The relationship between systemic inflammatory biomarkers, lung function and exercise in chronic obstructive pulmonary disease

Sheridan Beaumont BChiroSc, MChiroprac

A thesis presented in candidature for the degree of Master of Research.

Department of Chiropractic, Faculty of Science and Engineering,

Macquarie University, Sydney, Australia

14 April 2015

Table of Contents

Chapters

Chapter One: Introduction

Chapter Two: Inflammation in COPD

Chapter Three: Review of the Literature

Abstract

AIM

To evaluate CRP, leukocytes and exercise capacity in determining current FEV_1, in order to identify appropriate potential determinants for use in developing a predictive diagnostic model for people at risk of developing COPD.

METHODS

A literature review and meta-analysis was conducted to assess the influence of CRP, leukocytes and exercise in predicting current FEV_1 levels.

DISCUSSION

As much of the literature on systemic inflammatory biomarkers relates to the more advanced stages of COPD, assessing these levels across mild to severe COPD will establish whether systemic inflammation is lower for milder stages of the disease. This may provide new information about the value of using a combination of these assessments as an indicator of people at risk of developing COPD.

Candidate Statement

I certify that the work incorporated in this thesis has not been submitted for a higher degree to any other university or institution.

I certify that the work presented in this thesis is my own except as acknowledged in the text.

Signature ..... Date .....

Acknowledgements

I would like to thank my supervisor Roger Engel for his patience throughout this journey. Your support and encouragement has reduced the gradients, turned gravel into asphalt and transformed gapping chasms into minor crossings.

To the support staff at Macquarie university, in particular Ben Brown, thank you for your kind assistance and willingness to help at all times throughout the past year.

To my devoted husband Andrew, who has provided unlimited support and belief in my endeavors, made many sacrifices as a result. You are a pillar of strength.

And finally my thanks to my parents and Aunt Cathy, for their guidance and assistance.

List of Figures

List of Tables

Abbreviations

ADO: Age, dyspnoea and air-flow obstruction

BMI: Body mass index

BODE: BMI, airway obstruction, dyspnoea and exercise capacity

CRP: C-Reactive protein

COPD: Chronic obstructive pulmonary disease

FEV_1: Forced expiratory volume in the 1st second

FVC: Forced vital capacity

hs-CRP: High sensitivity CRP

IL: Interleukin

LTB4: Leukotriene B4

MMP: Matrix metalloproteinase

MPO: Myeloperoxidase

NE: Neutrophil elastase

NK: Natural killer

TNFα: Tumour necrosis factor α

Chapter One: Introduction

1.1 BACKGROUND

Chronic obstructive pulmonary disease (COPD) is a progressive and only partly reversible lung disease characterised by airflow limitation and difficulty breathing in and out fully (1, 2). Historically its origins can be traced back almost four centuries with the earliest reference to emphysema being in 1679. The foundations for understanding the pathophysiology of the disease did not occur until almost 140 years later when a description of the clinical components of chronic bronchitis appeared in the literature in 1814 (3). Simple lung function assessment was made possible with the invention of the spirometer in 1846. However, the first recorded evidence of individual components of the disease did not occur until 100 years later when Christie recorded that his patient had "physical signs of emphysema together with chronic bronchitis and asthma" (4). It took until 1965 before the collective term 'COPD' was first coined by William Biscoe at the 9th Aspen Emphysema Conference (5). In 2001, the global initiative for lung disease (GOLD) defined COPD as "chronic airflow obstruction that is progressive and only partly reversible", a definition that is still in use today (6).

COPD is a major burden of disease and is responsible for high rates of disability, hospital admission and premature death (7). In Australia, approximately one in thirteen people aged forty years and over have lung function consistent with the diagnosis, while in 2010 COPD was ranked as the third leading cause of death globally (8). With such a high impact on global morbidity and mortality, an increasing amount of research is being conducted on improving the diagnostic and therapeutic tools used for managing the disease. Airflow obstruction measured by spirometry is the main way of diagnosing COPD with forced expiratory volume in the 1st second (FEV_1) and its relationship to forced vital capacity (FVC) the primary method for classifying people into stages of COPD as well as for measuring the efficacy of treatment (9). Notwithstanding, these measures do not describe clinical aspects of the disease such as symptoms, quality of life or health status (10, 11), and they also fail to provide any information about the underlying pathological processes associated with COPD (12).

In an effort to improve prognostic accuracy a number of studies have investigated the effect of combining existing assessments into a single measure (13-17). This approach has yielded more comprehensive scoring systems with multi-dimensional assessments. The two most prominent of these are the BODE index which combines BMI (body mass index), airway obstruction, dyspnoea and exercise capacity (15) and the ADO index which combines age, dyspnoea and air-flow obstruction (18).

While the BODE and ADO have improved predictive values, their use is limited as many of the individual assessments are associated with the more advanced stages of the COPD and provide little benefit for interventions targeted at changing the natural progression of the disease in its earliest stages (19).

A retrospective study of 38,859 patient records collected over a nineteen year period highlighted this issue. While 85% of the patients in the study had reported relevant symptoms to their doctor at least once in the five years leading up to being diagnosed with COPD less than half of them had reported these symptoms in the eleven to fifteen years before diagnosis (20). The inadequacy in recognising the early signs and symptoms of COPD represents a missed opportunity for early intervention with the authors of the study recommending an improvement in the diagnostic tools used to detect COPD from routinely collected patient data.

Early diagnosis of COPD is important not only because of the limited reversibility of the disease but also because evidence has shown that the greatest loss of lung function occurs in the early stages of the disease (21, 22). The rationale for intervening earlier in the disease cycle is accompanied by a greater potential for altering the pattern of loss of lung function typically seen as COPD progresses.

In a bid to identify those who were at risk of developing COPD the Global Initiative for Chronic Obstructive Lung Disease (GOLD) added a fifth category (Stage 0) to its classification system of stages 1-4 in 2001. A person classified as being in Stage 0 had chronic cough and sputum but normal spirometry, while the other four stages are characterised by having FEV_1 / FVC < 0.70 and vary by decreasing ranges of FEV_1. In particular these stages are defined as: Stage 1 - Mild COPD ($FEV_1 \geq 80\%$ normal); Stage 2

- Moderate COPD (FEV_1 50-79% normal); Stage 3 – Severe (FEV_1 30-49% normal) and Stage 4 - Very severe (FEV_1< 30% normal) (7).

However, the Stage 0 category was removed in 2006 because data showed that many people in Stage 0 did not progress to GOLD Stage 1 or beyond. Since its removal, reliable prognostic determinants such as systemic inflammatory biomarkers that could be used to assist in identifying people at risk of developing COPD, have been reported in the literature. These measures are yet to be incorporated into multi-dimensional assessments for COPD.

With an increase in research aimed at developing more clinically relevant assessments of COPD, there has been an increasing interest in the use of inflammatory biomarkers as a measure of disease severity. A recent study combining the BODE index with the systemic inflammatory biomarker C-reactive protein (CRP) reported that the combination improved prognostic accuracy (23). Other markers and risk factors of COPD that could also be used in assessing the early stages of the disease include smoking history, age, history of exacerbation, the presence of comorbidities, physical activity levels and exercise intolerance.

1.2 SMOKING

Evidence that cigarette smoking is a major factor in the development of COPD is well documented (24) with the earliest reports of an association between smoking and clinical symptoms attributed to the physician Benjamin Rush in 1798 (25). However, the link between serious pathology and smoking was not confirmed until the late 1950s when multiple large health organisations around the world formally attributed lung cancer to smoking (24, 26-29).

In 1967 Petty and his colleagues uncovered the link between smoking and pathological change that could lead to the development of COPD. The link was mucous gland hyperplasia. Petty, in reporting on the findings from autopsies on 154 smokers noted the presence of lung lesions and emphysema in 50% of the cases (30). The association

between smoking and declining lung function was first described by Fletcher and Peto in their seminal study on male coal miners in the 1970s (31, 32).

Measuring airflow obstruction using the ratio FEV_1/FVC is currently the gold standard for diagnosing COPD (33) with an evaluation of disease progression measured by the rate of decline in FEV_1 (34). Estimates that 20% of smokers and 50% of lifelong smokers are likely to develop COPD later in life are based on these measures (35).

A systematic review of the epidemiological evidence related to declining FEV_1 and smoking confirmed their association (34). This review demonstrated that the number of cigarettes smoked per day was positively correlated with the rate of decline in FEV_1 and that current smokers had an accelerated rate of loss of more than 10mL/year compared to those that had never smoked. Results from this and other reviews also showed that over time the accelerated rate of decline in FEV_1 reported in ex-smokers tended to revert back to the rate of decline reported in people who had never smoked (34, 36, 37). This was confirmed by other reviews that reported similar decreases for people with and without COPD (36, 38-40). Given that FEV_1 is used to assess the severity and progression of COPD slowing the rate of decline should be interpreted as slowing the rate of disease progression (41). A study suggesting the risk of developing COPD could fall by as much as 50% by smoking cessation alone (42) further supports the conclusion that smoking cessation is more effective in the early stages of the disease compared to the later stages (31, 34, 37, 39, 43, 44).

Smoking has been associated with increased levels of inflammatory biomarkers in current smokers compared to those that have never smoked (45-49), while the number of cigarettes smoked per day has also been linked to increased levels of these biomarkers (46, 48, 50-52). As smoking plays a role in the inflammatory process, correlation with the level of inflammatory markers could act as an additional measure of its effect.

A landmark study using data from the 3rd National Health and Nutrition Examination Survey (NHANES III) clearly identified the effects of smoking cessation on inflammatory biomarkers (53). The survey analysed data from 15,489 current, ex- and non-smokers and found that the levels of CRP and leukocytes were positively correlated with smoking intensity and that there was a negative association between these biomarker levels and

smoking cessation. Furthermore, the elevated levels returned to baseline five years after smoking was stopped.

A study by Asthana *et al.* challenged part of these findings in reporting a correlation between smoking intensity and leukocyte count but not CRP levels, suggesting that any correlation between smoking and CRP was likely to be masked by a stronger relationship with adiposity (54). These studies highlight the complexities associated with investigating the link between systemic inflammatory biomarkers and COPD. Furthermore, the effect of age, sex and race can also influence biomarker levels and has the potential to further confound the results.

1.3 INFLAMMATION

Chronic inflammation

A core feature of COPD is an abnormal inflammatory response in the central and peripheral airways to environmental agents such as air pollution and cigarette smoke (55). The inflammatory response to inhaled stimulants is a normal protective reaction to smoking that appears to be modified and exaggerated in those that develop COPD. While there are elevated numbers of innate immune cells in the lungs, evidence suggests that their defense capabilities are suppressed by smoking (56). This abnormal inflammatory response is found to persist following smoking cessation despite the absence of the inflammatory stimulus (57, 58). Smoking also alters the repair mechanism of the lungs causing an abnormal repair (fibrosis of small airways) or inhibition of repair (seen in emphysema) (59-62).

Variability in airflow limitation in individuals can also be influenced by secretions and inflammation in response to inhaled noxious particles such as cigarette smoke (55). This results in differences in susceptibility of an individual to develop COPD. Results from a study found that despite the majority of individuals with COPD having a history of smoking, only 15-30% of all smokers developed COPD (31). It is also possible non-smokers may develop COPD. Additionally, fixed airway obstruction can be contributed to by asthma or other chronic disorders that cause inflammation in the lower respiratory tract.

Smoking is thought to cause COPD by inducing a chronic inflammatory response which increases inflammatory mediators, proteases and oxidative stress, all of which are thought to damage the lung. Oxidative stress is the dominant driver in this inflammatory process and in addition to cigarette smoke is also promoted by obesity and tissue hypoxia (63, 64). Oxidative stress occurs both locally in the lungs and systemically (65) and causes the circulating and intra-pulmonary leukocytes to increase the production of reactive oxygen species (ROS) (66).

Systemic inflammation

While COPD was traditionally considered to be a disease of the lungs, more recent research has led to the disease also being characterised as a systemic inflammatory disorder, indicated by the presence of elevated circulatory inflammatory biomarkers that are present even in periods of clinical stability (67-71). While a definitive COPD-specific biomarker is yet to be identified there are many local and general systemic inflammatory markers that are elevated in stable COPD (72-74). These include C-reactive protein (CRP), leukocytes (particularly neutrophils), fibrinogen (67, 69, 75, 76) and Interleukin (IL)-6 (1, 3). All have proven to be reliable as well as powerful diagnostic indicators for morbidity and mortality in COPD (75, 77). Reports that lung function (FEV_1) may be inversely related to CRP levels have also appeared in the literature (78-80). Furthermore, levels of these sytemic inflammatory biomarkers have been shown to become even higher during an acute exacerbation (67, 69).

The GOLD guidelines define an exacerbation as "An event in the natural course of the disease that is characterised by a sudden change in the patient's baseline dyspnoea, cough, and sputum that is beyond normal day-to-day variations" (81). Exacerbations become more frequent as COPD progresses (82). A history of exacerbations is used as a marker of disease prognosis in all stages of COPD with the risk of an exacerbation associated with smoking status (53).

Comorbidities and exercise intolerance

Poor regulation of the inflammatory process that leads to low-grade systemic inflammation in COPD is also linked to many of the comorbidities frequently associated with the disease (83-86). Comorbidities such as cardiovascular disease, diabetes, osteoporosis and geriatric cachexia are common in COPD and are known to have an inflammatory aetiology (87, 88).

More recently, physical activity was found to be a determinant of all-cause mortality in COPD (89) with higher levels of exercise correlated with fewer hospital admissions, better functional status and lower mortality (90-94). Prognosis can be predicted clinically by using a measure of exercise capacity (the six minute walking distance: 6MWD) as a predictor of future exacerbations and mortality (95-97). Increasing exercise performance and capacity is therefore an important target for therapeutic intervention in COPD.

Between 1990 and 2006 hospitalisation rates for COPD remained relatively stable (98) despite mortality rates for the disease having dropped by approximately half between 1980 and 2010 (99). The implication is that while management strategies designed to prolong life have improved, strategies designed to slow deterioration of the disease remain largely unsuccessful (100-103).

1.4 THE NEED FOR EARLY DIAGNOSIS

Despite increased awareness and improvements in diagnosis, COPD is still considered to be under-diagnosed and under-reported around the world. Although a large proportion of people that have COPD have been exposed to noxious agents such as cigarette smoke, only 50% of lifelong smokers end up developing the disease (35). As smoking is the greatest known risk factor for developing COPD later in life it seems appropriate to design a diagnostic assessment that includes alternative markers of susceptibility in the smoking population (104).

Diagnosing COPD begins with the presence of symptoms, meaning the disease has already begun to progress. There is need for more research focusing on earlier diagnosis which allows for earlier intervention. Reinstating the ‘at risk’ Stage 0 classification with

additional elements that improve the prognostic ability of the classification would increase the potential for slowing disease progression during the early stages of COPD when the greatest loss of lung function occurs.

A prospective study of participants comparing those considered to be 'at risk' of developing COPD and healthy controls considered GOLD Stage 0 to be a "subclinical disease" (105). They found that although spirometry measurements were worse, functional parameters such as dyspnoea, exercise capacity, oxygen saturation and quality of life were similar to those in the healthy population (105). While another study found that GOLD Stage 0 did have value as a screening tool and was associated with excess FEV_1 decline (133).

While only a small number of individuals in that study progressed from GOLD Stage 0 to GOLD Stage 1 (1.4%), it highlighted smoking as the major risk factor for developing COPD. The study also reported that improvement from GOLD Stage 0 to healthy was associated with smoking cessation, younger age and normal BMI, whereas persistent GOLD Stage 0 (39.8%) was associated with continued smoking, depressive symptoms, higher FEV_1 decline and metabolic syndrome in men and older age in women (106). Given these findings, there is support for reassessing the diagnostic criteria of GOLD Stage 0 and for reintroducing the classification to enable more accurate identification of those at risk of developing COPD later in life.

Although the use of systemic inflammatory biomarkers as early detectors of COPD is promising their value may be underutilised as assessment for COPD is usually not undertaken until a patient's symptoms are obvious. One possible solution may be to use these biomarkers as part of a screening process for all long-term smokers, given the higher probability they will go on to develop COPD. Notwithstanding the risk to smokers, the predictive value of this approach should include additional factors for people who are not smokers, as this disease also affects many non-smokers.

This approach may also have to take in to account a gap in the research with respect to investigating early detection of the disease. The majority of clinical trials to date have focused on moderate to severe COPD. This is particularly pertinent with respect to trials on exercise and inflammatory biomarkers. Investigating these measures in mild COPD will

improve our understanding of the inflammatory process during the earlier stages of the disease as well as the relationship between the inflammatory process, lung function and exercise capacity. Furthermore, as smoking is known to increase local and systemic inflammation in COPD, investigating inflammation in non-smokers may provide further insight into the value of using systemic inflammatory biomarkers as indicators of people at risk of developing COPD in the future.

1.5 AIMS OF THE THESIS

There are two aims of this thesis. The first is to evaluate the predictive capacity of systemic inflammatory biomarkers, lung function and exercise capacity in assessing current FEV_1 levels. The second aim is to identify the predictive capacity of these elements in assessing current FEV_1 levels, with a view to test these variables in a longitudinal study for their predictive capacity of future FEV_1 levels. The results from that study could then be used to develop a multivariate predictive model that assesses a patient's risk of developing COPD.

Chapter Two: Inflammation in COPD

2.1 BACKGROUND

From the perspective of inflammation, COPD is a chronic inflammatory disease of the lungs associated with exposure to cigarettes or other noxious particles and gasses (55, 81, 107). While the pathological changes associated with COPD can vary among individuals the main changes occur in the central and peripheral airways, lung parenchyma and pulmonary vasculature. The respiratory tract's response to these noxious stimuli is a normal inflammatory response that appears to be modified and exaggerated in some people, in particular those who go on to develop COPD. While smoking has been identified as the main risk factor associated with developing the disease other airborne particles also have the ability to contribute to the ongoing inflammatory process that leads to structural and functional changes in the airways and airflow limitation (59-62).

Genetic susceptibility can also influence disease development. It has been established that 1–2% of individuals who develop COPD have a genetically induced α-1 antitrypsin deficiency (108). While an estimated 50% of lifelong smokers develop COPD (35), only 15-30% of all smokers develop the disease (31) indicating an interplay between continuous smoking and the pathogenesis of COPD (62). The persistence of chronic inflammation after smoking cessation (57, 58) supports the view that an abnormal inflammatory reaction in response to smoking is the cause that leads to the development of COPD in genetically predisposed individuals.

2.2 RESPIRATORY INNATE IMMUNITY

Chronic lung inflammation is associated with an increase in the numbers of macrophages, neutrophils and lymphocytes across all stages of COPD (109, 110). Within the lung, exposure to inhaled noxious agents causes a chronic inflammatory response through activation of alveolar macrophages and epithelial cells (111). This causes them to release chemotactic mediators that perpetuate the inflammatory process by recruiting additional cells including neutrophils, monocytes and lymphocytes (particularly CD8+ T cells) (111).

Epithelial cells lining

Epithelial cells lining the trachea-bronchial tree are the initial cells to interact with inhaled substances. These cells are responsible for a number of actions. They act as a physical barrier to the outside air coming in (airway epithelium), they remove microbes and foreign particles through the muco-ciliary clearance mechanism and they produce antimicrobial and anti-inflammatory proteins (112, 113). Additionally, cytokines derived from these epithelial cells and host defense proteins have pro-inflammatory and immune-regulatory functions (114).

Ongoing irritation from smoking alters the architecture and function of the airway epithelium in other ways (115-118). It causes the cells to secrete inflammatory proteases and mediators including granulocyte-macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and interleukins (IL) IL-1b and IL-8 (119, 120) which activate fibroblasts and cause fibrosis (121-123). TGF-β in particular is believed to induce fibrosis in the small airways because of its ability to secrete connective tissue growth factor a mediator, which encourages collagen deposits (124-126).

Alveolar macrophages

Although the level of macrophages, neutrophils, T-lymphocytes and dendritic cells are related to the degree of airflow limitation (127-129) and are abnormally increased in all stages of COPD (40), the relationship between the inflammatory cell types and the mechanisms driving these increases remain unclear (56). Alveolar macrophages have been identified as playing a pivotal role in orchestrating the inflammatory response associated with COPD and have been linked to many features of the disease (130). Macrophages are situated on the surface of the respiratory epithelium, exposing them directly to the outside environment (130). They have a variety of defense functions that allow them to identify pathogenic material and engage in phagocytosis and apoptosis of cells (131).

Macrophages are believed to initiate the inflammatory process in COPD because they are potent producers of pro-inflammatory cytokines and chemotactic factors (including IL-1,

IL-6, IL-8, TNF-α, monocyte chemotactic peptide (MCP)-1 and leukotriene LTB-4) and because of their ability to respond to stimuli by secreting ROS, proteases, and proteins (109, 110, 132). In addition to the above secretions, the ability of macrophages to take up and release neutrophil elastase (NE) enables macrophages to breakdown a similar spectrum of proteins to that of neutrophils. Their chemotactic capacity also adds to the inflammatory process by promoting infiltration of leukocytes into damaged tissues (133). This secretion of chemotactic factors from macrophages is increased as a result of cigarette exposure. The enhanced release of chemo-attractants, particularly IL-8 and LTB-4 (134) facilitates neutrophil infiltration into the respiratory tract, which is thought to be a key driver in the development of COPD (135, 136). These effects are compounded by the fact that the number of macrophages are found to be 5-10 times higher in people with COPD (129).

Neutrophils

Neutrophils are frontline defensive cells that secrete inflammatory cytokines, antibacterial peptides, ROS, lipid mediators and proteases including NE, an enzyme that can degrade bacteria and proteins including collagen, elastin and a matrix of metallo-proteinase enzymes (MMP-8 and MMP-9) (137-139). These secretions are strongly linked to the destruction of lung tissue in emphysema and mucous metaplasia in chronic bronchitis (140). They have also been implicated as a cause of potent mucus production through the over-stimulation of sub-mucosal mucous glands and goblet cells (119, 141-144).

Proteolytic enzymes such as MMP-9 break down elastic tissue and contribute to the development of pulmonary emphysema (145, 146). Macrophages are also able to release NE which is taken-up from neutrophils, increasing their proteolytic potential and potentially causing secondary damage (133, 145-147).

Dendritic Cells

Dendritic cells form a dense network in the airways and lung parenchyma. They are located in close proximity to the outside environment which allows them to recognise and take up inhaled pathogenic substances (148, 149). They assist with adaptive immune

responses by migrating to lymphoid tissues carrying these pathogens. This activates B and T-lymphocytes (150), macrophages and neutrophils (151, 152).

Natural Killer Cells

Natural killer (NK) cells unlike other innate immune cells, do not respond to pathogens but instead detect host cells that are stressed or compromised (for example by infection or tumor) and assist in their destruction. NK cells secrete cytokines such as TNF-α, IL-12 and interferon gamma (IFN-γ) which mediate the inflammatory response to the compromised cell by activation of other immune cells. It is known that smokers have a reduction in NK cell activity with evidence that smoking inhibits production of IFN-γ, TNF-α, as well as the cytotoxic abilities of these cells (153).

The lung's innate immune system is comprised of the airway epithelial cell barrier, neutrophils, alveolar macrophages, dendritic cells and NK cells. It provides the first line of defense against microbes. Recent studies have indicated the role of inflammasome, a large intercellular multi-protein complex that is involved in innate immunity, in the response of the immune system to airway inflammation in COPD (154-156). While the level of many of the lung's innate immune defense cells are elevated in COPD evidence also suggests that they maybe suppressed in a smoking dependent manner (56). It is this defective innate immune response that predisposes individuals with COPD to respiratory infection. While the airways of healthy individuals are sterile, those with COPD are frequently colonized with bacteria such as Streptococcus pneumonia, Haemophilus influenzae and Moraxella catarrhalis. It has been reported that in stable COPD up to 30% of patients are colonized with pathogens while during an exacerbation this can rise to up to 50% (157).

This defective immune response may explain why patients with COPD frequently develop bacterial and viral infections, a common cause of exacerbations and a significant factor in disease progression (157). In an acute exacerbation patho-physiological pathways associated with stable COPD intensify as shown by raised levels of pulmonary and systemic inflammatory markers, lung hyperinflation and oxidative stress (66, 158-160)

2.3 ADAPTIVE IMMUNITY AND AUTOIMMUNITY

Chronic lung inflammation is present in mild to severe COPD (109, 110) with the number of circulating neutrophils remaining elevated even when the condition is stable (67, 70). Mechanisms other than chronic inflammation such as adaptive immune and autoimmune responses thought to be implicated in the development and progression of COPD.

The respiratory innate immune system is comprised of epithelial cells, macrophages, neutrophils, dendrites, and NK cells which mediate the initial defence against pathogens and are also able to activate cells that are part of the adaptive immune response. Adaptive immunity is made up of B-lymphocytes (that are able to produce antibodies) and T-lymphocytes such as CD8+ and CD4+ cells. The role of adaptive immunity in smokers with COPD is suggested by the presence of a specific inflammatory pattern in the respiratory tract of people with COPD. The pattern consists of CD8+ and CD4+ cells across all stages of COPD (161-163), while lymphoid follicles are found to contain B-lymphocytes and T-cells in the more severe stages of COPD (163).

Evidence has shown that COPD may also have an autoimmune component that plays a role in the pathogenesis of the disease through its contribution to inflammation in the respiratory tract even after smoking cessation (66, 67).

2.4 SYSTEMIC INFLAMMATORY BIOMARKERS

Our understanding of COPD has changed over the past ten years with the disease now being characterised by the presence of low-grade systemic inflammation, indicated by elevated levels of inflammatory biomarkers such as C-reactive protein (CRP), leukocytes, fibrinogen and interleukin 6 (IL-6) (67-70, 164). Systemic inflammation has also been identified as playing a role in exercise intolerance by inducing peripheral muscle wasting which can acts as a further deterrent to physical activity.

Although the pathophysiology underlying the disease process is reasonably well understood, the pathogenesis of the amplified inflammatory response, the systemic inflammation and the persistence of inflammation in the absence of noxious stimuli

(following smoking cessation) continue to remain unclear. In addition to the amplified inflammatory response, two other processes that may also contribute to the pathogenesis of COPD are oxidative stress and the imbalance between proteases and anti-proteases.

2.5 OXIDATIVE STRESS

In its response to cigarette smoke, NO_2, SO_2, particulates as well as inflammation itself, cause epithelial cells, macrophages, neutrophils and eosinophils to increase their production of ROS (165, 166). Oxidative stress occurs when the production of ROS exceeds the body's antioxidant capabilities. This process has been implicated as a potential amplifying mechanism in COPD making it central to the pathological processes that lead to damage in the lung (167, 168). ROS are natural byproducts of oxygen metabolism and include peroxides and oxygen ions. When in excess they result in damaging biological effects such as cell dysfunction and even cell death if the damage to cell DNA, proteins, lipids or enzymes is serious enough (166, 169-171).

2.6 PROTEASE AND ANTI-PROTEASE IMBALANCE

Imbalance between protease and anti-protease (a substance that inhibits the action of proteases), results from over-production of proteinases or underproduction of anti-proteases. Imbalance can also be triggered by the activation or inactivation of proteases and anti-proteases. Increased secretion of proteases is due to oxidative stress, which primes their release from both neutrophils (elastase, cathepsin G and proteinase-3) and macrophages (cathepsins B, L and S) as well as a variety of MMPs. Oxidation decreases and inactivates several anti-proteinases including secretory leukoproteinase inhibitor, α-1 antitrypsin and tissue inhibitors of MMPs and are all involved in the pathogenesis of COPD (172).

2.7 SUMMARY

The inflammatory processes that result from inhaling irritants such as cigarette smoke are thought to initiate and maintain the inflammatory response in the lungs. This results in systemic inflammation, skeletal muscle wasting and comorbidities (173). However, as non-smokers also develop COPD other factors must contribute to the pathogenesis of the disease.

In COPD the dominant driver of the inflammatory process is oxidative stress, which is promoted by cigarette smoke, obesity and tissue hypoxia (63, 64). Oxidative stress can occur both locally in the lungs and systemically and causes the circulating and intra-pulmonary leukocytes to increase production of ROS (65, 66).

CRP, an acute phase protein, is considered to be a marker of systemic inflammation and tissue damage. The exact starting point of the systemic inflammation in COPD is unknown. However, epithelial cells as well as circulating ROS, cytokines (TNF-α, IL-1, IL-6, IL-17) (78) and GM-CSF have the ability to elevate the level of CRP by stimulating the liver to increase hepatic production of CRP, fibrinogen and factor VIII or by stimulating the bone marrow to secrete additional inflammatory cells such as monocytes, leukocytes and platelets (172, 174) which can also cause systemic inflammation.

While systemic inflammatory biomarkers are produced by the liver in response to increased levels of cytokines and ROS (78), local production of CRP by monocytes and lymphocytes within the inflamed lung also occurs making it difficult to pinpoint the exact source that increases serum inflammatory markers (175). Indeed, many studies have tried to elucidate the direct cause of this systemic inflammation by focusing on the influence of the lung compared to other causes of systemic inflammation. These attempts have met with limited success. The concept of an ‘overspill effect’ of local lung inflammatory products into the systemic circulatory system during a stable state whilst controversial, is believed to be the primary mechanism causing elevated serum inflammatory biomarkers. Results from studies investigating this mechanism have been inconsistent and inconclusive with no correlation found between lung (sputum) and systemic (blood) levels of these markers (176). There is however evidence that movement of α-1 antitypsn and secretary

leukoprotease inhibitor does occur from the lungs to the blood in humans with COPD and lungs to the lymph in sheep (176).

Serum inflammatory biomarker levels were initially measured in patients with respiratory disease in the 1980's in an attempt to understand the association between smoking and lung function (177, 178). In 2004, Gan *et al* demonstrated that systemic inflammation was present in patients with stable COPD (67). This changed our understanding of the systemic nature of COPD and went some way to explaining the high prevalence of certain comorbidities.

Currently no COPD-specific inflammatory biomarkers have been isolated. However, many general systemic inflammatory markers such as CRP, leukocytes, fibrinogen (67, 69, 75) and interleukin-6 (IL-6) (1, 3) have been found to be powerful diagnostic tools for morbidity and mortality in COPD (75, 77). Levels of CRP, leukocytes and fibrinogen become even higher during an exacerbation (67, 69). An increased risk of a further exacerbation has also been associated with an increase in levels of these markers even in patients considered to be stable. This occurs regardless of the stage of the disease or whether there was a history of exacerbations (75).

In this thesis systemic inflammatory biomarkers were chosen to be measured as opposed to local lung measures to predict the risk of development of COPD for three main reasons. Firstly, systemic markers are more routinely tested therefore it is likely that the patient will have medical records that would include these levels. This makes it easier to compare past levels against current levels to see if they have altered. The second reason is because sputum measurement may be more difficult to collect than blood testing and maybe less accurate (not everyone has sputum). Finally, because all smokers are known to have inflammation in the lungs, local lung inflammation measured from sputum may not accurately identify the presence of COPD.

In COPD it appears that poor regulation of the inflammatory process leads to chronic low-grade systemic inflammation. This inflammation has been linked to comorbidities associated with COPD such as cardiovascular disease, diabetes, osteoporosis and geriatric cachexia (83-88, 179). Systemic inflammation in COPD also plays a role in exercise intolerance due to its ability to induce peripheral muscle wasting (180-182) and because of

its progressive effects on airflow obstruction, causing patients to become more sedentary (183). This makes exercise capacity a good measure for identifying those at risk of developing the disease, this will be evaluated chapter four. As a precursor to this evaluation the following chapter contains a review of the literature on the association between exercise and inflammatory biomarkers in COPD.

Chapter Three: Review of the literature

3.1 INTRODUCTION

The objective of this literature review is to investigate the association between exercise, stable COPD and systemic inflammatory biomarkers in the published literature.

The abnormally exaggerated inflammatory response to inhaled irritants in COPD (55, 72-74, 81, 107) has implications beyond airflow limitation due to fibrosis and remodelling of the airways and lung parenchyma (9, 59-66). In addition to being a chronic inflammatory disease of the lungs, COPD is now also considered a systemic inflammatory disease (67-71, 164, 184).

Clinically COPD presents as emphysema, chronic bronchitis or asthma with the main symptoms being declining lung function, increasing breathlessness, cough, sputum and chest tightness (185). Many comorbidities and systemic consequences of the disease such as cardiovascular impairment and skeletal muscle dysfunction (186) have an inflammatory aeitology and have been attributed to the systemic inflammation that also accompanies COPD (83-88, 173, 179, 186, 187) CRP levels in particular have been found to correlate with cardiovascular risk factors (88).

Furthermore, systemic inflammation plays a role in exercise intolerance by inducing peripheral muscle wasting (180-182, 188-190) and because of its progressive effects on airflow obstruction. Both of these reduce capacity for physical activity and cause a person to become more sedentary (183). This process can occur before COPD is even diagnosed as inactivity levels are more pronounced in people with only mild symptoms of dyspnoea but with low levels of oxygen diffusion and exercise capacity (191).

Muscle wasting is associated with an increase in the inflammatory biomarker TNF-α (188). The combination of inflammation and ROS provoking muscle breakdown is considered the most likely cause of this increase since COPD is strongly linked to an increase in oxidative stress (189, 190). Because TNF-α is able to increase hepatic

production of CRP, it is possible that muscle wasting may also influence systemic inflammation (78).

As COPD progresses, skeletal muscle wasting contributes to expiratory airflow limitation where airflow does not meet the increase in expiratory effort (192). This leads to air-trapping and hyperinflation (193). Expiratory flow limitation together with hyperinflation results in an increase in the effort required for ventilation during periods of higher demand such as when exercising. This produces exertional dyspnoea and fatigue, which curtail exercise performance and act as an additional factor in reducing exercise capacity (182, 193). Exercise-limiting dyspnoea prevents maximum exercise capacity from being achieved (194, 195). Evidence identifying chest wall compliance as a contributing factor in the aetiology of this exercise-limiting dyspnoea has been reported in the literature (196, 197).

Physical activity is a determinant of all-cause mortality in COPD (89) with higher levels correlated with fewer hospital admissions, better functional status and lower mortality (90-94). Prognosis can be predicted clinically by using a measure of exercise capacity (the six minute walking distance: 6MWD) as a predictor of future exacerbations and mortality (95-97). Improving physical activity and exercise capacity in patients with COPD is therefore an important target of therapeutic intervention.

While strategies designed to prolong life have improved, strategies designed to slow the progress of the disease remain largely unsuccessful, with pulmonary rehabilitation (PR) a key feature in this strategy (100-103). The utilisation of exercise as a therapy for COPD was first documented in 1952 by Alvan Barach, who found that two of his emphysemic patients had a reduction in exercise-induced dyspnoea following training that improved their exercise capacity (198). This ran contrary to the then current understanding that dyspnoea on exertion was a “major troubling symptom and avoiding dyspnoea was appropriate disease management” (198). Despite Barach’s finding, evidence of the beneficial effect of exercise training on COPD was not confirmed until forty years later (198).

The first report of a formalised PR program was published by Thomas Petty and his colleagues in the late 1960s. They reported that out of 124 patients with emphysema, 94 were deemed "better" following a program of exercise that showed improvements in exercise tolerance (199). Modern PR programs that include exercise as a core component are still modelled on Petty's original design. In 2000, a study was published showing PR resulted in fewer days in hospital and reduced exacerbations (200). More recently, lower levels of CRP and IL-6 have been reported in patients with COPD who have higher levels of walking (201).

Reports of a relationship between systemic inflammatory markers and the type and intensity of exercise training have begun to appear in the literature (202, 203), with evidence suggesting that biomarkers associated with chronic inflammation are reduced in people that are physically active as well as people that become physically active (87). Furthermore, other studies have shown that there is an inverse relationship between CRP and exercise capacity in those with (204) and without COPD (205).

Despite research showing that exercise reduces exercise intolerance, reverses muscle dysfunction and improves health related quality of life (206-208), the exact mechanisms that enables exercise to induce these beneficial and possibly anti-inflammatory effects in COPD it is yet to be established.

The aim of this literature review is to address this question by investigating the evidence of a link between serum inflammatory biomarkers, such as CRP and leukocytes, and exercise in individuals with COPD.

3.2 METHODS

Design

A literature search was conducted on all articles published between 1970 and November 2014 relating to exercise and the systemic inflammatory biomarkers C-reactive protein and leukocytes in stable COPD. Articles were excluded if the research was not on humans or if the participants had a history of exacerbation in the previous month.

Three separate searches were conducted using the following terms:

1. Chronic obstructive pulmonary disease, chronic obstructive lung disease, chronic airflow obstruction, chronic bronchitis, chronic asthma, chronic emphysema, obstructive lung disease.

2. Leukocytes, CRP.

3. Exercise.

Results from the three searches were then combined and the exclusion criteria applied.

Search Strategy

Searches were restricted to publications in English. The search for "COPD OR chronic obstructive lung disease" was limited to title only. MeSH and corresponding truncated terms were used with the operator "OR". The results from the two searches were combined using the term "AND".

The following keywords were searched:
'Chronic obstructive pulmonary disease' OR COPD, 'chronic obstructive lung disease' OR COLD, 'chronic airflow obstruction', 'chronic bronchitis', 'chronic asthma', 'chronic emphysema', 'obstructive lung disease' OR OLD were combined with "OR" and then with specific inflammatory biomarker terms: 'C-Reactive Protein' OR CRP and leukocytes OR leukocyte and white blood cell OR WBC. This list was then combined with AND exercise.

Information sources

Literature searches of all studies published up until November 2014, were conducted using Ovid MEDLINE, Cumulative Index to Nursing and Allied Health Literature (CINAHL).

Inclusion Criteria

The literature was confined to studies that were in English and published between 1970 and December 2014. Only studies relating to patients with stable COPD, that is no exacerbations in the previous month, were included. Studies had to include a description of serum levels of CRP or leukocytes in relation to exercise.

Exclusion Criteria

Exclusion criteria included animal studies, studies that included participants with an exacerbation in the previous month and studies that included participants with known comorbidities that had an inflammatory aetiology. Studies were assessed to confirm that FEV_1 values were consistent with a diagnosis of COPD, and if they did not meet this criteria they were removed.

Additionally, studies that only used sputum readings for CRP or leukocytes were excluded because this type of reading is a measure of local lung inflammation not necessarily systemic inflammation (176). For studies that included both sputum and serum inflammatory biomarkers, only the serum biomarker measurements were used. All review articles and studies that included pharmacological therapy as a primary intervention were also excluded because of the potential of these treatments to influence systemic inflammatory biomarkers (209-214).

Screening methods

Titles and abstracts were reviewed. Relevant articles were added from the reference lists from these original articles. Full-text articles were obtained for all studies that met the eligibility criteria.

Number of articles retrieved and number included in analysis

The combination of terms of COPD, inflammatory biomarkers and exercise, yielded 420 articles from the databases. The studies selected were imported into Endnote and the duplicates removed yielding a total of 28 articles (23, 180, 181, 202-204, 215-237). The abstracts from all of these articles were assessed by a single reviewer and grouped into categories represented in Figure 1 below.

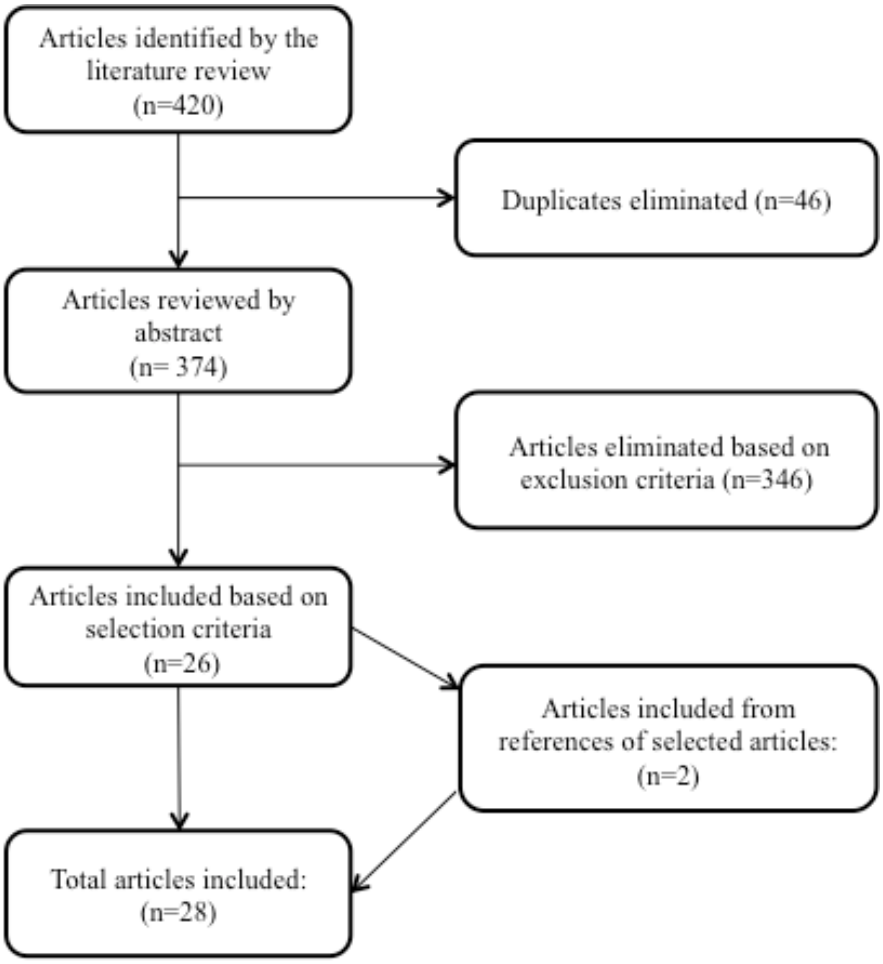


Figure 1- Articles identified from the literature review

Quality appraisal strategy

Full-text articles were reviewed by a single investigator. There was wide variability in the study designs, reporting methods used and results recorded in each of the studies, which meant they studies had to be grouped based on their different measurement methods and assessments performed. For example, measurements of biomarkers in relation to different exercise tasks were taken as either before and after measurements, or one off baseline measurements. This was further complicated by the different types and intensities of exercise tasks performed by the subgroups of this data. Papers were grouped into three categories based on the type and intensity of exercise described in the article (see Figure 2).

The three categories were:

1. Maximal exercise/sub-maximal exercise. Maximal exercise is exercise performed until exhaustion; sub-maximal exercise is exercise performed without exhaustion being reached and it is determined as a proportion of maximal exercise;

2. Low to moderate intensity exercise such as pulmonary rehabilitation (PR); or

3. Standardised exercise testing such as the 6 minute walking distance (6MWD), 12 minute walking distance (12MWD), long-distance corridor walk (LDCW), timed 400-metre walk test or short physical performance battery or daily step count.

Assessment and bias of associated instruments

A range of methods were used to test CRP and leukocyte levels across the studies. This may have been due in part to recent advances in testing technologies that have increased the sensitivity of biomarker testing, for example high sensitivity CRP (hs-CRP). As this technology was not available in the earlier studies, measurement of CRP levels may not have been as accurate. As mentioned previously, with regard to leukocyte levels only

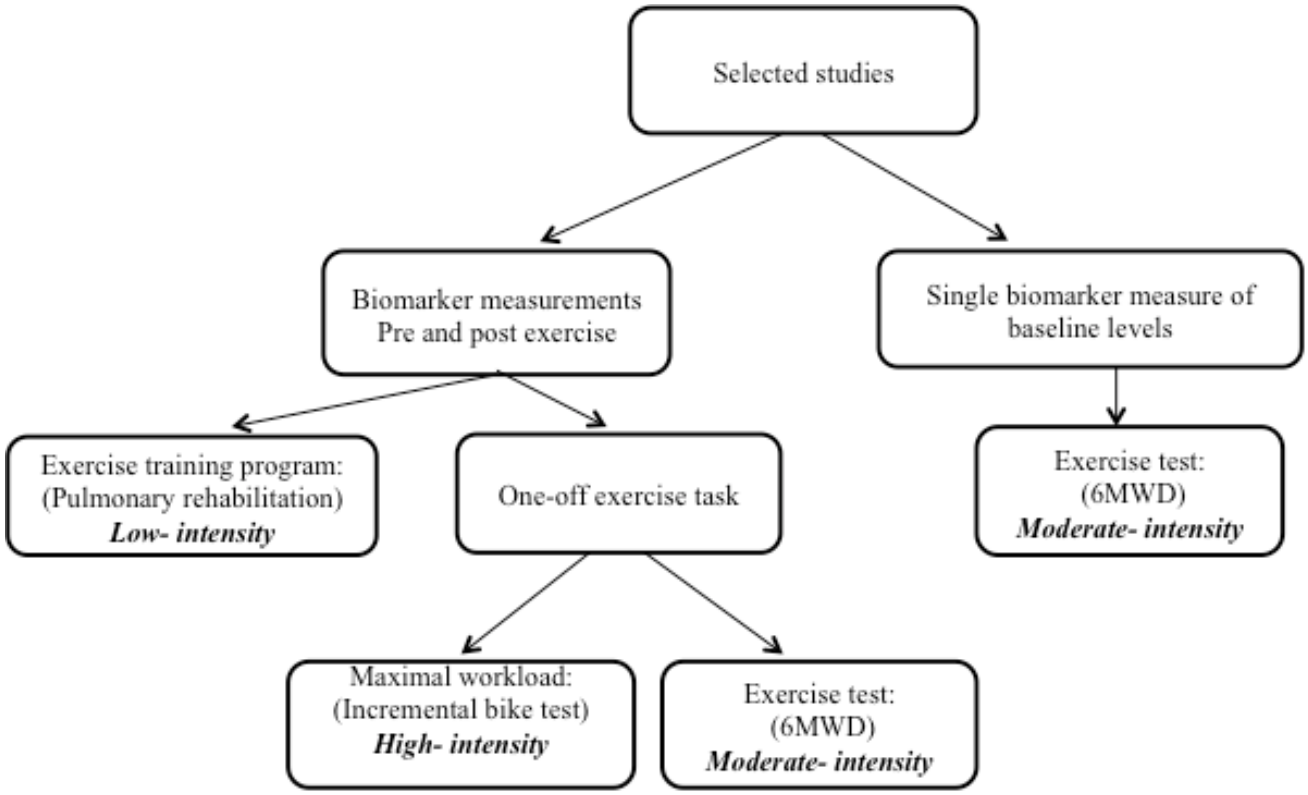
studies that measured serum leukocytes levels were included in the review as sputum leukocytes levels are not considered to be indicative of systemic inflammation levels.

Data description and associated extraction methods

After applying the exclusion criteria the combined results of the two searches were imported into Endnote and grouped into categories. Studies that performed exercise tests or pulmonary rehabilitation assessments were categorised into two groups based on the methodology of biomarker sampling. The first group measured associations with exercise, as these studies only presented baseline measurements. The second group measured the effect of exercise, by recording levels of inflammatory biomarkers before and after exercise.

Figure 2 describes how the studies were divided into two groups, with the first assessing the impact of exercise on levels of biomarkers ('Biomarker measurements Pre and post exercise'), and the second group assessing the association between exercise and biomarkers without investigating determinacy ('Single biomarker measure of baseline levels').

Analysis



Six minute walking distance; 6MWD

Figure 2- Study grouping based number of measurements, type and intensity of exercise

The studies were then further sub-categorised based on whether they used a one-off exercise test ('Maximal workload: Incremental bike test', 'Exercise test: 6MWD'), or a longitudinal exercise program ('Exercise training program: Pulmonary rehabilitation'). Intensity values ('Low-intensity', 'Moderate-intensity', 'High-intensity') of each training program were also assigned to these groups to facilitate comparison.

These groupings were then made into a table with categories, including: author and year of publication; number of subjects; exercise intensity or type of exercise test; what systemic inflammatory markers were recorded; and any association or responses these markers had with exercise (see Table 1).

3.3 RESULTS

Study selection and data abstraction

The first stage of the analysis was to determine the effect of different levels of exercise on CRP and leukocytes before and after intervention. This was achieved by analysing studies grouped under the 'maximal or sub-maximal exercise' and 'training program' groups. The third group, 'exercise tests', was not included in this analysis as exercise was not performed over a long enough period that would reliably reflect any change in biomarkers attributable to the exercise.

The second stage of the analysis was to determine if there was a relationship between exercise and CRP or leukocytes, by comparing results of measurements of biomarkers before and after exercise.

Study characteristics

Types and intensity of exercise included:

- Maximal and sub-maximal exercise.
-Training program and Training program with nutritional supplementation.
-Exercise tests (6MWD, 6MWD, 12MWD, LDCW, SPPB and daily step count).

Studies that included stable COPD mainly involved participants with mild to severe COPD. A small number of studies had specific sub-populations such as patients with muscle-wasting or participants with a previous exacerbation. In addition to this, although studies that used pharmacological therapy as a primary intervention were excluded, many of the studies included participants that were concurrently using statins and inhaled steroids. As both statins and inhaled steroids have been shown to have an anti-inflammatory effect in COPD their inclusion may have influenced the results of this review (209-214).

Quality assessment

All of the studies in the 'effects of exercise' category (single bout or training program) were of low quality with low sample sizes except the study by Broekhuizen et al. (216) which involved a doubled-blind randomised controlled trial (RCT) measuring the effect of exercise training and nutrition on COPD. Few studies had healthy controls as a comparator while the type and intensity of exercise varied making comparison between studies difficult. The configuration of the pulmonary rehabilitation programs also varied between trials. Some serum-marker measurements were taken immediately post exercise while others were measured at some later time. In summary, all of the 'effect of exercise' studies were considered to be of poor quality.

By comparison, studies in the 'associations with exercise' category were of better quality with larger sample sizes and comparable protocols. This made comparison between trials easier.

Table 1: Characteristics of studies (Part 1)

AFFECTS AND ASSOCIATIONS OF EXERCISE ON INFLAMMATORY BIOMARKERS (CRP and LEUKOCYTES)

EXERCISE GROUPING	STUDY: AUTHOR, YEAR		GROUP FEATURES	EXERCISE TYPE, INTENSITY	INFLAMMATORY BIOMARKERS RECORDED	RESPONSE OF BIOMARKERS TO EXERCISE TYPE
ACUTE BOUT OF EXERCISE	Canavan et al., 2007	17 (M/F) COPD 10 males, 7 females. Made up of 3 smokers, 8 ex-smokers and one- non smoker.	Bout, maximal-> then PR	CRP, IL-6, TNF-alpha	No changes to biomarkers
	Spruit et al., 2007	16- all stable (Stable grp 16 (14M), 14prior AE 14 (10M)-exacerbation grp. All had normal BMI, mm weakness and reduced peak ex capacity.	Bout, maximal cycling	CRP, IL-6, IL8, insulin-like growth factor 1	No changes to biomarkers
	Helvoort et al., 2006	10 muscle wasted COPD and 10 non-muscle wasted healthy	Bout, maximal +submaximal	CRP, leukocytes, cytokines...	Increase of IL6 for both groups
	Davidson et al., 2012	20 COPD	Bout, incremental	hsCRP,IL-6, IL-8,IL-10, WBC, neutrophils, leukocytes, monocytes ,basophils, eosinophils, CD4+, CD8+	Increase of WBC count, neutrophils, lymphocytes, monocytes and CD8+ (immediately following ex) Reduced CD4/CD8+ ratio, No change to CRP. because IL6, IL-8 reduced after exercise it suggest that a bout exercise has different response to an acute inflammatory exacerbation
	Helvoort et al., 2005	16 COPD, 10 healthy	Bout, maximal incremental (BIKE)	CRP, neutrophils, leukocytes, monocytes, lymphocytes (natural killer-cells, T and B lymphocytes	Increase of neutrophils, leukocytes, monocytes, lymphocytes, no changes to CRP (in both healthy and COPD)
	Helvoort et al., 2007	10 muscle wasted COPD	Bout (6MWT), submaximal	IL-6, leukocytes, neutrophils, monocytes ,lymphocytes (CD4+, CD8+)	increase of IL-6, leukocytes, neutrophils, monocytes ,lymphocytes (CD4+, CD8+)
	Helvoort et al., 2007	10 muscle wasted COPD	CPET (Bike) Maximal	" " "	increase of IL-6, leukocytes, neutrophils, monocytes ,lymphocytes (CD4+, CD8+)
	Koechlin et al.,2004	17 COPD	Maximal repetitive quadriceps test	CRP, IL-6 TNF	CRP, IL-6 TNF elevated at rest however no change in these markers following exercise; CRP inversely correlated exercise tolerance
EXERCISE TRAINING	Vogiatzis et al., 2007	15 COPD	high intensity 3x week for 10 weeks (PR)	CRP, IL6 and TNF-alpha	NO significant change to CRP, IL6 and TNF-alpha
	Spruit et al., 2005	78 COPD	6MWD, Peak exercise capacity (bike) 12 week program	CRP,TNF-alpha	No significant association or changes in CRP and TNF-alpha
	Petersen et al., 2008	19 severe COPD and 20 controls	Pulmonary rehabilitation-endurance training 2xweek for 7weeks	CRP, IL18	Training had no effects on CRP or IL-18
EXERCISE TRAINING and NUTRITIONAL SUPPLEMENTATION	Sugawara et al., 2010	32 COPD mod-severe	low intensity Ex(baseline-->3months after nutrition)	CRP, IL-6,TNF-alpha, IL-8	CRP, IL-6,TNF-alpha, IL-8
	Broekhuizen et al., 2005	80 COPD	submaximal and a incremental bike (exercise capacity) + 6MWT Placebo, double blind	CRP, IL-6,TNF-alpha (baseline-> 8weeks)	no change to CRP, IL-6, TNF-alpha; "therefore rehabilitation enhances the increase in exercise capacity which cannot be ascribed to changes in systemic inflammatory" response

Table 2: Characteristics of studies (Part 2)

AFFECTS AND ASSOCIATIONS OF EXERCISE ON INFLAMMATORY BIOMARKERS (CRP and LEUKOCYTES)

EXERCISE GROUPING	STUDY: AUTHOR, YEAR	GROUP FEATURES	EXERCISE TYPE, INTENSITY	INFLAMMATORY BIOMARKERS RECORDED	RESPONSE OF BIOMARKERS TO EXERCISE TYPE
EXERCISE TESTS for exercise capacity and physical performance/ Capacity (6MWT, 12MWT,LDCW, SPPB)	Ferrari et al., 2013	77 COPD	6MWT (physical performance) (baseline-> 3yrs)	CRP, IL-6	IL6 associated with reduced exercise tolerance
	Hallin et al., 2011	49 mod-severe COPD	12MWD (ex cap)- One off measurement	CRP and fibrinogen	no associations
	Yende et al., 2006	268 COPD	LDCW (exercise capacity)	CRP, IL-6 and TNF-alpha	IL-6 independent predictor for reduced exercise tolerance
	Brinkley at al., 2009	542 COPD and other comorbidities	SPPB (4min walk, repeated chair stands)	CRP, IL-6 and TNF-alpha	IL-6 associated with reduced ex tolerance and mortality
	Gorrod et al., 2007	41 COPD	6MWT (physical performance) one off	CRP, IL-6, TNF-alpha, neopterin	Ex cap inversely related to CRP and fibrinogen
	Broekhuizen et al., 2006	102 COPD	Bout -6MWT and maximal and sub maximal	hs CRP, IL-6	TNF and IL6 inverse associated with quad strength
	Pinto-Plata et al., 2006	88 COPD+33 smokers+38 non-smokers	6MWT (physical performance) one off	hs CRP	CRP negative correlation with 6MWT and CRP higher in COPD group(particularly mod-severe)-Elevated CRP and IL6 associated with poor physical performance (independent of disease status COPD or CHD)
	Garcia- Aymerich et al., 2009	341 COPD	6MWD	CRP TNF-alpha	regular physical activity previous to exacerbation had higher 6MWD and lower circulating CRP and TNF-alpha (specifically TNF better associated with 6MWD and CRP better associated with FEV)-- strong association between physical activity and exercise capacity (6MWD)
	Vilet et al., 2005	78 COPD 21 controls	6MWD	CRP	CRP inversely related to testosterone(which had an inverse relationship to exercise intolerance). Exercise capacity was more impaired in those with raised CRP
	Moy et al., 2014	171 COPD	6MWT and daily step count	CRP, IL6	Higher 6MWT inversely correlated with CRP and IL6, People who waked the most had lowest levels of plasma CRP and IL6.CRP higher in COPD group(particularly mod-severe)and associated with 6MWT
BODE SCORE (6MWD) DAILY STEP COUNT	Folchini et al., 2011	45 COPD	BODE (6MWD) + 6MWT	CRP	CRP negatively correlated with 6MWT CRP correlates better with BODE than 6MWT suggesting more associated with physical capacity
	Watz et al.,2008	170 COPD	BODE-exercise capacity and daily steps count (5days)-physical capacity	hs CRP and fibrinogen	higher fibrinogen and CRP and left cardiac dysfunction is associated with reduced physical capacity in patients with COPD, dependant of clinical stages(GOLD) or the BODE index.
	Lui SF., et al 2011	114 COPD	BODE score (6MWD- ex capacity) one off-longitudinal	CRP and fibrinogen	CRP and BODE are independent predictors for survival in COPD, higher CRP = poorer prognosis, combination has higher predictive value
	de Torres et al., 2008	218 COPD	BODE (6MWD)-longitudial	CRP	COPD (mod- severe) CRP levels not associated with with survival compared to BODE
	de Torres et al., 2006	130 COPD + 65 controls	BODE + 6MWD-longitudial	CRP	Strong inverse correlation between CRP and 6MWD, CRP correlates independently with BODE
	Gaki et al., 2011	222 COPD and 132 smokers/ex smokers	BODE (6MWD)	CRP, IL-6 and TNF-alpha	NO associations between CRP, IL-6 and TNF-alpha

Table 3: Summary of results (Part 1)

Study Ref	Source	COPD/Healthy	No. of Participants	FEV1 (% of predicted)	FEV/FVC	BMI	Age	6MWD (m)	CRP (microg/L)	FFMI	Maximal Workload (watts)	WBC (count pre nano Litre)
1	Canavan 2007	COPD	12	48.9	N/A	28.4	69.0	N/A	3.8	20.6	N/A	N/A
2	Spruit 2007	COPD	16	45.0	42.0	25.8	73.0	439.0	3.4	N/A	N/A	N/A
3	Van Helvoort 2006	Healthy	10	108.0	75.0	29.1	59.0	N/A	7.3	N/A	206.0	6.0
4	Van Helvoort 2006	COPD	10	54.0	43.0	26.7	66.0	N/A	10.8	19.7	106.0	9.2
5	Van Helvoort 2006	COPD	10	53.0	41.0	20.3	65.0	N/A	16.0	18.6	82.0	8.1
6	Davidson 2012	COPD	20	66.0	54.5	29.7	69.0	N/A	3.3	14.5	90.0	7.1
7	Van Helvoort 2005	Healthy	11	110.0	77.0	27.5	56.0	N/A	2.5	N/A	201.0	5.4
8	Van Helvoort 2005	COPD	16	42.0	39.0	25.3	60.0	N/A	10.0	N/A	90.0	7.6
9	Van Helvoort 2007	COPD	10	55.0	45.0	20.8	64.0	462.0	N/A	14.5	89.0	N/A
10	Koechlin 2004	Healthy	7	107.0	80.9	25.0	58.0	N/A	1.3	18.5	N/A	N/A
11	Koechlin 2004	COPD	10	41.0	51.0	26.0	60.0	N/A	9.3	18.8	N/A	N/A
12	Vogiatzis 2007	Healthy	10	94.7	77.0	28.8	61.0	N/A	1.5	N/A	N/A	N/A
13	Vogiatzis 2007	COPD	15	35.7	36.0	25.9	66.0	N/A	9.7	N/A	50.0	N/A
14	Spruit 2005	COPD	65	45.0	42.0	23.9	65.0	419.0	5.2	N/A	N/A	N/A
15	Peterson 2008	Healthy	20	96.0	77.0	25.0	64.0	N/A	3.0	N/A	N/A	N/A
16	Peterson 2008	COPD	19	31.0	56.0	25.0	66.0	N/A	7.1	N/A	N/A	N/A
17	Sugawara 2010	COPD	32	55.6	43.8	18.3	77.7	377.7	1.7	14.1	N/A	N/A
18	Matsuyama 2005	COPD	32	50.1	N/A	19.1	65.8	385.1	N/A	N/A	N/A	6.8
19	Matsuyama 2005	COPD	32	51.2	N/A	19.3	66.2	385.4	N/A	N/A	N/A	6.9
20	Broekhuizen 2005	COPD	51	38.2	49.2	22.5	64.0	N/A	7.8	16.1	N/A	N/A
21	Broekhuizen 2005	COPD	51	35.8	47.2	22.1	62.0	N/A	9.1	15.7	N/A	N/A
22	Yende 2006	COPD	268	65.1	N/A	25.4	73.6	N/A	3.5	N/A	N/A	N/A
23	Yende 2006	Healthy	2005	98.6	N/A	27.4	73.2	N/A	2.5	N/A	N/A	N/A

Table 4: Summary of results (Part 2)

Study Ref	Source	COPD/Healthy	No. of Participants	FEV1 (% of predicted)	FEV/FVC	BMI	Age	6MWD (m)	CRP (microg/L)	FFMI	Maximal Workload (watts)	WBC (count pre nano Litre)
24	Hallin 2011	COPD	27	31.0	N/A	20.7	64.0	N/A	4.9	N/A	59.5	N/A
25	Hallin 2011	COPD	22	32.0	N/A	26.4	64.0	N/A	6.6	N/A	73.2	N/A
26	Brinkley 2009	COPD	160	N/A	N/A	28.1	69.1	N/A	4.3	N/A	N/A	N/A
27	Broekhuizen 2006	COPD	54	35.9	36.0	21.9	61.3	355.0	1.5	15.8	63.0	N/A
28	Broekhuizen 2006	COPD	48	33.1	35.0	22.8	64.7	301.0	12.5	16.1	51.8	N/A
29	Pinto-Plata 2006	COPD	88	37.0	42.0	27.0	66.0	375.0	5.0	N/A	64.0	N/A
30	Pinto-Plata 2006	Healthy	33	91.0	73.0	26.5	62.0	546.0	2.0	N/A	119.0	N/A
31	Pinto-Plata 2006	Healthy	38	92.0	75.0	28.9	67.0	558.0	2.2	N/A	131.0	N/A
32	Vilet 2005	COPD	78	44.0	41.0	26.0	66.0	435.0	4.2	N/A	N/A	N/A
33	Vilet 2005	Healthy	21	108.0	76.0	26.0	63.0	679.0	N/A	N/A	N/A	N/A
34	Moy 2014	COPD	171	54.0	N/A	29.0	72.0	369.0	2.4	N/A	N/A	N/A
35	Watz 2008	Healthy	34	90.0	62.9	27.0	66.3	N/A	2.0	18.3	N/A	N/A
36	Watz 2008	COPD	57	63.0	53.2	27.8	63.3	N/A	3.0	18.6	N/A	N/A
37	Watz 2008	COPD	43	40.6	39.1	25.0	63.2	N/A	4.0	17.8	N/A	N/A
38	Watz 2008	COPD	36	32.8	36.2	24.6	63.7	N/A	2.1	17.4	N/A	N/A
39	Liu 2011	COPD	114	53.2	54.0	23.5	69.6	402.4	6.0	N/A	N/A	N/A
40	de Torres 2008	COPD	130	53.0	63.9	27.0	63.0	460.0	4.1	N/A	N/A	N/A
41	de Torres 2008	COPD	88	35.0	52.2	27.0	65.0	374.0	3.9	N/A	N/A	N/A
42	de Torres 2006	COPD	125	53.0	63.9	17.0	65.0	459.0	4.8	27.0	N/A	N/A
43	Gaki 2011	COPD	222	50.0	56.0	27.5	63.0	280.0	6.0	17.2	N/A	N/A
44	Gaki 2011	Healthy	132	94.0	82.0	28.0	60.0	490.0	2.0	20.6	N/A	N/A

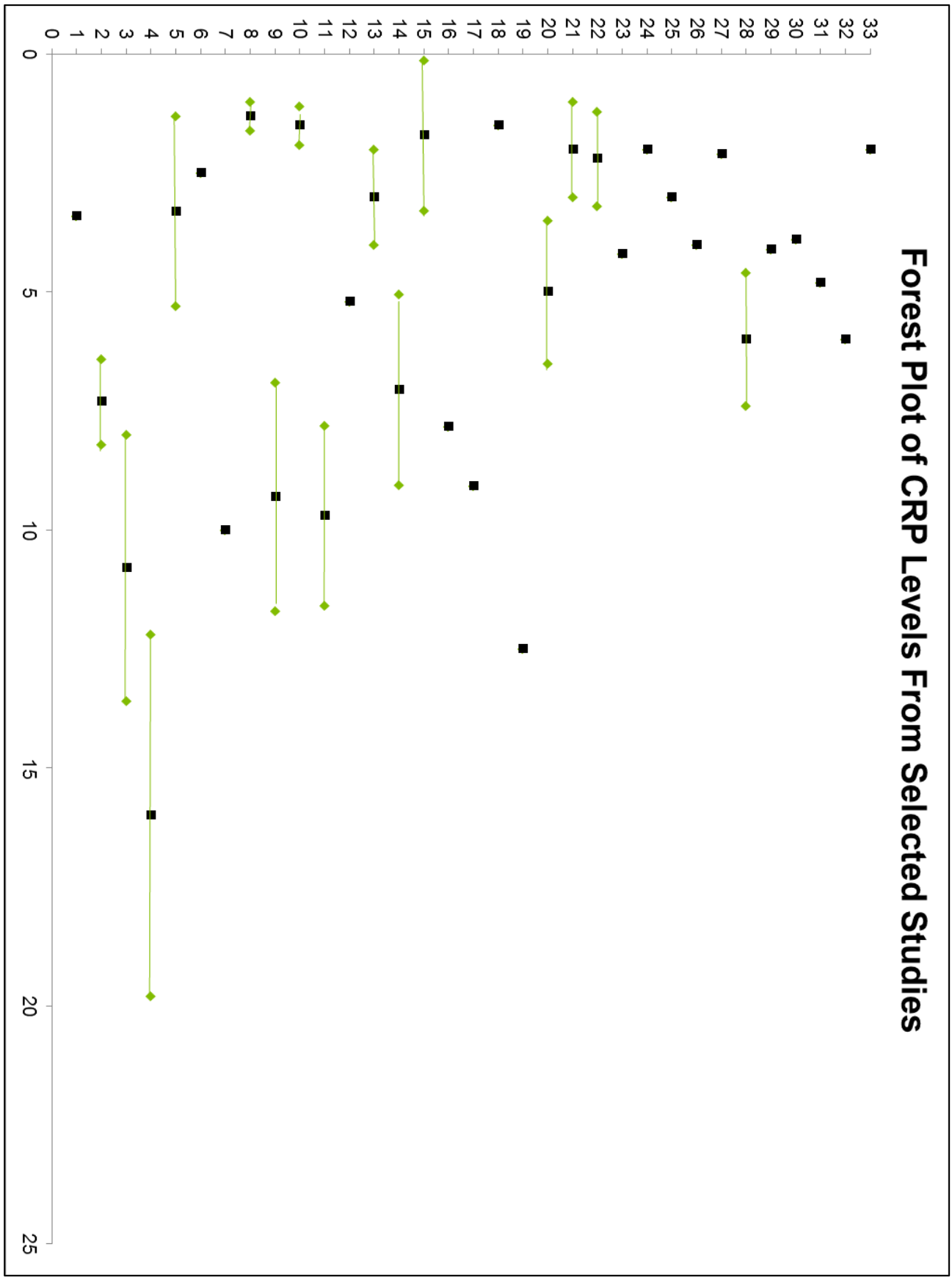


Figure 3: Forest plot

Table 5: Forest Plot data

Summary of COPD Data From Selected Studies

Test Group	Reference	N	Outcome	Lower	Mean CRP	Upper
1	Spruit 2007	16	COPD	N/A	3.4	N/A
2	Van Helvoort 2006	10	No COPD	6.4	7.3	8.2
3	Van Helvoort 2006	10	COPD	8	10.8	13.6
4	Van Helvoort 2006	10	COPD	12.2	16	19.8
5	Davidson 2012	20	COPD	1.3	3.3	5.3
6	Van Helvoort 2005	11	No COPD	N/A	2.5	N/A
7	Van Helvoort 2005	16	COPD	N/A	10	N/A
8	Koechlin 2004	7	No COPD	1	1.3	1.6
9	Koechlin 2004	10	COPD	6.9	9.3	11.7
10	Vogiatzis 2007	10	No COPD	1.1	1.5	1.9
11	Vogiatzis 2007	15	COPD	7.8	9.7	11.6
12	Spruit 2005	65	COPD	N/A	5.2	N/A
13	Peterson 2008	20	No COPD	2	3	4
14	Peterson 2008	19	COPD	5.1	7.1	9.1
15	Sugawara 2010	32	COPD	0.1	1.7	3.3
16	Broekhuizen 2005	51	COPD	N/A	7.8	N/A
17	Broekhuizen 2005	51	COPD	N/A	9.1	N/A
18	Ferrari 2013	24	COPD	-2.8	9.7	22.2
19	Ferrari 2013	53	COPD	-3.8	8	19.8
20	Broekhuizen 2006	54	COPD	N/A	1.49	N/A
21	Broekhuizen 2006	48	COPD	N/A	12.5	N/A
22	Pinto-Plata 2006	88	COPD	3.5	5	6.5
23	Pinto-Plata 2006	33	No COPD	1	2	3
24	Pinto-Plata 2006	38	No COPD	1.2	2.2	3.2
25	Vilet 2005	78	COPD	N/A	4.2	N/A
26	Folchini 2011	45	COPD	-8.3	7.7	23.7
27	Watz 2008	34	No COPD	N/A	2	N/A
28	Watz 2008	57	COPD	N/A	3	N/A
29	Watz 2008	43	COPD	N/A	4	N/A
30	Watz 2008	36	COPD	N/A	2.1	N/A
31	Liu 2011	114	COPD	4.6	6	7.4
32	de Torres 2008	130	COPD	N/A	4.1	N/A
33	de Torres 2008	88	COPD	N/A	3.9	N/A
34	de Torres 2006	125	COPD	N/A	4.8	N/A
35	Gaki 2011	222	COPD	N/A	6	N/A
36	Gaki 2011	132	No COPD	N/A	2	N/A

Table 1- 4 represent the collated data from all the studies in the literature review. Figure 3 and Table 5 represent the CRP data collated from each test group within the 27 studies, graphically represented in the forest plot above. The 27 studies resulted in 36 test groups, as some studies had multiple test groups.

Each selected group was classified as being designated as having COPD or not having COPD, as listed in the 'Outcome' column of Table 5. The figures listed under column 'N' represent the number of subjects in that test group. The column 'Mean CRP' represents the mean micrograms of CRP per litre for that test group, and the 'Lower' and 'Upper' columns represent one standard deviation less than and greater than the mean, respectively.

Some studies did not report CRP data standard deviations, preferring to report medians and interquartile ranges. In these cases, the standard deviations were reported presented as N/A in Table 5, such that the graph in Figure 3 will show a range of nil for these test groups.

It should also be noted that test group number 35 (Watz 2008) was classified as COPD GOLD I in the original study. However, this test group actually had an average FEV_1 of 90% predicted, and was classified as 'No COPD' in accordance with the classification criteria applied throughout this paper (FEV_1 < 70% of predicted; FEV_1/FVC < 70%) for these analyses.

Risk of bias

Selection bias was present in many of the studies as the groups were enrolled based on pre-determined features such as muscle wasting, severity of COPD and history of exacerbation (see Table 6). Inconsistencies between studies persisted as not all studies adjusted data for confounders such as medication use, smoking status, age, sex, obesity or muscle wasting. It is also possible that undiagnosed comorbidities with inflammatory aetiologies may have gone unreported in some of the trials. Assessment of the risk of bias found that most of the studies appeared to have a high rick of bias.

Table 6: Risk of Bias

STUDY	Allocation concealment (selection bias)	Random sequence generation (selection bias)	Group similarity at baseline (selection bias)	Timing of outcome assessments (detection bias)	Selective reporting (reporting bias)	Incomplete outcome data (attrition bias)	Compliance (performance bias)	Blinding of participants/ personel (Performance bias)	Intention-to-treat-analysis	Other bias	TOTAL
Caravel et al., 2007	-	-	+	N/A	-	-	-	+	+	?	3
Spruit et al., 2007	-	-	+	N/A	+	+	+	+	N/A	?	5
Helvoort et al., 2006	-	-	-	N/A	+	+	+	-	N/A	?	3
Davidson et al., 2012	-	-	-	+	+	+	+	+	N/A	?	5
Helvoort et al., 2005	-	-	-	-	+	+	+	+	N/A	?	4
Helvoort et al., 2007	-	+	-	-	+	+	+	-	N/A	?	4
Koechlin et al.,2004	-	-	-	N/A	+	+	+	+	N/A	?	4
Vogiatzis et al., 2007	-	-	+	-	+	+	+	+	N/A	?	5
Spruit et al., 2005	-	-	+	N/A	-	-	-	-	-	?	1
Petersen et al., 2008	-	-	-	+	+	-	+	?	N/A	?	3
Sugawara et al., 2010	-	-	+	+	+	?	+	-	N/A	?	4
Broekhuizen et al., 2005	+	+	+	+	+	-	N/A	+	+	?	7
Ferrari et al., 2013	-	+	-	+	+	-	-	+	-	?	4
Hallin et al., 2011	-	N/A	+	+	+	+	N/A	+	+	?	6
Yende et al., 2006	-	-	+	+	+	-	N/A	+	+	?	5
Brinkley at al., 2009	-	-	-	+	+	+	N/A	?	+	?	4
Gorrod et al., 2007	-	-	+	-	-	-	N/A	+	+	?	3
Broekhuizen et al., 2006	-	-	+	+	+	+	N/A	+	+	?	6
Pinto-Plata et al., 2006	-	-	-	+	+	+	N/A	-	+	?	4
Garcia- Aymerich et al., 2009	+	-	+	+	+	?	N/A	+	+	?	6
Vilet et al., 2005	-	-	-	+	+	?	N/A	-	+	?	3
Moy et al., 2014	-	-	+	+	-	-	+	+	+	?	5
Folchini et al., 2011	-	-	+	+	+	+	N/A	+	+	?	6
Watz et al.,2008	-	-	+	+	+	-	-	+	+	?	5
Lui FS., et al 2011	-	-	+	+	+	-	-	+	+	?	5
de Torres et al., 2008	-	-	+	+	+	-	-	+	+	?	5
de Torres et al., 2006	-	-	-	+	+	-	-	+	+	?	4
Gaki et al., 2011	-	-	-	+	+	-	N/A	+	+	?	4

An overall score of 6 or more indicates an overall low risk of bias for that study		
	+	Low risk of bias
	-	High risk of bias
	?	Unclear risk of bias
	N/A	Not applicable

3.4 DISCUSSION

The main findings from the analyses conducted on published studies investigating the link between serum inflammatory biomarkers and exercise in individuals with COPD showed a relationship between lung function and systemic inflammation, and lung function and exercise. CRP levels measured before and after exercise tests did not change, and therefore reflected that CRP was not influenced by exercise (202, 203, 217-219, 221). This contrasted with leukocyte levels, which were found to increase following maximal and sub-maximal exercise (203, 220). All studies that measured CRP consistently found that it was inversely related to FEV_1. CRP levels were originally measured in the literature because it has been demonstrated that exercise is found to have anti-inflammatory effects in healthy individuals as well as in those with comorbidities with an inflammatory aetiology, whereas in long-term training (such as pulmonary rehabilitation) no inflammatory or anti-inflammatory response to exercise has been reported (216, 225). However one of the studies did report that CRP levels reduced with pulmonary rehabilitation when accompanied by omega-3 and omega-6 supplementation (225).

An acute bout of exercise in COPD, as with in healthy individuals, has been shown to be pro-inflammatory provided the intensity is high enough (238, 239). Reports that an intense bout of either maximal or incremental exercise produced an immediate increase in serum levels of leukocytes, neutrophils, lymphocytes and monocytes (202, 203, 219) are contradicted by a report of no change in leukocyte levels following exercise (224). Helvoort *et al*., (220) reasoned that as baseline levels of leukocytes were already elevated in patients with COPD, the increase in leukocyte levels measured immediately following exercise could not be directly attributed to exercise.

A similar inconsistency has also been recorded for IL-6 in COPD where reports of no change in IL-6 levels following a single bout of maximal exercise (187, 217, 218, 221) are contradicted by reports of both a decrease (219) and an increase (202, 220) in levels. This inconsistency needs to be considered in light of reports that IL-6 levels increase in healthy subjects following maximal intensity exercise (245).

Given the ability of IL-6 to up-regulate hepatic production of CRP (240), an increase in IL-6 could be responsible for elevating the level of CRP. As intense exercise performed by otherwise healthy patients with muscle-damage is known to cause an increase in IL-6 (241), the link between it and exercise may take on greater importance in COPD where muscle wasting and dysfunction are a regular feature. This could partly explain the high levels of CRP commonly reported in COPD.

Furthermore, one of the studies reported that non-invasive ventilation (which has been reported to unload the respiratory muscles), completely abolished the increase of IL-6 following exercise in COPD patients, while healthy controls without ventilation continued to have increased IL-6 levels (236). This indicates that the respiratory muscles are implicated in the release of IL-6 in COPD patients during exercise, however this may also be influenced by other factors such as the hypothalamic pituitary adrenal axis. As respiratory muscles in people with COPD are constantly under load, this may indicate a potential mechanism for driving systemic inflammation in COPD as it can increase in hepatic production of CRP.

The association between acute exercise COPD and TNF-α is inconsistent. However this is also true for studies on healthy individuals. Three studies on patients with COPD found that levels of TNF-α were not changed by acute exercise (217, 218, 221). In the study performed by Canavan *et al.* (217), one patient that had a high level of TNF-α (following intense exercise) also had lower lean mass and nutrition compared to the control group that did not have depleted muscle mass. This suggests that muscle status may be an influencing factor in the inflammatory response to exercise in COPD. The association between loss of muscle mass (shown by reduced BMI and fat-free mass) and elevated TNF-α has been shown in other studies (242-244). These findings were further highlighted by Rabinvich et al. (187), who found that TNF-α levels were elevated in muscle-wasted COPD patients but not in healthy controls. This was supported by Edi *et al*., (244), who found that circulating TNF-α was higher in COPD patients with muscle depletion than COPD patients without muscle depletion. Yende et al. (180) similarly reported that COPD patients with lower quadriceps strength also had high TNF-α. These findings were later challenged by findings of muscle wasted patients that showed no change in quadriceps strength or TNF-α levels, however they did report an increase in ROS which is known to increase TNF-α levels (189, 190, 202).

Cytokine response to acute exercise is affected by age, gender (245), carbohydrate load (246) and timing of exercise relative to prior bouts of activity (247) in healthy people. In people with COPD, body mass composition was found to influence acute exercise-induced cytokine levels (248). These are all possible factors that may have influenced the above results of the literature review.

Exercise Training-

While CRP levels remain unchanged following exercise (202, 203, 217-219, 221), no inflammatory biomarkers were found to change following long-term exercise training (187, 222-224), apart from training with the addition of nutritional supplementation. Sugawara *et al*.(249) found that the combination of low intensity exercise with nutritional supplementation of polyunsaturated fatty acid (PUFA) improved exercise capacity and reduced the elevated levels of hs-CRP, IL-6, IL-8 and TNF-α compared to controls. A similar association was reported by Matsuyama *et al*. (250) who showed that supplementation with PUFA decreased both serum and sputum cytokines and improved exercise capacity, although CRP was not measured in that study. However, a double-blind RCT by Broekhuizen *et al*. (216) reported no change in inflammatory biomarkers following exercise with PUFA supplementation. They claimed that the beneficial effects of PUFA on exercise were not related to a reduction in systemic inflammatory cytokines.

Exercise Tests-

The BODE index, a clinical parameter that incorporates a multidimensional scoring system is a better predictor of severity in COPD compared to FEV_1 (15). In addition to this, CRP levels have been shown to be reliable predictors of mortality in stable COPD (23). Liu F.S *et al*. (23) found that both CRP levels and the BODE score were independent prognostic indicators for survival in stable COPD. By combining both measures, patients were able to be stratified into different mortality risk levels using a system that was shown to have a high predictive value in clinical practice. However, earlier studies gave conflicting results about the correlation of CRP and BODE index and their independent and combined ability to predict survival in COPD (234, 235).

In 2006, Torres *et al*. (204) reported that in patients with stable COPD, CRP levels were inversely correlated with FEV_1, 6MWD, BMI and scoring systems that included these measures such as the BODE index. Two years after their original study Torres *et al*. (234) reported that outcomes in patients with stable COPD could be predicted by BODE index and its components. The inverse association between CRP and 6MWD (204) was confirmed by two other studies by Pino-Plata *et al*. (229) and Folchini *et al*. (232).

An interesting element of this association is that there is a stronger correlation between CRP and the BODE index (which uses the 6MWD) than between CRP and the 6MWD itself, demonstrating that CRP is strongly associated with overall physical capacity rather than a single performance of physical activity. While there is a strong association between physical activity and exercise capacity in COPD patients (91, 230, 251), those who were more physically active had a reduced risk of elevated levels of CRP and TNF-α (230). Although exercise training programs were found to not influence CRP levels, it still remains that those that have higher physical activity levels also have lower levels of CRP and TNF-α, making it difficult to elucidate the effects of exercise on systemic inflammation in COPD. Furthermore, Moy *et al*. (201) reported that patients with COPD who had a higher daily step count had lower levels of CRP and IL-6 and that these biomarkers were also strongly associated with exercise capacity (6MWD) and endurance time (181, 221, 226).

A recent study measuring CRP and IL-6 levels at baseline and again after three years in people with COPD found IL-6 levels were raised and inversely correlated with 6MWD. However, no changes in CRP levels were reported (215), suggesting that systemic inflammation (demonstrated by elevated levels of IL-6) had a negative effect on physical performance.

In line with the above study are the results from Yende *et al*. (180) who found that IL-6 was predictive of exercise capacity in elderly patients with and without obstructive lung disease. Similarly, Brinkley *et al*. (227) showed an association between IL-6 and physical performance that was independent of race, gender, age and body composition in an elderly cohort with multiple comorbidities (including COPD). This indicates that IL-6 based systemic inflammation in COPD may be progressive, persistent and associated with reduced exercise tolerance over time.

Limitations

There are a number of limitations that need to be considered when assessing the results of this review. Pulmonary rehabilitation has only been included in COPD management guidelines since the 1990s. For a long time exercise was thought to have a negative effect on COPD with concern about its appropriateness given the presence of airflow obstruction. As a result, the body of evidence relating to the use of exercise as a therapeutic intervention for COPD spans a relatively brief period (only twenty-five years), making assessment of any long-term trends difficult. In addition, the use of inflammatory biomarkers is an even newer field of research in COPD. The combination of these two factors limits the scope of any conclusions.

Many of the 30 articles used in this review had variable sample sizes and included data that may have confounded the results, for instance participants with muscle wasting, variable smoking status, and gender imbalance (almost half the studies included only male participants). In addition to these, the instruments used to measure CRP levels varied over time with only a limited number of studies including a measure of high sensitivity CRP (hs CRP). The majority of the included studies were small with only 10 containing more than 100 participants. Many studies did not adjust for variables such as age, gender, medication use and smoking status, which was poorly defined and in some cases not even mentioned.

Significance of the study findings

The findings of this review indicate that more detailed research is required before any conclusion about the ability of exercise to influence inflammatory biomarkers in COPD can be made with confidence. The review highlighted the need for a trial for those at risk of developing COPD as the majority of trials have been on current COPD sufferers.

Generalisability

Despite statistically significant findings in some studies reviewed, the limited number of studies, small cohorts and frequently conflicting results reduce the reliability of the conclusions about the ability of exercise to influence leukocytes, TNF-α and IL-6. However, consistent reports of CRP levels remaining unchanged following exercise appear to be reliable. The review also highlighted that high intensity exercise can produce leukocytosis which may lead to an exacerbation, something that represents a deterioration in prognosis clinically.

Future research

With the burden of disease predicted to increase over the next 20 years, more research into the ability of exercise to reduce systemic inflammation in COPD is needed. Larger studies with lower levels of bias that include multi-dimensional scoring systems such as the BODE index may provide new approaches in disease management. Given the relatively short time frame that systemic inflammation has been associated with COPD, a longitudinal study investigating the long-term effects of exercise and systemic inflammation on those at risk of developing COPD is warranted.

Despite the limitations outlined in this literature review, the analysis is a valuable addition to the body of knowledge regarding the relationship between exercise and systemic inflammation in COPD.

3.5 CONCLUSIONS

Even though almost all of the studies used in this review had small sample sizes, it consistently appears as though CRP levels are not influenced by exercise of any type or intensity, for those with and without COPD. In spite of this, the literature confirms that physical activity levels are associated with a reduced risk of high levels of CRP. Additionally, given that CRP is inversely correlated with FEV_1 it is possible that improvements in lung function through non-pharmacological interventions such as manual therapy could influence CRP levels.

Chapter Four: Statistical Analysis

4.1 OBJECTIVE

The objective was to determine suitable potential determinants for testing in a future study that will ultimately be assessed for inclusion in a predictive model for patients at risk of developing COPD.

4.2 STATISTICAL METHODS

Following a review of the literature described in Chapter 3, data from twenty-seven of the studies identified in that review were selected and their data collated in preparation for meta-analysis. Sub-groups of participants were identified from these studies based on a range of attributes including demographics, smoking history, exercise capacity, maximal and sub-maximal exercise performance, lung capacity and performance, oxygen levels, body mass, BODE score, dyspnoea levels, C-reactive protein levels and levels of leukocytes.

Some studies presented data on comparison groups within the study (for example a group with COPD and a control group without COPD). In these cases, these distinct groups were recorded separately. This was done to better represent the differences in attributes between groups with different COPD outcomes, and to facilitate the collection of more data points.

In line with accepted clinical understanding of identification of COPD (Body-mass index, airflow Obstruction, Dyspnoea, and Exercise – BODE Index) (15), COPD is understood for the purposes of this study to be evident when an individual has an FEV_1 of less than 80% of predicted, and an FEV_1/FVC of less than 70%. There was one group of subjects in the Watz *et al* study (233) that had been classified as GOLD Stage 1 despite having an FEV_1 of 90% predicted and an FEV_1/FVC ratio of less than 0.7. For the purposes of the meta-analysis this group was deemed not to have COPD. All other groups were classified

as either having or not having COPD with no discrepancies between classifications applied in this study and those applied by the original researchers.

Two of the twenty-seven studies selected did not disclose the FEV_1 level as a percentage of predicted for the subjects, and were therefore not used in the final analysis. This was because this was the core statistic used to determine prevalence of COPD across the different populations, and the main statistic against which other variables were assessed for the impact as determinants.

The Broekhuizen 2005 study (216) performed measurements and undertook discussion on the CRP levels of its subjects, however the data was only presented numerically in graphical format. As the data was presented graphically to scale and with adequate granularity, an approximation was taken using graphical measurement aids, after which numerical data was gathered which was equivalently detailed as that present in numerical format in the other studies.

Nine statistical analyses were performed on the data. These are listed below-

a) Correlation of mean FEV_1 (% predicted) and mean CRP (micrograms /Litre);
b) Regression with mean CRP (micrograms per Litre) as the independent variable and FEV_1 (% predicted) as the dependent variable;
c) Correlation of mean FEV_1 (% predicted) and mean 6MWD (metres);
d) Regression with mean 6MWD (metres) as the independent variable and FEV_1 (% predicted) as the dependent variable;
e) Correlation of mean FEV_1 (% predicted) and mean maximal workload (watts);
f) Regression with mean maximal workload (watts) as the independent variable and FEV_1 (% predicted) as the dependent variable;
g) Correlation of mean FEV_1 (% predicted) and white blood count (count per nanolitre);
h) Regression with white blood count (count per nanolitre) as the independent variable and FEV_1 as the dependent variable;
i) Odds ratio of having COPD (as defined as an FEV_1 of less than 80% predicted and an FEV_1/FVC of less than 70%) based on a CRP threshold of more than or less than 3 micrograms per Litre.

Correlation Analyses (Pearson's coefficient)

The correlation analyses were performed to independently identify the Pearson correlation coefficient between the four variables (CRP, 6MWD, maximal workload, and WBC) and FEV_1 at a statistically significant level. These analyses were performed to identify the strength of the relationship when FEV_1 and each variable move together, such that when either moves the other can be said to move by a proportional amount. By identifying the strength of the correlation between the two FEV_1 and each variable, both can be assessed for their relative importance to each other in terms of ultimately developing a more accurate and timely predictive model for the development of COPD. The correlation tests were performed using the inbuilt 'Data Analysis' function within Microsoft Excel 2010, and was assessed from the output of the 'Regression' function with a significance level of 0.05. While the same results could be achieved using the 'CORREL' function, using the 'Regression' data analysis function facilitated assurance that the probability value (p-value) was below the 0.05 significance level. This significance level was chosen to simultaneously minimise Type I and Type II errors, and is a widely used significance level in statistical analysis of biomedical data.

Regression Analyses

The relationship between each of the four independent variables (CRP, 6MWD, maximal workload, and WBC) and the dependent variable (FEV_1) at a statistically significant level. These analyses were performed to identify the proportion of predicted movement in the dependent variable (FEV_1) from any change in the level of each independent variable. By understanding these statistically significant drivers of changes in the level of FEV_1, a more targeted approach to testing and developing a predictive model can be achieved. It should be noted that these analyses of dependence are between the independent variables and current FEV_1 levels in subjects. As the ultimate model intended to be developed from future studies is to predict future FEV_1 (and therefore a subject's likelihood of developing COPD in the future) from current independent variables, the understanding provided from these tests is one of preliminary assessment of the more direct relationship (current FEV_1 levels) underpinning the ultimate dependent variable (future FEV_1).

Due to the scattered nature of the data across the different test groups, a multivariate regression with all of the independent variables being assessed within the one test was not viable, as the number of studies that measured all of these variables was extremely limited. A multivariate regression that incorporated all of these four variables (CRP, 6MWD, maximal workload, and WBC) would need to draw from the same populations, to ensure the validity of the analysis.

Furthermore, the ultimate predictive model to be developed from future studies will not be employing current FEV_1 levels as the dependent variable, so in any case the value of this type of analysis would be limited. As such, the approach to assess each of the four variables independently of each other is the most appropriate.

The regression tests were performed using the inbuilt 'Data Analysis' function within Microsoft Excel 2010, and was assessed from the output of the 'Regression' function with a significance level of 0.05. This significance level was chosen to simultaneously minimise type I and type II errors, and is a widely used significance level in statistical analysis of biomedical data.

Odds Ratio

The odds ratio analysis, was designed to evaluate the ability of CRP, a key variable identified in this study, to predict FEV_1. It should be noted that current FEV_1 values were used despite the overall goal of predicting the likelihood of developing COPD in the future based on the current level of independent variables. This was due to the need to better understand the current drivers of COPD so that future research can be effectively targeted. An odds ratio was calculated so as to gauge the impact of CRP levels on COPD. A CRP level of 3 micrograms per Litre was chosen as the threshold for this analysis as it reflected the level identified in the literature as being associated with an increase in the prevalence of a number of chronic inflammatory diseases including COPD (254).

The odds ratio was calculated manually using Microsoft Excel 2010. As mentioned previously, one test group from the Watz *et al* study (233) was reclassified in this analysis as not having COPD. The risk factor in the odds ratio calculation was the threshold of

greater than or equal to a mean CRP of 3 micrograms per Litre for each test group (CRP≥ 3 micrograms/L). The outcome classification of the odds ratio calculation was therefore whether a test group had COPD or not, as defined by a mean (or median) FEV_1 of less than 80% of predicted, and a mean (or median) FEV_1/FVC of less than 70.

4.3 RESULTS

Nine separate statistical analyses were performed on the grouped data. Correlation and regression analyses were performed on the relationship between FEV_1 and the four variables CRP, White Blood Count (WBC), maximal workload, and 6MWD. The correlation and regression for each of the four variables were assessed together for each of the four variables below. The ninth analysis was an odds ratio centred on CRP levels.

Of the twenty-five studies selected for analysis, one (235) stipulated that the measurements of the airways (FEV_1, FEVL, and FVC) were performed after subjects had used bronchodilators. The exercise tests performed in this study (6MWD) and therefore the subsequent measurements were also post-bronchodilator. Although this was included in the analysis, it may be considered as a potential confounder.

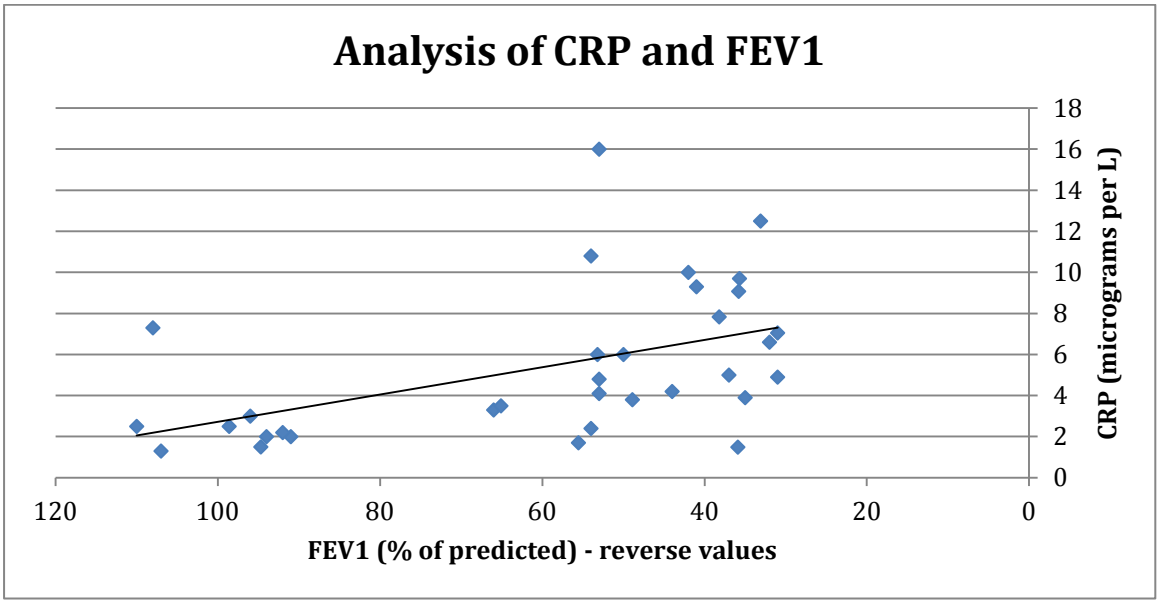
The 6MWD data presented in the Matsuyama (250) article was actually 6MWD results as a percentage of predicted, as opposed to metres achieved on actual tests performed. As outlined in Nury 2014 (252), the predicted 6MWD values are accurate estimates of actual performance achieved in healthy adults with similar demographic attributes. It was therefore necessary to determine predicted 6MWD values based on the average attributes of these test groups. It has been shown that equations for predicted 6MWD in Caucasian populations do not correlate well with other ethnic groups such as Asian populations (252) (253).

Two studies were identified that provide equations for predicted 6MWD results in Asian populations, being Nury and Poh (252, 254). Out of 9 studies reviewed by Dourado, Poh was the only study that did not find a statistically significant difference in 6MWD results between men and women, resulting in Poh's ultimate predicted 6MWD formula was not being gender-specific. Given that this result was an outlier when compared to the

prevailing literature, this approach and its associated formula were rejected. The formula presented by Nury for Asian populations was selected as it delineates between males and females and is purpose-built for Asian populations. The limitations of using Nury's formula were noted in that they were tested on populations much younger than the average age of the test groups in Matsuyama's study, and that they were based on Indonesian subjects as opposed to Japanese subjects. Despite these limitations, Nury's formulas presented the most accurate assessment of predicted 6MWD figures available, upon which the '% of predicted' results were converted back to metres to facilitate comparison to the other reviewed studies.

CRP and FEV_1

Thirty-six test groups were identified from the selected studies that contained sufficient data on FEV_1 and CRP levels to enable correlation and regression analyses Figure 4. A significance level of 0.05 was applied for both tests. The probability that the correlative and predictive relationships between CRP and FEV_1 can be explained by random chance was 0.004196, indicating a highly significant result. The Pearson correlation coefficient was 0.485348 which represented a moderate correlation, while the coefficient of determination in the regression was 0.235562 indicating that CRP is correlated with and is a statistically significant predictor of current FEV_1.

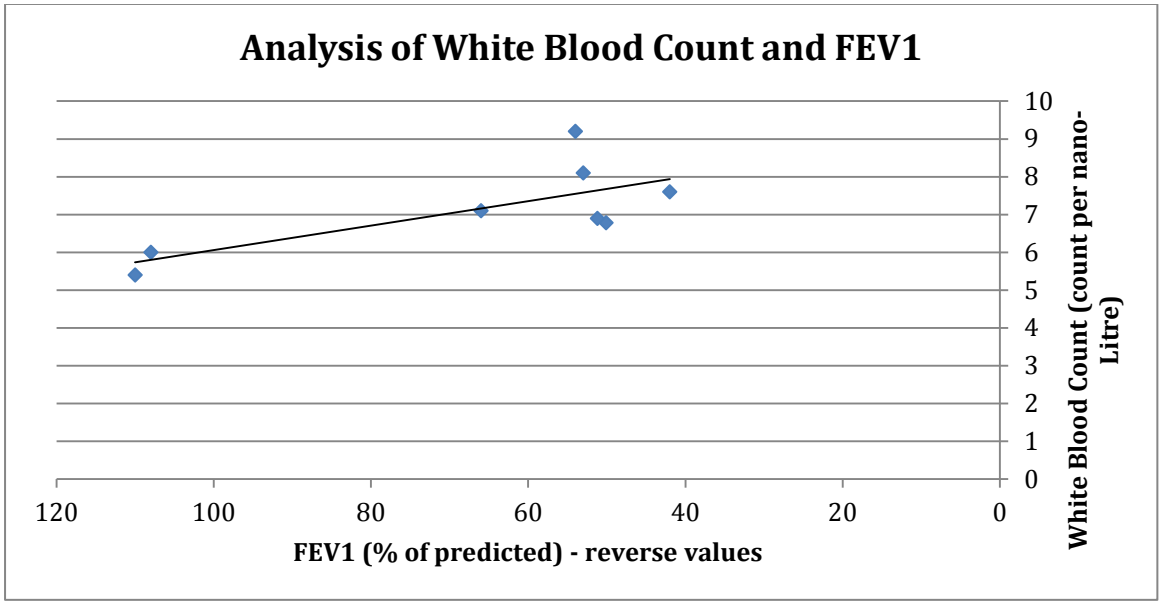


CRP: C-Reactive protein; FEV_1 % predicted: Forced expiratory volume % predicted

Figure 4: Analysis of CRP and FEV_1

FEV_1 and White Blood Count

Eight test groups were identified in the literature that contained sufficient data on FEV_1 and WBC levels to enable correlation and regression analyses see Figure 5. A significance level of 0.05 was applied for both tests. The probability that the correlative and predictive relationships between WBC and FEV_1 can be explained by random chance was 0.039598, indicating a statistically significant result. The Pearson correlation coefficient for these two data sets was 0.730448 representing a strong correlation. The coefficient of determination in the regression analysis was 0.533554 indicating that white blood count is strongly correlated with and is a strong predictor of current FEV_1.

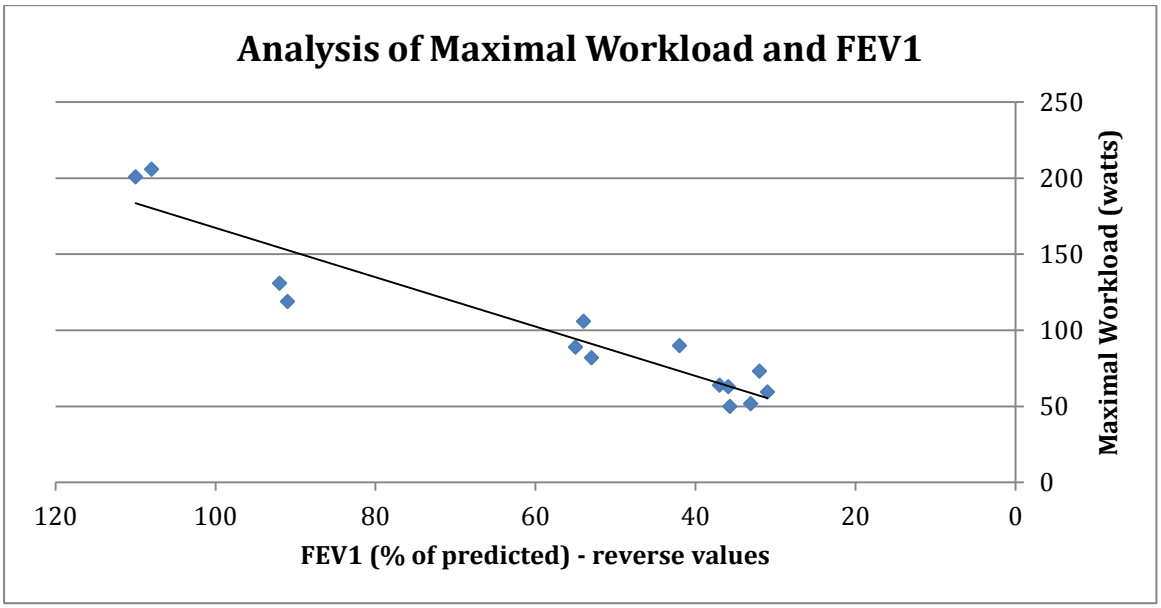


WBC: white blood cell count; FEV_1 % predicted: Forced expiratory volume % predicted

Figure 5: Analysis of White Blood Count and FEV_1

FEV_1 and Maximal Workload

Fourteen test groups were identified in the literature that contained sufficient data on FEV_1 and maximal workload to enable correlation and regression analyses see Figure 6. A significance level of 0.05 was applied for both tests. The probability that the correlative and predictive relationships between maximal workload and FEV_1 can be explained by random chance was less than 0.0000005, indicating a highly significant result. The Pearson correlation coefficient was 0.942346, representing a strong correlation. The coefficient of determination in the regression was 0.888016, indicating that maximal workload is strongly correlated with and is a strong predictor of current FEV_1.

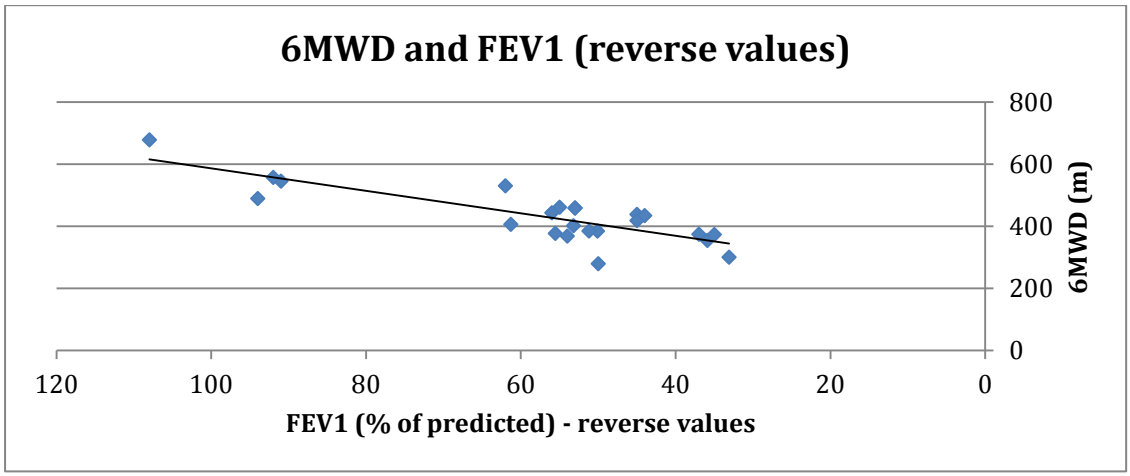


FEV_1 % predicted: Forced expiratory volume % predicted

Figure 6: Analysis of Maximal Workload and FEV_1

FEV_1 and 6MWD

Nineteen test groups were identified in the literature that contained sufficient data on FEV_1 and 6MWD to enable correlation and regression analyses see Figure 7. A significance level of 0.05 was applied for both tests. The probability that the correlative and predictive relationships between 6MWD and FEV_1 can be explained by random chance was 0.000003, indicating a highly significant result. The Pearson correlation coefficient was 0.857146 representing a strong correlation while the coefficient of determination in the regression of 0.734699, indicating that 6MWD is strongly correlated with and is a strong predictor of current FEV_1.



CRP: C-Reactive protein; FEV_1 % predicted: Forced expiratory volume % predicted

Figure 7: Analysis of 6MWD and FEV_1

Odds Ratio

Applying the risk factor of a CRP level of 3 micrograms per Litre to the twenty-seven test groups identified as suitable for the odds ratio analysis with COPD, nineteen test groups had a CRP level equal to or higher than this level and eight test groups had a CRP level lower than this. Applying the standard to eight healthy test groups, two had CRP levels equal to or greater than 3 micrograms per Litre while the remaining six groups had CRP levels below this (see Table 7). The resulting odds ratio was 26, indicating that from the studies analysed a test group with a CRP level equal to or above 3 micrograms per Litre was 26 times more likely to be a current COPD sufferer than a group with a CRP level below 3 micrograms per Litre. With a confidence interval of 95% the odds ratio calculation was statistically significant ($p<0.05$).

Table 7: Odds Ratio Analysis

	CRP<3	CRP>=3	Odds of Condition and <CRP=3:	Odds of Condition and >CRP=3:	Odds:
Healthy	6	2	0.75	0.25	3
COPD	2	17	0.11	0.89	0.12

Odds Ratio	26

Chapter Five: Discussion

5.1 DISCUSSION OF RESULTS

A meta-analysis of the results from studies previously published in the past twenty-five years on inflammatory biomarkers and exercise in people with COPD evaluated the relationship between specific inflammatory biomarkers, exercise capacity and lung function. The aim of the study was to confirm if any of the four variables tested (CRP, WBC, 6MWD and maximal workload) were strong predictors of current lung function (FEV_1). This process is the first step in developing a predictive model that incorporates inflammatory biomarkers as one of the components, a process that is set to continue in future studies.

The results from this study confirm that CRP, WBC, 6MWD and maximal workload are correlated with and are predictors of current lung function (FEV_1) in COPD. It is therefore reasonable to assume that when combined in a multivariate analysis, the same variables will also contribute to a strong predictive model for assessing future FEV_1 levels. These results confirm the appropriateness of including these variables in a longitudinal study that is designed to determine factors that can predict future FEV_1. If found to be reliable in that study they could be included in a predictive model for assessing those considered 'at risk' of developing COPD. The data from this study also enables a determination of the expected levels of biomarkers and exercise performance at different FEV_1 ranges (% predicted) for those with COPD as well as for healthy controls.

Our findings are consistent with previous studies that examined biomarker levels in people with COPD. Previous studies in the literature confirm the individual association of CRP, 6MWD, peak workload, and leukocytes and their associations with current FEV_1 levels. However, this meta-analysis spanned all FEV_1 % predicted ranges including above 80% *i.e.* the group 'at risk'. Although there are a number of studies that compare biomarker levels and FEV_1 in stable COPD, many of them did not include FEV_1 levels in the non-COPD range.

These results extend the literature by highlighting the relevance of assessing these markers in the earlier stages of the disease in a future study, prior to the development of COPD. The assessment derived from this proposed model could then be used as a basis for early intervention.

Results from healthy individuals and those with stable COPD provided sufficient data to enable the testing of a predictive model that could indicate the expected values of FEV_1 for people with COPD. From these linear graphs it was possible to identify abnormal levels of biomarkers and exercise capacity at high current FEV_1 levels.

The most striking result was that although 6MWD was more prevalent in the literature than maximal workload, its predictive ability of current FEV_1 levels was noticeably lower (R^2= 0.735 and R^2 = 0.888 respectively). 6MWD is also a feature of the BODE index and is generally more widely used as an indicator of exercise capacity. Maximal workload would also be expected to be a stronger predictor of future FEV_1 levels and will therefore be a better predictive tool for assessing the development of COPD than the 6MWD.

Perhaps the inclusion of peak workload performance is considered dangerous in a cohort of people with advanced COPD. This may possibly be the reason why 6MWD is more frequently used than maximal workload. However, in regards to assessing those who have not yet developed COPD, it would be suitable to use maximal workload as part of a screening tool for those considered to be 'at risk' (Stage 0) of developing COPD as their performance capabilities would be higher than those with more advanced COPD.

Despite the relationship being statistically significant CRP proved to be a less reliable predictor of current FEV_1 levels than initially expected (R^2 = 0.236). Therefore, in the predictive model it may be necessary to include CRP levels as part of a multivariate analysis to ensure the predictive model has the highest prognostic accuracy possible.

This study adds to the body of knowledge in the field because it is the first study to show that exercise capacity and systemic inflammatory biomarkers could potentially be used to predict whether those at risk of developing COPD whether due to genetic or environmental factors with an FEV_1 > 80% will develop the disease.

This is an important finding as systemic inflammation may begin before other symptoms of COPD such as cough or sputum manifest. Evidence already exists to show that manual therapy intervention increases exercise capacity and lung function in COPD (197). The presence of predictors such as CRP and leukocytes may also be useful as triggers for applying interventions designed to increase exercise capacity and reduce inflammation. This approach is currently being tested in trials designed to unload the respiratory muscles during exercise improving chest wall compliance through manual therapy intervention (personal communication).

The purpose of this study was to identify the relationship between current lung function and potential drivers of COPD such as CRP, WBC, maximal workload and 6MWD. As they were all shown to be predictors of current lung function they should be included in studies designed to test and develop a model to predict future lung function. Ultimately, a predictive model that incorporates these variables could help to identify those at risk of developing COPD in the future.

These results also inform the levels of CRP, WBC, 6MWD and peak workload expected in those who have an FEV_1 range above 80% predicted (*i.e.* non-COPD) thereby establishing levels in the group considered 'at risk' of developing COPD.

Using CRP levels and leukocyte counts is convenient as these biomarkers are routinely tested and would therefore have a history of previous readings for comparison over time.

Combining the level of biomarkers with exercise performance in individuals with risk factors such as smoking, cough, sputum and low levels of physical activity to predict whether an individual with an FEV_1 reading above 80% will go on to develop COPD in the future could be used by doctors as the threshold for early intervention.

Early detection of COPD is important because the greatest loss of lung function occurs in the earlier stages of the disease. The rationale for administering treatment earlier before a large proportion of lung function is lost enhances the potential to alter the typical pattern of loss of lung function associated with COPD progression. These results support the re-introduction of an 'at risk' COPD stage for people with a history of smoking, and/or respiratory disease and/or cough and sputum and a $FEV_1 > 80\%$. Combined with the

information provided in this thesis about the correlation between these factors and developing COPD an ‘at risk’ category would increase predictive ability.

The literature reports associations between biomarkers and lung function (COPD severity). Multidimensional scoring systems such as the BODE and ADO are comprised of signs and symptoms that manifest in the more advanced stages of the disease. Despite the independent predictive value of CRP to determine prognosis, there are no multi-dimensional measures that incorporate systemic inflammatory biomarkers. While some studies have found that combining the BODE index and CRP improves predictive power, these studies have only looked at the more advanced stages of COPD, leading to a call for the creation of a multidimensional score that is clinically relevant during the earlier stages of the disease (255-258).

Furthermore, as smoking is known to increase local and systemic inflammation in COPD, we recommend studying a cohort of smokers as this may provide new information about the value of using systemic inflammatory biomarkers as indicators of people ‘at risk’ of developing the disease.

Although the use of systemic biomarkers is promising with respect to detecting people at risk of developing COPD in the future their value may be underutilised, as assessing a patient for COPD is usually not undertaken until symptoms are present. One possible solution may be to include systemic inflammatory biomarkers as part of a screening tool for long-term smokers over the age of forty with FEV_1 above 80% who show symptoms such as cough and sputum. Routine biomarker testing could also be used in conjunction with other diagnostic measures such as the 6MWD and physical activity levels.

Given the probability that 50% of life-long smokers will go on to develop COPD (35) these measures could serve as an appropriate screening process for long-term smokers. However, the value of this approach may have to be considered for people with other aetiologies. It may be possible to adapt this approach to non-smokers by combining systemic inflammatory biomarker testing with using other measures such as history of respiratory illness and genetic testing.

5.2 LIMITATIONS

Given that the next stage in this work is to develop a multivariate model that can predict future FEV_1, it would have been ideal in this study to use a multivariate regression model with current FEV_1 as the dependent variable and CRP, WBC, 6MWD and peak workload as independent variables. However, filtering the studies that contained all of these data points resulted in a dataset that was too limited to provide statistically significant results. This outcome reinforces the need for future studies in the field.

There are a number of limitations that need to be considered in this study. The majority of studies that reported on the association between inflammatory biomarkers and exercise had small sample sizes and were of poor quality. Due to the size of the combined datasets, a multivariate regression analysis was not appropriate, requiring each variable to be assessed independently. These factors may have reduced the generalisability of the results.

The analyses may also have been influenced by the effect of a number of confounding variables. As smoking is known to influence the level of systemic inflammatory biomarkers such as CRP and leukocytes (46, 48, 50-52, 63), the absence of smoking status in some of the studies may have affected the results of the biomarker analyses. As smoking plays a role in the inflammatory process correlation with the level of inflammatory markers could act as an additional measure of its effect. Smoking has been associated with increased levels of inflammatory biomarkers in current smokers compared to those that have never smoked (45-49) while the number of cigarettes smoked per day has also been linked to increased levels of both CRP and leukocytes (46, 48, 50-52).

Obesity and low blood oxygen saturation (hypoxia) promote oxidative stress which has been associated with increasing systemic inflammation in COPD (259). The absence of both of these measures in all of the included studies may have confounded the reported levels of CRP and leukocytes because it prevented adjustment for them. Fat free mass index is a measure of muscle bulk and is an indicator of the level of muscle wasting, a common comorbidity in COPD. As muscle wasting has been associated with an increase in the production of TNF-α, this may be a potential confounder of systemic inflammatory biomarkers levels because of its ability to increase hepatic production of CRP and bone

marrow production of leukocytes. Medications such as statins and corticosteroids are commonly prescribed for conditions associated with COPD. We were unable to adjust for these due to incomplete medication history data, which may have confounded the results of our analyses as statins (212-214) and corticosteroids are known to influence systemic inflammatory biomarker levels (209-211).

While most studies attempted to exclude inflammatory comorbidities, the likelihood that people with undiagnosed inflammatory conditions were included in these studies is quite high given their prevalence in COPD (67, 173, 260-263). Furthermore, as cardiovascular disease is a common comorbidity of COPD and CRP levels are known to be increased in the presence of this disease, it is possible that our analyses of CRP may have been affected. In order to minimise the effect that exacerbations have on raising leukocyte levels only studies that included patients with stable COPD were selected.

Additionally, only some studies reported the use of a bronchodilator challenge when measuring lung function while others omitted reporting on this element altogether. Although the use of FEV_1 % predicted accounts for this difference, it is unclear whether 6MWD and maximal workload measures were affected by this factor as exertional dyspnoea (which is known to prevent maximal exercise capacity from being achieved) may have been influenced by the presence of a short-acting bronchodilator.

As a COPD-specific biomarker has not yet been identified in the literature, the effects of these confounders are unable to be fully adjusted for.

Chapter Six: Conclusion

Smoking is recognised as the main causative factor of COPD with estimates that tobacco use will be linked to 1 billion deaths by the end of the 21st century (42). There is therefore a need for more research into earlier diagnosis especially in people who are at risk of developing COPD later in life, previously referred to as GOLD 'Stage 0'. Earlier diagnosis enables interventions to be implemented which have the potential to influence morbidity and mortality rates, aimed at slowing the natural progression of the disease

In COPD, the inflammatory response to inhaled stimulants is exaggerated and the lung's defence capabilities suppressed. These abnormal responses persist even after removal of the stimulus and result in alterations to the repair mechanisms of the lungs. Oxidative stress is thought to be the dominant driver in this inflammatory process, which causes leukocytes to increase the production of reactive oxygen species both locally and systemically. The evidence for the concept of the 'overspill effect' where local lung inflammatory products spill over in to the systemic circulatory system is inconsistent and may not fully explain the primary mechanism behind elevated levels of serum inflammatory biomarkers.

While our understanding of COPD is rapidly changing, systemic inflammatory biomarkers offer a new possibility for assessing disease severity. However, they are yet to be adequately explored in those at risk of developing COPD as well as during the early stages of COPD. Results from a series of analyses on the relationship between systemic inflammatory biomarkers, lung function and exercise capacity show an inverse relationship between inflammatory biomarkers and current lung function. A future study is proposed in which these findings could be used to test and develop a predictive model for assessing those at risk of developing COPD.

References

1. World Health Organisation 2014 [cited 2014 20 April]. Available from: http://www.who.int/respiratory/copd/definition/en/.

2. Australia LF. The COPDX Plan: Australian and New Zealand guidelines for the management of chronic obstructive pulmonary disease 2014. Available from: http://www.copdx.org.au/the-copd-guidelines.

3. Petty TL. The history of COPD. Int J Chron Obstruct Pulmon Dis. 2006;1(1):3-14.

4. Christie RV. Emphysema of the Lungs-II. Br Med J. 1944;1(4334):143-6.

5. Richard Russell PF, Peter Barnes. Managing COPD: Springer Healthcare UK; 2011.

6. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med. 2001;163(5):1256-76.

7. DISEASE GIFCOL. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease 2010 [20 April 2014]. Available from: http://www.goldcopd.org/uploads/users/files/GOLDReport_April112011.pdf.

8. Evaluation IHME. Global Burden of Disease, Visualizations, GBD Arrow Diagram 2013. Available from: http://www.healthmetricsandevaluation.org/gbd/visualizations/gbd-arrow-diagram.

9. Celli BR, MacNee W, Force AET. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J. 2004;23(6):932-46.

10. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. American Journal of Respiratory & Critical Care Medicine. 2007;176(6):532-55.

11. Antonelli-Incalzi R, Imperiale C, Bellia V, Catalano F, Scichilone N, Pistelli R, et al. Do GOLD stages of COPD severity really correspond to differences in health status? Eur Respir J. 2003;22(3):444-9.

12. Yoon HI, Sin DD. Biomarkers of therapeutic response in patients with chronic obstructive pulmonary disease: a critical review of the literature. Drugs. 2011;71(14):1821-37.

13. Price DB, Tinkelman Dg Fau - Nordyke RJ, Nordyke Rj Fau - Isonaka S, Isonaka S Fau - Halbert RJ, Halbert RJ. Scoring system and clinical application of COPD diagnostic questionnaires. Chest. 2006;129(6):1531-9.

14. Puhan MA, Garcia-Aymerich J Fau - Frey M, Frey M Fau - ter Riet G, ter Riet G Fau - Anto JM, Anto Jm Fau - Agusti AG, Agusti Ag Fau - Gomez FP, et al. Expansion of the prognostic assessment of patients with chronic obstructive pulmonary disease: the updated BODE index and the ADO index. Lancet. 2009; 374(9691):704-11.

15. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(10):1005-12.

16. Cote CG, Celli BR. BODE index: a new tool to stage and monitor progression of chronic obstructive pulmonary disease. Pneumonol Alergol Pol. 2009;77(3):305-13.

17. Ong KC, Lu Sj Fau - Soh CS-C, Soh CS. Does the multidimensional grading system (BODE) correspond to differences in health status of patients with COPD? Int J Chron Obstruct Pulmon Dis. 2006;1(1):91-6.

18. Puhan MA, Garcia-Aymerich J, Frey M, ter Riet G, Anto JM, Agusti AG, et al. Expansion of the prognostic assessment of patients with chronic obstructive pulmonary disease: the updated BODE index and the ADO index. Lancet. 2009;374(9691):704-11. doi: 10.1016/S0140-6736(09)61301-5.

19. Kelly E, Owen CA, Pinto-Plata V, Celli BR. The role of systemic inflammatory biomarkers to predict mortality in chronic obstructive pulmonary disease. Expert Rev Respir Med. 2013;7(1):57-64.

20. Jones RC, Price D, Ryan D, Sims EJ, von Ziegenweidt J, Mascarenhas L, et al. Opportunities to diagnose chronic obstructive pulmonary disease in routine care in the UK: a retrospective study of a clinical cohort. Lancet Respir Med. 2014;2(4):267-76. doi: 10.1016/S2213-600(14)70008-6. Epub 2014 Feb 13.

21. Jenkins CR, Jones PW, Calverley PM, Celli B, Anderson JA, Ferguson GT, et al. Efficacy of salmeterol/fluticasone propionate by GOLD stage of chronic obstructive pulmonary disease: analysis from the randomised, placebo-controlled TORCH study. Respir Res. 2009;10:59.(doi):10.1186/465-9921-10-59.

22. Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, et al. Changes in forced expiratory volume in 1 second over time in COPD. N Engl J Med. 2011;365(13):1184-92.

23. Liu SF, Wang CC, Chin CH, Chen YC, Lin MC. High value of combined serum C-reactive protein and BODE score for mortality prediction in patients with stable COPD. Arch Bronconeumol. 2011;47(9):427-32.

24. US Department of Health and Human Services. The health consequences of smoking—50 years of progress: a report of the Surgeon General. Atlanta, GA2014. 355-91]. Available from: http://www.surgeongeneral.gov/library/reports/50-years-of-progress/full-report.pdf.

25. Goldberg R. Drugs Across the Spectrum. 5th ed ed. Belmont, USA: Thompson Wadsworth; 2006.

26. The Medical Research C. Tobacco Smoking and Lung Cancer. British Medical Journal. 1957;1(5034):1523-4.

27. Doll R. Tobacco: A medical history. Journal of Urban Health : Bulletin of the New York Academy of Medicine. 1999;76(3):289-313.

28. Burney LE. Smoking and lung cancer: A statement of the public health service. Journal of the American Medical Association. 1959;171(13):1829-37.

29. World Health Organization. Epidemiology of cancer of the lung. Report of a study group. World Health Organization technical report series 192. Geneva: World Health Organization, 1960.

30. Petty TL, Ryan SF, Mitchell RS. Cigarette smoking and the lungs. Relation to postmortem evidence of emphysema, chronic bronchitis, and black lung pigmentation. Arch Environ Health. 1967;14(1):172-7.

31. Fletcher C, Peto R. The natural history of chronic airflow obstruction. British Medical Journal. 1977;1(6077):1645-8.

32. Fletcher G PR, Tinker C, et al. The natural history of chronic bronchitis and emphysema. New York: Oxford; 1976.

33. World Health Organization. COPD management: World Health Organization; 2014. Available from: http://www.who.int/respiratory/copd/management/en/.

34. Lee PN, Fry JS. Systematic review of the evidence relating FEV_1 decline to giving up smoking. BMC Med. 2010;8:84.(doi):10.1186/741-7015-8-84.

35. Lundback B, Lindberg A, Lindstrom M, Ronmark E, Jonsson AC, Jonsson E, et al. Not 15 but 50% of smokers develop COPD?--Report from the Obstructive Lung Disease in Northern Sweden Studies. Respir Med. 2003;97(2):115-22.

36. Peto R, Speizer FE, Cochrane AL, Moore F, Fletcher CM, Tinker CM, et al. The relevance in adults of air-flow obstruction, but not of mucus hypersecretion, to mortality from chronic lung disease. Results from 20 years of prospective observation. Am Rev Respir Dis. 1983;128(3):491-500.

37. Anthonisen NR, Connett JE, Kiley JP, Altose MD, Bailey WC, Buist AS, et al. Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV_1. The Lung Health Study. JAMA. 1994;272(19):1497-505.

38. Willemse BW, Postma DS, Timens W, ten Hacken NH. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. Eur Respir J. 2004;23(3):464-76.

39. Xu X, Dockery DW, Ware JH, Speizer FE, Ferris BG, Jr. Effects of cigarette smoking on rate of loss of pulmonary function in adults: a longitudinal assessment. Am Rev Respir Dis. 1992;146(5 Pt 1):1345-8.

40. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, et al. Severity of airflow limitation is associated with severity of airway inflammation in smokers. Am J Respir Crit Care Med. 1998;158(4):1277-85.

41. van Schayck CP, Kaper J. Smoking and COPD: will they ever vanish into smoke? Prim Care Respir J. 2006;15(2):81-3. Epub 2006 Mar 9.

42. Laniado-Laborin R. Smoking and chronic obstructive pulmonary disease (COPD). Parallel epidemics of the 21 century. Int J Environ Res Public Health. 2009;6(1):209-24. doi: 10.3390/ijerph6010209. Epub 2009 Jan 9.

43. Anthonisen NR, Skeans MA, Wise RA, Manfreda J, Kanner RE, Connett JE. The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. Ann Intern Med. 2005;142(4):233-9.

44. Wagena EJ, Knipschild PG, Huibers MJ, Wouters EF, van Schayck CP. Efficacy of bupropion and nortriptyline for smoking cessation among people at risk for or with chronic obstructive pulmonary disease. Arch Intern Med. 2005;165(19):2286-92.

45. Yasue H, Hirai N, Mizuno Y, Harada E, Itoh T, Yoshimura M, et al. Low-grade inflammation, thrombogenicity, and atherogenic lipid profile in cigarette smokers. Circ J. 2006;70(1):8-13.

46. Hansen LK, Grimm RH, Jr., Neaton JD. The relationship of white blood cell count to other cardiovascular risk factors. Int J Epidemiol. 1990;19(4):881-8.

47. Hastie CE, Haw S, Pell JP. Impact of smoking cessation and lifetime exposure on C-reactive protein. Nicotine Tob Res. 2008;10(4):637-42. doi: 10.1080/14622200801978722.

48. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med. 2003;138(11):891-7.

49. Ohsawa M, Okayama A, Nakamura M, Onoda T, Kato K, Itai K, et al. CRP levels are elevated in smokers but unrelated to the number of cigarettes and are decreased by long-term smoking cessation in male smokers. Prev Med. 2005;41(2):651-6.

50. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107(3):499-511.

51. Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. Eur Heart J. 2005;26(17):1765-73. Epub 2005 Apr 7.

52. Rohde LE, Hennekens CH, Ridker PM. Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. Am J Cardiol. 1999;84(9):1018-22.

53. Bakhru A, Erlinger TP. Smoking Cessation and Cardiovascular Disease Risk Factors: Results from the Third National Health and Nutrition Examination Survey. PLoS Medicine. 2005;2(6):e160.

54. Asthana A, Johnson HM, Piper ME, Fiore MC, Baker TB, Stein JH. Effects of smoking intensity and cessation on inflammatory markers in a large cohort of active smokers. Am Heart J. 2010;160(3):458-63. doi: 10.1016/j.ahj.2010.06.006.

55. Groneberg DA, Chung KF. Models of chronic obstructive pulmonary disease. Respir Res. 2004;5:18.

56. Shaykhiev R, Crystal RG. Innate immunity and chronic obstructive pulmonary disease: a mini-review. Gerontology. 2013;59(6):481-9. doi: 10.1159/000354173. Epub 2013 Sep 3.

57. Ind PW. COPD disease progression and airway inflammation: uncoupled by smoking cessation. Eur Respir J. 2005;26(5):764-6.

58. Simmons MS, Connett JE, Nides MA, Lindgren PG, Kleerup EC, Murray RP, et al. Smoking reduction and the rate of decline in FEV(1): results from the Lung Health Study. Eur Respir J. 2005;25(6):1011-7.

59. Wouters EF. Local and systemic inflammation in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2005;2(1):26-33.

60. Barnes PJ. Mediators of chronic obstructive pulmonary disease. Pharmacol Rev. 2004;56(4):515-48.

61. Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2004;1(3):176-83.

62. Decramer M, Janssens W Fau - Miravitlles M, Miravitlles M. Chronic obstructive pulmonary disease. Lancet. 2012;379(9823):1341-51.

63. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest. 2007;131(5):1557-66.

64. Khan NI, Naz L, Yasmeen G. Obesity: an independent risk factor for systemic oxidative stress. Pakistan journal of pharmaceutical sciences. 2006;19(1):62-5.

65. MacNee W. Oxidants/antioxidants and COPD. Chest. 2000;117(5 Suppl 1):303S-17S.

66. McNicholas WT. Chronic obstructive pulmonary disease and obstructive sleep apnea: overlaps in pathophysiology, systemic inflammation, and cardiovascular disease. Am J Respir Crit Care Med. 2009;180(8):692-700.

67. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax. 2004;59(7):574-80.

68. Agusti A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. PLoS ONE. 2012;7(5):e37483.

69. Dickens JA, Miller BE, Edwards LD, Silverman EK, Lomas DA, Tal-Singer R, et al. COPD association and repeatability of blood biomarkers in the ECLIPSE cohort. Respir Res. 2011;12:146.

70. Zhang Y, Bunjhoo H, Xiong W, Xu Y, Yang D. Association between C-reactive protein concentration and chronic obstructive pulmonary disease: a systematic review and meta-analysis. J Int Med Res. 2012;40(5):1629-35.

71. Kostikas K. Systemic Biomarkers in the Evaluation and Management of COPD Patients: Are We Getting Closer to Clinical Application? Curr Drug Targets. 2013;14(2):177-91.

72. Nussbaumer-Ochsner Y, Rabe KF. Systemic manifestations of COPD. Chest. 2011;139(1):165-73. doi: 10.1378/chest.10-252.

73. Doyle TJ, Washko GR, Fernandez IE, Nishino M, Okajima Y, Yamashiro T, et al. Interstitial lung abnormalities and reduced exercise capacity. Am J Respir Crit Care Med. 2012;185(7):756-62. doi: 10.1164/rccm.201109-1618OC. Epub 2012 Jan 20.

74. Pinto-Plata V, Casanova C Fau - Mullerova H, Mullerova H Fau - de Torres JP, de Torres Jp Fau - Corado H, Corado H Fau - Varo N, Varo N Fau - Cordoba E, et al. Inflammatory and repair serum biomarker pattern: association to clinical outcomes in COPD. (1465-993X (Electronic)). Respir Res. 2012;13:71. doi: 10.1186/1465-9921-13-71

75. Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. Jama. 2013;309(22):2353-61.

76. Dentener MA, Creutzberg EC, Schols AM, Mantovani A, van't Veer C, Buurman WA, et al. Systemic anti-inflammatory mediators in COPD: increase in soluble interleukin 1 receptor II during treatment of exacerbations. Thorax. 2001;56(9):721-6.

77. Celli BR, Locantore N, Yates J, Tal-Singer R, Miller BE, Bakke P, et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;185(10):1065-72.

78. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. The Journal of clinical investigation. 2003;111(12):1805-12.

79. Margretardottir OB, Thorleifsson SJ, Gudmundsson G, Olafsson I, Benediktsdottir B, Janson C, et al. Hypertension, systemic inflammation and body weight in relation to lung function impairment-an epidemiological study. COPD. 2009;6(4):250-5.

80. Higashimoto Y, Iwata T, Okada M, Satoh H, Fukuda K, Tohda Y. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. Respir Med. 2009;103(8):1231-8.

81. (GOLD) GIFCOLD. Global Strategy for the Diagnosis, Management and Prevention of COPD 2014. Available from: http://www.goldcopd.org/guidelines-global-strategy-for-diagnosis-management.html.

82. Hoogendoorn M, Feenstra TL, Hoogenveen RT, Al M, Mölken MR-v. Association between lung function and exacerbation frequency in patients with COPD. International Journal of Chronic Obstructive Pulmonary Disease. 2010;5:435-44.

83. de Godoy I, Donahoe M, Calhoun WJ, Mancino J, Rogers RM. Elevated TNF-alpha production by peripheral blood monocytes of weight-losing COPD patients. Am J Respir Crit Care Med. 1996;153(2):633-7.

84. Fabbri LM, Rabe KF. From COPD to chronic systemic inflammatory syndrome? Lancet. 2007;370(9589):797-9.

85. Li YP, Schwartz RJ, Waddell ID, Holloway BR, Reid MB. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha. J Fed Am Soc Ex Biol. 1998;12(10):871-80.

86. McGarvey LP, John M, Anderson JA, Zvarich M, Wise RA, Committee TCE. Ascertainment of cause-specific mortality in COPD: operations of the TORCH Clinical Endpoint Committee. Thorax. 2007;62(5):411-5.

87. Flynn MGM, B. K. ; Markofski, M. M. State of the Art Reviews: The Anti-Inflammatory Actions of Exercise Training. American Journal of Lifestyle Medicine. 2007;1 (3):220-35

88. Ray KK, Cannon CP, Cairns R, Morrow DA, Rifai N, Kirtane AJ, et al. Relationship between uncontrolled risk factors and C-reactive protein levels in patients receiving standard or intensive statin therapy for acute coronary syndromes in the PROVE IT-TIMI 22 trial. J Am Coll Cardiol. 2005;46(8):1417-24.

89. Waschki B, Kirsten A, Holz O, Muller KC, Meyer T, Watz H, et al. Physical activity is the strongest predictor of all-cause mortality in patients with COPD: a prospective cohort study. Chest. 2011;140(2):331-42.

90. Moy ML, Matthess K, Stolzmann K, Reilly J, Garshick E. Free-living physical activity in COPD: assessment with accelerometer and activity checklist. Journal of Rehabilitation Research and Development. 2009;46(2):277-86.

91. Pitta F, Troosters T, Spruit MA, Probst VS, Decramer M, Gosselink R. Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;171(9):972-7. Epub 2005 Jan 21.

92. Watz H, Waschki B, Meyer T, Magnussen H. Physical activity in patients with COPD. Eur Respir J. 2009;33(2):262-72. doi: 10.1183/09031936.0024608. Epub 2008 Nov 14.

93. Garcia-Aymerich J, Lange P, Benet M, Schnohr P, Anto JM. Regular physical activity reduces hospital admission and mortality in chronic obstructive pulmonary disease: a population based cohort study. Thorax. 2006;61(9):772-8.

94. Garcia-Rio F, Rojo B, Casitas R, Lores V, Madero R, Romero D, et al. Prognostic value of the objective measurement of daily physical activity in patients with COPD. Chest. 2012;142(2):338-46.

95. Emtner MI, Arnardottir HR, Hallin R, Lindberg E, Janson C. Walking distance is a predictor of exacerbations in patients with chronic obstructive pulmonary disease. Respir Med. 2007;101(5):1037-40. Epub 2006 Nov 7.

96. Bowen JB, Votto JJ, Thrall RS, Haggerty MC, Stockdale-Woolley R, Bandyopadhyay T, et al. Functional status and survival following pulmonary rehabilitation. Chest. 2000;118(3):697-703.

97. Pinto-Plata VM, Cote C, Cabral H, Taylor J, Celli BR. The 6-min walk distance: change over time and value as a predictor of survival in severe COPD. Eur Respir J. 2004;23(1):28-33.

98. Australian Institute of Health & Welfare. Australia's Health: The eleventh biennial health report of the Australian Institute of Health and Welfare 2008.

99. Australian Institute of Health & Welfare. Australian Hospital Statistics 2005-2006. 2007 (in Health services series no. 30).

100. Pitta F, Troosters T, Probst VS, Langer D, Decramer M, Gosselink R. Are patients with COPD more active after pulmonary rehabilitation? Chest. 2008;134(2):273-80.

101.Laviolette L, Bourbeau J, Bernard S, Lacasse Y, Pepin V, Breton MJ, et al. Assessing the impact of pulmonary rehabilitation on functional status in COPD. Thorax. 2008;63(2):115-21.

102.Oga T, Nishimura K, Tsukino M, Hajiro T, Sato S, Ikeda A, et al. Longitudinal changes in health status using the chronic respiratory disease questionnaire and pulmonary function in patients with stable chronic obstructive pulmonary disease. Quality of Life Research. 2004;13(6):1109-16.

103.Bestall JC, Paul EA, Garrod R, Garnham R, Jones RW, Wedzicha AJ. Longitudinal trends in exercise capacity and health status after pulmonary rehabilitation in patients with COPD. Respir Med. 2003;97(2):173-80.

104.Vestbo J, Lange P. Can GOLD Stage 0 provide information of prognostic value in chronic obstructive pulmonary disease? Am J Respir Crit Care Med. 2002;166(3):329-32.

105.Komus N, Tertemiz KC, Sevinc C. [The importance of the at risk COPD patients (Stage 0) and clinical differences]. Tuberk Toraks. 2008;56(4):382-9.

106.Brito-Mutunayagam R, Appleton SL, Wilson DH, Ruffin RE, Adams RJ. Global Initiative for Chronic Obstructive Lung Disease stage 0 is associated with excess FEV(1) decline in a representative population sample. Chest. 2010;138(3):605-13. doi: 10.1378/chest.09-2607. Epub 010 Apr 23.

107.MacNee W. Pathogenesis of Chronic Obstructive Pulmonary Disease. Clinics in Chest Medicine. 2007;28(3):479-513.

108.Gooptu B, Ekeowa UI, Lomas DA. Mechanisms of emphysema in alpha1-antitrypsin deficiency: molecular and cellular insights. Eur Respir J. 2009;34(2):475-88. doi: 10.1183/09031936.0096508.

109.Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet. 2004;364(9435):709-21.

110.Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(26):2645-53.

111.Stockley RA, Mannino D, Barnes PJ. Burden and pathogenesis of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2009;6(6):524-6. doi: 10.1513/pats.200904-016DS.

112.Adler KB, Li Y. Airway epithelium and mucus: intracellular signaling pathways for gene expression and secretion. Am J Respir Cell Mol Biol. 2001;25(4):397-400.

113.Aarbiou J, Rabe KF, Hiemstra PS. Role of defensins in inflammatory lung disease. Ann Med. 2002;34(2):96-101.

114.Shaykhiev R, Otaki F, Bonsu P, Dang DT, Teater M, Strulovici-Barel Y, et al. Cigarette smoking reprograms apical junctional complex molecular architecture in the human airway epithelium in vivo. Cell Mol Life Sci. 2011;68(5):877-92. doi: 10.1007/s00018-010-0500-x. Epub 2010 Sep 6.

115.Auerbach O, Forman JB, Gere JB, Kassouny DY, Muehsam GE, Petrick TG, et al. Changes in the bronchial epithelium in relation to smoking and cancer of the lung; a report of progress. N Engl J Med. 1957;256(3):97-104.

116.Heijink IH, Brandenburg SM, Postma DS, van Oosterhout AJ. Cigarette smoke impairs airway epithelial barrier function and cell-cell contact recovery. Eur Respir J. 2012;39(2):419-28. doi: 10.1183/09031936.0193810. Epub 2011 Jul 20.

117.Kennedy SM, Elwood RK, Wiggs BJ, Pare PD, Hogg JC. Increased airway mucosal permeability of smokers. Relationship to airway reactivity. Am Rev Respir Dis. 1984;129(1):143-8.

118.Pilette C, Godding V, Kiss R, Delos M, Verbeken E, Decaestecker C, et al. Reduced epithelial expression of secretory component in small airways correlates with airflow obstruction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;163(1):185-94.

119.Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur Respir J. 2003;22(4):672-88.

120.Baraldo S, Turato G, Badin C, Bazzan E, Beghe B, Zuin R, et al. Neutrophilic infiltration within the airway smooth muscle in patients with COPD. Thorax. 2004;59(4):308-12.

121.Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, Rennard SI. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. Am J Respir Crit Care Med. 1997;155(5):1770-6.

122.Hellermann GR, Nagy SB, Kong X, Lockey RF, Mohapatra SS. Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. Respir Res. 2002;3(1):22.

123.Floreani AA, Wyatt TA, Stoner J, Sanderson SD, Thompson EG, Allen-Gipson D, et al. Smoke and C5a induce airway epithelial intercellular adhesion molecule-1 and cell adhesion. Am J Respir Cell Mol Biol. 2003;29(4):472-82. Epub 2003 Apr 24.

124.Bartram U, Speer CP. The role of transforming growth factor beta in lung development and disease. Chest. 2004;125(2):754-65.

125.Ihn H. Pathogenesis of fibrosis: role of TGF-beta and CTGF. Curr Opin Rheumatol. 2002;14(6):681-5.

126.Chen G, Grotendorst G, Eichholtz T, Khalil N. GM-CSF increases airway smooth muscle cell connective tissue expression by inducing TGF-beta receptors. Am J Physiol Lung Cell Mol Physiol. 2003;284(3):L548-56. Epub 2002 Dec 6.

127.Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;157(3 Pt 1):822-6.

128.Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, et al. Airways obstruction, chronic expectoration, and rapid decline of FEV_1 in smokers are associated with increased levels of sputum neutrophils. Thorax. 1996;51(3):267-71.

129.Finkelstein R, Fraser RS, Ghezzo H, Cosio MG. Alveolar inflammation and its relation to emphysema in smokers. Am J Respir Crit Care Med. 1995;152(5 Pt 1):1666-72.

130.Shapiro SD. The macrophage in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1999;160(5 Pt 2):S29-32.

131.Shaykhiev R, Krause A, Salit J, Strulovici-Barel Y, Harvey BG, O'Connor TP, et al. Smoking-dependent reprogramming of alveolar macrophage polarization: implication for pathogenesis of chronic obstructive pulmonary disease. J Immunol. 2009;183(4):2867-83. doi: 10.4049/jimmunol.0900473. Epub 2009 Jul 27.

132.Boyle JJ. Macrophage activation in atherosclerosis: pathogenesis and pharmacology of plaque rupture. Current Vascular Pharmacology. 2005;3(1):63-8.

133.Kumagai K, Ohno I, Okada S, Ohkawara Y, Suzuki K, Shinya T, et al. Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma. J Immunol. 1999;162(7):4212-9.

134.Traves S, Culpitt S, Russell R, Barnes P, Donnelly L. Increased levels of the chemokines GROα and MCP-1 in sputum samples from patients with COPD. Thorax. 2002;57(7):590-5.

135.Tanino M, Betsuyaku T, Takeyabu K, Tanino Y, Yamaguchi E, Miyamoto K, et al. Increased levels of interleukin-8 in BAL fluid from smokers susceptible to pulmonary emphysema. Thorax. 2002;57(5):405-11.

136.Di Stefano A, Maestrelli P, Roggeri A, Turato G, Calabro S, Potena A, et al. Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. Am J Respir Crit Care Med. 1994;149(3 Pt 1):803-10.

137.Hiemstra PS, van Wetering S, Stolk J. Neutrophil serine proteinases and defensins in chronic obstructive pulmonary disease: effects on pulmonary epithelium. Eur Respir J. 1998;12(5):1200-8.

138.Dubravec DB, Spriggs DR, Mannick JA, Rodrick ML. Circulating human peripheral blood granulocytes synthesize and secrete tumor necrosis factor alpha. Proc Natl Acad Sci U S A. 1990;87(17):6758-61.

139.Tetley TD. New perspectives on basic mechanisms in lung disease. 6. Proteinase imbalance: its role in lung disease. Thorax. 1993;48(5):560-5.

140.Stockley RA. Neutrophils and the pathogenesis of COPD. Chest. 2002;121(5 Suppl):151S-5S.

141.Witko-Sarsat V, Halbwachs-Mecarelli L, Schuster A, Nusbaum P, Ueki I, Canteloup S, et al. Proteinase 3, a potent secretagogue in airways, is present in cystic fibrosis sputum. Am J Respir Cell Mol Biol. 1999;20(4):729-36.

142.Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. J Clin Invest. 1990;85(3):682-9.

143.Barnes PJ. Mechanisms in COPD: differences from asthma. Chest. 2000;117(2 Suppl):10S-4S.

144.Bucchioni E, Kharitonov SA, Allegra L, Barnes PJ. High levels of interleukin-6 in the exhaled breath condensate of patients with COPD. Respir Med. 2003;97(12):1299-302.

145.Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, et al. Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases. Am J Physiol Lung Cell Mol Physiol. 2002;283(4):L867-73.

146.Punturieri A, Filippov S, Allen E, Caras I, Murray R, Reddy V, et al. Regulation of elastinolytic cysteine proteinase activity in normal and cathepsin K-deficient human macrophages. J Exp Med. 2000;192(6):789-99.

147.O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. Thorax. 2006;61(5):448-54.

148.Holt PG, Stumbles PA. Regulation of immunologic homeostasis in peripheral tissues by dendritic cells: the respiratory tract as a paradigm. J Allergy Clin Immunol. 2000;105(3):421-9.

149.Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, et al. The plasticity of dendritic cell responses to pathogens and their components. Science. 2001;294(5543):870-5.

150.Van Pottelberge GR, Bracke KR, Demedts IK, De Rijck K, Reinartz SM, van Drunen CM, et al. Selective accumulation of langerhans-type dendritic cells in small airways of patients with COPD. Respir Res. 2010;11:35.(doi):10.1186/465-9921-11-35.

151.Robbins CS, Dawe DE, Goncharova SI, Pouladi MA, Drannik AG, Swirski FK, et al. Cigarette smoke decreases pulmonary dendritic cells and impacts antiviral immune responsiveness. Am J Respir Cell Mol Biol. 2004;30(2):202-11. Epub 2003 Aug 14.

152.Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000;18:767-811.

153.Mian MF, Lauzon Nm Fau - Stampfli MR, Stampfli Mr Fau - Mossman KL, Mossman Kl Fau - Ashkar AA, Ashkar AA. Impairment of human NK cell cytotoxic activity and cytokine release by cigarette smoke. J Leukoc Biol. 2008;83(3):774-84.

154.Birrell MA, Eltom S. The role of the NLRP3 inflammasome in the pathogenesis of airway disease. Pharmacol Ther. 2011;130(3):364-70. doi: 10.1016/j.pharmthera.2011.03.007. Epub Mar 21.

155.Wanderer AA. Interleukin-1beta targeted therapy in severe persistent asthma (SPA) and chronic obstructive pulmonary disease (COPD): proposed similarities between biphasic pathobiology of SPA/COPD and ischemia-reperfusion injury. Isr Med Assoc J. 2008;10(12):837-42.

156.Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469(7329):221-5. doi: 10.1038/nature09663. Epub 2010 Dec 1.

157.Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med. 2008;359(22):2355-65.

158.Hurst JR, Perera WR, Wilkinson TM, Donaldson GC, Wedzicha JA. Systemic and upper and lower airway inflammation at exacerbation of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2006;173(1):71-8.

159.Stanojkovic I, Kotur-Stevuljevic J, Milenkovic B, Spasic S, Vujic T, Stefanovic A, et al. Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation. Respir Med. 2011;105 Suppl 1:S31-7.

160.O'Donnell DE, Laveneziana P. The clinical importance of dynamic lung hyperinflation in COPD. Copd. 2006;3(4):219-32.

161.Motz GT, Eppert Bl Fau - Sun G, Sun G Fau - Wesselkamper SC, Wesselkamper Sc Fau - Linke MJ, Linke Mj Fau - Deka R, Deka R Fau - Borchers MT, et al. Persistence of lung CD8 T cell oligoclonal expansions upon smoking cessation in a mouse model of cigarette smoke-induced emphysema. J Immunol. 2008;181(11):8036-43.

162.Sullivan AK, Simonian Pl Fau - Falta MT, Falta Mt Fau - Mitchell JD, Mitchell Jd Fau - Cosgrove GP, Cosgrove Gp Fau - Brown KK, Brown Kk Fau - Kotzin BL, et al. Oligoclonal CD4+ T cells in the lungs of patients with severe emphysema. J Res Crit Care Med. 2005;172(5):590-6.

163.Motz GT, Eppert Bl Fau - Wesselkamper SC, Wesselkamper Sc Fau - Flury JL, Flury Jl Fau - Borchers MT, Borchers MT. Chronic cigarette smoke exposure generates pathogenic T cells capable of driving COPD-like disease in Rag2-/- mice. Am J Resp Care Med. 2010;181(11):1223-33.

164.Kostikas K, Bakakos P, Papiris S, Stolz D, Celli BR. Systemic biomarkers in the evaluation and management of COPD patients: are we getting closer to clinical application? Curr Drug Targets. 2013;14(2):177-91.

165.MacNee W. Oxidative stress and lung inflammation in airways disease. Eur J Pharmacol. 2001;429(1-3):195-207.

166.Angelis N, Porpodis K, Zarogoulidis P, Spyratos D, Kioumis I, Papaiwannou A, et al. Airway inflammation in chronic obstructive pulmonary disease. Journal of Thoracic Disease. 2014;6(Suppl 1):S167-S72.

167.Cavalcante AG, de Bruin PF. The role of oxidative stress in COPD: current concepts and perspectives. J Bras Pneumol. 2009;35(12):1227-37.

168.Rahman I. The role of oxidative stress in the pathogenesis of COPD: implications for therapy. Treatments in Respiratory Medicine. 2005;4(3):175-200.

169.MacNee W, Rahman I. Is oxidative stress central to the pathogenesis of chronic obstructive pulmonary disease? Trends Mol Med. 2001;7(2):55-62.

170.Bargagli E, Olivieri C, Bennett D, Prasse A, Muller-Quernheim J, Rottoli P. Oxidative stress in the pathogenesis of diffuse lung diseases: a review. Respir Med. 2009;103(9):1245-56. doi: 10.016/j.rmed.2009.04.014. Epub May 22.

171.Holguin F. Oxidative stress in airway diseases. Ann Am Thorac Soc. 2013;10(Suppl):S150-7. doi: 10.1513/AnnalsATS.201305-116AW.

172.Eklund CM. Proinflammatory cytokines in CRP baseline regulation. Adv Clin Chem. 2009;48:111-36.

173.Fabbri LM, Luppi F, Beghe B, Rabe KF. Complex chronic comorbidities of COPD. Eur Respir J. 2008;31(1):204-12.

174.Bhatt SP, Dransfield MT. Chronic obstructive pulmonary disease and cardiovascular disease. Transl Res. 2013;162(4):237-51.

175.Shameem M, Bhargava R, Ahmad Z, Saad T, Fatima N, Malik A. Association between serum C- reactive protein levels and other important predictive markers of outcome in COPD. Acta Med Iran. 2011;49(1):18-20.

176.Sinden NJ, Stockley RA. Systemic inflammation and comorbidity in COPD: a result of 'overspill' of inflammatory mediators from the lungs? Review of the evidence. Thorax. 2010;65(10):930-6. doi: 10.1136/thx.2009.130260. Epub 2010 Jul 13.

177.Bridges RB, Wyatt RJ, Rehm SR. Effects of smoking on inflammatory mediators and their relationship to pulmonary dysfunction. Eur J Respir Dis Suppl. 1986;146:145-52.

178.Yeung MC, Buncio AD. Leukocyte count, smoking, and lung function. Am J Med. 1984;76(1):31-7.

179.Ridker PM. Cardiology Patient Page. C-reactive protein: a simple test to help predict risk of heart attack and stroke. Circulation. 2003;108(12): e81-5.

180.Yende S, Waterer GW, Tolley EA, Newman AB, Bauer DC, Taaffe DR, et al. Inflammatory markers are associated with ventilatory limitation and muscle dysfunction in obstructive lung disease in well functioning elderly subjects. Thorax. 2006;61(1):10-6.

181.Broekhuizen R, Wouters EF, Creutzberg EC, Schols AM. Raised CRP levels mark metabolic and functional impairment in advanced COPD. Thorax. 2006;61(1):17-22.

182.Cooper CB. Airflow obstruction and exercise. Respir Med. 2009;103(3):325-34.

183.Donaire-Gonzalez D, Gimeno-Santos E, Balcells E, Rodriguez DA, Farrero E, de Batlle J, et al. Physical activity in COPD patients: patterns and bouts. Eur Respir J. 2013;42(4):993-1002.

184.Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). Eur Respir J. 2008;31(4):869-73. doi: 10.1183/09031936.0111707. Epub 2008 Jan 23.

185.Vestbo J HS, Agusti A, Jones P, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD, Rodriguez-Roisin R. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. American Journal of Respiratory and Critical Care Medicine. 2013;187(4):347-65.

186.Sidney S, Sorel M, Quesenberry CP, Jr., DeLuise C, Lanes S, Eisner MD. COPD and incident cardiovascular disease hospitalizations and mortality: Kaiser Permanente Medical Care Program. Chest. 2005;128(4):2068-75.

187.Rabinovich RA, Figueras M, Ardite E, Carbo N, Troosters T, Filella X, et al. Increased tumour necrosis factor-alpha plasma levels during moderate-intensity exercise in COPD patients. Eur Respir J. 2003;21(5):789-94.

188.Oudijk EJ, Lammers JW, Koenderman L. Systemic inflammation in chronic obstructive pulmonary disease. Eur Respir J Suppl. 2003;46:5s-13s.

189.Debigare R, Cote CH, Maltais F. Peripheral muscle wasting in chronic obstructive pulmonary disease. Clinical relevance and mechanisms. Am J Respir Crit Care Med. 2001;164(9):1712-7.

190.Macallan DC. Wasting in HIV infection and AIDS. J Nutr. 1999;129(1S Suppl):238S-42S.

191.Van Remoortel H, Miek Hornikx, Heleen Demeyer, Daniel Langer, Chris Burtin, Marc Decramer, Rik Gosselink, Wim Janssens, and Thierry Troosters. Daily physical activity in subjects with newly diagnosed COPD. Thorax. 2013;68(10):962-3.

192.Pedersen OF, Butler JP. Expiratory flow limitation. Comprehensive Physiology. 2011;1(4):1861-82.

193.Troosters T, van der Molen T, Polkey M, Rabinovich RA, Vogiatzis I, Weisman I, et al. Improving physical activity in COPD: towards a new paradigm. Respir Res. 2013;14:115.

194.O'Donnell DE, Laveneziana P. Dyspnea and activity limitation in COPD: mechanical factors. COPD. 2007;4(3):225-36.

195.O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D. Mechanisms of activity-related dyspnea in pulmonary diseases. Respiratory Physiology & Neurobiology. 2009;167(1):116-32.

196.Engel RM, Vemulpad SR, Beath K. Short-term effects of a course of manual therapy and exercise in people with moderate chronic obstructive pulmonary disease: a preliminary clinical trial. J Manipulative Physiol Ther. 2013;36(8):490-6. doi: 10.1016/j.jmpt.2013.05.028. Epub Sep 17.

197.Engel RM GP, Beath K, Vemulpad S. Medium term effects of including manual therapy in a pulmonary rehabilitation program for chronic obstructive pulmonary disease (COPD): a randomized controlled pilot trial. J Man Manip Ther 2014. doi: 10.1179/2042618614Y.0000000074.

198.Casaburi R. A brief history of pulmonary rehabilitation. Respir Care. 2008;53(9):1185-9.

199.Petty TL, Nett LM, Finigan MM, Brink GA, Corsello PR. A comprehensive care program for chronic airway obstruction. Methods and preliminary evaluation of symptomatic and functional improvement. Ann Intern Med. 1969;70(6):1109-20.

200.Griffiths TL, Burr ML, Campbell IA, Lewis-Jenkins V, Mullins J, Shiels K, et al. Results at 1 year of outpatient multidisciplinary pulmonary rehabilitation: a randomised controlled trial. Lancet. 2000;355(9201):362-8.

201.Moy ML, Teylan M, Weston NA, Gagnon DR, Danilack VA, Garshick E. Daily step count is associated with plasma C-reactive protein and IL-6 in a US cohort with COPD. Chest. 2014;145(3):542-50.

202.Van Helvoort HA, Heijdra YF, Thijs HM, Vina J, Wanten GJ, Dekhuijzen PN. Exercise-induced systemic effects in muscle-wasted patients with COPD. Med Sci Sports Exerc. 2006;38(9):1543-52.

203.van Helvoort HA, van de Pol MH, Heijdra YF, Dekhuijzen PN. Systemic inflammatory response to exhaustive exercise in patients with chronic obstructive pulmonary disease. Respir Med. 2005;99(12):1555-67.

204.de Torres JP, Cordoba-Lanus E, Lopez-Aguilar C, Muros de Fuentes M, Montejo de Garcini A, Aguirre-Jaime A, et al. C-reactive protein levels and clinically important predictive outcomes in stable COPD patients. Eur Respir J. 2006;27(5):902-7.

205.Kuo HK, Yen CJ, Chen JH, Yu YH, Bean JF. Association of cardiorespiratory fitness and levels of C-reactive protein: data from the National Health and Nutrition Examination Survey 1999-2002. Int J Cardiol. 2007;114(1):28-33. Epub 2006 May 5.

206.Ambrosino N, Strambi S. New strategies to improve exercise tolerance in chronic obstructive pulmonary disease. Eur Respir J. 2004;24(2):313-22.

207.O'Shea SD, Taylor NF, Paratz J. Peripheral muscle strength training in COPD: a systematic review. Chest. 2004;126(3):903-14.

208.Troosters T, Gosselink R, Decramer M. Exercise training in COPD: how to distinguish responders from nonresponders. J Cardiopulm Rehabil. 2001;21(1):10-7.

209.Sin DD, Lacy P, York E, Man SF. Effects of fluticasone on systemic markers of inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2004;170(7):760-5.

210.Adcock IM, Maneechotesuwan K, Usmani O. Molecular interactions between glucocorticoids and long-acting beta2-agonists. J Allergy Clin Immunol. 2002;110(6 Suppl):S261-8.

211.Sin DD, Man SF. Corticosteroids and adrenoceptor agonists: the compliments for combination therapy in chronic airways diseases. Eur J Pharmacol. 2006;533(1-3):28-35. Epub 2006 Feb 7.

212.Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med. 2005;352(1):20-8.

213.Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA. 2001;286(1):64-70.

214.Davignon J, Jacob RF, Mason RP. The antioxidant effects of statins. Coron Artery Dis. 2004;15(5):251-8.

215.Ferrari R, Tanni SE, Caram LM, Correa C, Correa CR, Godoy I. Three-year follow-up of Interleukin 6 and C-reactive protein in chronic obstructive pulmonary disease. Respir Res. 2013;14:24.

216.Broekhuizen R, Wouters EF, Creutzberg EC, Weling-Scheepers CA, Schols AM. Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. Thorax. 2005;60(5):376-82.

217.Canavan J, Garrod R, Marshall J, Jackson D, Ansley P, Jewell A. Measurement of the acute inflammatory response to walking exercise in COPD: effects of pulmonary rehabilitation. Int J Chron Obstruct Pulmon Dis. 2007;2(3):347-53.

218.Spruit MA, Troosters T, Gosselink R, Kasran A, Decramer M. Acute inflammatory and anabolic systemic responses to peak and constant-work-rate exercise bout in hospitalized patients with COPD. Int J Chron Obstruct Pulmon Dis. 2007;2(4):575-83.

219.Davidson WJ, Verity WS, Traves SL, Leigh R, Ford GT, Eves ND. Effect of incremental exercise on airway and systemic inflammation in patients with COPD. J Appl Physiol. 2012;112(12):2049-56.

220.van Helvoort HA, Heijdra YF, de Boer RC, Swinkels A, Thijs HM, Dekhuijzen PN. Six-minute walking-induced systemic inflammation and oxidative stress in muscle-wasted COPD patients. Chest. 2007;131(2):439-45.

221.Koechlin C, Couillard A, Cristol JP, Chanez P, Hayot M, Le Gallais D, et al. Does systemic inflammation trigger local exercise-induced oxidative stress in COPD? Eur Respir J. 2004;23(4):538-44.

222.Vogiatzis I, Stratakos G, Simoes DC, Terzis G, Georgiadou O, Roussos C, et al. Effects of rehabilitative exercise on peripheral muscle TNFalpha, IL-6, IGF-I and MyoD expression in patients with COPD. Thorax. 2007;62(11):950-6.

223.Spruit MA, Gosselink R, Troosters T, Kasran A, Van Vliet M, Decramer M. Low-grade systemic inflammation and the response to exercise training in patients with advanced COPD. Chest. 2005;128(5):3183-90.

224.Petersen AM, Mittendorfer B, Magkos F, Iversen M, Pedersen BK. Physical activity counteracts increased whole-body protein breakdown in chronic obstructive pulmonary disease patients. Scand J Med Sci Sports. 2008;18(5):557-64.

225.Sugawara K, Takahashi H, Kasai C, Kiyokawa N, Watanabe T, Fujii S, et al. Effects of nutritional supplementation combined with low-intensity exercise in malnourished patients with COPD. Respir Med. 2010;104(12):1883-9.

226.Hallin R, Janson C, Arnardottir RH, Olsson R, Emtner M, Branth S, et al. Relation between physical capacity, nutritional status and systemic inflammation in COPD. Clin Respir J. 2011;5(3):136-42.

227.Brinkley TE, Leng X, Miller ME, Kitzman DW, Pahor M, Berry MJ, et al. Chronic inflammation is associated with low physical function in older adults across multiple comorbidities. J Gerontol A Biol Sci Med Sci. 2009;64(4):455-61. doi: 10.1093/gerona/gln038. Epub 2009 Feb 4.

228.Garrod R, Marshall J, Barley E, Fredericks S, Hagan G. The relationship between inflammatory markers and disability in chronic obstructive pulmonary disease (COPD). Prim Care Res J. 2007;16(4):236-40.

229.Pinto-Plata VM, Mullerova H, Toso JF, Feudjo-Tepie M, Soriano JB, Vessey RS, et al. C-reactive protein in patients with COPD, control smokers and non-smokers. Thorax. 2006;61(1):23-8.

230.Garcia-Aymerich J, Serra I, Gomez FP, Farrero E, Balcells E, Rodriguez DA, et al. Physical activity and clinical and functional status in COPD. Chest. 2009;136(1):62-70.

231.Van Vliet M, Spruit MA, Verleden G, Kasran A, Van Herck E, Pitta F, et al. Hypogonadism, quadriceps weakness, and exercise intolerance in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;172(9):1105-11.

232.Folchini F, Nonato NL, Feofiloff E, D'Almeida V, Nascimento O, Jardim JR. Association of oxidative stress markers and C-reactive protein with multidimensional indexes in COPD. Chron Resp Dis. 2011;8(2):101-8.

233.Watz H, Waschki B, Boehme C, Claussen M, Meyer T, Magnussen H. Extrapulmonary effects of chronic obstructive pulmonary disease on physical activity: a cross-sectional study. Am J Respir Crit Care Med. 2008;177(7):743-51.

234.de Torres JP, Pinto-Plata V, Casanova C, Mullerova H, Cordoba-Lanus E, Muros de Fuentes M, et al. C-reactive protein levels and survival in patients with moderate to very severe COPD. Chest. 2008;133(6):1336-43.

235.Gaki E, Kontogianni K, Papaioannou AI, Bakakos P, Gourgoulianis KI, Kostikas K, et al. Associations between BODE index and systemic inflammatory biomarkers in COPD. Copd: Journal of Chronic Obstructive Pulmonary Disease. 2011;8(6):408-13.

236.Hannink JD, van Hees HW, Dekhuijzen PN, van Helvoort HA, Heijdra YF. Non-invasive ventilation abolishes the IL-6 response to exercise in muscle-wasted COPD patients: a pilot study. Scand J Med Sci Sports. 2014;24(1):136-43. doi: 10.1111/j.600-0838.2012.01484.x. Epub 2012 Jun 19.

237.Di Marco F, Terraneo S, Roggi MA, Repossi AC, Pellegrino GM, Veronelli A, et al. Physical activity impairment in depressed COPD subjects. Respir Care. 2014;59(5):726-34. doi: 10.4187/respcare.02848. Epub 2013 Nov 12.

238.Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol. 1999;515(Pt 1):287-91.

239.van Helvoort HA, Heijdra YF, Dekhuijzen PN. Systemic immunological response to exercise in patients with chronic obstructive pulmonary disease: what does it mean? Respiration. 2006;73(2):255-64. Epub 2006 Jan 19.

240.Kishimoto T. The biology of interleukin-6. Blood. 1989;74(1):1-10.

241.Philippou A, Bogdanis G, Maridaki M, Halapas A, Sourla A, Koutsilieris M. Systemic cytokine response following exercise-induced muscle damage in humans. Clin Chem Lab Med. 2009;47(6):777-82. doi: 10.1515/CCLM.2009.163.

242.Di Francia M, Barbier D, Mege JL, Orehek J. Tumor necrosis factor-alpha levels and weight loss in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1994;150(5 Pt 1):1453-5.

243.Pitsiou G, Kyriazis G, Hatzizisi O, Argyropoulou P, Mavrofridis E, Patakas D. Tumor necrosis factor-alpha serum levels, weight loss and tissue oxygenation in chronic obstructive pulmonary disease. Respir Med. 2002;96(8):594-8.

244.Eid AA, Ionescu AA, Nixon LS, Lewis-Jenkins V, Matthews SB, Griffiths TL, et al. Inflammatory response and body composition in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;164(8 Pt 1):1414-8.

245.Timmons BW, Tarnopolsky MA, Snider DP, Bar-Or O. Immunological changes in response to exercise: influence of age, puberty, and gender. Med Sci Sports Exerc. 2006;38(2):293-304.

246.Nieman DC, Davis JM, Henson DA, Gross SJ, Dumke CL, Utter AC, et al. Muscle cytokine mRNA changes after 2.5 h of cycling: influence of carbohydrate. Med Sci Sports Exerc. 2005;37(8):1283-90.

247.Li TL, Gleeson M. The effect of single and repeated bouts of prolonged cycling on leukocyte redistribution, neutrophil degranulation, IL-6, and plasma stress hormone responses. Int J Sport Nutr Exerc Metab. 2004;14(5):501-16.

248.Schols AM, Buurman WA, Staal van den Brekel AJ, Dentener MA, Wouters EF. Evidence for a relation between metabolic derangements and increased levels of inflammatory mediators in a subgroup of patients with chronic obstructive pulmonary disease. Thorax. 1996;51(8):819-24.

249.Sugawara K, Takahashi H, Kasai C, Kiyokawa N, Watanabe T, Fujii S, et al. Effects of nutritional supplementation combined with low-intensity exercise in malnourished patients with COPD. Respir Med. 2010;104(12):1883-9. doi: 10.016/j.rmed.2010.05.008. Epub Jun 8.

250.Matsuyama W, Mitsuyama H, Watanabe M, Oonakahara K, Higashimoto I, Osame M, et al. Effects of omega-3 polyunsaturated fatty acids on inflammatory markers in COPD. Chest. 2005;128(6):3817-27.

251.Steele BG, Holt L, Belza B, Ferris S, Lakshminaryan S, Buchner DM. Quantitating physical activity in COPD using a triaxial accelerometer. Chest. 2000;117(5):1359-67.

252.Nusdwinuringtyas N, Widjajalaksmi, Yunus F, Alwi I. Reference equation for prediction of a total distance during six-minute walk test using Indonesian anthropometrics. Acta Med Indones. 2014;46(2):90-6.

253.Dourado VZ. [Reference Equations for the 6-Minute Walk Test in Healthy Individuals.]. Arq Bras Cardiol. 2011;25:S0066-782X2011005000024.

254.Poh H, Eastwood PR, Cecins NM, Ho KT, Jenkins SC. Six-minute walk distance in healthy Singaporean adults cannot be predicted using reference equations derived from Caucasian populations. Respirology. 2006;11(2):211-6.

255.Dijk WD, Bemt L, Haak-Rongen S, Bischoff E, Weel C, Veen JC, et al. Multidimensional prognostic indices for use in COPD patient care. A systematic review. Respir Res. 2011;12:151.(doi):10.1186/465-9921-12-151.

256.Oga T, Tsukino M, Hajiro T, Ikeda A, Nishimura K. Predictive properties of different multidimensional staging systems in patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2011;6:521-6.(doi):10.2147/COPD.S24420. Epub 2011 Oct 11.

257.Vestbo J, Prescott E, Almdal T, Dahl M, Nordestgaard BG, Andersen T, et al. Body mass, fat-free body mass, and prognosis in patients with chronic obstructive pulmonary disease from a random population sample: findings from the Copenhagen City Heart Study. Am J Respir Crit Care Med. 2006;173(1):79-83.

258.Ischaki E, Papatheodorou G, Gaki E, Papa I, Koulouris N, Loukides S. Body mass and fat-free mass indices in COPD: relation with variables expressing disease severity. Chest. 2007;132(1):164-9. Epub 2007 May 15.

259.Keaney JF, Jr., Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol. 2003;23(3):434-9.

260.Agusti A. Systemic effects of chronic obstructive pulmonary disease: what we know and what we don't know (but should). Proc Am Thorac Soc. 2007;4(7):522-5.

261.Agusti AG, Noguera A, Sauleda J, Sala E, Pons J, Busquets X. Systemic effects of chronic obstructive pulmonary disease. Eur Respir J. 2003;21(2):347-60.

262.Sin DD, Anthonisen NR, Soriano JB, Agusti AG. Mortality in COPD: Role of comorbidities. Eur Respir J. 2006;28(6):1245-57.

263.Wouters EF, Creutzberg EC, Schols AM. Systemic effects in COPD. Chest. 2002;121(5 Suppl):127S-30S.