.g. walking, gardening, playing darts, general housework)	☐	☐	☐	☐
b) Moderately energetic (e.g. scrubbing, polishing car, dancing, golf, cycling, decorating, lawn mowing, leisurely swimming)	☐	☐	☐	☐
c) Vigorous (e.g. running, hard swimming, tennis, squash, digging, cycle racing)	☐	☐	☐	☐

Please give the average number of hours per week that you spend in such activities:

d) Mildly energetic *Hours per week* ☐

e) Moderately energetic *Hours per week* ☐

f) Vigorous *Hours per week* ☐

30 a) In the **past week** – on average, for how long did you walk outside your home/workplace? (If you did not walk please enter '0' in the boxes).

On each weekday [Hours][Minutes]   On each weekend day [Hours][Minutes]

b) In the **past week** – on average, for how long did you cycle? (If you did not cycle please enter '0' in the boxes).

On each weekday [Hours][Minutes]   On each weekend day [Hours][Minutes]

31 How would you describe your usual walking pace? *Please tick one box only.*

Slow pace (less than 3 mph) ☐   Steady average pace ☐

Brisk pace ☐   Fast pace (over 4 mph) ☐

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32 Have you ever broken/fractured a bone?   Yes ☐   No ☐

If Yes, please give details of your injuries: What age were you?

First injury:

. . . .   ☐

Second injury:

. . . .   ☐

Third injury:

. . . .   ☐

Please specify what caused the bone(s) to break/fracture?

	First injury	Second injury	Third injury
1) Fall from greater than standing height (e.g. from a chair or stairs)	☐	☐	☐
2) Fall from a standing height (e.g. walking)	☐	☐	☐
3) Fall from less than standing height (e.g. getting out of a chair)	☐	☐	☐
4) Road traffic accident	☐	☐	☐
5) High energy trauma (e.g. sports injury)	☐	☐	☐
6) Other (please specify)	☐	☐	☐

. . . .

33 On average, how much alcohol do you consume in a week?

> Note: **1 unit** = 1 small glass of wine (125ml)
>   = ½ pint of beer/lagar/cider
>   = 25ml pub measure of spirit

Enter number of units: ☐

34   **a)** Do you smoke cigarettes?   Yes ☐   No ☐   Past-smoker ☐

How many do/did you smoke each day?   ☐

How many years have you/did you smoke(d)?   ☐

b) If you are a past-smoker,
how many years has it been since you quit smoking?   ☐

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Appendix D: Consent Form

Dennis J Shale MD
Professor of Respiratory Medicine

Nichola S Gale PhD
Research Associate

Department of Respiratory Medicine
Cardiff University School of Medicine
Level 1, Wales Heart Research Institute
Heath park
Cardiff
CF14 4XN

Cardiff and Vale University Health Board

CONSENT FORM

LREC Reference Number:

Assessment of risk in chronic airways disease evaluation (ARCADE)

Name of Lead Investigators: Professor D J Shale and Professor J R Cockroft

Please initial box

1. I confirm that I have read and understand the information sheet dated 23/03/11 (version 3) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected if I withdraw.

3. I agree that if my capacity to consent is lost during the study, I will be withdrawn from the study and data or tissue already collected under consent will be anonymised and utilised for study purposes.

4. I am willing that my GP is informed of my participation in the project as well as any clinically relevant results.

5. I understand that information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact me and provide information about my health status.

6. I agree to be contacted in 2 and 5 years time after the baseline assessment for a further follow-up visit.

7. I agree to take part in the above study

_____   _____   _____
Name of Research Subject   Date   Signature
(Please print)

_____   _____   _____
Name of Research Member   Date   Signature
(Please print)

3 copies required: top copy for researcher; one copy for patient; one copy to be kept with research subject's notes.
LREC Number **11/WSE02/7**   Version 3   23/03/2011

Appendix E: St. George's Respiratory Questionnaire

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you the most problem.

Please read the instructions carefully and ask if you do not understand something.

Do not spend too long deciding about your answer.

PART 1

Please tick 1 of the following

	Most days a week	Several days a week	A few days a month	Only with chest infection	Not at all
1) Over the last year, I have coughed					
2) Over the last year, I have brought up phlegm (sputum):					
3) Over the last year, I have had shortness of breath:					
4) Over the last year, I have had attacks of wheezing:					

5)   During the last year, how many severe or very bad unpleasant attacks of chest trouble have you had? Please tick 1 of the following

More than three	
3 attacks	
2 attacks	
1 attack	
None	

6) **How long did the worst attack of chest trouble last?** Please tick 1 of the following

a week or more	
3 or more days	
1 or 2 days	
less than a day	

7) **Over the last year, in an average week, how many good days (with little chest trouble) have you had?** Please tick 1 of the following

None	
1 or 2	
3 or 4	
nearly every day 1	

8) **If you have a wheeze, is it worse in the morning? Yes/**No

   PART 2

9) **How would you describe your chest condition?** Please tick 1 of the following

The most important problem I have	
Causes me quite a lot of problems	
Causes me a few problems	
Causes no problem	

10) **If you have ever had paid employment?** Please tick 1 of the following

My chest trouble made me stop work	
My chest trouble interferes with my work or made me change my work	
My chest trouble does not affect my work	

11) **Questions about what activities usually make you feel breathless.**

Sitting or lying still	Yes/No
Getting washed or dressed	Yes/No
Walking around the home	Yes/No
Walking outside on the level	Yes/No
Walking up a flight of stairs	Yes/No
Walking up hills	Yes/No
Playing sports or games	Yes/No

12) **More questions about your cough and breathlessness.**

My cough hurts	Yes/No
My cough makes me tired	Yes/No
I get breathless when I talk	Yes/No
I get breathless when I bend over	Yes/No
My cough or breathing disturbs my sleep	Yes/No
I get exhausted easily	Yes/No

13)   Questions about other effects your chest trouble may have on you.

My cough or breathing is embarrassing in public	Yes/No
My chest trouble is a nuisance to my family, friends or neighbours	Yes/No
I get afraid or panic when I cannot get my breath	Yes/No
I feel that I am not in control of my chest problem	Yes/No
I do not expect my chest to get any better	Yes/No
I have become frail or an invalid because of my chest	Yes/No
Exercise is not safe for me	Yes/No
Everything seems too much of an effort	Yes/No

14)   Questions about your medication.

My medication does not help me very much	Yes/No
I get embarrassed using my medication in public	Yes/No
I have unpleasant side effects from my medication	Yes/No
My medication interferes with my life a lot	Yes/No

15) **Questions about how activities may be affected by your breathing.**

I take a long time to get washed or dressed	Yes/No
I cannot take a bath or shower, or I take a long time	Yes/No
I walk more slowly than other people, or I stop for rests	Yes/No
Jobs such as housework take a long time, or I have to stop for rests	Yes/No
If I walk up one flight of stairs, I have to go slowly or stop	Yes/No
If I hurry or walk fast, I have to stop or slow down	Yes/No
My breathing makes it difficult to do things such as walk up hills, carry things up stairs, light gardening such as weeding, dance, play bowls or play golf	Yes/No
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	Yes/No
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	Yes/No

16) We would like to know how your chest trouble usually affects your daily life.

Please tick which of the following apply to you

I cannot play sports or games	
I cannot go out for entertainment or recreation	
I cannot go out of the house to do the shopping	
I cannot do housework	
I cannot move far from my bed or chair	

17)   Tick the statement which you think best describes how your chest affects you.

Please tick 1 of the following

It does not stop me doing anything I would like to do	
It stops me doing one or two things I would like to do	
It stops me doing most of the things I would like to do	
It stops me doing everything I would like to do	

For Office use only

Symptom _____   Activity _____   Impact _____   Total _____

Appendix F: The COPD Assessment Test

Appendix G: Comprehensive Geriatric Assessment-Short Form

CGA-SF
Comprehensive Geriatric Assessment
Short Form

Patient ID

Completed by .... Date ....

Motivation	circle one:	High	Usual	Low	
Self-rated health	circle one:	Excellent	Good	Fair	Poor	Couldn't' say
Cognition	circle one:	Normal	Mild cognitive impairment	Dementia	

circle all that apply: Agitation/wandering Delusions/hallucinations Delirium

Emotional Normal OR **circle all that apply:** Anxiety Bereavement Depression Fatigue

Sleep Normal OR **circle all that apply:** Poor or disrupted Daytime drowsiness

Communication circle one for each

Speech Normal Impaired **Hearing** Normal Impaired **Vision** Normal Impaired

Strength **Grip strength** Normal Weak **Proximal muscle strength** Normal Weak

Hemiparesis: **Arm** Yes No **Leg** Yes No

Mobility	Transfer	Ind	Asst	Dep	
	Walking	Ind	Asst	Dep	**Slow ?** Yes No
	Aid used?	Walking stick	Frame		
Balance	Balance	Normal	Impaired		
	Falls	None	Yes		
Elimination	Bowel	Cont	Occas accident	Incont	
	Bladder	Cont	Occas accident	Incont	
Nutrition	Weight change	Stable	Loss	Gain	
	Appetite	Normal	Fair	Poor	
	Weight	Normal	Under	Obese	
ADLs	Feeding	Ind	Asst	Dep	
	Bathing	Ind	Asst	Dep	
	Dressing	Ind	Asst	Dep	
	Toileting	Ind	Asst	Dep	
IADLs	Cooking	Ind	Asst	Dep	
	Cleaning	Ind	Asst	Dep	
	Shopping	Ind	Asst	Dep	
	Medications	Ind	Asst	Dep	
	Driving	Ind	Asst	Dep	
	Banking	Ind	Asst	Dep	

Social Engagement

☐ Frequent
☐ Occasional
☐ Rarely

Medical History

☐ Hypertension
☐ COPD
☐ TIA/Stroke
☐ Angina/MI
☐ CHF
☐ Diabetes
☐ Cancer
☐ Alcohol excess
☐ Pressure sores
☐ Hip Fracture
☐ OA/RA
☐ Osteoporosis
☐ Parkinson's dis.

Problems

1.
2.
3.
4.
5.
6.
7.

Current medication

Notes: