Clot microstructure (d_f) as a biomarker and measurement of thrombogenicity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)

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Summary

Introduction: Chronic obstructive pulmonary disease is an inflammatory condition of the lungs characterised by irreversible airway obstruction and impairment of gas exchange. Acute exacerbation is associated with an increased incidence of venous thromboembolism. The main aim of the study was to investigate whether patients with acute exacerbation were thrombogenic utilising the functional biomarker of clot microstructure, the fractal dimension (d_f).

Methodology: The study recruited 30 stable patients from the chest clinic and 85 patients with acute exacerbation from the Emergency Department of a tertiary teaching hospital. One sample of blood was taken from stable group. Acute exacerbation group had four sampling points at 0 hours, 4-6 hours, 24 hours and 3-7 days.

Results: The biomarker, d_f was significantly elevated in patients presenting with acute exacerbation when compared to stable group (1.71 ± 0.06 vs 1.69 ± 0.05, p=0.03). There was no significant increase in d_f across the four time points (p=0.28) in the acute exacerbation group. All inflammatory markers and fibrinolytic markers such as D-dimer were significantly higher in acute exacerbation group. Those who died during admission in the acute exacerbation group had significantly elevated d_f when compared to those who survived (1.76 ± 0.03 vs 1.71 ± 0.06, p=0.02) and binary regression analysis showed that d_f was a significant predictor of mortality (p=0.024).

Conclusions: Patients with chronic obstructive pulmonary disease during exacerbation had denser and tighter clot microstructure as demonstrated by significantly elevated d_f when compared to stable group indicating that they were thrombogenic. This was due to profound inflammatory response and increased fibrin production. However, with appropriate treatments and prophylactic anticoagulation, there was no further increase in d_f which might explain low incidence of venous thromboembolism during admissions. Therefore, d_f is a useful biomarker that measures thrombogenicity, effect of treatment and predicts mortality in patients with acute exacerbation.

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Abbreviations

AA	Arachidonic acid
AAT	Alpha 1 Antitrypsin
ADP	Adenosine diphosphate
AECOPD	Acute Exacerbation of Chronic obstructive pulmonary disease
AF	Atrial fibrillation
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
APC	Activated Protein C
APTT	Activated partial thromboplastin time
AQI	Air Quality Index
ASPI	Arachidonic acid induced platelet aggregation
AT	Antithrombin
ATP	Adenosine triphosphate
BMI	Body Mass Index
BOLD	Burden of Obstructive Lung Disease
CD	Cluster of Differentiation
CFT	Clot formation time
CHRNA	Cholinergic Receptor Nicotinic Alpha
CO	Carbon monoxide
CO2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxygenase
CRF	Case report form
CRP	C-reactive protein
CT	Clotting time
CTPA	Computed tomography pulmonary angiogram
CVA	Cerebrovascular accident
CXCR2	CXC chemokine receptor 2

DAMP	Damage-associated molecular patterns
DIC	Disseminated intravascular coagulation
DKA	Diabetic ketoacidosis
DNA	Deoxyribonucleic acid
DVT	Deep Vein Thrombosis
ECLIPSE	Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic acid
ELIZA	Enzyme-linked immunosorbent assay
EPCR	Endothelial cell Protein C receptor
EPSRC	Engineering and Physical Sciences Research Council
ERV	Expiratory Reserve Volume
FDP	Fibrin degrading products
FEV1	Forced Expiratory Volume at one second
FRC	Functional Residual Capacity
FXIII	Factor XIII
GBD	Global Burden of Disease
GCP	Good Medical Practice
G-CSF	Granulocyte colony stimulating factor
GDPR	General Data protection Regulation
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GP	Gel point
GPR	Gly-Pro-Arg
GWAS	Genome Wide Association Studies
H^+	Hydrogen ions
H20	Water
H2CO3	Carbonic acid
HAT	Hospital acquired thrombosis
Hb	Haemoglobin

HBRU	Haemostatic Biomedical Research Unit
$HCO3^-$	Bicarbonate
HCT	Haematocrit
Hg	Mercury
HK	High molecular weight kininogen
HRQoL	Health Related Quality of Life
HTN	Hypertension
I:E ratio	Inspiratory: Expiratory ratio
ICS	Inhaled corticosteroids
ICU	Intensive Care Unit
IHD	Ischemic Heart Disease
IL	Interleukin
ILC	Innate Lymphoid Cells
INR	International normalised ratio
IQR	Interquartile ranges
IREB	Iron Responsive Element Binding protein
IRV	Inspiratory Reserve Volume
KDa	Kilo Daltons
LABA	Long-acting beta-2 agonist
LLN	Lower Limit of Normal
LMWH	Low Molecular Weight Heparin
LTOT	Long term oxygen therapy
MCF	Maximum clot firmness
ML	Maximum lysis
MCP-1	Monocyte Chemoattractant Protein-1
MMPs	Matrix metalloproteinases
MPO	Myeloperoxidase
MUC5AC	Mucin 5AC, Oligomeric Mucus/Gel-Forming
Myd88	Myeloid differentiation primary response 88
NASA	National Aeronautics and Space Administration

NE	Neutrophil Elastase
NET	Neutrophil Extracellular Traps
NEWS	National Early Warning Score
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NISCHR	National Institute for Social Care and Health Research
NIV	Non-invasive ventilation
OSA	Obstructive sleep apnoea
P2RY12	Purinergic Receptor P2Y12
PAI	Plasminogen activator inhibitor
PAMP	Pathogen-associated molecular patterns
PAR	Protease-activated receptor
PCI	Primary coronary intervention
PCT	Procalcitonin
PE	Pulmonary Embolism
PFT	Pulmonary Function Tests
PGG2	Prostaglandin G2
PGH2	Prostaglandin H2
PH	Pulmonary hypertension
PHW	Public Health Wales
PIS	Participant information sheet
PK	Prekallikrein
PLT	Platelets
PM	Particulate Matter
PPE	Personal protective equipment
PPV	Pneumococcal Polysaccharide Vaccine
PR	Pulmonary rehabilitation
PT	Prothrombin time
R&D	Research & Development
RBC	Red blood cells

RCT	Randomised Controlled Trial
REC	Research Ethics Committee
REDUCE	Reduction in the Use of Corticosteroids in Exacerbated COPD
ROC	Receiver operating characteristics
ROTEM	Rotational thromboelastometry
RV	Residual Volume
SABA	Short acting beta-2 agonist
SABA	Short acting bronchodilators
SAIL	Secure Anonymised Information Linkage
SAMA	Short acting muscarinic agonist
SBUHB	Swansea Bay University Health Board
SCOPD	Stable Chronic obstructive pulmonary disease
SD	Standard deviation
SERPINA	Serpin Family A Member
SIRS	Systemic inflammatory response syndrome
SPIROMICS	Subpopulations and Intermediate Outcomes in COPD Study
SPSS	Statistical Package for Social Sciences
STARD	Standards for Reporting Diagnostic accuracy studies
STEMI	ST elevation myocardial infarction
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SUMMIT	Study to Understand Mortality and MorbidITy
TBXA2	Thromboxane A2
Tc1	cytotoxic T lymphocytes 1
Tc2	cytotoxic T lymphocytes 2
TEG	Thromboelastography
TF	Tissue Factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming Growth Factor
T_{GP}	Time to gel point

Th17	T-helper type
TLC	Total Lung Capacity
TLR	Toll-like receptors
TNF-α	Tumour Necrosis Factor-α
tPA	Tissue plasminogen activator
TRIF	TIR-domain-containing adapter-inducing interferon-β
TV	Tidal Volume
UK	United Kingdom
Upa	Urokinase plasminogen activator
USA	United States of America
V/Q	Ventilation/ Perfusion
VC	Vital Capacity
VH	Venous Hypertensive
VTE	Venous thromboembolism
vWF	Von Willibrand Factor
WBC	White blood cells
WCEMR	Welsh Centre for Emergency Medicine Research
WHO	World Health Organisation
WIMD	Welsh Index of Multiple Deprivation

Chapter 1: Literature review

1.1. Introduction

1.1.1 Background to the research

Chronic obstructive pulmonary disease (COPD) is a disease of the lungs characterised by chronic airflow obstruction and impairment of gas exchange. In COPD patients, there is an abnormal inflammatory response to smoking and exposure to other noxious particles (MacNee, 2006). This profound inflammatory response can lead to mucous hypersecretion as in chronic bronchitis, permanent destruction of the alveolar walls with resultant loss of elastin as in emphysema and inadequate repair mechanisms of the damaged tissues as in bronchiolitis (MacNee, 2006). COPD patients frequently develop acute exacerbations that can be triggered with or without an infection and are potentially life threatening (Burge & Wedzicha, 2003).

The enhanced inflammatory response in COPD patients leads to local tissue damage releasing various inflammatory cytokines. The tissue factor released from the vascular endothelium triggers the coagulation pathways (Grover & Mackman, 2018). In addition, the inflammatory cytokines released cause systemic inflammation (Oudijk, Lammers, & Koenderman, 2003). All these factors collectively increase the thromboembolic risk in COPD patients. Venous thromboembolism (VTE) is a condition where a blood clot formed in deep veins (deep vein thrombosis- DVT) can travel and get lodged into the pulmonary arteries (pulmonary embolism-PE). There was a high prevalence of PE (15-30%) during acute exacerbations (Børvik et al., 2016) and a recent systematic review by Han et al. (2022) showed the prevalence to be 11%. The incidence of thromboembolism in the general population was 0.8 to 2.7/1000 person years (ISTH Steering Committee for World Thrombosis Day, 2014) and the incidence of thromboembolism in COPD appears to be slightly higher at 3.8/ 1000 person years (Børvik et al., 2016).

Previous studies have attempted to assess the effect of COPD on thrombogenicity and the risk of developing VTE (Børvik et al., 2016), however no markers are found to accurately assess the global haemostasis. Point of care tests such as thromboelastography (TEG) are increasingly used to detect clinically significant defect in the coagulation system. The advantages of point

of care tests are that they can be carried out in bedside, are less expensive and can help to guide therapy (Meybohm, Zacharowski, & Weber, 2013). Therefore, there is a pressing clinical requirement to develop a simple and rapid method of assessing thrombogenicity in AECOPD to identify those most at risk and initiate appropriate treatment.

Fractal dimension (d_f) is a functional biomarker of coagulation and haemostasis that can be used as a point of care test to assess thrombogenicity. It scientifically measures the elasticity of the blood clot in real time in whole blood which other tests cannot measure. It detects abnormalities in the clot microstructure of the incipient blood clot (Evans et al., 2010a). It was demonstrated that higher values of d_f are associated with denser and stronger clots which are more elastic and lower values represent less dense and weaker clots (Davies et al., 2016). It was demonstrated in the previous studies that d_f can reliably measure thrombogenicity in several disease conditions.

This thesis aims to determine the changes in clot microstructure in COPD patients as a potential marker of thrombogenicity, treatment and progression of the disease. We therefore recruited two groups of patients, one with stable disease from the respiratory clinic and the other with acute exacerbation presenting to the Emergency Department. It was hypothesised that COPD patients during acute exacerbation form blood clots with a tighter and denser clot microstructure (high d_f).

1.2. Chronic obstructive pulmonary disease (COPD)

1.2.1. Epidemiology

COPD is a chronic irreversible airway disease characterised by progressive decline of the lung function that causes significant morbidity and mortality. The social and economic burden to the society from COPD is therefore substantial. COPD is prevalent worldwide and varies in different countries depending upon their social and economic status (Rabe et al., 2007). It is estimated that approximately 384 million people suffered from COPD worldwide in 2010 with the global prevalence of 11.7% (Adeloye et al., 2015). COPD is the third leading cause of death in the world with 3.23 million deaths in 2019 according to World Health Organisation (WHO). It is expected that this number is going to rise substantially (World Health Organisation, 2020).

There are several large-scale epidemiological studies such as the Burden of Obstructive Lung Disease (BOLD) program that examined the prevalence of COPD worldwide (Buist et al., 2005). The BOLD programme BOLD-1 project recruited 30,000 people over 42 different sites in middle- and low-income countries. They used standardised methods such as questionnaires and pre and post bronchodilator spirometry and found that the prevalence of COPD grade 2 or higher was 10.1% (Buist et al., 2007). The prevalence increased with age and smoking and other factors contributing to airway obstruction include tuberculosis, exposure to fumes in occupational settings and cooking (Buist, Vollmer, & McBurnie, 2008). However, the household pollution did not explain the cause of COPD in low-income countries. There was high prevalence of COPD in never smokers (Lamprecht et al., 2011). The people in low-income areas of South Asia and Sub-Saharan Africa had smaller lungs when compared to people in Europe (Meghji et al., 2016).

In the UK, COPD is the second most common disease after asthma, and it is estimated that there are approximately 1.2 million people with diagnosed COPD and 2 million with undiagnosed COPD. The prevalence of COPD in the UK has increased by 27% as of 2016. Each year there are 115,000 new diagnoses of COPD which is equal to one case diagnosed every five minutes. As of 2012, there are 10% more male patients with diagnosed COPD than females. In addition, there is two-fold increase in the prevalence and incidence of COPD in the most deprived population. Most of the patients diagnosed with COPD in the UK are more

than 40 years of age (British Lung Foundation, 2022). There are approximately 30,000 deaths per year from COPD which represents 26.1% death from a lung disease (Rayner, Sherlock, Creagh-Brown, Williams, & deLusignan, 2017) and it is unclear how many of them die of thromboembolic disease. The economic burden from COPD is estimated to be over £1.9 billion annually (British Lung Foundation, n.d.).

It is estimated that 117,000 patients suffer with COPD in Wales (British Lung Foundation, 2019) and it is estimated that approximately 1500 people die of COPD every year (Public Health Wales, 2011). It is however not clear how many patients died of VTE or other vascular causes.

1.2.2. Definition of COPD

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) is a collaboration launched in 1977 by National Heart, Lung, and Blood Institute, National Institutes of Health, United States of America (USA), and the World Health Organization. The aim of this project was to raise awareness of COPD and to improve its prevention and treatment. This is achieved by working with health professionals and public health officials worldwide.

> According to the Global Initiative for Chronic Obstructive Lung Disease 2022 report (GOLD, 2022, p. 4), COPD is defined as "a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases and influenced by host factors including abnormal lung development".

1.2.3. Risk factors

There are several factors that increase the risk of developing COPD.

1.2.3.1 Smoking (Tobacco, Marijuana, Vaping)

Cigarette smoking is known to be the most common risk factor for developing COPD worldwide (Burney et al., 2020). It is known to be the cause in 9 out of 10 COPD patients, however only 20% of smokers develop COPD in their lifetime. One of the reasons found by Smith et al. (2020) is the asymmetry in airway tree calibre to lung size which is a lung development abnormality. According to Burney et al. (2014), smoking correlates with airflow obstruction but not with mortality. Bhatt et al. (2018) showed that smoking duration itself provides an increased risk than pack years. According to the World Health Organisation (2022), tobacco caused 8 million deaths worldwide and cigarette smoking is the most common form of tobacco use. Over 7 million of these deaths are due to direct tobacco use and 1.2 million deaths due to passive smoking. There is no evidence to suggest that cannabis smoking causes COPD, however it does cause a degree of hyperinflation of the lungs (Tashkin, 2010). According to Tan et al. (2009), concurrent use of marijuana and tobacco increased the COPD risk by nearly three-fold. There are more than 7000 chemicals released when a cigarette is burned, however electronic cigarettes (vaping) are known to have less chemicals and is estimated to be 95% safe (Nutt et al., 2014). The effects of smoking using electronic cigarettes (vaping) are not known (Gotts, Jordt, McConnell, & Tarran, 2019), however according to Garcia-Arcos et al. (2016) chronic nicotine use causes features of COPD in mice. Even though switching from cigarette smoking to e-cigarettes reduced the incidence of exacerbations (Polosa et al., 2020), concurrent use is associated with developing COPD in males (Kim and Kang, 2021).

1.2.3.2 Occupational exposure

Undoubtedly, there is an increased risk of developing COPD in people with occupational exposures such as coal mining, silica, cotton dust or cadmium fume (Cullinan, 2012). The severity of emphysema was significantly elevated in coal miners (Kuempel, Wheeler, Smith, Vallyathan, & Green, 2009). A study conducted by Reynolds et al. (2017) showed that

exposure to crystalline silica in Welsh slate miners was associated with increased risk of COPD by nearly 1.5 fold. Long term exposure to dust from cotton can lead to obstructive airway disease called byssinosis (Lai & Christiani, 2013). Hutchinson et al. (2018) found that cadmium nanoparticles cause increased citrullinated proteins which may contribute to developing COPD.

1.2.3.3 Air pollution

The outdoor air pollutants include particulate matter (PM), sulphur dioxide, ozone, nitrogen dioxide, carbon monoxide and lead released from industries, forest and crop fires, garbage burning and automobile exhaust. Particulate matter of the size 2.5 micrometre (PM2.5) is widely used as the main indicator of risk to health because 90% of it is released by diesel engines. The indoor air pollutants are mostly the same in different concentrations from combustion of solid fuels, smoking, emissions from construction materials and poor ventilation (Jiang, Mei & Feng, 2016). The multicentre study by the BOLD collaborative research group showed that there was no association between PM2.5 and chronic airway obstruction (Amaral, 2021).

1.2.3.4 Genetics

The first known genetic cause for developing COPD was alpha 1 antitrypsin (AAT) deficiency which is caused by the mutations in SERPINA 1 (Serpin Family A Member 1) gene. AAT inhibits the elastase enzyme which can damage alveoli and cause COPD (Silverman, 2020). This disease is inherited in an autosomal codominant fashion, and it is estimated that approximately 3.3 million people suffer worldwide according to GBD 2013 Mortality and Causes of Death Collaborators (2015). McCloskey et al. (2001) showed that there is a significant risk of developing COPD in smoking first degree relatives of patients with severe disease. Genome wide association studies (GWAS) conducted by Pillai et al. (2009) showed that COPD had a significant association and CHRNA3 (Cholinergic Receptor Nicotinic Alpha 3)/CHRNA5/IREB2 (Iron responsive element binding protein 2) region on chromosome 15q25. Two large studies have identified 22 (Hobbs et al., 2017) and 82 (Sakornsakolpat et al., 2019) genome wide significant loci associated with COPD GWAS. There is an International COPD Genetics Consortium to facilitate research.

1.2.3.5 Age

COPD is considered as the disease of the elderly. In a Canadian study, Osman et al. (2017) found that COPD was nearly four times higher in individuals over 65 years old. The maximum lung function is reached at 20-25 years in humans and then declines, this is caused by an increase in size of the alveoli and is called senile emphysema or aging lung. Senile emphysema is physiological and there is no inflammatory destruction of the airways (Brandsma, 2017). However, in smokers this process can be accelerated leading to early onset COPD (Fukuchi, 2009). In addition, cigarette smoking can diminish the antioxidant and autophagic defences, antiaging molecules, and DNA (Deoxyribonucleic acid) repair mechanism of cells (Mercado, Ito, & Barnes, 2015). However, COPD can manifest in the 20's in patients with alpha 1 antitrypsin deficiency (Holm et al., 2014). In the UK, the NICE (2021) has recommended that COPD should be suspected in anyone who is more than 35 years old and has risk factors and symptoms suggestive of COPD.

1.2.3.6 Socio-economic status

According to a large multicentre study by Townsend et al. (2017), poverty is significantly associated with COPD. This might be due to multiple factors. According to the World Health Organisation (2022), over 80% of the 1.3 tobacco users live in low and middle-income countries and the main mode of tobacco use is through cigarette smoking. COPD leads to increased levels of unemployment thereby exacerbating the poverty (Grønseth et al., 2017). Deprivation refers to the inability to access resources and opportunities in a community and in the UK this is assessed using Index of Multiple Deprivation. The study by Collins et al. 2018 showed that deprivation in COPD patients in England is associated with emergency hospitalisation, health care costs and mortality. The study by Steiner et al. (2017) showed that COPD patients from the most deprived areas are less likely to complete a pulmonary rehabilitation programme, therefore these patients are more prone to develop frequent exacerbations. The Welsh Index of Multiple Deprivation (WIMD, 2019) is an official measure of deprivation produced by the Welsh Government and has eight domains such as Income, Employment, Health, Education, Access to Services, Community safety, Physical environment, and Housing. Each domains have several indicators of deprivation and there are currently 47 indicators. WIMD 2019 ranks the small areas in Wales to 1 (most derived) to 1909

(least deprived). The score is grouped into quintile ranges 1-191, 192-382, 383-573, 574-955 and 956-1909 (Welsh Index of Multiple Deprivation (WIMD), 2019) **[Figure 1.1]**.

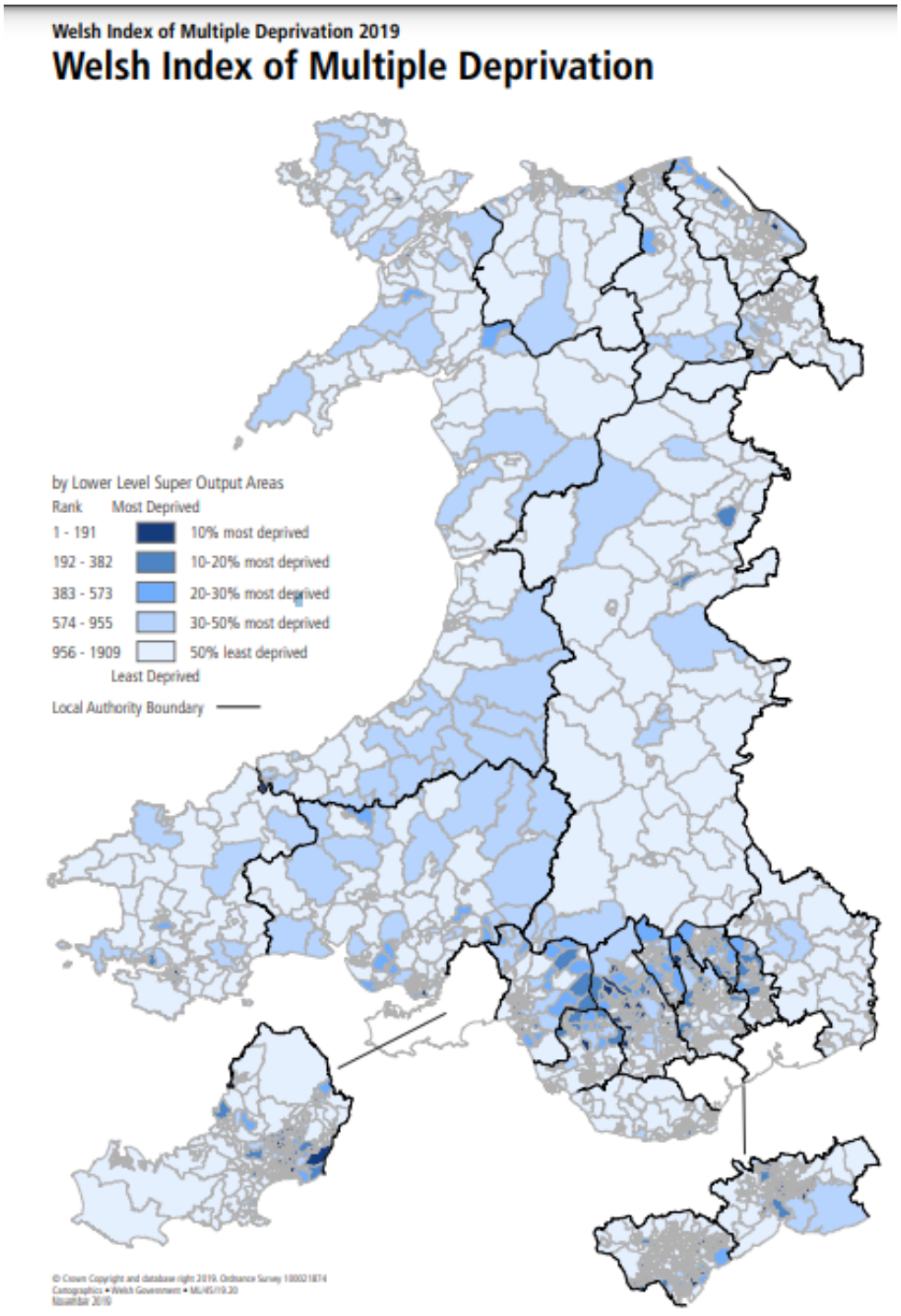

Figure 1.1: Shows the Welsh Index of Multiple deprivation 2019 map of Wales. Accessed on 17th November 2022 from https://statswales.gov.wales/Catalogue/Community-Safety-and-Social-Inclusion/Welsh-Index-of-Multiple-Deprivation/WIMD-maps-2019.

1.2.3.7 Asthma

COPD and asthma are both obstructive airway diseases, however there are a lot of similarities and differences between these two diseases. Asthma typically starts from childhood and is mostly caused by an exaggerated allergic response leading to airflow obstruction that is reversible with treatment. COPD on the other hand starts mid to late life and is characterised by permanent destruction of the airways leading to airway obstruction that is not completely reversible. The symptoms such as wheezing, cough and treatment for both asthma and COPD can overlap (Gibson & Simpson, 2009). The presence of symptoms of both asthma and COPD in the same patient is called asthma-COPD overlap syndrome (Bonten et al. 2017). Spirometry is considered as the one of the most reliable tests in diagnosing airway obstruction. However, Janson et al. (2019) argued that the there is significant difference in the reversibility, therefore careful history and physical examination should be carried out along with other investigation such as radiology to differentiate between asthma and COPD (Yawn, 2009). A history of asthma increases the risk of developing COPD by 10-30 fold (McGeachie, 2017). According to Tai et al. (2014), children with severe asthma are at increased risk of developing COPD.

1.2.3.8 Ethnicity

There is no evidence to suggest that ethnicity has increased risk of developing COPD. However, Martin et al. (2012) in their study in London found that differences do exist in prevalence and severity by ethnicity. In addition, there is variation in respiratory symptoms and other respiratory illnesses in severe COPD in different ethnicities (Kim et al., 2017).

1.2.3.9 Sex

In a large multinational study by Burney et al. (2021), it was shown that COPD is more prevalent in men (11.2%) than women (8.6%). This might be due to smoking habits, however smoking habits in women have significantly increased. There are over 100 million female smokers in the world according to World Health Organisation (2022). Female smokers tend to get a more severe form of disease with early onset of disease or low smoking exposure (Sørheim, 2010). Recent studies have shown that there is narrowing of the gap of gender mortality with COPD (Jovičić Burić, Erceg, & Antoljak, 2022).

1.2.3.10 Pregnancy and lung development

A large study done by Magnus, Henderson, Tilling, Howe, and Fraser (2018) found that maternal smoking was associated with significant reduction in lung function and developing COPD. A meta-analysis by Doyle et al. (2019) showed that individuals born very preterm or with low birth weight are at increased risk of developing COPD. A study on 10,192 smokers by Hayden et al. (2015) found that childhood pneumonia was associated with development of COPD by nearly 1.5-fold. However, the reason for this is not fully understood.

1.2.3.11 Other factors

A worldwide population-based study by Valfleteren et al. (2016) showed that COPD patients had low body mass index (BMI). According to Horner et al. (2017), there is no association between living in high altitude and prevalence of COPD when individual risk factors are considered. Amaral et al. (2015) showed that in a tuberculosis endemic area, the history of tuberculosis is a potential cause for developing COPD. According to Emilsson, Janson, Benediktsdóttir, Júlíusson, and Gíslason (2012), there is a strong prevalence of COPD in patients with nocturnal oesophageal reflux disease.

1.2.4. Normal lung structure and physiology

1.2.4.1 Normal lung structure

To understand the pathological process in COPD, it is imperative to understand the anatomy of the respiratory tract. The upper respiratory tract is the conducting zone that includes nose, nasal cavities, sinuses, pharynx and part of larynx above the vocal cords **(Figure 1.2)**. The air enters the body through the nose and there are millions of hairs that trap dirt and particles. In addition, there are four pairs of sinuses that secrete mucus which drains into the nasal cavity which also helps to trap dirt and particles. There are three pairs of turbinates which are the folded structures on the lateral side of the nasal cavity to warm and moisten the air before it enters the lungs and helps with mucous drainage. The initial one third of the nasal cavity is lined by stratified columnar epithelium and the posterior one third by pseudostratified ciliated columnar epithelium and mucous producing goblet cells. In addition, the entire nasal cavity has a rich blood supply. All these anatomical adaptation helps to trap particles, warm and moisten the air before reaching the lungs. The pharynx or throat carries and regulates the flow of air from nose to larynx. The larynx is a hollow tube that helps air to pass from pharynx to trachea and prevents food and other particles entering the trachea (Lumb & Thomas, 2019).

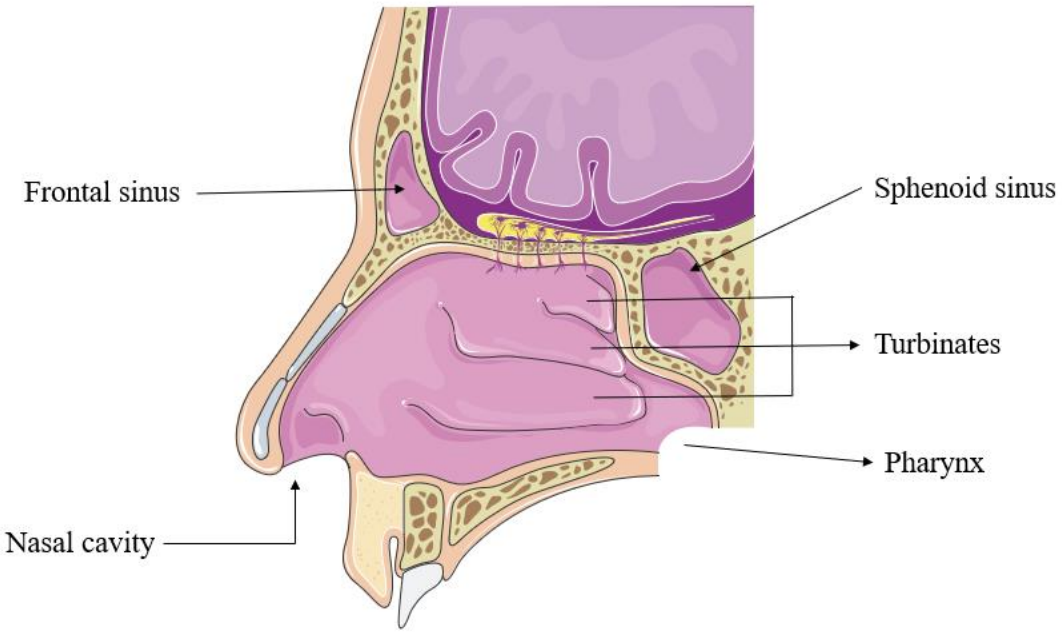

Figure 1.2: This figure shows the cross-section anatomy of the nasal cavity and pharynx. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by

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The lower respiratory tract is the respiratory zone that includes part of the larynx below the vocal cords, trachea, bronchi, bronchioles, alveolar duct and alveoli. The human lungs are divided into right which has three lobes and left which has two lobes **(Figure 1.3)**. The trachea or the windpipe is a D-shaped tube that carries air from larynx to lungs and is 10-11cm long dividing into right and left main bronchus supplying each lung at the level of thoracic vertebra 5 (T5).

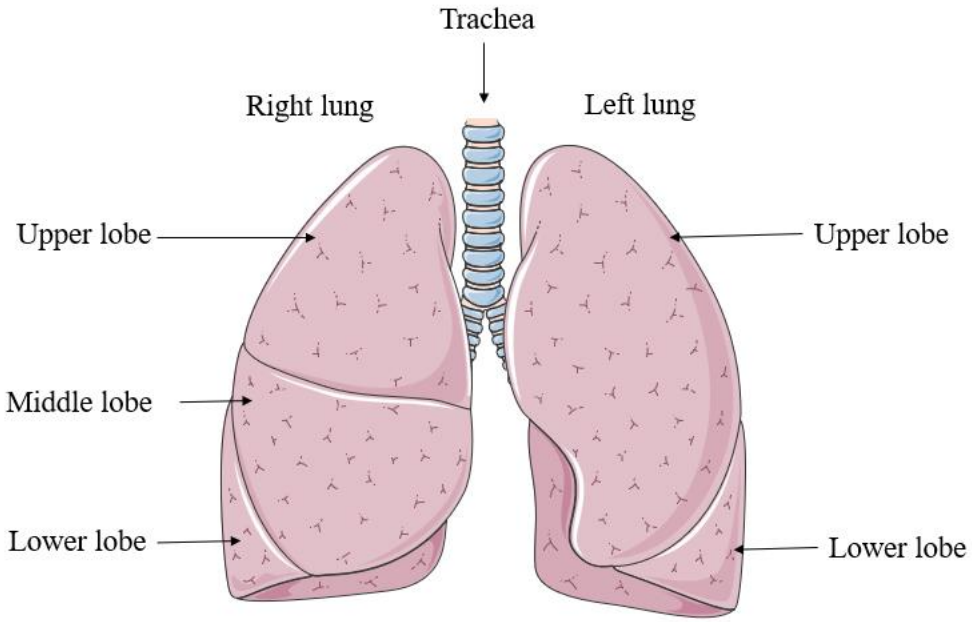


Figure 1.3: Human lungs are divided into right with three lobes and left with two lobes. Trachea which is the continuation of larynx is divided into right and left main bronchus. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)].

The right main bronchi divide into three lobar bronchi (secondary bronchi) supplying three lobes of the right lung and the left main bronchi divides into two lobar bronchi supplying two lobes of the left lung. There are in total 23 generations of divisions from the trachea **(Figure 1.4)**. The first 0-14 generations are called conducting zone or airways and is from trachea to terminal bronchioles. From 6^{th} generation the branches are called bronchioles because it is

approximately 1mm in diameter. The bronchiole generation from 15-23 is involved in gas exchange and therefore called the respiratory zone or lung parenchyma. The respiratory bronchioles (15-18 generations) which are the branch from the terminal bronchioles give rise to alveolar duct (19-22 generations) and finally alveoli (23rd generation) [Lumb & Thomas, 2019].

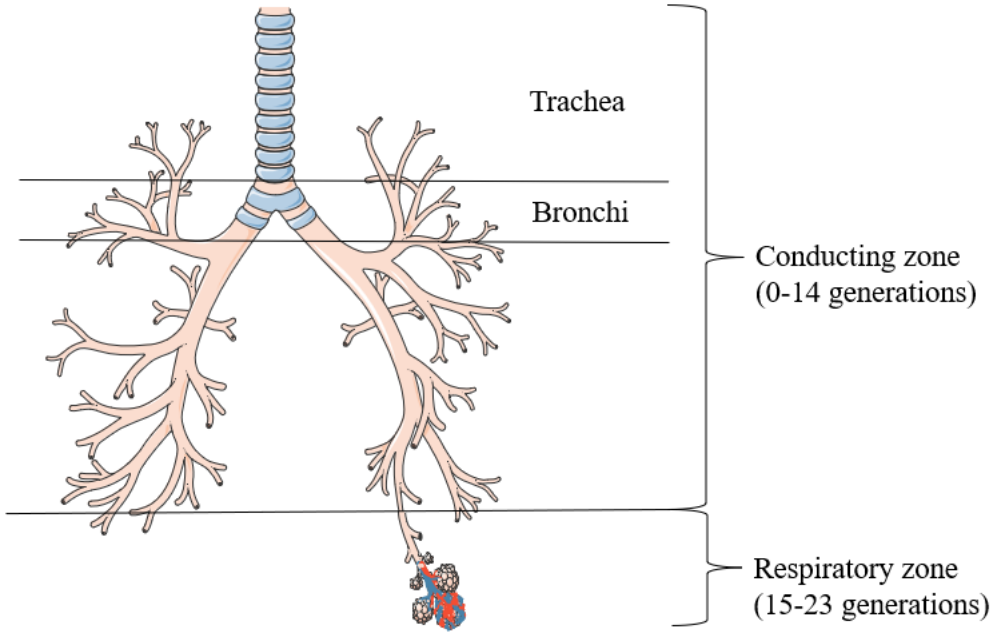


Figure 1.4: Human trachea has 23 generations of divisions. The 0-14 generations form the conducting zone and 15-23 generations form the respiratory zone. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)].

The trachea contains 16-20 semi-circular cartilages which prevents it collapsing during breathing. There are four histological layers in trachea, the innermost mucosa layer is lined by pseudostratified ciliated columnar epithelium. The second submucosa layer has mucous glands, smooth muscles, blood vessels, nerves and lymphatics. The mucous produced by goblet cells helps to trap particles and microorganism and the rhythmic upward movement of cilia enables the mucus to be expelled by coughing. The third layer is the fibrocartilaginous layer, and the fourth layer is the fibroelastic adventitia. The bronchi have similar histological layers as the trachea, however the semi-circular hyaline cartilage becomes less, and the smooth muscles

increase till the sixth-generation bronchi. Therefore, bronchioles have a cartilage free wall with plenty of smooth muscles that help to control the airflow by constriction and relaxation. Similarly, there is transition of epithelium from pseudostratified ciliated columnar epithelium in bronchi to simple cuboidal ciliated epithelium in the bronchioles (6^{th} to 14^{th} generation). There are no goblet cells in bronchioles (Lumb & Thomas, 2019; Ramos, Krahnke, & Kim, 2014).

The lining of the respiratory bronchioles (15^{th} to 18^{th} generations) is by simple squamous epithelium and there are club cells (Clara cells) which produce surfactant. The alveoli are a balloon like structure which is lined by a single layer of cells and there are approximately 500 million alveoli in adult human lungs (Ochs et al., 2004). There are three types of cells in the alveoli, type 1 pneumocytes, type 2 pneumocytes and alveolar macrophages (Kia'i & Bajaj, 2022). The type 1 pneumocytes constitute 95% of the alveolar cells and are made up of thin squamous cells through which gas exchange occurs. The type 2 pneumocytes are larger cuboid cells containing phospholipid multilamellar bodies, which is the precursor of pulmonary surfactant that reduces surface tension thereby preventing lung collapse. In addition, it expresses immunomodulatory proteins, transepithelial movement of water and facilitates alveolar epithelial regeneration post injury (Brandt & Mandiga, 2021). The alveolar macrophages are found in close contact with type 1 & 2 pneumocytes and are derived from monocytes (Hu & Christman, 2019). They are the first defence against pathogens, pollutants, help in clearing surfactant/ cell debri and initiate immune response in the lungs (Joshi, Walter, & Misharin, 2018).

The bronchial artery supplies the lung's conducting zone and pulmonary artery supplies the respiratory zone. The bronchial artery which is a small branch from the aorta supplies oxygenated blood to lung parenchyma (Jain, Bordes, & Bhardwaj, 2021). Pulmonary artery is the only artery in the body that carries deoxygenated blood from the heart to lungs and pulmonary vein is the only vein that carries oxygenated blood back to the heart from the lungs. The pulmonary vasculature therefore comprises of arterial tree, extensive capillary network around each alveolus and venular tree. All these ensure large surface area for gas exchange and low resistance to blood flow (Townsley, 2012). In addition, there are lymphatics which are found close to terminal bronchioles and prevent accumulation of fluid and keep alveolar membrane dry.

1.2.4.2 Normal lung physiology

The lungs are the fundamental organs of the human respiratory system with over 500 million alveoli. This equates to a total surface area of 140 m^2 which is the size of a tennis court. The lungs help humans to breath air in and out facilitating gas exchange. The main components of air are nitrogen (78%), oxygen (21%) and the rest of the 1% of air constitutes gases such as argon, carbon dioxide (CO2) and carbon monoxide (CO). There are several tiny particles floating on the air called aerosols and the commonest of them are dust and pollen. Both dust and pollen are well known factors that induce an allergic response in asthma patients (Baxi & Phipatanakul, 2010) and may be a factor contributing to the COPD exacerbation (Jamieson et al., 2013). In addition, there are noxious particles such as soot, smoke, exhaust from car and industry contributes to air pollution (NASA [National Aeronautics and Space Administration], 2016). The exposure to these noxious particles can cause inflammation in a normal lung. However, in COPD patients there is an abnormal inflammatory response which can lead to the development of COPD and can cause exacerbations in those with established COPD. Air pollution is measured using air quality index (AQI) at 0-500 and an AQI of more than 100 is unhealthy (Airnow, n. d.). The amount of water that air can hold before it rains is called relative humidity which is 100%. Air pressure decreases when height or altitude increases (NASA, 2016). Being an inert gas, nitrogen in the air that is inspired comes out during expiration without being absorbed into the body. However, oxygen in the air diffuses into the blood and is carried by haemoglobin to different parts of the body. The carbon dioxide that is formed as a waste product in the cells is carried in the blood as carbonic acid, which then forms carbon dioxide in the lungs and diffuses into the air and is exhaled. The primary muscle that is involved in breathing is the diaphragm and other muscles that facilitate breathing are intercostal and abdominal muscles (Aliverti, 2016).

The amount of air that goes in and out of the lungs can be measured using spirometry. The time to inhale is called inspiratory time and time to exhale is called expiratory time. The expiratory time is normally double the inspiratory time which is represented as I:E (Inspiratory: Expiratory) ratio of 1:2. The amount of air that moves in and out during normal breathing or a respiratory cycle is the Tidal Volume (TV) which is approximately 500mls in adult males and 400mls in adult females. The amount of air that can be breathed in after a maximum inspiration is Inspiratory Reserve Volume (IRV) and the amount of air that can be breathed out during a

maximum expiration after a normal inspiration is Expiratory Reserve Volume (ERV). The air that is left in the lung after maximum expiration is called Residual Volume (RV) which is 2-2.5 litres. The maximum volume of air that can be inspired and expired is called Vital Capacity (VC) which is 4-6 litres (therefore, VC= IRV+TV+ERV). The volume of air in the lung after a normal expiration is called Functional Residual Capacity (FRC) which is 3-4 litres (FRC=ERV+RV). The gas volume in the lung after maximum inspiration is called Total Lung Capacity (TLC) which is 6-8 litres. Therefore, Total Lung Capacity (TLC)= Vital Capacity (VC) + Residual Volume (RV) **[Figure 1.5]**. It is estimated that approximately 100-150mls of air (i.e. 30% of the Tidal Volume) does not take part in gas exchange and remain in the conducting zone or in the anatomical dead space. Therefore, the amount of air that participates in gas exchange is 5L/min which is equal to cardiac output, therefore the alveolar ventilation-perfusion ratio is 1 (Hall & Hall, 2021; Haddad & Sharma, 2021).

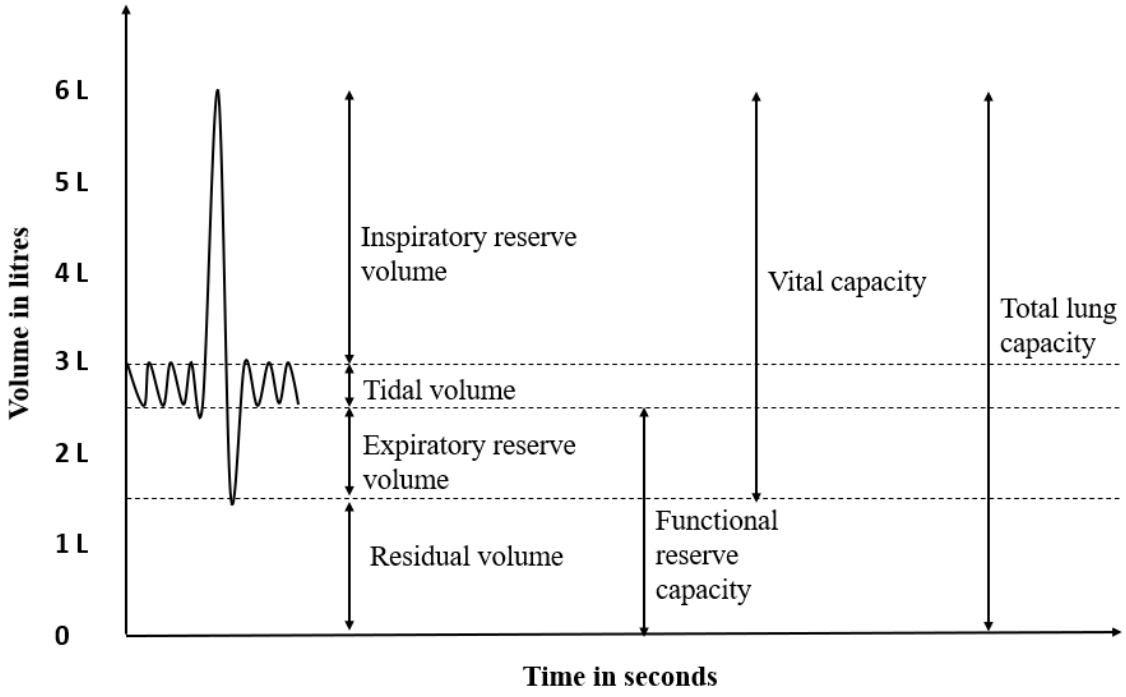


Figure 1.5: Normal spirometry showing the volume of air in litres versus time in seconds.

For an effective gas exchange, air should move in and out of the lungs effortlessly. However, in COPD, there is obstruction to the air flow. Pulmonary function tests (PFT) such as spirometry is used to assess the amount (volume) and speed (flow) of air that goes in and out of the lungs. Spirometry is non-invasive, readily available and can be performed within 15 minutes in adults (Johnson & Theurer, 2014). The test is performed by asking participants to take a deep breath and then blow into a tube with maximum force as long as possible. However,

the study by Lamprecht et al. (2007) showed that six seconds spirometry is as sensitive and specific in diagnosing COPD. This test depends upon the patient's compliance and the tests are performed at least three times to achieve reproducibility and the largest values are then reported (Graham et al., 2019). Spirometry gives several measurements, however in COPD patients, the most important are FEV1 (Forced Expiratory Volume in one second) and Forced Vital Capacity (FVC). The amount of air that is forcefully expired in 1 second is Forced Expiratory Volume 1 (FEV1) and the amount of air that is breathed in and out forcefully is Forced Vital Capacity (FVC). The normal values of FEV1 and FVC is 80-120% and in COPD patients FEV1 and FEV1/FVC are reduced (Barreiro & Perillo, 2004) **[Figure 1.6]**.

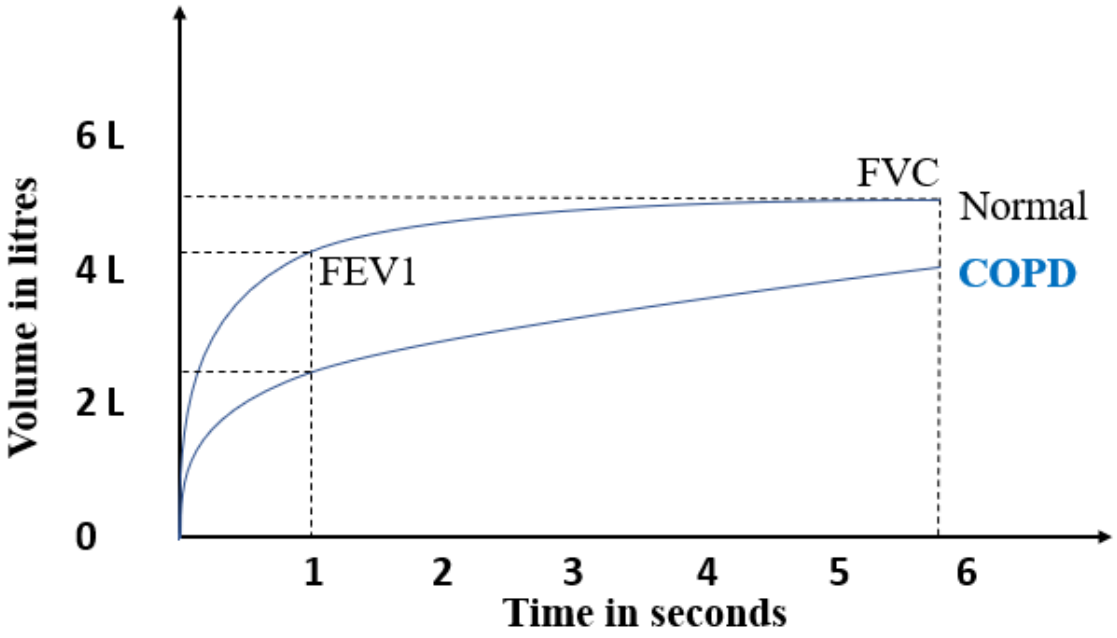


Figure 1.6: The volume time graph spirometry changes in COPD. The graph demonstrates that both FEV1 and FVC are significantly reduced in COPD which is due to airway obstruction.

Alveoli are balloon like structures and once they are inflated, they recoil. Pulmonary compliance is the extent to which lung expands per unit increase in transpulmonary pressure and is normally 200ml/cm H_2O (Jonson & Svantesson, 1999). In COPD, because of airway obstruction, there is air trapping leading to hyperinflation and lung compliance increases (Papandrinopoulou, Tzouda, & Tsoukalas, 2012). Airway resistance is the friction caused by the movement of air through the respiratory system and is normally 2 to 3 cm H_2O/ L/ sec. It peaks at the 5^{th} generation and decreases with each airway generation. The airway resistance is increased in COPD because of chronic airway obstruction (Jalusic-Gluncic, 2011). The gas

exchange happens through a single celled alveoli into the blood stream and vice versa through simple diffusion. Diffusion is a process where a substance moves from higher concentration to lower concentration. During gas exchange oxygen diffuses into the blood stream and carbon dioxide diffuses from the blood into the air (Wagner, 2015). The oxygen that diffuses into the blood stream binds to the haemoglobin in the red blood cells and is transported to the cells. Carbon dioxide (CO_2) which is formed as a waste product of metabolism in the cells combines with water (H20) to form carbonic acid H2CO3 which then dissociates into bicarbonate ($HCO3^-$) and hydrogen ion (H^+) in the plasma. This chemical reaction reverses when the blood passes through the alveolar blood vessels and the CO_2 formed diffuses into the alveoli and is exhaled (Hall & Hall , 2021).

$CO2 + H2O \rightleftharpoons H2CO3 \rightleftharpoons HCO3^- + H^+$

There are two requirements for gas exchange to take place in the alveoli. Firstly, there should be adequate flow of air in and out of alveoli known as ventilation and secondly, there should be adequate blood flow through alveolar capillaries called perfusion. Therefore, the ratio of the amount of air reaching the alveoli per minute to the amount of perfusion to the alveoli per minute is called ventilation/ perfusion (V/Q) ratio and the value is 1. Any change in this value is called ventilation/ perfusion mismatch (Hall & Hall, 2021). For effective oxygenation delivery to different organs both lungs and heart need to work together. The deoxygenated blood from the body reaches the right atrium of the heart through superior and inferior venacava. Superior venacava drains the upper half of the body while inferior venacava drains the lower part of the body. When right atria contract, the blood reaches the right ventricle. The pressure in the right atrium is 2 mm of Hg and in the right ventricle it is 25mm of Hg. From the right ventricle the blood is pumped into both the lungs via the pulmonary artery. The blood pressure in the pulmonary artery is 25/8 mm of Hg, when compared to systemic blood pressure of 120/80 mm of Hg. The pressure at the start of pulmonary capillaries is 12 mm of Hg and at the end it is 8 mm of Hg. The pulmonary vein carries oxygenated blood from the lungs to the left atrium and its pressure is 5mm of Hg. This blood is drained to the right ventricle (120 mm of Hg) and is pumped via the aorta to different parts of the body (Hall & Hall, 2021).

1.2.5. Pathological/ structural changes and pathophysiology of the lungs in COPD

1.2.5.1 Pathological/ structural changes of the lungs in COPD

The pathological changes in COPD can occur in the airways, lung parenchyma and pulmonary vasculature. Pathological changes occurring in the lungs in COPD depend upon the risk factors. As discussed before cigarette smoking is the most common risk factor in developing COPD. It was proposed that in COPD there is remodelling of the airways. Chronic exposure to smoke and other noxious particles induces inflammation in the lungs and in COPD, there is an abnormal inflammatory response. This is one of the reasons why not all smokers develop COPD. This inflammatory process can lead to 3 different pathological changes in the lungs namely emphysema, chronic bronchitis, and obstructive bronchiolitis.

1.2.5.1.1 Changes in the airways (from nose to terminal bronchioles)

COPD patients have frequent upper airway diseases. According to Roberts et al. (2003), COPD patients had high prevalence of several nasal symptoms (75%). These symptoms were particularly common in patients who had frequent exacerbation. In addition, Piotrowska et al. (2010) found that there was inflammation of the nasal mucous and sinuses (rhinosinusitis) and is common in smokers possibly from the irritation from smoke and other particles. Hamdan et al. (2016) reported that COPD patients have higher incidence of laryngopharyngeal symptoms such as hoarseness, excessive throat mucus and cough. The study by Tsao & Shieh (1994) showed that COPD patients have a lesser tracheal index. Tracheal index is the ratio between coronal diameter by sagittal diameter. Eom et al. (2013) found similar observations and found that tracheal index is significantly lower in mild-moderate COPD patients. Chronic bronchitis is a type of COPD where there is productive cough for three months for two consecutive years without any other respiratory or cardiac cause. The main pathology is mucous hypersecretion and goblet cell hypertrophy in the bronchi. The goblet cells expand in the expense of ciliated epithelial cells and Clara cells (Szilasi, Dolinay, Nemes, & Strausz, 2006). The intact bronchial epithelium undergoes atrophy and squamous cell metaplasia. Gohy et al. (2019) in their invitro studies found that in COPD there may be reduction in ciliated cells through dysregulation of epithelial differentiation by transforming growth factor (TGF)- β1. There is inflammation of

the submucosa layer with hypertrophy of the smooth muscles and mucous glands. There is infiltration of macrophages, mononuclear monocytes, $CD8^+$ (Cluster of Differentiation 8) T lymphocytes and plasma cells. Neutrophils are found in submucosa and in the mucous only during exacerbations. Due to increased mucous production, there is high chance of mucous plug formation which leads to airflow blockage and inflammation which then lead to infections (Szilasi, Dolinay, Nemes, & Strausz, 2006).

1.2.5.1.2 Changes in lung parenchyma (respiratory bronchioles, alveolar duct and alveoli)

In emphysema there is permanent destruction of the acinus that includes respiratory bronchioles, alveolar sacs, alveolar duct, and alveoli. The main pathological process is the unregulated inflammation due to large amounts of proteolytic enzymes released into the lung parenchyma (Szilasi, Dolinay, Nemes, & Strausz, 2006). Inflammation in emphysema is dominated by $CD8^+$ T lymphocytes, however macrophages and neutrophils are also involved. The enzymes such as leukocyte elastase, cathepsin G, proteinase 3, MMPs (Matrix metalloproteinases), cystein proteinases and plasminogen activator destroy the elastin and alveolar walls (Saetta, Turato, Maestrelli, Mapp, & Fabbri, 2001). This leads to loss of elastic recoil and thereby reduction in lung function and forced expiratory volume at one second (FEV1). There are three types of emphysema, centrilobular (proximal acinar), pan-acinar, paraseptal (distal acinar) emphysema. In centrilobular emphysema which is common in cigarette smoking there is destruction of the respiratory bronchioles and is predominantly found in the upper lobes. In pan acinar which is common in α-1 antitrypsin deficiency, the respiratory bronchioles, alveolar ducts and sacs are equally involved and predominantly found in the lower lobes. On the other hand, in distal acinar which may coexist with other two types, leads to bullous disease **(Figure 1.7)**. In obstructive bronchiolitis, there is mucous hypersecretion secondary to goblet cell metaplasia leading to obstructed and collapsed bronchioles. Goblet cells replaces Clara cells thereby less surfactant production and airway collapse (Szilasi, Dolinay, Nemes, & Strausz, 2006).

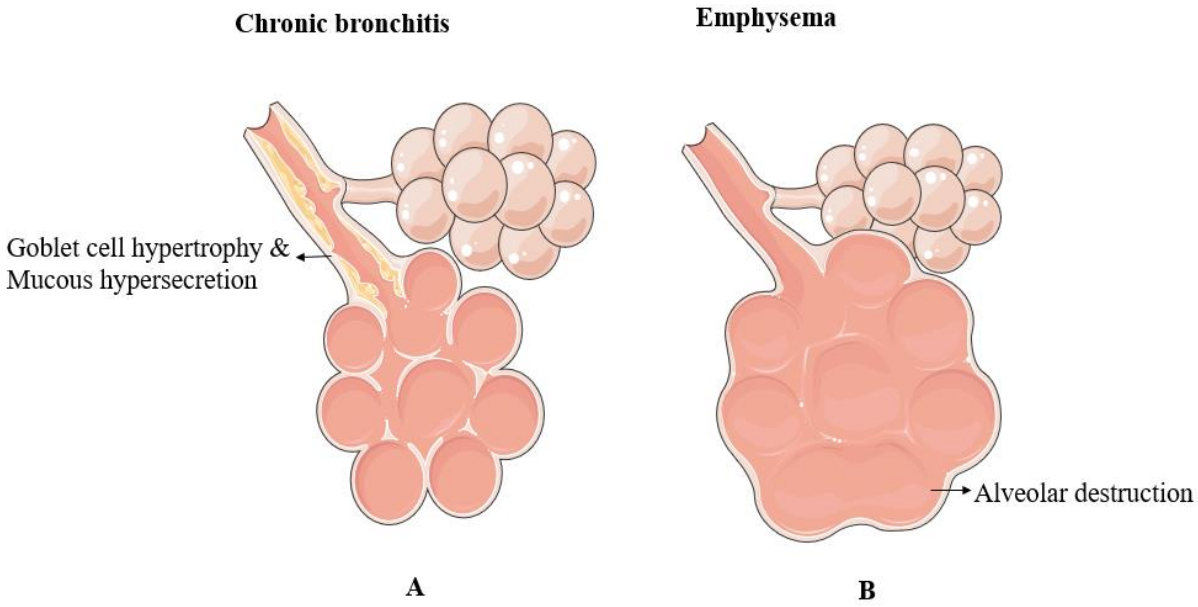


Figure 1.7: The figure A shows changes in chronic bronchitis where there is goblet cell hypertrophy and mucous hypersecretion that leads to chronic airway obstruction. Figure B shows the changes in emphysema where there is alveolar destruction which limits gas exchange. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)].

1.2.5.1.3 Inflammatory cells and their role in airway remodelling

The lungs are constantly exposed to the environment and there are several defence mechanisms to counteract a threat. The respiratory system is protected by the epithelium which itself is a physical barrier but can secrete mucous that acts as an additional physical barrier. The mucous traps microorganisms including bacteria, dust, pollen and other particles which then is expelled out by upward cilia movements. Furthermore, there are complex specialist immune cells that can kill pathogens, neutralise, and remove toxins by releasing a variety of enzymes. However, repeated exposures to toxic particles can cause persistent inflammation and permanent accumulation of these specialist cells into different parts of the respiratory system (Borger, Lau, & Hibbs, 2019). Therefore, it is now understood that the main pathological process in COPD is the chronic inflammation mediated by the inflammatory cells such as neutrophils, monocytes/macrophages and lymphocytes that gets infiltrated into the lung tissues. Neutrophil being the most active inflammatory cell, is found in incresed numbers in the sputum and blood (neutrophilia) of COPD patients (Wang, Xu, Meng, Adcock, & Yao, 2018) **[Figure 1.8]**.

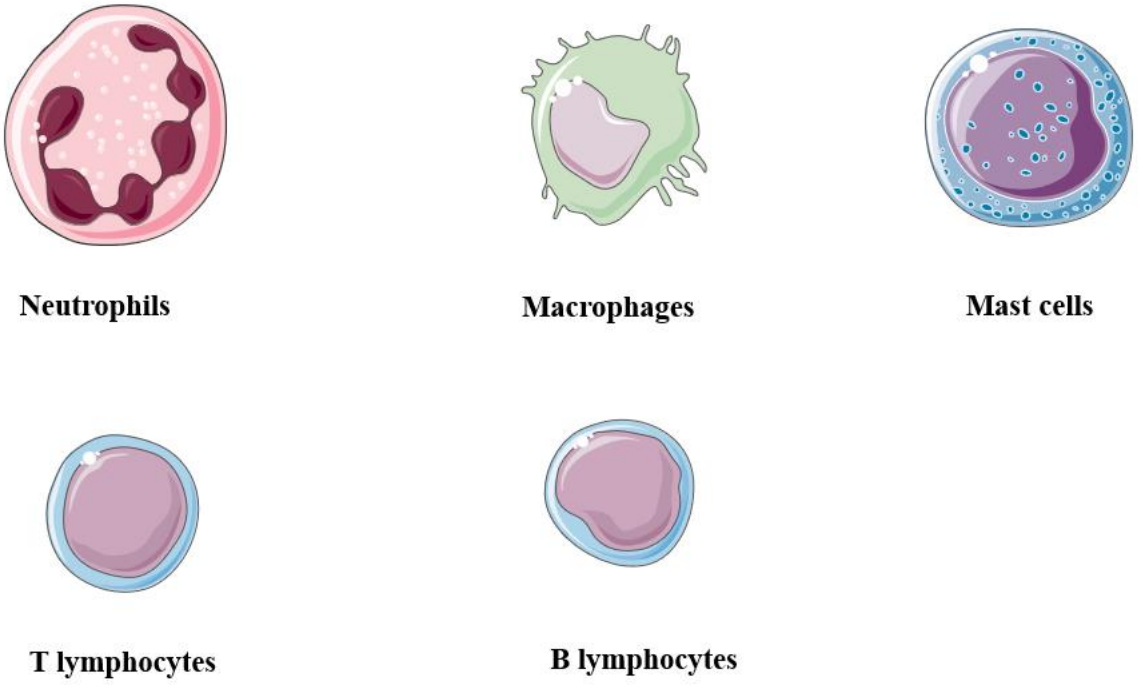


Figure 1.8: Different white blood cells that are involved in the inflammatory process in COPD. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)].

Neutrophils secrete three types of proteases (enzymes that cleaves proteins) such as neutrophil elastase (NE), matrix metalloproteinase (MMP) and myeloperoxidase (MPO). NE degrades the structural components of extracellular matrix (Lerman & Hammes, 2018), enhance fibroblasts proliferation causing peribronchial fibrosis (Gregory et al. 2015) and is a potent stimulant of mucous secretion from submucosal cells and goblet cells (Arai, Kondo, Izumo, Tamaoki, & Nagai, 2010) . This combined effect leads to small airway obstruction. The MMP's that are involved in the pathogenesis of COPD are collagenase MMP-1, the gelatinase MMP-9, and the metalloelastase MMP-12 (Hendrix & Kheradmand, 2017). MMP's are known to destroy extracellular matrix. MPO are stored in both neutrophils and macrophages and they are inflammatory mediators (Nauseef, 2018). The 3-Chlorotyrosine expression is strongly associated with MPO activity in the sputum of COPD patients, therefore it can be used as a biomarker (O'Donnell et al. 2010). Large amounts of neutrophil extracellular traps (NETs) are observed in the airways of COPD patient. They help to entangle bacteria, however excessive

amounts can produce tissue damage ((Qui et al. 2017)). In their study, Dicker et al. 2018 found that NETs formation was increased in the airway of severe COPD patients and those with frequent exacerbations.

Macrophages that are derived from monocytes produce several inflammatory mediators such as IL-8 (Interleukin-8), IL-1β, tumour necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), reactive oxygen species, and MMPs (Barnes, 2014). IL-8 enhances the expression of MUC5AC (Mucin 5AC, Oligomeric Mucus/Gel-Forming) directly or indirectly by inducing the secretion of NE from neutrophils, leading to mucin overproduction and airway obstruction (Jundi & Greene, 2015). According to Simpson et al. 2014, the antibiotic azithromycin inhibits IL-8 and reduces severe exacerbation rate. Therefore Azithromycin us used as a prophylactic antibiotic to prevent exacerbations in certain group of COPD patients. The study by Lerner, Lei, Sundar, and Rahman (2016) found that knockout of IL-8 receptor CXCR2 (CXC chemokine receptor 2) protected mice from cigarette smoke-induced lung inflammation and DNA damage in COPD pathogenesis. IL-1β is known to upregulate the expression of neutrophilic cytokines and MMPs such as MMP-9 and MMP-12 (Lappalainen, Whitsett, Wert, Tichelaar, & Bry, 2005). The study my Reynaert et al. (2015) in mice showed that, TNF-α induces the stimulation of MMPs and NE and the activation of CD8+ T lymphocytes. . Mast cells have been implicated with asthma; however new evidence shows that it is associated in patients with centrilobular emphysema. According to Soltani et al. (2012) there was increased deposition of mast cells in lamina propria in COPD patients.

Lymphocytes such as T-cell, B-cell and T-helper type 17 (Th17) infiltrate the lung (Shaykhiev & Crystal, 2013). T-Lymphocytes are increased in the smokers compared to those who have never smoked with increased CD8+ cells compared with CD4+ cells (Mikko et al. 2013). According to Gadgil and Duncan (2008), the lung tissue destruction is caused directly by T-cell-induced cytotoxicity or indirectly by activating macrophages. There are two types of CD8+ cells based on cytokines they secrete called Tc1 (cytotoxic T lymphocytes 1) cells and Tc2 (cytotoxic T lymphocytes). CD8+ TC2 cells which mainly produce IL-4 and IL-5 cytokines, were significantly increased in COPD lungs and might promote tissue damage and the development of emphysema during exacerbations (Barczyk et al. 2006). According to Hogg et al. 2004, in advanced stages of COPD, there are increased numbers of B-cells within the lymphoid follicles. The study by Duan et al. (2006) found that because Th17 are major sources of the cytokine IL-17, they enhance airway smooth muscle contraction and proliferation. Innate

lymphoid cells (ILC) are a type of lymphocyte and are critical mediators of mucosal immunity (Hsu, Gottschalk, Tsantikos, & Hibbs, 2021). Upon response to noxious particles, ILC2 exhibited phenotype plasticity to form ILC1. The frequency of ILC1 correlated with disease severity and exacerbations in COPD (Silver et al., 2016).

1.2.5.1.4 Changes in pulmonary vasculature

The study by Anderson, Anderson, Gustafsson, and Carlsen (2017) showed that there was significant venous remodelling in COPD patients with pulmonary hypertension which was assessed using venous hypertensive (VH) grade scores. VH scores indicates the extent of intimal smooth muscle proliferation (arterialisation) and intimal fibrosis **(Figure 1.9)**. The inflammation is predominantly mediated by $CD8^+$ T lymphocytes and macrophages. The change in pulmonary vasculature is associated with smoking (Wright, Levy, Churg, 2005). Because of the increased pulmonary vessel thickness, the distensibility is reduced and can cause pulmonary hypertension (Kubo et al. 2000). Hueper et al. 2015 demonstrated that pulmonary microvascular blood flow was reduced in mild COPD patients.

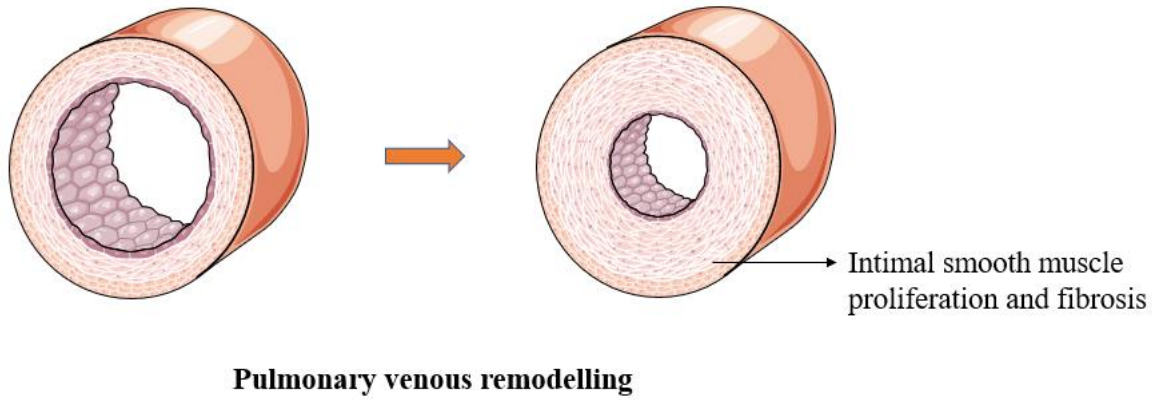


Figure 1.9: The changes in pulmonary blood vessels in COPD demonstrating that there is intimal smooth muscle proliferation and fibrosis. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)].

1.2.5.2 Pathophysiology in COPD

Patients with COPD have several pathophysiological changes in the lungs. In chronic bronchitis, there is mucous hypersecretion resulting in chronic productive cough. Leopold et al. (2009) found that there were cilia shortening in smokers that can cause reduced mucous clearance. Because 90% of COPD patients have a history of smoking and nearly 30-40% of them are current smokers, COPD patients will have a degree of impaired mucous clearance. The ongoing inflammatory process and exudates in the small airways lead to airflow obstruction. Therefore, COPD patients take longer time to breath air out (prolonged expiration) and this leads to air trapping. The I:E (Inspiratory: Expiratory) ratio increases from 1:2 (normal) to 1:3-1:5. In addition, air trapping results in hyperinflation of the lungs which is a characteristic of emphysema in chest x-ray. Guerra et al. (2009) found that in patients <50 years, chronic bronchitis significantly increased the risk of airflow limitation and all-cause mortality by nearly two-fold. Spirometry differentiates obstructive and restrictive lung disease and in obstructive lung disease such as COPD, the FEV1/ FVC ratio is <0.7 (70%). It also helps to differentiate between obstructive lung diseases such as COPD and asthma where the airflow limitation is reversible in asthma. Spirometry is normally done in a pulmonary function laboratory that is normally part of a respiratory clinic. If there is a clinical diagnosis of COPD from history, examination and other radiological evidence, patients are referred for spirometry. If spirometry confirms a non-reversible obstructive pattern, then COPD diagnosis is confirmed. The severity of COPD according to GOLD criteria is based on post-bronchodilator FEV1 in those patients who has FEV1/ FVC ratio <0.7 (GOLD, 2022) **[Table 1.1]**.

Table 1.1: GOLD (2022) criteria for COPD severity based on Forced Expiratory Volume in one second) FEV1 values. As the severity increase, the FEV1 values decreases.

GOLD criteria	**Severity**	**FEV1**
GOLD1	Mild	FEV1 ≥ 80% predicted
GOLD2	Moderate	50% ≤ FEV1 < 80% predicted
GOLD3	Severe	30% ≤ FEV1 <50% predicted
GOLD4	Very severe	FEV1 <30% predicted

Enright et al. (2011) in their study found that spirometry met 90% of the test quality for the diagnosis of COPD in a multicentre study in 14 countries. Shneider et al. (2009) argued that spirometry had a better diagnostic accuracy for COPD when compared to asthma. Therefore, it is vital that patients with suspected COPD should undergo spirometry. However, Lamprecht et al. (2013) found that clinical diagnosis was made without spirometry in 75% of COPD. Therefore, there is a need of change in practice and according to a study by Schirnhofer et al. (2011), targeted spirometry in individuals with respiratory symptoms could identify half of underdiagnosed COPD patients. In their study involving 31 general practices, Walters et al. (2011) found that 56% of the people having treatment for COPD had a normal lung function test. This however is dependent on the availability of adequate resources and use of handheld spirometry devices is a useful tool to be available to general practitioners (Derom et al., 2008). Appropriate training should be available to the staff performing spirometry in general practice. Akhtar and Wilson (2005) observed that the spirometry results obtained by the general practice nurses was lower when compared to that done in the pulmonary function laboratory leading to over diagnosis of COPD. Lamprecht et al. (2011) observed that the prevalence of COPD was very high in never smokers who were >40 years old, however when lower limit of normal (LLN) was used instead of rigid FEV1/ FVC ration of 0.7, the prevalence of COPD was lower. Similar observations were noted by Vollmer et al. (2009) and in addition noted that FEV1/ FEV6 can be used as a substitute for FEV1/ FVC.

As disease progresses, the gas exchange in COPD patients deteriorates. This is due to airway remodelling caused by chronic inflammation leading to extensive destruction of the alveoli and alveolar capillaries. There is ventilation/ perfusion (V/Q) mismatch leading to hypoxia and hypercapnia (type 2 respiratory failure). Rodríguez-Roisin et al. (2009) in their study found that V/Q mismatch was disproportionately worse only in mild (GOLD 1) COPD. This may be due to the fact that in mild COPD, there is minimal airflow obstruction which means that lung parenchyma is more affected than the conducting zone. Hajian et al. (2018) found that V/Q mismatch significantly worsens during COPD exacerbation. Because of this, advanced COPD patients become oxygen dependent and will have high carbon dioxide levels in their blood gases. The high carbon dioxide levels increase the acid load of the lungs; however, the body compensates this by increasing bicarbonate absorption from the kidneys resulting in high bicarbonate levels in blood gases which is called respiratory acidosis. However, during exacerbation (flare up) of COPD, this compensatory mechanism fails, and patients develop respiratory acidosis.

COPD is one of the main causes for Group 3 pulmonary hypertension. COPD patients develop pulmonary hypertension because of the hypoxic vasoconstriction resulting in intimal and smooth muscle hypertrophy and hyperplasia. Hypoxia in COPD is caused by destruction of alveoli and alveolar capillaries (Chaouat, Naeije & Weitzenblum. 2008). Pulmonary hypertension (PH) is defined as mean pulmonary arterial pressure >20 mm of Hg at rest (Simonneau & Hoeper, 2019) or 30mm of Hg during exercise (Kovacs, Berghold, Scheidl, & Olschewski, 2009). Transthoracic echocardiography is used as a non-invasive method to diagnose PH; however, the gold standard test is right heart catheterisation (Augustine et al., 2018). Right heart catheterisation however is an invasive procedure therefore is not widely preferred. Hoeper et al. (2006) argued that right heart catheterisation performed in experienced centres were safe. However, several studies found that pulmonary artery pressure measurements by echocardiography is comparable to right heart catheterisation (D'Alto et al., 2013; Oxley et al., 2017). Therefore, current clinical practice is to measure pulmonary artery pressure with echocardiography. The study by Thabut et al. (2005) showed that approximately 50% of the severe COPD patients had PH who had been referred for lung reduction/ transplantation surgery, however only a small percentage had severe pulmonary hypertension. Because of pulmonary vasoconstriction, the right ventricle needs to contract harder which ultimately leads to right ventricular hypertrophy and subsequently right ventricular failure. This is called cor pulmonale and COPD patients presents with features of right heart failure such as leg oedema, hepatomegaly (liver enlargement) and ascites (Shujaat, Minkin, & Eden, 2007).

1.2.6. Diagnosis of COPD

1.2.6.1 Clinical diagnosis

The diagnosis of COPD should be considered in individuals who present with certain key indicators (Vogelmeier et al., 2017). Shortness of breath or dyspnoea is the cardinal symptom of COPD which is progressive, irreversible and exertional which causes disability and has significant impact on the quality of life (Marciniuk, 2011). Chronic cough is another indicator of COPD which can be intermittent with or without sputum production. Cough is often disregarded as the consequence of smoking or exposure to irritants; however, it may be the first indicator of COPD (Smith & Woodcock, 2006). Another indicator for COPD is chronic sputum production. As described before patients with cough and sputum production for three months on two consecutive years is diagnosis of chronic bronchitis. If there is large sputum production, then patients might have an underlying bronchiectasis (Ramos et al., 2014). The sputum is normally clear, however during infections the sputum can be coloured. Blood in sputum (haemoptysis) in COPD patients may indicate serious pathologies such as infection, pulmonary embolism or malignancy (Earwood & Thompson, 2015). Recurrent lower respiratory tract infections are another indicator of COPD. Because of chronic airflow obstruction, there is air trapping or stagnation of air which leads to longer exposure of pathogens to lung tissue. In addition, excessive mucous production and damage to the epithelial lining, means there is an increased opportunity for the microorganisms to colonise and thereby cause recurrent infections. It is estimated that 75% of all acute exacerbation of COPD is caused by an identifiable bacterial or viral pathogen (Sethi & Murphy, 2008). COPD patients often have wheezing which is an airway sound caused by narrowing that can be intermittent or persistent. Wheezing substantially increases during exacerbations. Alongside, there is chest tightness that is a subjective feeling when there is an increased airflow obstruction that gets relieved with bronchodilator therapy. Another symptom often underdiagnosed is the fatigue (Goërtz et al., 2018). It is estimated that approximately 50% of moderate to severe COPD patients suffer from fatigue. Fatigue is a subjective feeling of physical exhaustion (Peters et al., 2010) and is a characteristic feature of chronic illness. The study by Prescott et al. (2002) and Vanfleteren et al. (2016) found that COPD patients had lower BMI.

A detailed medical, occupational, family and travel history should be undertaken that helps as an aid in the diagnosis of COPD. The history should have emphasis on occupational history and social history where exposure to individual risk factors is ascertained. It is vital to obtain key information such as the type, duration of exposure and there might be particulate history pertaining to a certain region. It is difficult to find physical signs on examination during the initial stages of COPD, therefore this is not diagnostic. During later stages, patients have persistent wheeze, muscle wasting, cachexia, features of heart failure. COPD patients may appear cyanotic particularly in emphysema called blue bloaters and may be puffing for breath in chronic bronchitis called pink puffers. During exacerbations patients might have fever and may be septic. National Institute of Clinical Excellence has given clear guidelines regarding the diagnosis of COPD. Patients who are more than 35 years and presenting with longstanding history of shortness of breath, productive cough, frequent chest infections and wheeze with history of risk factors such as of smoking, occupational or environmental exposure should be investigated for COPD (NICE, 2021).

1.2.6.2 Investigations that helps in the diagnosis of COPD

There are several investigations that help in the diagnosis of COPD. As explained before spirometry is the gold standard test that is used to confirm and assess the severity of COPD. Another most popular test that is used is chest x-ray. Chest x-ray is not diagnostic; however, it gives valuable information regarding the state of the lungs and to rule out other alternative diagnosis. Patients with moderate to severe emphysema have a typical hyperinflated lungs, horizontal ribs, flattened diaphragm and narrow mediastinum. Other findings are bullae in emphysema, pneumothorax (air outside lungs), consolidation in pneumonia and opacities in malignancy. Computed tomography is not recommended for routine use in COPD; however, it can help to diagnose concomitant diseases and cancer especially those patients at risk of developing it. The study by Couturaud et al. (2021) found that 5.9% of AECOPD patients suffered from pulmonary embolism that was confirmed using Computer Tomography Pulmonary Angiogram (CTPA). Blood tests are not diagnostic but help to identify α-1 antitrypsin deficiency the other cause of shortness of breath. During infective exacerbations, the white cell count, inflammatory markers such as CRP (C-reactive protein) and PCT (procalcitonin) will be elevated. To evaluate patients' blood oxygen saturation, a pulse oximetry can be used. It will also aid in making a decision whether the patient requires supplemental oxygen. Arterial blood gas measurements are mostly used in acute settings where

patients present with acute exacerbations. On occasions D-dimer is used as a ‘rule out test’ to detect pulmonary embolism among COPD patients. The study by Zhang et al. (2016) demonstrated that D-dimer can be used as a potential biomarker for the progression of COPD. Echocardiography is used to diagnose pulmonary hypertension, right ventricular hypertrophy and right ventricular failure in COPD patients (Sujaat, Minkin, & Eden, 2007).

1.2.7 Acute exacerbation of COPD

COPD is a chronic respiratory illness and an exacerbation is the worsening or flare up of the COPD symptoms that require an intervention (Wedzicha & Seemungal, 2007). There were multiple attempts to define exacerbations in terms of symptoms alone or symptoms and an intervention. Both methods have their own advantages and disadvantages (Pauwels et al., 2004). GOLD (2022) has defined exacerbations based on interventions alone which is now widely accepted as mild, moderate and severe **(Table 1.2)**.

Table 1.2: GOLD 2022 classification of COPD exacerbation into mild, moderate and severe and its management.

Severity	Management
Mild	Short acting bronchodilators (SABD) that includes, short acting beta-2 agonist (SABA) and short acting muscarinic agonist (SAMA)
Moderate	SABD + antibiotics ± oral corticosteroids
Severe	Exacerbation requiring emergency department or hospital admission.

Exacerbations occur commonly in moderate to severe COPD group. It is estimated that 22-40% of patients experience at least one episode and 9-16% experience more than one episode of moderate to severe exacerbations (Gayle et al., 2018). In UK, COPD is the second common cause for emergency admissions (Hunter et al. 2016). The prevalence of exacerbation in moderate to severe COPD group varied in studies by Miravitlles et al. (2004) [1.5 to 2 episodes per year] and Wedzicha & Donaldson (2003) [2.3-3 exacerbations per year]. Another study by Sadatsafavi et. (2016) found that the average rate of exacerbations was 1.53 episodes per year. Studies show that frequent exacerbations reduce quality of life (Seemungal et al., 1998) and contribute to lung function decline (Donaldson et al., 2002). The results from ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) study showed that the rate at which exacerbations occur is dependent on a frequent-exacerbation phenotype (Hurst et al., 2010). Analysis of 25,857 patients by Donaldson et al. (2010) found that COPD exacerbations increase the risk of myocardial infarction and stroke. The SUMMIT (Study to Understand Mortality and MorbidITy) randomised control study conducted in 43 countries involving 16,485 patients showed that there is an increased risk of cardiovascular disease within 30 days of exacerbation in those who are hospitalised. Mortality increased with

frequency of COPD exacerbations (Soler-Cataluña et al., 2005) and meta-analysis by Hoogendoorn et al. (2011) showed that the average case fatality rate was 15.6% (95% CI 10.9-20.3). The study by García-Sanz et al. 2017 showed a high one-year mortality (26.2 %) and five-year mortality (64.3%) in COPD exacerbation. There is no single biomarker that can assess the severity of exacerbation. SPIROMICS (Subpopulations and Intermediate Outcomes in COPD Study) study found that high concentrations of sputum eosinophils can identify smokers with high severity, more frequent exacerbation, and increased emphysema (Hastie et al., 2017).

1.2.8 Management of COPD

The management of COPD patients is very complex. The primary aim is improving the quality of life by reducing the debilitating symptoms that are mentioned above and to prevent exacerbations. The flaring up of symptoms is called exacerbation and is caused by either bacterial or viral infection (50-70%), exposure to irritants or other unknown factors. The interventions in the management of COPD are therefore broadly divided into non-pharmacological and pharmacological.

1.2.8.1 Non-pharmacological interventions

One of the most important non-pharmacological interventions is to identify and reduce the exposure of risk factors. Approximately 50% of the COPD patients are current smokers. Therefore, cessation of smoking is a key intervention for all COPD patients who continue to smoke (Tashkin, 2015). All COPD patients who are actively smoking should be referred to a smoking counselling programme. This includes patient education, behavioural therapies, and lifestyle changes. Cochrane systematic review by van Eerd, van der Meer, van Schayck, and Kotz (2016) found that a combination of behavioural and pharmacotherapy in smoking cessation in COPD patients is very effective. Patients should be encouraged to reduce exposure to indoor and outdoor air pollutants. In addition, patients should also be advised to prevent occupational exposure.

Vaccinations are recommended for COPD patients to prevent exacerbations or serious infections such as pneumonia. As mentioned above 50-70% of the exacerbations are caused by infections and the commonest being rhinovirus, influenza, Haemophilus influenzae and Streptococcus pneumonea. The vaccines routinely given to COPD patients are influenza and pneumococcal vaccines. Meta-analysis by Kopsaftis, Wood-Baker & Poole (2018), found that ‘killed’ influenza vaccines reduced exacerbations in COPD patients, however there was no change in mortality. Intra-nasal live attenuated influenza vaccine did not improve the number of exacerbations. Systematic review by Bekkat-Berkani et al. (2017) supports the evidence regarding the risk-benefit ratio of seasonal influenza vaccinations. RCT (Randomised Controlled Trial) by Alfageme (2006) found that pneumococcal vaccine [23-valent pneumococcal polysaccharide vaccine (PPV)] reduced community acquired pneumonia in

COPD patients if they are less than 65 years old and those with severe airflow obstruction. Cochrane review of 12 RCT's by Walters, Tang, Poole, and Wood-Baker (2017), found that injectable polyvalent pneumococcal vaccination gave significant protection against community acquired pneumonia and reduction in exacerbations. In addition, COPD patients are in the priority list for Covid-19 vaccinations.

Pulmonary rehabilitation (PR) helps to improve physicopathological and psychopatholgical manifestation of COPD. It is a multidisciplinary approach aimed to improve exercise capacity, health related quality of life (HRQoL) and dyspnoea. A meta-analysis of 13 RCT's by Ryrsø et al. (2018) found that early PR in COPD patients admitted to hospital with exacerbations reduces mortality, hospital length of stay and readmissions with exacerbations. Another meta-analysis of 20 RCT's by Lee & Kim (2019) found that PR significantly improved the respiratory muscle strength measured by maximal expiratory pressure and maximal inspiratory pressure. McCarthey et al. (2015) did a Cochrane systematic review of 65 RCT's and found that PR significantly improved HRQoL, functional and maximal exercise capacity in COPD patients. Blervaque et al. (2021) found that 5-yr survival probability was more in the COPD patients who received long term pulmonary rehabilitation.

1.2.8.2 Pharmacological interventions

The drug treatment options available for COPD patients includes bronchodilators (beta-2 agonists, muscarinic antagonist, phosphodiesterase inhibitors and Leukotriene receptor antagonists), anti-inflammatory (corticosteroids), mucolytics and antibiotics. These drugs are used individually or as combination therapy. Beta-2 agonist acts by stimulating beta-2 receptors and cause bronchial smooth muscle relaxation thereby dilatation of the airways. The short acting beta-2 agonist (SABA) such as salbutamol and terbutaline acts within 15 minutes and last for 4-6 hours. It is therefore used for immediate relief of symptoms. The long-acting beta-2 agonist (LABA) such as salmeterol and formoterol have an action lasting for 12 hours or more. Muscarinic antagonist or anticholinergics competitively binds to acetyl choline receptors there by blocking the smooth muscle contraction mediated by acetyl choline resulting in bronchodilation. Short-acting muscarinic antagonist (SAMA) such as ipratropium helps in immediate relief of symptoms, and it acts at 30-60 minutes to 3-6 hours. Long-acting muscarinic antagonists (LAMA) such as tiotropium has an onset of action at 30 minutes and lasts for over 24 hours, therefore it can be used once-daily. Phosphodiesterase inhibitors acts

by inhibiting the phosphodiesterase enzyme, thereby causing smooth muscle relaxation in airways and blood vessels. Methylxanthines such as theophylline and aminophylline (theophylline+ ethylenediamine) are non-selective phosphodiesterase inhibitors and therefore they can cause significant systemic side effects. Leukotriene receptor antagonists such as montelukast and zafirlukast reduce inflammation and cause smooth muscle relaxation in airways (Wiffen, P., Mitchell, M., Snelling, M., & Stoner, N., 2017).

Corticosteroids switch off multiple inflammatory genes that get activated during the inflammatory process (Barnes, 2006). In COPD, steroids are given either in an inhaled form, orally or intravenously. Carbocysteine is a mucolytic that can be given orally or by inhalation and they act by opening the disulphide bonds in mucus using their free sulphydryl groups. N-acetyl cysteine which is used as an antidote for paracetamol overdose is a mucolytic given by inhalation act by hydrolysing the disulphide bonds in mucus. Antibiotics are used if the exacerbation is known to be caused by bacterial infection (Wiffen, P., Mitchell, M., Snelling, M., & Stoner, N., 2017) **[Table 1.3]**.

Table 1.3: The list of different medications that can used in the management of COPD

$Beta_2$ agonists	Short-acting (SABA)	Salbutamol, Terbutaline
	Long-acting (LABA)	Salmeterol, Formoterol
Muscarinic antagonist	Short-acting (SAMA)	Ipratropium bromide
	Long-acting (LAMA)	Tiotropium
Phosphodiesterase inhibitors	Methylxanthines	Theophylline, Aminophylline
Leukotriene receptor antagonists		Montelukast and zafirlukast
Corticosteroids	Oral	Prednisolone
	Intravenous	Hydrocortisone
	Inhaled	Fluticasone, Budesonide, Beclomethasone, Mometasone
Mucolytic agents		Carbocysteine, N-acetyl cysteine
Antibiotics		Amoxicillin, Doxycycline

Pharmacological interventions differ in stable and acute exacerbation of COPD (AECOPD).

1.2.8.2.1 Pharmacological interventions in stable COPD

Patients who are diagnosed with COPD are normally started on a long-acting bronchodilator for symptom control and to prevent exacerbation. There are controversies regarding the selection of long-acting bronchodilator therapy and whether it is better to use monotherapy or in combination. The three main inhaled therapies used are LABA, LAMA and ICS (Inhaled corticosteroids). The Cochrane review of 26 studies showed that LABA are effective over medium- and long-term COPD patients with moderate to severe form of the disease. Even though LABA improves quality of life and reduces exacerbations, there is no improvement in mortality (Appleton, Smith, Veale, & Bara, 2000). LABA/ LAMA combination therapy in COPD patients reduced exacerbations, increased FEV1, reduces occurrence of pneumonia and improved quality of life in a systematic review of 11 studies (Horita et al., 2017). Oba, Keeney, Ghatehorde, and Dias (2018) in their systematic review of 99 studies and 101,311 patients found that a combination therapy of LABA/ LAMA was the most effective therapy followed by LAMA monotherapy in preventing COPD exacerbations. According to Barrecheguren, Monteagudo, and Miravitlles (2018), in mild to moderate COPD, LAMA monotherapy is equally effective when compared to starting LABA/ LAMA combination therapy. LABA/ ICS combination is better than LAMA alone in COPD patients with eosinophil count more than 4% and LABA/ ICS was associated with higher incidence of pneumonia (Suissa, Dell'Aniello, and Ernst, 2018). Another meta-analysis of 212 RCTs and 19 observational studies showed that triple therapy of LABA/ LAMA/ ICS when compared to LABA/LAMA was significantly more effective in reducing exacerbations and mortality. However, there was increased incidence of pneumonia, no changes in lung function (Axson et al., 2000). The study by Suissa, Patenaude, Lapi, and Ernst (2013) on 163, 514 patients showed that current use of ICS was associated with a 69% increased chance of developing serious pneumonia, however, this risk disappears after 6 months of stopping ICS. Low dose oral theophylline is used in the treatment of COPD. According to Ram et al. (2002), a systematic review of 20 RCTs showed that addition of theophylline along with other therapies increased FEV1 and FVC and increased the arterial blood gas tensions (PaO_2 and PCO_2) in COPD. However, Devereux et al. (2018) in their RCT showed that addition of theophylline to ICS therapy in severe COPD patients did not improve the frequency of exacerbation in one year. Leukotriene receptor antagonists inhibitors are very effective in asthma, however a systematic review of 7 studies and 342 patients showed there is

reduction in the frequency of dyspnoea and sputum production, but not improvement in FEV1 or FVC (Lee, Kim, & Kim, 2015). Mucolytics such as carbocysteine helps in reduction of exacerbation and hospital admission (Poole, Sathananthan, & Fortescue, 2019). Longer term use of carbocysteine is associated with decreased incidence of COPD exacerbations as per the metanalysis by Zeng, Yang, Huang, and Xiao (2017). There is an ongoing debate regarding the use of long-term use of prophylactic antibiotics in COPD. According to Huckle, Fairclough, and Todd (2018), analysis of 12 RCTs showed that use of long-term small dose antibiotics improved exacerbations and quality of life. Macrolide antibiotics (azithromycin and erythromycin) showed greatest effect and anti-inflammatory properties. Similar finding was found by the Cochrane systematic reviews analysing nine studies. However, issues such as antibiotic resistance should be considered before initiating such therapies and therefore, it should be reserved for selected COPD patients (Janjua et al., 2021).

1.2.8.2.2 Pharmacological interventions in AECOPD

Nebulised salbutamol and ipratropium bromide is the bronchodilator of choice for AECOPD. A systematic review of 10 studies by Kopsaftis et al. (2018) found that there is no evidence in using higher doses of SABA when compared to lower doses, delivery method (inhaler vs nebuliser) and there were significantly increased cardiac side effects. According to McCrory and Brown (2002), the systematic review of four trials found that there is no significant difference in the degree of bronchodilatation between beta-2 agonist or ipratropium and there is no difference if used as a combination or individually. Suissa, Assimes, and Ernst (2003) found that SABA did not increase the risk of fatal or non-fatal myocardial infarction. In clinical practice, systemic corticosteroids in AECOPD can be given either orally (prednisolone) or intravenously/ parenteral (hydrocortisone). According to Walters et al. (2014), systemic corticosteroids reduced the risk of treatment failure, relapse in one month, reduction in hospital length of stay and improvement of the symptoms, however there is no benefit in 30-day mortality. In addition, this systematic review found that there is no added benefit in giving parenteral steroids. The REDUCE (Reduction in the Use of Corticosteroids in Exacerbated COPD) randomised controlled trial found that 5-day steroid treatment is not inferior to the conventional 14-day regimen (Leuppi et al., 2013). Therefore, in clinical practice a 5-day course of steroids is widely used. Aminophylline infusion is used in refractory bronchoconstriction in AECOPD. According to Duffy, Walker, Diamantea, Calverley, and Davies (2005), even though aminophylline marginally improves acid-base balance, there is no

evidence to suggest that it improves outcomes. Mucolytics are shown to be effective in exacerbations, however this may not be true due to the bias involved in the studies (Poole Sathananthan, & Fortescue, 2019). About 50-70% of AECOPD is caused by an infection and if there is evidence of bacterial infection, then it is beneficial to prescribe antibiotics. AECOPD patients can present with a wide variety of bacterial infection such as upper or lower respiratory tract infections, pneumonia and can present with sepsis, severe sepsis and septic shock (Wang et al., 2019). Antibiotics administered within the first hour are associated with increased survival and delay is associated with decrease in the survival by 7.6% each hour according to Kumar et al. (2006) in patients with documented hypotension. Therefore, COPD patients with sepsis should be administered antibiotics within one hour of arrival to the hospital. Viral infections can also present similarly, and antivirals are recommended for infections with H1 N1 virus (Rewar, Mirdha, and Rewar, 2015) and COVID-19 (Beigel et al., 2020).

As the disease progresses, there is increased destruction of alveoli leading to ventilation perfusion mismatch in COPD patients. This leads to alveolar hypoxia and consequently systemic hypoxia. Oxygen and glucose are the main substrate for cellular respiration and the cells may not function properly in hypoxia (Dziurla et al., 2010). Therefore, COPD patients who are hypoxic experience tiredness, reduced exercise tolerance, decrease in quality of life and increased risk of death (Kim, Benditt, Wise, & Sharafkhaneh, 2008). Two landmark trials by the Nocturnal Oxygen Therapy Trial Group (Nocturnal Oxygen Therapy Trial Group, 1980) and Medical Research Council Working Party (Stuart et al., 1981) demonstrated that continuous oxygen therapy improves mortality in COPD patients who require oxygen. Long term oxygen therapy (LTOT) should be given minimum 15hrs/day to those with stable COPD disease with resting PaO_2 of $\leq$ 7.3 kPa and those with a resting PaO_2 of $\leq$ 8kPa with evidence of peripheral oedema, polycythaemia (HCT $\geq$ 55%) or pulmonary hypertension (Hardinge et al., 2015).

Non-invasive ventilation (NIV) is a mode of ventilation where patients are supported when they breath using a mask. Osadnik et al. (2017) in their meta-analysis of 17 clinical trials found that NIV reduced the risk of dying by 46% and the risk of needing intubation by 65% in AECOPD patients. According to Raveling et al. (2021), in stable patients, NIV reduced daytime hypercapnia, survival and HRQoL and in patients with persistent hypercapnia, there is improvement in survival but not quality of life. The current guidelines are to commence on NIV if the patient is having respiratory acidosis (pH<7.35).

1.2.8.2.3 Self-management

Self-management is a concept of taking responsibility and engaging with the health care provider in the treatment of chronic illnesses (Grady & Gough, 2014). In COPD patients this will help to improve the quality of life and prevent exacerbations. There were multiple randomised controlled studies that showed there was reduction in exacerbations, hospital admissions and health care visits with a self-management programme (Gadoury et al., 2005; Bourbeau et al., 2003; Rice et al., 2010), however some studies showed no benefit (Bischoff et al., 2012; Bucknall et al., 2012; Fan et al., 2012). Meta-analysis by Zwerink et al. (2014) and Lenferink et al. (2016) showed that self-management interventions improve the quality of life in COPD patients and reduce the number of hospital admissions with exacerbation. It is now a common practice to offer rescue packs for COPD patients that includes a short course of steroids and antibiotics. In clinical practice, most of the patients who attend the emergency department with an exacerbation of COPD have already been commenced on their rescue pack. In the UK, most of the GP surgeries have a COPD nurse and patients have got direct access to them.

1.2.9 Comorbidities in COPD and its management

COPD patients often have concomitant chronic diseases (comorbidities) and there are several comorbidities associated with COPD. These include cardiovascular diseases, diabetes mellitus, hypertension, venous thromboembolism, heart failure and cancer (Chatila, Thomashow, Minai, Criner, & Make, 2008). The prevalence of these co-morbidities is however variable among different studies. The study by Chetty et al. (2017) showed that 86% of the COPD patients have at least one co-morbidity, however patients with two or more comorbidities are significantly higher according to Raherison et al. (2018). COPD in itself can increase the risk for other diseases or vice versa, therefore all the disease conditions should be identified and treated appropriately. The presence of comorbidities in COPD patients increase the risk of hospital admissions, reduced HRQoL and mortality (Cavaillès et al., 2013).

1.2.9.1 Systemic Hypertension

Hypertension is very common and affects 1 in 4 adults in England (The National Institute for Health and Care Excellence, 2019). Several large studies have shown that hypertension is the most frequently occurring co-morbidity in COPD which varied from 35-50% (Divo et al., 2012, Chetty et al., 2017). One of the reasons for this would be that most of the COPD patients remain smokers and there is a strong association between smoking and hypertension. Smoking causes hypertension by stimulating sympathetic system, impairing endothelial function and increasing arterial stiffness (Virdis, Giannarelli, Neves, Taddei, and Ghiadoni, 2010). Another cause would be the use of salbutamol which is a beta-2 agonist that increases heart rate and blood pressure. However, a RCT by Cekici, Valipour, Kohansal, and Burghuber (2009) demonstrated that there is no difference in blood pressure in healthy individuals on using salbutamol. This might not be the case in smokers who have stiffened arterial system. As COPD progresses, there is increased physical inactivity (Shin, 2018), and this might contribute to hypertension (Hegde & Solomon, 2015). There are no specific guidelines in the treatment of systemic hypertension in COPD, therefore patients should be treated as per the national guidelines for the treatment of hypertension (National Institute for Health and Care Excellence, 2019). It is to be noted that hypertension itself is not an independent risk factor for VTE (Holst, Jensen, and Prescott, 2010), therefore hypertensives are not routinely anticoagulated.

1.2.9.2 Ischemic heart disease

COPD patients have high incidence of developing ischemic heart disease (IHD). As discussed above the most frequent comorbidity associated with COPD is hypertension and hypertension itself is a significant risk factor for IHD (Brunström & Carlberg, 2018). In addition, smoking is an important another risk factor for IHD, and current smokers have 2 times increased risk of developing IHD (Banks et al., 2019). COPD patients who have IHD have increased risk of developing myocardial infarction with the use of salbutamol (de Vries et al. 2008). One of the main causes for deaths in mild- moderate COPD is cardiovascular disease, next to cancer according to Berry & Wise (2010). The management of IHD is similar to how non-COPD patients are managed. In their study Barsoum et al. 2014 found that myocardial infarction is not a risk factor for VTE. Patients with IHD are often commenced on an antiplatelet therapy.

1.2.9.3 Diabetes mellitus

Diabetes mellitus is a comorbidity that is known to exist in COPD patients. There are several reasons that is suggested, the first one being that COPD is a chronic systemic inflammatory disease. There is evidence from ATTICA study by Pitsavos et al. (2007) to demonstrate that there is association between low grade systemic inflammation and diabetes. Secondly, the oxidative stress in COPD patients due to chronic hypoxia can be another contributing factor (Evans, Goldfine, Maddux, and Grodsky, 2002). Thirdly, steroids are used as inhalers in stable COPD patients and systemically during acute exacerbations. Steroid use is a well-known factor for development of diabetes (Hwang & Weiss, 2014). According to Ho et al. (2017), a COPD patient with diabetes has worse outcomes, therefore diabetes in this group should be treated aggressively. Holst, Jensen, and Prescott (2010) in their epidemiological study found that diabetes mellitus was not an independent risk factor for VTE.

1.2.9.4 Cerebrovascular accidents

Morgan et al. (2017) argued that COPD patients are at higher risk of developing cerebrovascular accidents (CVA). There are several reasons for this and the most important one is smoking. In their meta-analysis Pan et al. (2019) found a strong relationship between smoking and CVA. Smokers had increased risk of developing CVA than non-smokers with current smokers having the most risk. In addition, passive smoking increased the risk of CVA

by 45% and with every increment of smoking by five cigarettes, there was a 12% increase in stroke. It is worth remembering that about 30-40% COPD patients are current smokers. Other shared comorbidities such as hypertension (Perticone et al., 2021), ischaemic heart disease and diabetes may increase the risk of CVA. The study by Corlateanu et al. (2018) demonstrates that COPD patients are prone in developing haemorrhagic strokes. According to Windsor, Herrett, Smeeth, and Quint (2016), there is no association between COPD exacerbation and stroke. The management of CVA in COPD is not different to non-COPD patients. The Tromsø study found that there was an increased risk of VTE during the first 3 months after having an ischaemic stroke (Rinde et al. 2016).

1.2.9.5 Atrial fibrillation (AF)

AF is a type of arrythmia of the heart, where the atria will be fibrillating instead of contracting. This leads to stagnation of the blood in atria causing thrombus formation which can then embolise to different parts of the body. The heart rate can go above 150 beats per minute and the pulse is typically irregular. AF is therefore a serious medical condition and if not treated can increase the risk of stroke by 5-fold and mortality by 2-fold (Markides & Shilling, 2003). In addition, the Tromsø study demonstrated that the risk of VTE is increased 8-fold in patients with AF ((Rinde et al. 2016). The treatment for AF is therefore rate and rhythm control initially, however most of them require lifelong anti-coagulation. Hypertension and IHD are two shared important risk factors for developing AF in COPD. According to Chamberlain et al. (2011), smokers have a 2-fold increased risk of developing AF. Similar findings were observed by Aune, Schlesinger, Norat, & Riboli (2018) in their meta-analysis. Pulmonary hypertension, cor-pulmonale and use of salbutamol are known to cause atrial fibrillation in COPD patients.

1.2.9.6 Pulmonary hypertension (PH)

In COPD as the disease progresses there is pulmonary vascular remodelling which is due to chronic hypoxia and ongoing inflammation from cigarette smoking and other noxious particles. This is known to be the main cause of developing pulmonary hypertension in these patients (Chaouat, Naeije & Weitzenblum, 2008). According to a large study by Lutsey et al. 2022, within two years of developing VTE, 3.5% developed PH and those with PE, 6.2% developed PH within two years.

1.2.9.7 Cor-pulmonale

Cor-pulmonale is acute right ventricular failure which is caused by primary pulmonary problem. Acute cor-pulmonale is caused by massive pulmonary embolism and chronic cor-pulmonale is caused by pulmonary hypertension. COPD is the most common cause of chronic cor-pulmonale (Garrison, Pendela, and Memon, 2022).

1.2.9.8 Cancer

Lung cancer is the most common cancer in COPD patients and COPD is associated with a 2-fold increase in lung cancer (Young et al., 2015). The lifetime chance of developing lung cancer in smokers is more than 10 times than in non-smokers (Villeneuve & Mao, 1994) and 20% of the smokers develop COPD. Smokers with airflow obstruction have a 5-fold increased incidence of lung cancer (Young & Hopkins, 2010). However, COPD is an independent risk factor for developing lung cancer irrespective of smoking as per Park et al. (2020). Another interesting finding from this study was that never smokers with COPD have a 2.6 times increased chance of developing cancer when compared to those without COPD. According to Heit, Spencer, and White (2016), 20% of all VTE incidence is caused by active cancer.

COPD patients develop several concomitant diseases (co-morbidities) during their lifetime. The risk factors mentioned before (section 1.2.3) that are associated with COPD can also contribute to the development of some of these comorbidities. For example, smoking is a risk factor for developing both hypertension and COPD. Patients with hypertension are treated with antihypertensives and not anticoagulated because hypertension is not an independent risk factor for VTE. Therefore, in COPD patients with hypertension, the increased risk of VTE might be from the ongoing inflammatory process that worsens with ongoing smoking. COPD patients with comorbidities including atrial fibrillation, cerebrovascular accidents, pulmonary hypertension, and lung cancer may have an increased risk of developing VTE. Therefore, it is vital to address these risks and initiate appropriate treatment in the form anticoagulation therapy.

1.3. Normal haemostatic system

1.3.1. Introduction

Blood is essential for the body to deliver nutrients and oxygen to tissues and cells. Large volumes of blood can be lost after an injury and therefore the body adopts several mechanisms to stop the bleeding for its survival. This process is called haemostasis and it is the first stage of wound healing (Periayah, Halim, and Mat Saad, 2017). This is achieved by three mechanisms, firstly there is vasoconstriction and platelet plug formation at the site of the injury to stop bleeding, secondly there is fibrin mesh to stabilise the clot and thirdly formation of plasmin and fibrinolysis (McRae, S., 2011) **[Table 1.4; Figure 1.10]**.

Table 1.4: Classification of haemostasis into primary, secondary and tertiary haemostasis

Primary haemostasis	Local vasoconstriction and platelet plug formation
Secondary haemostasis	Formation of fibrin that stabilises the platelet plug
Tertiary haemostasis	Formation of plasmin and fibrinolysis

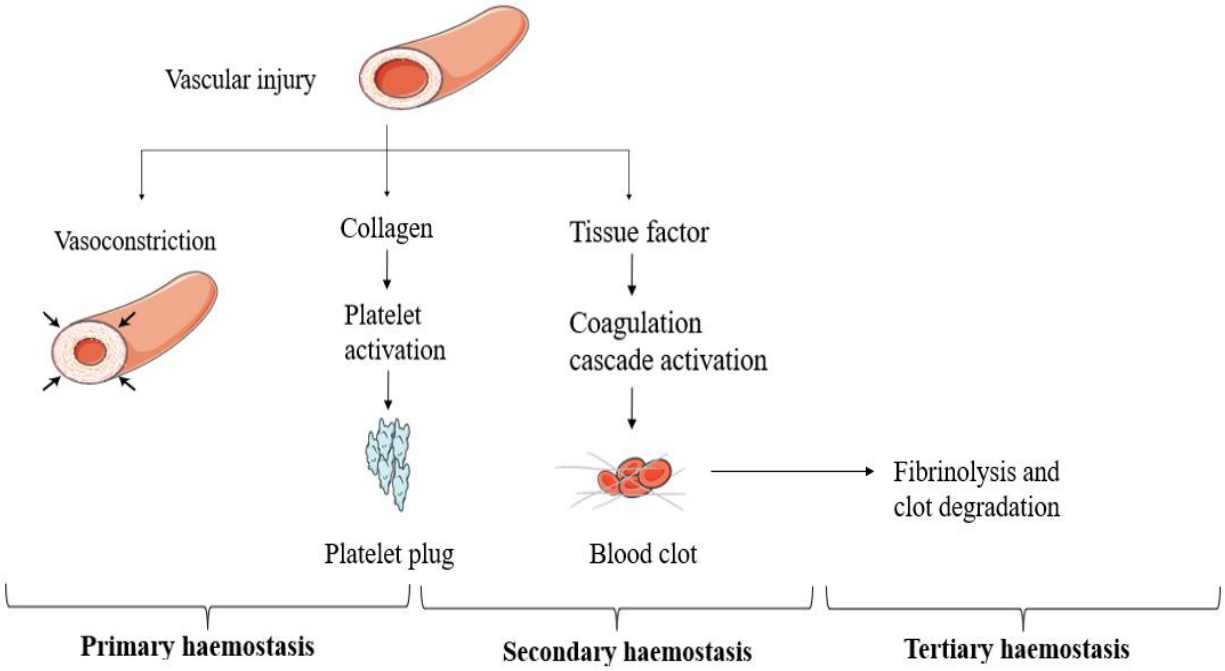


Figure 1.10: Different stages of haemostasis. After vascular injury the first response of the body is vasoconstriction. The collagen that is exposed leads to platelet activation which then forms the platelet plug. This is called primary haemostasis. The tissue factor released activates the coagulation pathway and the fibrin formed stabilises the blood clot. This process is called secondary haemostasis. Finally, in tertiary haemostasis, the blood clot undergoes lysis. [Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)].

1.3.2. Primary haemostasis

Soon after an injury, there is arterial vasoconstriction which is a reflex mechanism of the local sympathetic pain receptors. In addition, the vascular endothelial cells release a potent vasoconstrictor called endothelin-1 which enhances the vasoconstriction. Endothelin-1 is found in all vascular endothelium and is continuously synthesised and released, however this process is amplified in vascular injury (Davenport et al., 2016). This mechanism is thought to last for approximately 30 minutes.

The outer layer of the blood vessel (tunica externa) is made mainly of collagen which get exposed after a vascular injury. Collagen which is the primary component of connective tissue forms 25-35% of the whole-body protein content and the main structural protein in the body (Di Lullo, Sweeney, Korkko, Ala-Kokko, and San Antonio, 2002). Von Willibrand Factor (vWF) is a glycoprotein that liberally moves in the blood then binds to the collagen through its A1 and A3 collagen binding domains. This subsequently activates the A1 domain which binds to N-terminal domain of glycoprotein-Ib_{α} of the platelets (Springer, 2014). Platelets are colourless cells that originate from the bone marrow and circulate in the blood in an inactivated form for a period of 10 days. The normal platelet concentration in the blood is 150-400 x 10^9/L (Schulze & Shivdasani, 2005). However, the vWF- glycoprotein-Ib_{α} interaction is not sufficient to arrest platelets. There are two platelet receptors, integrin α_2 β_1 and glycoprotein VI that binds to collagen and facilitates 'platelet adhesion' at the area of vascular injury (Denorme, Vanhoorelbeke, and De Meyer, 2019). Another platelet receptor, integrin α_{2b} β_3 after binding to collagen sends outside-in signals leading to reorganisation of the cytoskeleton which causes flattening of the platelets. This process is called 'platelet spreading' which enables increase in the surface area of contact on the damaged endothelium (Lee, Fong, King, Brass, and Hammer, 2012). In addition, collagen activates phospholipase $C_{\gamma}2$ enzyme which cleaves phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate. This induces the release of calcium and 1,2-diacylglycerol which activates protein kinase C. This leads to platelet shape change, granule secretion, and aggregation. Platelets are disc shaped at rest and when activated, the cytoskeleton reorganises to form numerous pseudopods and appear to give its dendritic appearance (Periayah, Halim, and Mat Saad, 2017). In addition to platelet adhesion and spreading during the activation process, platelets release several granules that contain important factors of haemostasis, the most important among alpha (α) and dense granules. α-

granules form 10% of the platelet volume and has large polypeptides such as vWf and fibrinogen (Blair & Flaumenhaft, 2010). As discussed before vWF circulates in blood (plasma vWF) as well as being stored in α-granules (platelet vWF) [McGrath, McRae, Smith, and O'Donnell, 2010]. The dense granules contain high concentrations of low molecular weight compounds that potentiate platelet activation such as ADP (Adenosine diphosphate), ATP (Adenosine triphosphate), serotonin, and calcium.

'Platelet aggregation' or platelet- platelet adhesion is the clumping of platelets together and is essential for the formation of platelet plug to arrest bleeding. This complex process is mediated by three aggregating agents such as ADP, Thromboxane A2 (TBXA2) and Thrombin (Sangkuhl, Shuldiner, Klein, & Altman, 2011). ADP was the first agent that is recognised to cause platelet aggregation. It is found in dense granules and released upon platelet activation (Daniel et al., 1998). ADP causes change in shape, aggregation and further release of dense granules from platelets. ADP binds to P2RY12 (Purinergic Receptor P2Y12) receptor which couples with Gi inhibiting adenylate cyclase (Hardy, Jones, Mundell, & Poole, 2004). Because of this cyclic AMP (Adenosine monophosphate) is not formed which inhibits inositol 1,4,5-trisphosphate-mediated calcium release (Eigenthaler, Nolte, & Halbrügge, 1992; Jakobs, Watanabe, & Bauer, 1986). This leads to calcium release and thereby shape change (Varga-Szabo, Braun, & Nieswandt, 2009). Antiplatelet drugs such as clopidogrel and prasugrel irreversibly bind to P2RY12 blocking ADP induced activation and platelet aggregation.

The activated phospholipase A2 enzymes hydrolyse the membrane gylcerophospholipids to release arachidonic acid (AA). Cyclooxygenase 1 and 2 (COX-1 & 2) catalyses AA to prostaglandin G2 (PGG2) which is then immediately converted into prostaglandin H2 (PGH2). Thromboxane synthase then converts PGH2 to TBXA2 (Wang et al. 2021). Aspirin inhibits cyclooxygenase enzyme, thereby inhibiting TBXA2 synthesis. TBXA2 causes platelet aggregation by binding to thromboxane A2 receptor (TBXA2R) [Sangkuhl, Shuldiner, Klein, & Altman, 2011]. Thrombin is another potent aggregating agent formed by activation of the coagulation cascade which is mentioned in secondary haemostasis. Thrombin binds to protease-activated receptor (PAR) and facilitates aggregation and degranulation (Anderson et al., 1999). The binding of ADP, TBXA2 and thrombin to their respective receptors P2RY12, TBXA2R and PAR receptors and the release of calcium activates glycoprotein IIb/IIIa (GPIIb/IIIa) receptor expressed on platelet cells. GPIIb/IIIa receptor binds to fibrin and vWF which acts as bridges to bind to GPIIb/IIIa receptors of other platelets causing aggregation. The

platelet plug that is formed is then reinforced by fibrin which is formed by the activation of fibrinogen by thrombin. GPIIb/GPIIIa inhibitors such as Abciximab, Eptifibatide and Tirofiban bind to these receptors thereby blocking platelet aggregation (Sangkuhl, Shuldiner, Klein, & Altman, 2011).

1.3.3. Secondary haemostasis

The platelet plug that is formed during the primary haemostasis needs to be stabilised. This is achieved by the formation of fibrin meshwork that interlink the platelets. This process constitutes blood coagulation. Blood coagulation involves activation of several clotting factors which is called coagulation cascade. This happens simultaneously to complement primary haemostasis. When endothelium is exposed after an injury the collagen that is exposed activates the conversion of Factor XII to Factor XIIa and Prekallikrein to Kallikrein. Kallikrein along with kininogen further activates the Factor XII. This is followed by a series of blood coagulation factor activation which leads to the formation of Factor Xa. This pathway is called contact activation pathway or intrinsic pathway and can be measured using the test activated partial thromboplastin time (APTT) [Simão & Freener, 2017)]. Damaged endothelium releases tissue factor which activates Factor VII to Factor VIIa. Factor VIIa activates Factor X to Factor Xa. This pathway is therefore called tissue factor pathway or extrinsic pathway and can be measured using the test prothrombin time (PT). Both intrinsic and extrinsic pathway results in the formation of Factor Xa which then cleaves prothrombin to thrombin which then converts fibrinogen to fibrin. This is therefore called the common pathway (Palta, Saroa, & Palta, 2014) **[Figure 1.11]**.

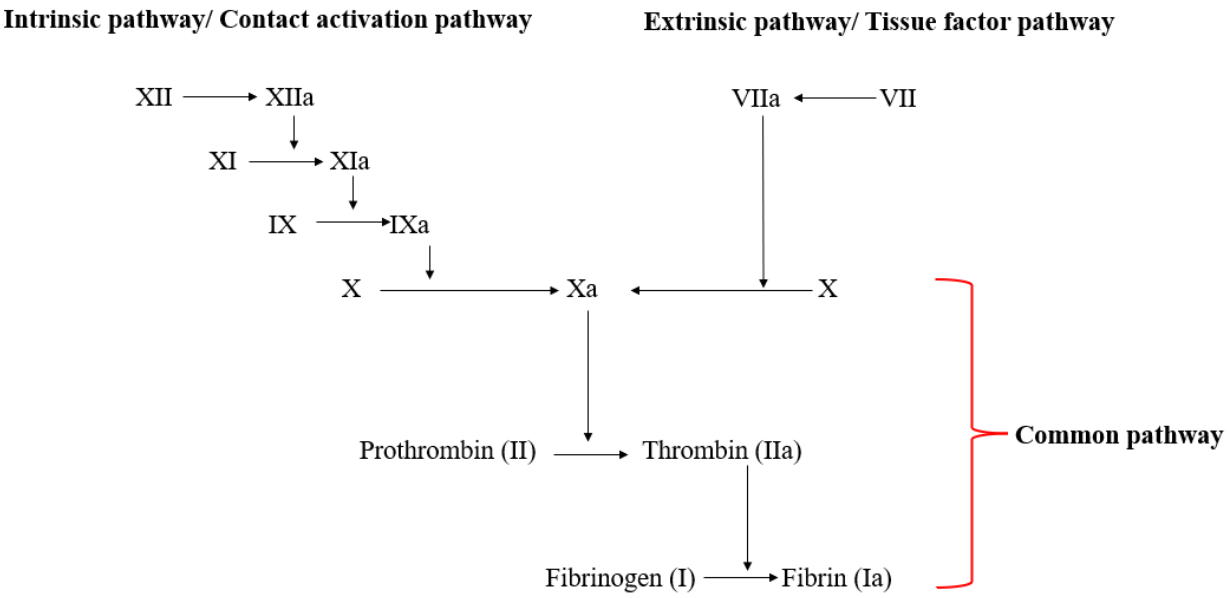


Figure 1.11: Illustration of the coagulation pathway. Coagulation is initiated through the activation of factors either through intrinsic (XII, XI, IX) or extrinsic pathways (VII) which ultimately activates Factor X to Xa. Factor Xa activates Prothrombin to Thrombin which then activates Fibrinogen to form Fibrin which is the common pathway.

The coagulation profile test therefore involves checking PT or international normalised ratio (INR), APTT and Fibrinogen. Deficiency of the coagulation factors can cause several bleeding disorders. Because most of the coagulation factors are synthesised in the liver, disorders/ failure of the liver can lead to bleeding disorders. Vitamin K deficiency can cause bleeding because it activates Factors II, VII, IX and X in its active form. Warfarin blocks the enzyme vitamin K epoxide reductase that activates Vitamin K.

Fibrinogen, which is a soluble glycoprotein complex, is the precursor of fibrin and is synthesised in the liver and circulated in the blood. Fibrinogen concentration can be measured in the blood and is normally 2.0 to 4.0 g/L. The half-life of fibrinogen is 3-5 days and is also found in α-granules. Fibrinogen has three pairs of polypeptide chains designated Aα, Bβ and γ arranged as 2 lateral domains (D) and one central domain (E) giving the classic pattern D:E:D with a molecular mass of 340 KDa (Hardy et al., 2020). Thrombin is a serine protease that cleaves FpA and FpB from the N-terminal portions of the Aα and Bβ chains in the central domain (E domain) of fibrinogen to form insoluble fibrin monomer. The cleavage of FpA result in new N-terminal sequence Gly-Pro-Arg-(GPR) named knobs 'A' which attaches to the

hole in γ chain called ‘a’ of another fibrin monomer and forms A-a interaction. Similarly, the third monomer is attached to form a double stranded protofibril. Protofibril is made of approximately 20-25 monomers and is the intermediate step in the fibrin polymerisation. In addition, the protruding αC terminals interact (αC-αC) within and between each protofibril to form polymers and is reinforced by Factor XIIIa (FXIIIa) and forms the fibrin (Soria et al., 2019). The fibrin fibres therefore grow laterally gaining length and thickness and on occasions they branch. The branching happens through two mechanisms, firstly there is incomplete lateral aggregation so that the protofibril diverge into two protofibrils (bilateral junction) and secondly there is only one ‘A-a’ bond so that fibrin monomer can grow independently into a new branch (trimolecular junction). The lateral aggregation and branching compete, therefore where there is more thickness, there is less branching and where there is less thickening, there is more branching. This is very similar to what happens with the airways in the lungs, which is known as fractal pattern. This process of fibrin lengthening, thickening and branching forms a 3D network strengthening the platelet plug (Weisel & Litvinov, 2017). Thrombin in the presence of calcium activates Factor XIII (FXIII) to Factor XIIIa which crosslinks γ-γ and α-γ chains. These cross links are strong covalent bonds making a clot durable and protect from premature fibrinolysis (Rosenfeld et al., 2015). Therefore, FXIII deficiency can cause bleeding disorders **(Figure 1.12)**.

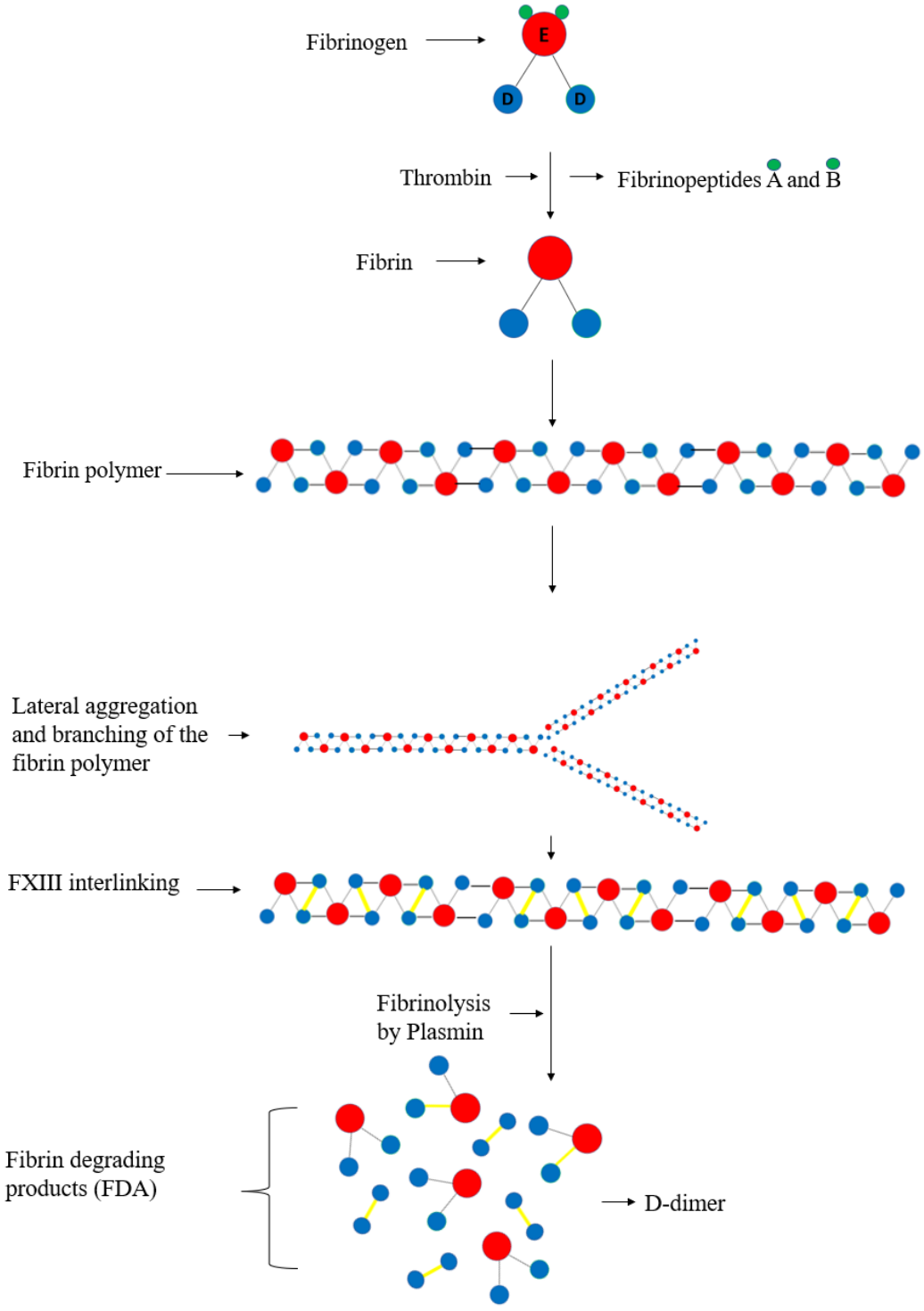


Figure 1.12: Fibrin polymers are formed from monomers which then branches three dimensionally. This is then interlinked by FXIII and finally undergo lysis to form fibrin degrading products.

Even though PT/ APTT is very useful in vitro, the cascade model does not explain some of the coagulation issues happening in a patient in vivo. Therefore, a cell-based model was suggested by Hoffman and Monroe (2001). According to this model instead of intrinsic, extrinsic and common pathway, there are three steps such as initiation, amplification and propagation. During the initiation phase, after a vascular injury, tissue factor bearing cells release tissue factor (TF) which is similar to extrinsic pathway. TF activates Factors IX, X and VII to form Factor IXa, Factor Xa and thrombin by TF-Factor VII complex. In amplification phase platelets are activated by Factor IXa, Factor Xa and thrombin by TF-Factor VII complex. There is activation of glycoprotein IIb/IIIa receptors and release of von Willebrand factor from endothelial cells. In addition, there is activation of Factors V, VIII, XI on the platelet surface. This is followed by propagation phase where activated platelet generates Factor Xa, and Factor IXa-Factor VIIIa complex & prothrombinase (Factor Xa-Va) complex on the platelet surface. This results in massive generation of thrombin which is a powerful platelet aggregating agent (Hoffman & Monroe, 2001; Ho & Pavey, 2017).

1.3.4. Tertiary haemostasis

Tertiary haemostasis refers to the breakdown of fibrin meshwork and restoration of blood flow to that area, therefore it is also called fibrinolysis. This process is achieved by a serine protease enzyme called plasmin. Plasmin is derived from inactive plasminogen, which is a glycoprotein that is synthesised in the liver and circulates in the blood. The conversion of plasminogen to plasmin is facilitated by tissue plasminogen activator (tPA) which is released by the damaged vascular endothelium (Loscalzo & Brunwald, 1998). Plasminogen activator inhibitor- 1 (PAI-1) is a potent endogenous inhibitor of tPA (Tjärnlund-Wolf, Brogren, Lo, & Wang, 2012). Plasminogen has high affinity to fibrin and gets attached via lysine binding sites on the kringle portions and remains entangled in the fibrin mesh. When tPA is released from the endothelium it also binds on the kringle portions of fibrin and activates plasminogen to plasmin. Plasmin that is formed therefore cleaves that fibrin site. Any free plasmin is immediately acted upon by α-2 antiplasmin which is a potent plasmin inhibitor found in large quantities in blood. The fibrinolysis is therefore regulated by PAI-1 and α-2 antiplasmin. The dose of alteplase which is a tPA given for stroke thrombolysis is given sufficient to overcome the action of PAI-1. Because plasmin is immediately inhibited by α-2 antiplasmin, there is no effect if plasmin is given intravenously (Novokhatny, 2008). Thus, the clot formed inside the body is continuously subjected to fibrinolysis, therefore for the treatment of VTE, long term anticoagulants are used to prevent further accumulation of the blood clot.

Plasmin cuts fibrin polymers into several small pieces (dimers and monomers) making the clot more permeable to tPA thereby dissolving the fibrin blood clot. This process is continued until all the fibrin is removed. These small fragments of dimers and monomers are called fibrin degrading products (FDP) which are finally cleared from the circulation by other proteases or by the liver and kidneys. The cross link between the two D fragments remains intact and this structure is called D-dimer and can be measured. D-dimer is tested when there is clinical suspicion of venous thromboembolism (DVT/ PE) and disseminated intravascular coagulation. D-dimer alongside Wells score is highly effective in detecting VTE (Chapin & Hajjar, 2015).

A defective fibrinolytic system causes abnormal blood clot formation, i.e. in hyperfibrinolysis there are looser and weaker clots which lead to bleeding and in hypofibrinolysis there will be denser clots and stronger clots that can lead to thrombosis.

1.3.5 Current measurements of haemostasis and its limitations

As discussed above haemostasis is a complex process that occurs in stages and involves co-ordinated action of different cells and enzymes simultaneously to arrest bleeding. This occurs in four compartments such as vasculature, platelets, coagulation factors and fibrinolytic system (Chee, 2014). It would have been ideal to have one test that can assess all these steps together and over the years scientists have developed several tests that help to detect different stages in haemostasis.

1.3.5.1 Tests of primary haemostasis

Platelet count

Platelets are an essential component for primary haemostasis and get activated when there is a vessel injury. The activated platelets then form a plug which controls the bleeding. If the number of platelets is significantly low (thrombocytopaenia), then there will be an increase in the bleeding time. Thrombocytopaenia occurs when platelet count is $<150\ 10^9$/L and the reasons are decreased production, increased destruction and splenic sequestration. Bone marrow failure and other haematological malignancies can cause decreased production of platelets. The increased destruction of platelets can occur as immune mediated (antibodies against platelets as in Idiopathic Thrombocytopenic Purpura) and non-immune causes (disseminated intravascular coagulation). When there is splenomegaly (increase in the size of spleen), platelets get trapped which is known as splenic sequestration as in myeloproliferative disorders (Vinholt, 2019).

Platelet aggregometry

Because primary haemostasis involves platelet plug formations it is good to assess platelet aggregation using this test. Anticoagulated whole blood using either citrate or hirudin is placed on a cup with electrodes. Then ADP or arachidonic acid (ASPI) or collagen is added to activate platelets which then aggregate to the electrodes. The impedance caused by platelet aggregation is then obtained using a graph (Tynngård, Lindahl, & Ramström, 2015). This test can also be used in research for therapy focussed applications (Tsoupras, Zabetakis, & Lordan, 2018). The

limitations of platelet aggregometry includes artefact due to sample collection or contamination, use of different anticoagulants (citrate or hirudin) and difference in the use of reagents.

1.3.5.2 Tests for secondary haemostasis

Clotting screen

This includes performing three types of tests Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT) and Fibrinogen. PT and APTT are measured by incubating platelet poor plasma at 37^{o}C and adding reagents. PT measures extrinsic (Factor VII, Factor VII) and common pathway (Factors X, V, II & I) by adding tissue factor, phospholipid and calcium. The time taken for a fibrin clot to form in seconds is PT and from this INR can be derived which is used to monitor Warfarin dosing. APTT measures intrinsic (Factors XII, XI, IX & VIII) and common pathway (Factors X, V, II & I) by adding a contact activator, phospholipid and calcium. The time taken to form fibrin clot in seconds is detected using a photo-optical clot detector and APTT ratio (APTTr) can be derived from this and is used to monitor heparin infusion dosing (Ho & Pavey, 2016). PT/ APTT assesses a pathway where there are multiple factors involved, therefore to find an actual problem, then multiple tests need to be conducted. Other issues with PT/ APTT tests are there are plenty of artefact due to sample collection or contamination, insensitivity to clinically relevant bleeding disorders such as mild von Willebrand disease or haemophilia A, certain disease can prolong PT/APTT without bleeding (lupus) and difficulty in reproducing the same results in the same sample in different laboratories due to different reagents (Chee, 2014). Fibrinogen is an acute phase protein (Jain, Gautam & Naseem, 2011) that is increased in infections such as Covid-19 (Wool & Miller, 2021).

Thromboelastography (TEG)/ Rotational thromboelastometry (ROTEM)

Thromboelastography (TEG) was the first viscoelastic technique developed by Dr Hartert in 1948 in the University of Heidelberg (Hartert, 1948). In this method, whole blood is placed into a heated cup at 37^{o}C and a pin is suspended via a tension wire which is connected to a mechanical-electrical transducer. The cup is then rotated around the pin on a limited arc (±4°45′ every 5s). As the blood clots, the resistance applied on the tension wire is captured as a graph.

From this graph it is possible to determine the onset of blood clot formations, maximum clot stability and fibrinolysis **(Table 1.5; Figure 1.13)**.

Table 1.5: Thromboelastometry (TEG) parameters, what it means and the normal values

Parameter	**What it means?**	**Normal value**
R value (reaction time)	Time until clot firmness reaches an amplitude of 2mm	4-8 minutes
K time (kinetics)	Time taken to achieve a clot firmness at 20mm	1-4 minutes
α- angle	Rate of clot formation	47-74^{o}
Maximum amplitude	Ultimate strength or overall stability of blood clot	55-73mm
A30 or LY30	Amplitude at 30 minutes	0-8%

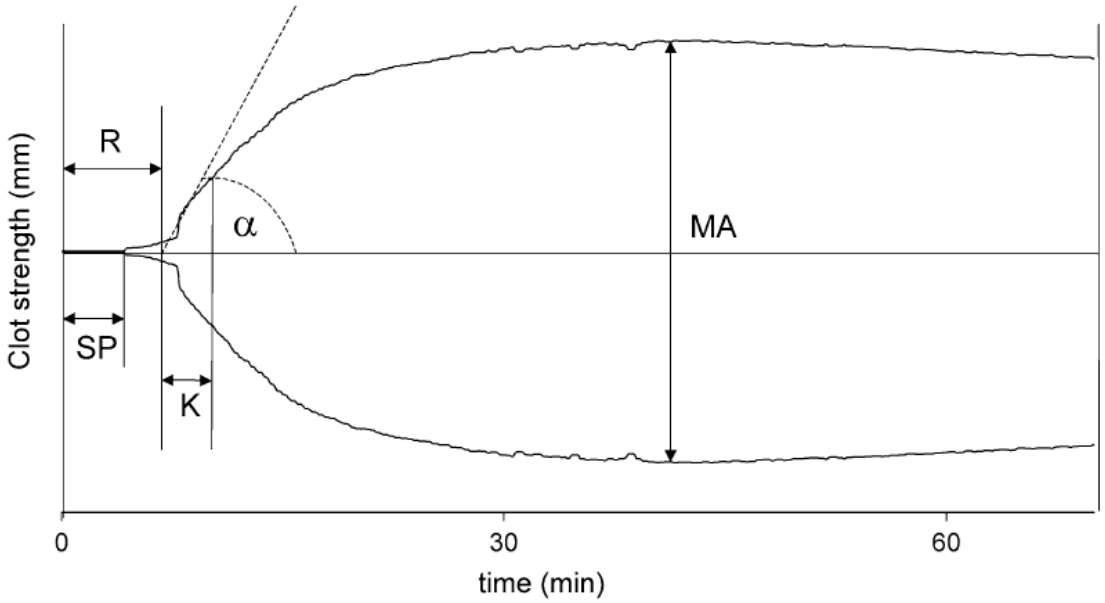


Figure 1.13: Thromboelastometry (TEG) graph. Adapted from Evans et al. 2008 with permission. The split point (SP) is the time elapsed for the formation of initial fibrin formation, the reaction time (R time) is the time till until clot firmness reach an amplitude of 2mm which indicates the formation of fibrin networks, K value indicates the time to achieve 20mm clot

that represents thrombin-platelet interaction, α-angle indicates the rate at which fibrin cross linking occurs, mean amplitude (MA) indicates maximum strength of clot.

Rotational thromboelastography (ROTEM) works on the same principle, but in this technique the cup is stationery, and the pin rotates (±4°45′every 6s). The parameters are named differently; however, it indicates almost the same. TEG initially was using unadulterated blood, but now citrated blood is used in both TEG and ROTEM with reagents added to speed up the clotting process. Both the techniques are fully automated, and both have several channels for the test to be performed simultaneously. Both these tests can be performed with 15 minutes; therefore, they are used as point of care tests where the results can be obtained real time. This is particularly important in performing surgeries such as cardiothoracic, liver and gynaecological procedures (Shen, Tabaie, & Ivascu, 2017). According to Hunt et al. 2015, the systematic review into the studies show there is no accuracy found in TEG results and little accuracy in ROTEM results and recommend that these tests are used only for research. The systematic review of 17 RCTs by Wikkelsø, Wetterslev, Møller, and Afshari (2016) found that there is evidence that use of TEG and ROTEM guided transfusion strategies may reduce the need for blood products and improve morbidity from bleeding in elective cardiac surgery patients. Therefore, they advised further research for the use of TEG/ ROTEM in acute settings **(Table 1.6)**.

Table 1.6: Thromboelastometry (TEG) parameters and its respective parameters in rotational thromboelastography (ROTEM)

TEG	**ROTEM**
R value (reaction time)	Clotting time (CT)
K time (kinetics)	Clot formation time (CFT)
α- angle	α- angle
Maximum amplitude	Maximum clot firmness (MCF)
A30 or LY30	Lysis index at 30 minutes (LI30)

1.3.5.3 Tests for tertiary haemostasis

D-dimer

There is a balance between the coagulation and fibrinolytic system. The human body is subjected to various external injuries and inflammatory processes that cause vascular endothelial damage, thereby activating the coagulation pathway. Subsequently, tiny non-significant blood clots that are formed are removed by the fibrinolytic system. Therefore, fibrin degrading products are detected in blood at any given point. A typical example is the endothelial dysfunction in sepsis (Boisramé-Helms, Kremer, Schini-Kerth, & Meziani, 2013). This is the reason why D-dimer is raised in infections and inflammatory conditions. According to the systematic review by Crawford et al. (2016), D-dimer is useful to rule out pulmonary embolism in patients who present to the emergency department with a low probability. However, one study showed that there was less utility in older people (>65 years). Recently, Covid-19 infection is associated with very high levels of D-dimer, therefore it has very limited use in ruling out VTE (Logothetis et al., 2021).

1.4 The effect of inflammation on coagulation

When there is damage to a part of the body, the response of the body is to initiate a repair mechanism known as inflammation. The damage can occur because of an injury or infection and is mediated by the immune system. At the site of inflammation there is release of cytokines from the damaged cells, migration of immune cells, vasodilatation leading to increased blood flow, increased cellular metabolism and extravasation of fluid. All these causes an increase in temperature, pain, redness, swelling and loss of function. This was first described by Cornelius Celsus as *calor, dolor, rubor, tumor* respectively and later Rudolf Virchow added *functio laesa* that means loss of function (Ferrero-Miliani, Nielsen, Andersen, & Girardin, 2007). Inflammation can be acute when it is self-limiting and lasting for a few days to six weeks or chronic when it lasts from several weeks to years. Acute inflammation starts after an insult which may be external (exogenous) or internal (endogenous) such as injuries, infections, allergens or irritants (Hannoodee & Nasuruddin, 2021). There is migration of neutrophils and macrophages to the site which releases cytokines, chemokines and acute phase proteins which is sufficient to remove foreign bodies and damaged cells/ tissues and to start the healing process (Germolec, Shipkowski, Frawley, and Evans, 2018). If the inflammatory process takes longer, then there will be infiltration of monocytes and macrophages and when the inflammatory process takes a longer course as in chronic inflammation, T-lymphocytes and plasma cells get infiltrated in the site (Ferrero-Miliani, Nielsen, Andersen, & Girardin, 2007).

There are many mediators that initiate the acute inflammatory cascade. Toll-like receptors (TLRs) are found on the surface of most of the immune cells and bind to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). This binding leads to interaction with adapter protein, myeloid differentiation primary response 88 (MyD88) which leads to the production of inflammatory cytokines or TIR-domain-containing adapter-inducing interferon-β (TRIF) leading to the production of interferon type-1 (El-Zayat, Sibaii, & Mannaa, 2019). Infection or tissue damage can activate phospholipase A2 enzymes which then produce arachidonic acid (AA) and finally prostaglandins and thromboxane A2 which cause platelet aggregation (Wenzel, 1997). Tissue damage activates mast cells releasing histamine, leukotrienes, TNF and kinins (Theoharides et el., 2012). The complement system gets activated by antigen-antibody complex (classical pathway), PAMP recognition by lectin (lectin pathway) or pathogenic surface (alternate pathway) releasing several complements.

They act by direct lysis or release of proinflammatory anaphylatoxins to prime the immune system or directing phagocytosis (Dunkelberger & Song, 2010). Hageman factor or Factor XII (Factor XII) is a coagulation factor which gets activated by tissue damage or pathogens to Factor XIIa which then activates the kallikrein-kinin pathway to form bradykinin. Bradykinin increases blood vessel permeability leading to swelling (Renné & Stavrou, 2019). Cytokines are cell signalling proteins that are secreted by almost every cell. They can be measured and therefore used as biomarkers to diagnose, prognosticate or to aid therapies (Kany, Vollrath, & Relja, 2019). The cytokines that are involved in inflammation (proinflammatory) are predominantly produced by macrophages, T- helper cells, CD4 cells and dendritic cells. They are interleukins (IL-1, IL-2, IL-6, IL-12, IL-17, IL-18), tumour necrosis factor (TNF-α) and γ-interferon and granulocyte colony stimulating factor (G-CSF). Out of the above cytokines IL-1, IL-6 and TNF-α are the key proinflammatory cytokines (Zhang & An, 2007).

Inflammation can lead to several coagulation abnormalities ranging from clinically non-significant changes in the blood tests to disseminated intravascular coagulation (DIC). Several mediators are involved; however, activation of tissue factor (TF) is thought to be the most important one (Levi, Keller, van Gorp, and ten Cate, 2003). As mentioned in secondary haemostasis, damaged endothelium release TF, which triggers the extrinsic pathway of blood coagulation. TF-Factor VIIa complex activates Factor X to Factor Xa which then converts prothrombin to thrombin that subsequently converts fibrinogen to fibrin. Therefore, in inflammation-induced coagulation activation, there is widespread TF mediated fibrin deposition. Inflammatory cytokines such as interleukins and TNF-α that are released at the site of injury induce increased expression of full-length TF and alternatively spliced TF in endothelial and blood cells (Witkowski, Landmesser, and Rauch, 2016). Furthermore, TF-Factor VIIa complex stimulates protease activated receptor (PAR) signalling thereby increasing the release of inflammatory cytokines (Demetz et al., 2010). The intrinsic pathway of coagulation is normally activated upon exposure to vessel wall collagen; however, it can also get activated through the contact system. The contact system includes Factor XII (Hageman factor), Factor XI, plasma prekallikrein (PK) and high molecular weight kininogen (HK). Tissue injury or exposure to pathogens activates the plasma kallikrein-kinin system which activates the intrinsic pathway which again leads to fibrin production. Bradykinin which is formed activates tPA subsequently activating the fibrinolysis system (Wu, 2015). There is always a balance between the coagulation and anti-coagulation system within the body. Therefore, this enhanced activation of coagulation by inflammation is counteracted by the

physiological anticoagulants such as antithrombin (AT), Protein C/ S and tissue factor pathway inhibitor (TFPI). AT which is synthesised in the liver directly inhibits thrombin forming thrombin-antithrombin complex (Hepner & Karlaftis, 2013). Protein C is synthesised in the liver and is a vitamin-K dependant glycoprotein and gets activated to activated Protein C (APC) when thrombin binds to thrombomodulin and is enhanced when Protein C is bound to endothelial cell Protein C receptor (EPCR). APC inhibits Factor IXa-Factor XIIIa complex and with Protein S it inhibits Factor Va (Esmon, 2003). Protein S is also synthesised in the liver and is a vitamin-K dependant glycoprotein which acts as a cofactor for Protein C to inactivate Factors Va and VIIIa. In addition, Protein S is a cofactor for TFPI (Zhang et al., 2021). TFPI inhibits TF-FVIIa complex and prevents activation of extrinsic pathway (Mast, 2016). Inflammation downregulates this physiological anticoagulant mechanism. Because of the formation of widespread fibrin in inflammation, the fibrinolysis pathways get activated initially. However, proinflammatory cytokines such as TNF-α and IL-1 reduce free tPA and increase the release of PAI-1, which subsequently leads to reduced fibrinolysis and therefore creating a procoagulant situation. In addition, there is TNF-α induced increased uPA production. Platelets get activated normally by certain endotoxins which then leads to platelet adhesion and aggregation (Levi, Keller, van Gorp, and ten Cate, 2003). Overall, inflammation causes increased thrombin generation, downregulation of physiologic anticoagulation and inhibition of thrombolysis which is the main reason for thrombogenicity in inflammatory conditions. There can be alterations in the above mechanisms and that might be the possible explanation that in certain infections, there are distinct features such as microangiopathy, haemorrhagic fever and most recently Covid-19.

1.5 Coagulation dysfunction in COPD

It is known that patient with COPD develop coagulation abnormalities that may contribute to increased risk of developing venous thromboembolism (VTE), myocardial infarction, stroke (cerebrovascular accidents) and other thrombotic events. The risk of thrombosis and cardiac abnormalities are higher in COPD when compared to non-COPD patients (Liu, Hu, Jiang, & Mei, 2021). There are several reasons, firstly, the primary underlying problem in COPD is the progressive inflammation leading to irreversible airway obstruction. As discussed above inflammation triggers coagulation pathways. In COPD exacerbation, the inflammatory process is amplified due to infection or other trigger factors which increase the risk of activating the coagulation pathway. This might be one of the reasons for increased incidence of VTE during exacerbation. Secondly, severe exacerbations cause type 2 respiratory failure which is characterised by high carbon dioxide and low oxygen in the blood. This leads to respiratory acidosis and acidosis itself is a risk factor for developing coagulation abnormality (Engström, Schött, Romner, and Reinstrup, 2006). All enzymes in the body requires optimal pH (7.35 to 7.45) to function properly. Thirdly, COPD patients are prone to develop recurrent infections. This can range from simple respiratory tract infections to life threatening pneumonia. Often, they develop sepsis which is an enhanced inflammatory response to infection and if there is associated organ disfunction, it is called severe sepsis and septic shock, if there is refractory hypotension. Sepsis can therefore activate coagulation pathways causing simple blood test abnormalities to severe form which is disseminated intravascular coagulation (DIC). In DIC, because of the profound inflammatory response, there is an unregulated activation of the coagulation pathways causing widespread microvascular thrombosis that leads to multiorgan failure. In addition, because of the ongoing consumption of coagulation factors and platelets, ultimately this leads to widespread bleeding (Tsao, Ho, and Wu, 2015; Simmons & Pittet, 2015). Finally, as the disease progresses, COPD patients becomes less mobile (Medina-Mirapeix et al., 2018) which increase the risk for VTE. In addition, patients with advanced or end stage COPD develop right heart failure (chronic cor pulmonale) which itself is a risk factor for developing VTE.

According to Børvik et al. (2016), patients with severe COPD (stage III/ IV) had two-fold higher risk of VTE when compared to patients without COPD and COPD patients with PE had higher risk of dying when compared to COPD patients without VTE (50.2% versus 5.6% per

year). Tromso study recruited COPD patients with first episode of VTE and the follow up showed that the overall mortality was 11.9%. According to Børvik et al. (2020), there is fivefold increase in the risk of developing VTE in severe (stage III/ IV) COPD and 50% of the severe COPD patients died within 3.5 months of diagnosing VTE. Bertoletti et al. (2013) in their study looking into 4036 COPD patient who presented with VTE found that 61% had PE as first presentation and those COPD patients who had PE as first presentation had higher incidence of recurrent PE and fatal PE. The common clinical symptom of PE includes shortness of breath, low oxygen saturation and tachycardia (high heart rate). COPD patients during exacerbation present with similar symptoms, therefore the diagnosis of PE is overlooked. Stein, Beemath, Meyers, and Olson (2007) in their retrospective review of over 58 million COPD admissions over a period of 24 years in USA showed that PE is underdiagnosed in these group of patients. However, several studies showed that the prevalence of PE is high (2-29%) in COPD (Lankeit & Held, 2016). There were several new studies that showed high prevalence of PE in AECOPD **(Table 1.7)**.

Table 1.7: Studies, study type, number of patients included and the prevalence of pulmonary embolism (PE) in COPD

Study	Study type	Number of patients	Prevalence
Fu et al. 2021	Systematic review	3170	17.2%
Sato et al. 2021	Systematic review	4093	12%
Couturaud et al. 2021	Prospective	740	11.7%
Rizkallah, Man, & Sin, 2009	Systematic review	550	19.9%

Therefore, the question is whether to investigate every patient who attend ED with exacerbation for PE which may put a lot of strain into already stretched system. Jiménez et al. (2021) conducted a RCT in 18 Spanish hospitals including 746 hospitalised AECOPD patients to evaluate an active strategy to identify PE by doing D-dimer in every patient and if positive performing CTPA. The study did not find any significant difference in the incidence of non-fatal symptomatic PE, readmission with COPD or 90 days mortality. The study concluded that there is no need to actively investigate for VTE.

1.6 New hemorheological markers

The term rheology was first coined by Eugene Bingham in 1920 who later founded the Society of Rheology (Wilson, 2018). The word rheology came from Greek words 'rheo' meaning flow and 'logia' meaning 'the study of'. Rheology is a branch of science dealing with the deformation and flow of materials and the understanding how materials change shape when a force is applied. The applied force per unit area is termed stress and the deformation that results from this stress is called strain (Cossa, 2019). If the force is acting perpendicular to an area, it is called normal stress and if acting parallel it is called shear stress. The strain caused by shear stress is known as the shear strain which represents the amount of deformation relative to the objects original dimension. Rheology therefore applies to solids, liquids and gases. Rheology is widely used in various industries to study materials having complex microstructure. Paint rheology is widely used to improve performance of the paints and improve customer satisfaction (Eley, 2019). Rheology is used in the food industry to study juices, jams and gels (Tabilo-Munizaga & Barbosa-Cánovas, 2005). In addition, rheology is used to characterise pharmaceutical and cosmetic formulations for cutaneous applications (Huang, 2019). In material science, two properties such as viscosity and elasticity determine deformation and flow. The viscosity is the measure of its resistance to deformation at a given rate and elasticity is the property to recover from deformation when the applied stress is removed. The materials that are purely viscous are termed Newtonian fluids (e.g. air and water) and materials that are purely elastic are termed Hookean solids (steel spring). However, most materials have both viscous and elastic properties and therefore termed non-Newtonian or viscoelastic materials. Shear thinning is a phenomenon where viscosity of certain non-Newtonian fluid gradually reduces with increasing levels of shear stress (Wilson, 2018). Blood is an example of a non-Newtonian fluid which exhibits shear thinning behaviour whilst flowing through the blood vessels (Lanotte et al., 2016).

Hemorheology is the study of flow properties of the blood and its components. The blood is made up of approximately 55% plasma and 45% blood cells. The blood cells include red blood cells (RBC-40 to 45%), white blood cells (WBC) and platelets (PLT). The blood plasma is made up of water (92%), proteins, glucose, mineral ions, hormones, and carbon dioxide. RBCs have a peculiar property where they clump together to form rouleaux because of its biconvex shape and is known as RBC aggregation. Haematocrit (HCT) measures the proportion of red

blood cells in a blood sample and because of the high proportion (40-45% of the blood), RBCs strongly influence the rheological behaviour of the blood under flow (Cho & Cho, 2011). Shear thinning behaviour of the blood is mainly attributed to the dynamics and interaction of the RBCs (Nader et al., 2019). When shear stresses are applied, RBCs undergo shape change from its normal biconvex shape which helps in the faster displacement of RBCs across capillaries (Lanotte et al., 2016). In addition, there is a decrease in RBC aggregation with increasing shear stress that reduces viscosity. In other words, more blood flow, more shear stress, less aggregation therefore less viscosity (Baskurt & Meiselman, 2013).

1.6.1 Quantifying viscosity and elasticity

Non-Newtonian fluids such as blood are viscoelastic, therefore blood combines both properties of a viscous liquid and an elastic solid, depending on the timescale of deformation (Lanotte et al., 2016). Viscoelasticity occurs due to the interaction of different components in the fluid and is characterised by subjecting the material to small amplitude sinusoidal deformations at a given frequency that is representative of the timescale. The elastic response obtained is called the storage modulus G' (G prime) and the viscous response is called the loss modulus G'' (G double prime) and the relationship between these two can be represented by phase angle (δ). A phase angle of $\delta = 0^o$ means purely elastic and phase angle of $\delta = 90^o$ means purely viscous (Wilson, 2018). Therefore, for viscoelastic materials, the phase angle is the lag between the applied stress (σ) and resultant strain (γ) and lies in between purely elastic and purely viscous ($0^o < \delta < 90^o$). To characterise different timescales, the material can be subjected to sinusoidal deformations at different frequencies; and G', G'' and phase angle can be measured.

1.6.2 The (Chambon-Winter) Gel point

The human body has approximately five litres of blood which is constantly circulated in the form of liquid. However, when exposed to tissue factor or a foreign surface, the clotting pathway is initiated which converts liquid blood to a solid form (jelly like consistency). This is due to the formation of insoluble fibrin from fibrinogen that then polymerise to form a fibrin 3D network with Factor XIIIa crosslinks that entraps the blood cells together to form a clot. Clotting time (CT) is the time taken by blood to form this 3D network under standard conditions in vitro. The gelling process is a structural change of a material from viscoelastic liquid to

viscoelastic solid and this transition is marked by the gel point (GP) [Evans, Hawkins, Williams, & Williams, 2008]. The terms clotting time and gel point have been used synonymously in several oscillatory shear studies using the dynamic rigidity (G') at a particular frequency of oscillation (Ryan, Mockros, Weisel, & Lorand, 1999). However, to rigorously define GP, it should be independent of absolute value of G' and oscillatory frequency (Chambon & Winter, 1987; Evans, Hawkins, Williams, & Williams, 2008). Therefore, at GP, the elastic (G') and viscous (G'') components of the complex shear modulus G^* scale as per the power laws in frequency [$(G'(\omega) \sim G''(\omega) \sim \omega^{\alpha}$]. This feature enables the detection of the GP by measuring a frequency independent loss tangent ($\tan \delta = G'/G''$) [Evans, Hawkins, Williams, & Williams, 2008]. Chambon and Winter (1987) mathematically described the GP of a cross linking polymer.

Similarly, when an oscillatory shear is applied to the blood, resistance increases when the clotting begins (fibrin polymerisation) **[Figure 1.14]**.

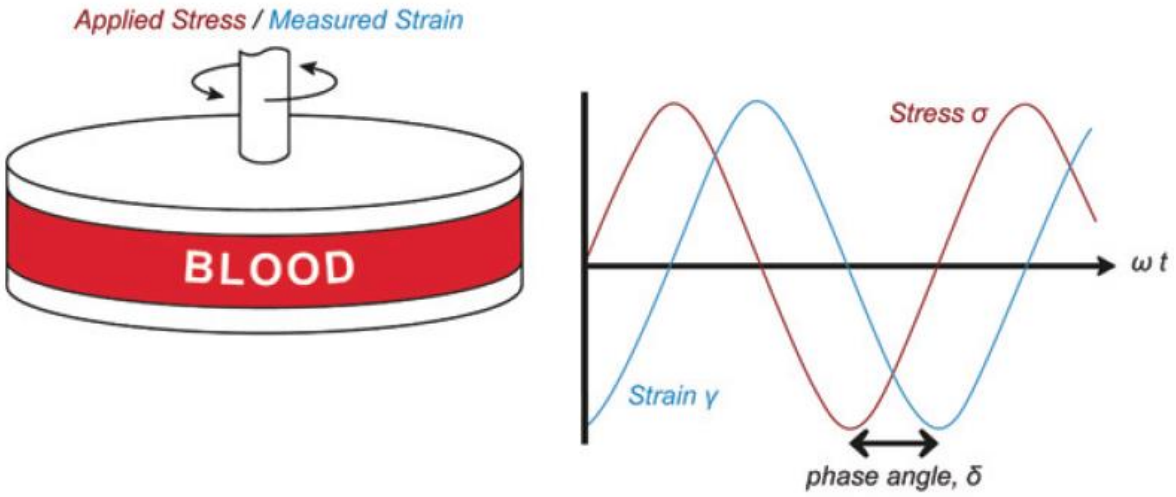


Figure 1.14: Demonstrates how stress (σ) is applied to a thin layer of blood between two surfaces and how strain (γ) is measured. The resulting sinusoidal wave pattern showing the stress (red) and strain (blue) waves. The difference between the stress and the strain is the phase angle (δ). Adapted from Evans et al. (2022) with permission.

This is achieved by using a controlled stress rheometer and there are several geometries available such as concentric cylinders, cone and plate, and parallel plates. Concentric cylinder is used for semi-viscous materials, cone and plate for very low/high viscosity materials and parallel plates for low viscosity to soft solid materials. Therefore, to test blood concentric

cylinder with double gap (360 microns) geometry is used. This double gap concentric stainless-steel cylinder (cup) can be fixed to a modulus through which temperature can be adjusted. The upper rotor is attached to a shaft that can be rotated with a pre-determined frequency which can be increased or decreased as required. The blood sample is placed in the gap of the double concentric cylinder and the rotor is then inserted into this gap **(Figure 1.15)**. The software programme then sends commands to the instrument rotor, which applies sinusoidal oscillations to the sample The resulting deformation is then measured during the clotting process. The blood should be allowed to clot, therefore unadulterated blood (no addition of anticoagulants) is used for this technique.

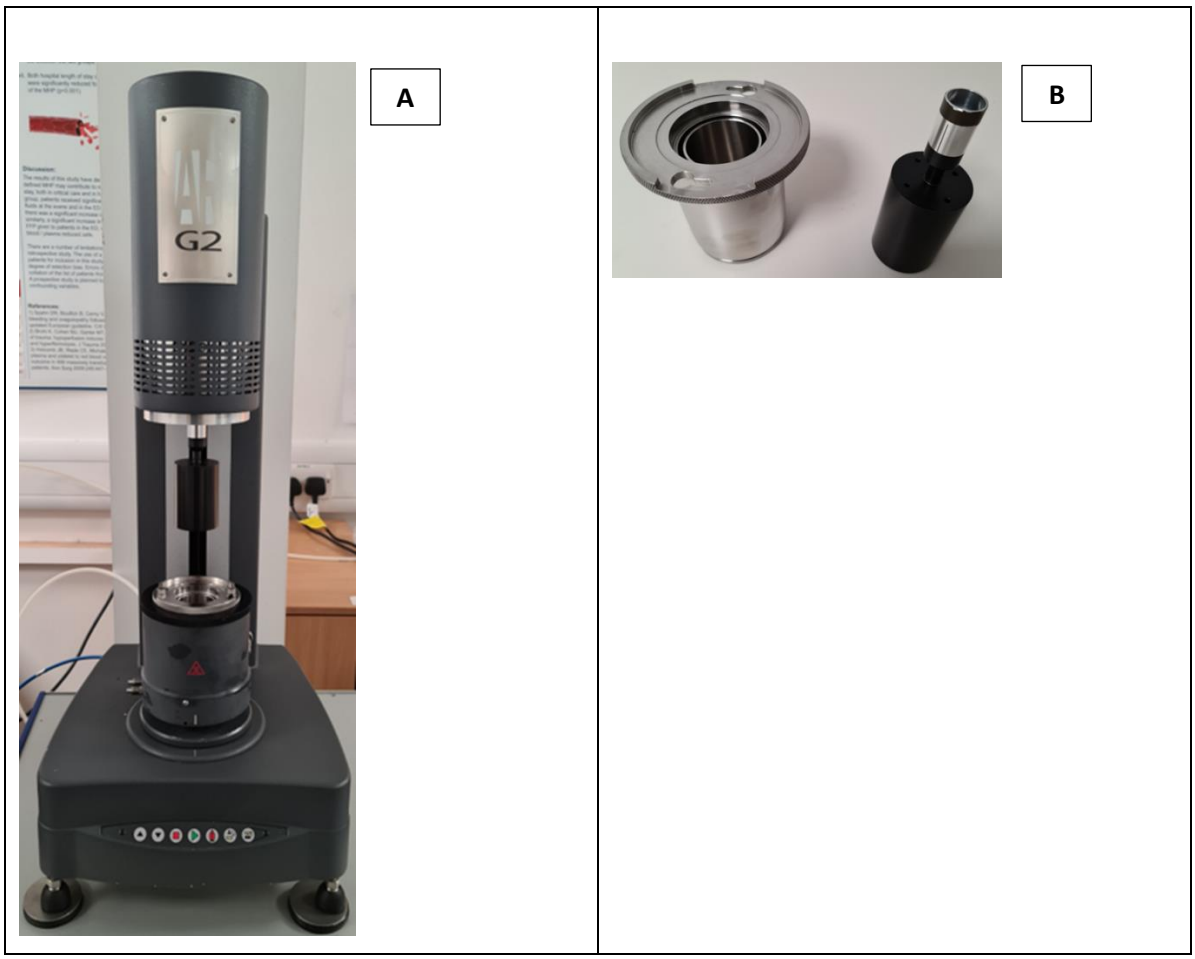


Figure 1.15: AR-G2 rheometer by TA instruments (A). B shows the double concentric cylinder and rotor of the geometry. The double concentric stainless-steel cylinder is attached to the lower Peltier unit and remains fixed throughout the experiment and blood sample is placed into the gap of the double concentric cylinder. The rotor is attached to the shaft which is then introduced into the lower part after (B).

Once the blood is placed in the geometry, then the stress is applied in an increasing frequency of 0.2 Hz, 0.6 Hz, 1.2 Hz and 2 Hz (Evans et al., 2010a). The point where phase angle becomes independent of the frequency of oscillation is called gel point (GP). Therefore, GP is the point at which blood becomes viscoelastic liquid to viscoelastic solid and time to gel point (T_{GP}) is the time taken for this process. In terms of a haemostatic function, the GP marks the establishment of an incipient clot. (Evans et al., 2010a) **[Figure 1.16]**.

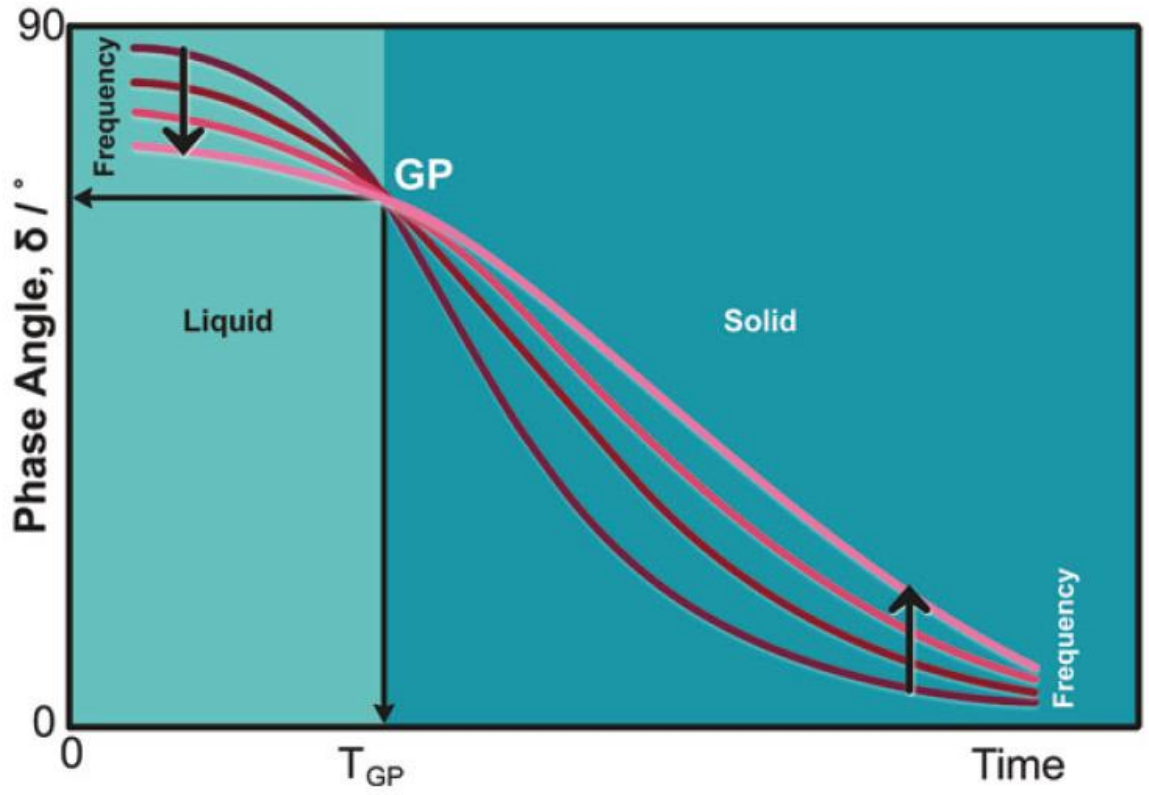


Figure 1.16: This figure shows that the gel point (GP) is where the phase angle at all four frequencies crosses over which means that this is the transition point between viscoelastic solid and viscoelastic liquid. Adapted from Evans et al. (2022) with permission.

1.6.3 Fractal dimension (d_f)

In nature, there are several structures that are arranged in certain patterns that repeat at different scales. This was first explained in detail by a mathematician called Mandelbrot, who first termed these repeating patterns as fractals in his theory of 'theory of roughness'. Mandelbrot discovered these patterns in mountains, coastline, river basins, structure of plants and clustering of galaxies. The reason for these interesting patterns is thought to be to use maximum area in

a defined space. These complex repeating patterns can be measured mathematically and the measurement of how complicated these patterns are is called Fractal dimension (Mandelbrot, 1982). In the human body, lungs (Tanabe, Sato, Suki, & Hirai, 2020), blood vessels (Li et al., 2021) and brain (Smith et al., 2021) display this repeating pattern and by applying this theory, scientists can understand how fractals help to maximise the organ function. Similarly, when fibrin polymers are formed from fibrinogen, it branches out three dimensionally within a blood clot. Because of this repeated branching, the distal branches get thinner and thinner, however, fibrin is present throughout the blood clot **(Figure 1.17)**. This arrangement of fibrin in a blood clot is called clot microstructure. This is further strengthened by FXIII crosslinks which enable fibrin to entangle RBC's and other blood cells to form a firm blood clot that is resistant to shear. Utilising the principles of fractal dimension, the complex branching pattern of fibrin in a blood clot can be measured mathematically. Therefore, both clot microstructure and fractal dimension (d_f) can be used synonymously.

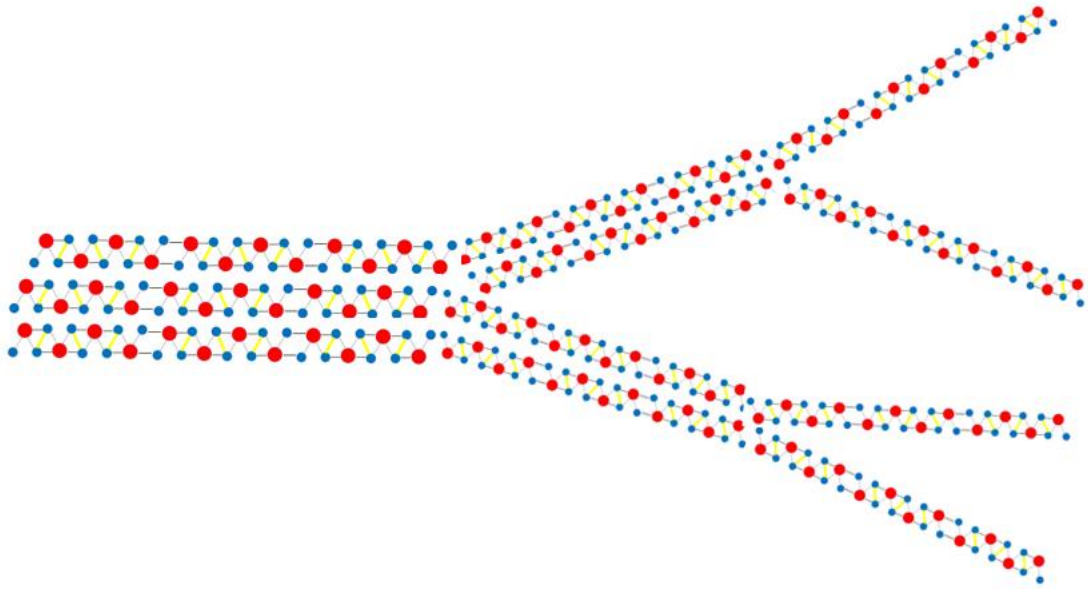


Figure 1.17: Branching of fibrin polymers three dimensionally that strengthens the blood clot.

It is possible to determine the fractal dimension (d_f) by fractal analysis. According to Muthukumar (1989), the stress relaxation exponent of a material 'α' is related to fractal dimension (d_f) as per the formula,

$$\boldsymbol{\alpha = d\,(d + 2 - 2D_f)/\,2(d + 2 - D_f)}$$

On rearranging the above formula,

$$\mathbf{D_f = (d + 2)\ (2\alpha\text{-}d)/\ 2(\alpha\text{-}d)}$$

As explained before phase angle (δ) is the difference between the stress (σ) and the strain (γ). The relationship between stress relaxation exponent (α) and phase angle (δ) is given by the formula,

$$\mathbf{\alpha = 2\delta/\pi}$$

d is the space dimension (d=3 for blood)

Therefore, by determining gel point and phase angle, fractal dimension of an incipient clot can be determined (Evans et al., 2010a).

1.7 Hypothesis and aims and objectives

The evidence presented demonstrates that COPD is a chronic inflammatory condition characterised by irreversible airway obstruction and impairment of gas exchange. Inflammation triggers activation of the coagulation pathway causing increased fibrin production which can cause VTE. In exacerbation of COPD there is flare of the inflammatory process and therefore can make patients thrombogenic. The functional biomarker of clot microstructure, d_f has been studied in several disease conditions and shown to predict thrombogenicity. A high d_f means that there is denser and stronger clot microstructure and a low d_f means weaker and loosely arranged clot microstructure. The hypothesis of this thesis was that COPD patients presenting to the emergency department with exacerbation have high d_f, meaning they are thrombogenic.

Therefore, the aims and objectives of this study are summarized below.

Aims

1. To determine changes in d_f in acute exacerbation of COPD when compared to stable group
2. To determine the changes in d_f with treatment in acute exacerbation of COPD across four time points
3. To determine the relationship between d_f and other conventional markers of coagulation
4. to determine the effect of COPD severity on d_f

Objectives

1. To undertake a retrospective study to understand the prevalence of venous thromboembolism (VTE) in the population studied
2. To undertake a prospective observational study by recruiting 30 stable COPD (SCOPD) patients from respiratory clinic and 85 acute exacerbation of COPD (AECOPD) patients from the emergency department
3. To perform sub analysis to evaluate the effect of infection and pH on d_f in AECOPD patients
4. To investigate whether d_f predicts mortality in AECOPD patients by comparing those who died and survived.

Chapter 2: Methodology

2.1 Introduction

This chapter describes all the methodologies required to validate the hypothesis that d_f, the biomarker for clot microstructure, is increased in patients with acute exacerbation of COPD (AECOPD) presenting to the emergency department. Biomarkers or biological markers are extensively used in medicine. Biomarkers help to measure a process objectively and accurately in the human body that can be physiological (normal) or pathological (abnormal). A biomarker should be reproducible and should undergo a continuous re-evaluation to improve its validity. In addition, it also helps to assess the effectiveness of treatment and predict or measure outcome (Strimbu & Tavel, 2010). According to World Health Organisation 2001, "A biomarker is any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease." There are two types of biomarkers, biomarkers of exposure that measure biological or pathogenic process leading to a disease and biomarkers of disease that are used in screening, diagnosis and monitoring of disease progression (Mayeux, 2004). There is a high interest in the development of biomarkers because they help in improvement of morbidity and mortality and make therapeutic interventions cost effective (Hunter et al., 2010). For the effective use of a biomarker in the clinical setting, it should be validated. Any new biomarker goes through three phases of development, biomarker discovery, analytical validation and clinical validation (Ou, Michiels, Shyr, Adjei, and Oberg, 2021). During analytical validation, a biomarker should demonstrate that it is reliable, affordable/ sustainable and fit for purpose. Clinical validation is to demonstrate that there is an association between a biomarker and endpoint of interest. The utilisation of d_f as a biomarker was discovered in 2010 (Evans et al., 2010a) and has undergone analytical validation and is currently in the phase of clinical validation. In determining the best principles in methodological research in both evaluating and validating a new biomarker, it is important to adhere to principles set out in Standards for Reporting Diagnostic accuracy studies (STARD) and Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. STARD consist of 30 essential items that are required to report a diagnostic accuracy study (Bossuyt et al., 2015). STROBE is an initiative that consists of a checklist of 22 items that should be included in reporting an observational study (STROBE, 2022).

2.2 AECOPD study design and population group

This study was designed to be a prospective observational study recruiting two groups of patients. Patients with stable COPD disease (SCOPD) who were attending the Chest Clinic and Pulmonary Rehabilitation programme at Morriston Hospital were recruited as the control group. The second group, which is the acute exacerbation of COPD (AECOPD), was recruited from Morriston Hospital's Emergency Department.

2.2.1 Study site

The study was undertaken at the Welsh Centre for Emergency Medicine Research (WCEMR) that is located within the Emergency Department (ED) at Morriston Hospital, Swansea **(Figure 2.1)**.

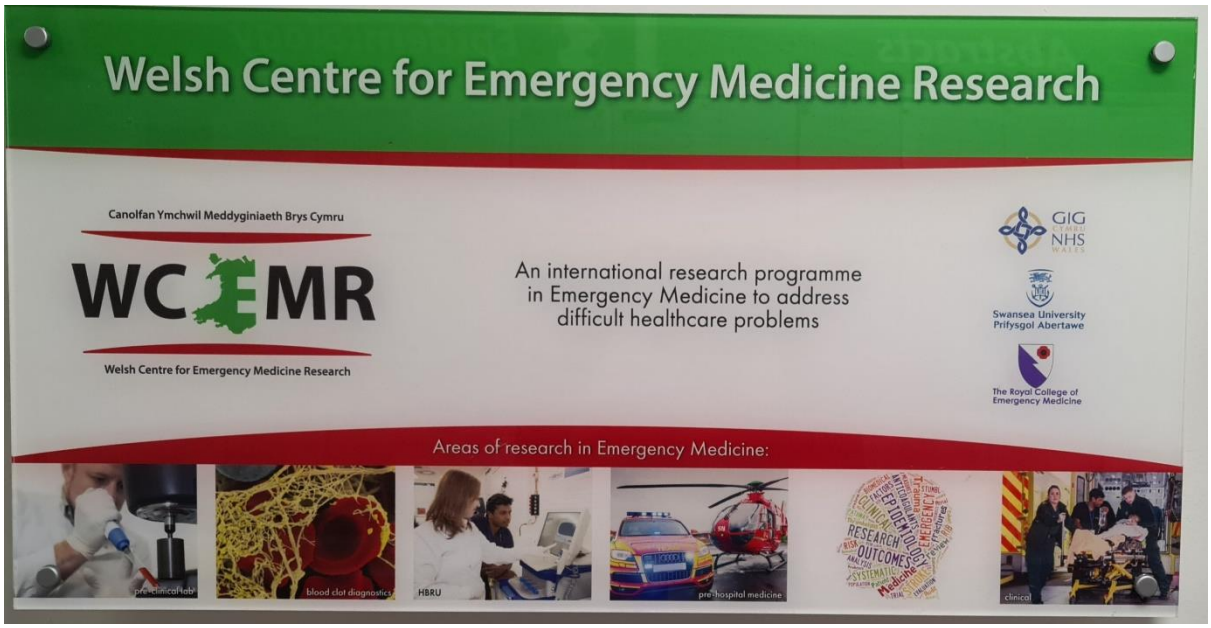


Figure 2.1: Welsh Centre of Emergency Medicine Research name board in Morriston Hospital Emergency Department

Morriston Hospital is a tertiary referral centre and the second biggest hospital in Wales. Morriston Emergency Department is one of the busiest ED's in Wales that sees over 85,000 new patients per year, including over 500 COPD patients. WCEMR is a successful partnership

with Swansea Bay University Health Board and Swansea University and was officially launched in March 2019 by the Health Minister of Wales. It has a fully equipped laboratory (Haemostasis Biomedical Research Unit - HBRU) with rheometers, platelet aggregometer, rotational thromboelastometry (ROTEM) and all the facilities to take blood samples on-site and to perform venepuncture under strict infection control measures **(Figure 2.2)**. Its proximity to the ED, intensive care unit (ICU) and wards makes it an ideal location to conduct clinical research. The research from WCEMR has produced over 100 publications and the Centre has international collaborations with research centres in Denmark, New Zealand and United States. The centre has secured prestigious funding from the National Institute for Social Care and Health Research (NISCHR) and the Engineering and Physical Sciences Research Council (EPSRC). These grants contributed to this research project. Because the chest clinic is located a few hundred yards from the research centre, to avoid delay in performing the rheological tests, the rheometer and other necessary equipment was transported to the clinic after obtaining approval from the Respiratory Department. The respiratory clinic runs in the morning and once the clinics are finished, the rheometer was transported back to HBRU. The AECOPD group were recruited from the Emergency Department in Morriston Hospital. If the recruited patients are admitted to medical wards or ICU, then subsequent samples taken from these areas were immediately conveyed to HBRU. To carry out the tests, there is a requirement of three people, one to collect the sample, one to convey the samples to HBRU and the third to perform the tests whenever it was possible. The rheology tests become void if the clotting process starts, therefore it is imperative to immediately transport the blood samples taken to the HBRU.

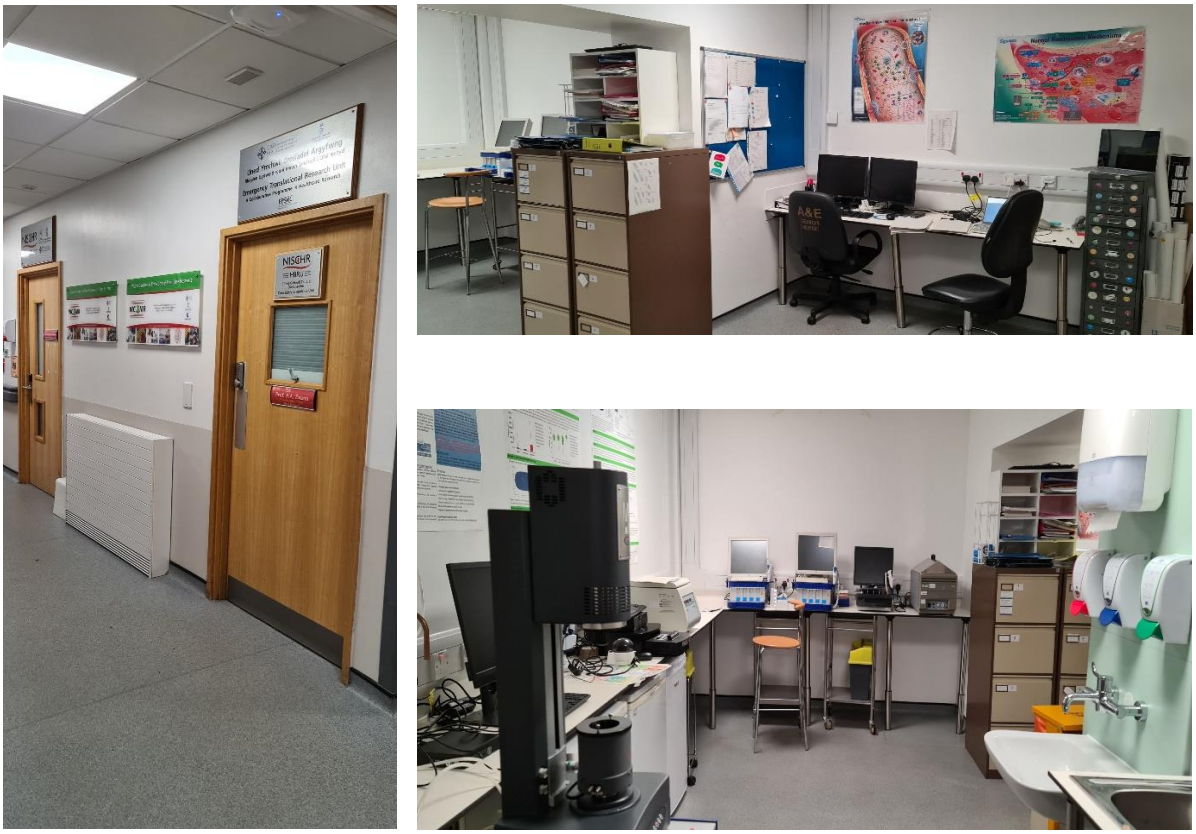


Figure 2.2: Haemostasis Biomedical Research Unit laboratory which is part of the Welsh Centre for Emergency Medicine Research situated in Morriston Hospital Emergency Department

2.2.2 Sample size and power calculation

The research into fractal dimension has demonstrated that d_f was normally distributed in the local population of healthy volunteers (Evans et al., 2010a). As per the data collected from a range of previous studies involving sepsis and acute inflammatory diseases (Davies et al., 2016; Stanford et al., 2015) it was expected that AECOPD patients may have a mean d_f of 1.79 (±SD 0.06). AECOPD being an acute inflammatory response, this was an appropriate estimate in this group. Firstly, this study aims to determine if d_f was significantly higher in AECOPD (inflammatory condition) when compared to SCOPD (non-inflammatory condition). Assuming that SCOPD patients have d_f similar to healthy individuals which is 1.73 (±SD 0.04), using a 2-sample t-test to detect a difference in these two groups with $\alpha = 0.05$, and a power of 0.8, the mean difference of 0.05 and a combined SD of 0.06, the number needed to recruit to this study was a minimum of 25. Taking into consideration of dropouts, it was decided to recruit

30 patients in each group (SCOPD and AECOPD) to undertake the comparison. Secondly, it will be determined whether a significant difference in d_f occurs in patients with AECOPD during standard treatment and whether changes in d_f relate to standard markers of haemostasis and inflammation collected at the same time. To determine if there are any changes in d_f at four sampling points (0 hours, 4-6 hours, 24 hours, and 3-7 days), one-way ANOVA will be utilised, as d_f is a normally distributed variable. Given previous data on the response of d_f to a wide range of therapeutic interventions and resuscitation methods, that d_f will change by 0.04 with a SD of 0.06, using $\alpha = 0.05$, power of 0.8 and one-way ANOVA to detect within group differences at four levels, it was determined that a minimum of 51 subjects would be required for this part of the study. Some of the AECOPD patients recruited may dramatically improve with treatment and will therefore be discharged without having all the four sampling points. Allowing for attrition, it was decided to recruit a maximum of 85 patients in AECOPD group. There were no studies to date that investigated changes in d_f in COPD patients and therefore, the clinical utility of d_f in COPD is not known. The new knowledge that will be gained from this study will inform the utility of this biomarker in COPD and future studies can be designed determining clinical outcome.

2.2.3 Ethical considerations

The SCOPD patients were recruited from the Chest Clinic and Pulmonary Rehabilitation programme at Morriston Hospital. Those who appeared suitable were identified by the Consultant Chest Physicians, who then referred these patients to the research team present in the Chest Clinic. The research team went through the inclusion/ exclusion criteria and if appropriate, the research team explained the study and offered patients a participant information sheet (PIS) and allowed them time to ask questions. If patients agreed, they were asked to sign the consent form. After obtaining the signed consent form, a single blood sample (up to maximum of 30ml) was taken under strict infection control measures. Patients were given an option to opt out at any stage during the study. All research team members were GCP (Good Medical Practice) trained and those who were trained to perform venepuncture were allowed to take blood samples. Health and Care Research Wales recommend undertaking GCP training to all health professionals who are involved in research in Wales (Health and Care Research Wales, n.d.).

AECOPD patients who require emergency treatment were recruited from Morriston Hospital Emergency Department. The hypothesis of this study was that the AECOPD group may have high d_f when compared to the SCOPD group. AECOPD patients were identified by Consultants in the Emergency Department who then referred them to the research team. The research team went through the inclusion/ exclusion criteria and if appropriate approached the patients. After explaining the study, enough time was given to read the PIS and if the patients agreed to participate in the research, a written consent was obtained. Four blood samples (up to maximum of 30mls each time) were collected at 0 hours (time of recruitment), 4-6 hours, 24-36 hours and 3-7days.There were occasions when some of the AECOPD patients were not able to give an informed consent and that was due to several reasons. Firstly, some of the AECOPD patients who presented to the emergency department were unconscious. This was due to either severe type 1 (reduced oxygen level in the blood) or type 2 (reduced oxygen level and increased carbon dioxide in the blood) respiratory failure. The reduced oxygen level in the blood is called hypoxia and increased levels of carbon dioxide is called hypercapnia. These patients therefore required immediate intubation and ventilation and were admitted to ICU. Secondly, depending on the severity of the respiratory failure, some of the AECOPD patients were confused and lacked capacity to provide an informed consent. These patients required non-invasive ventilation (NIV) via a mask. Under these circumstances, where consent cannot be obtained, a consultee declaration (presumed consent) was obtained from the direct care team or next-of-kin if present. When the patients recover from the acute illness, then appropriate consent was obtained where possible. If they don't recover or continued to lack capacity, then consultee declaration stays unless an objection was raised by the direct care team or next-of-kin. Unfortunately, some of the AECOPD patients died during the admission and if there were no objection from the direct care team or next-of-kin, then these patients remained in the study.

The hypothesis of this thesis was that COPD patients presenting to the emergency department with exacerbation have high d_f when compared to the stable group. Therefore, patients who are most unwell are likely to have the biggest change in abnormal clot microstructure due to the severity of the inflammatory response. Similarly, the patients with the most severe form of diseases are more likely to lack capacity. If these group of patients are excluded, then it will negatively impact on the spectrum of intensity of the inflammatory response of participants in the study. Patients should be able to understand the given information, remember that information, weigh the risk and use that information to make a decision and finally communicate their decision by talking, using sign language or by any other means. If patients

cannot do any of the above, then they lack capacity (NHS, 2021). All patients were provided with a Participant Information Sheet (PIS) and a copy of the consent from explaining that they can opt out at any time after they consent. The research team included emergency department clinicians, therefore, to avoid conflict of interest, the clinician involved in providing direct clinical care to an AECOPD patient was not able to obtain consent. A copy of the signed consent or declaration form was attached to patients' clinical notes.

This study was a prospective observational study, therefore, all precautions were taken not to influence the patient's routine care. The blood sample for the study was taken at the same time as blood required for clinical purposes to minimise the number of venepunctures and thereby patient discomfort. Participants were consenting to an additional amount of blood being taken, which was a routine low risk procedure. Venesection can cause some discomfort, therefore all members of the research team involved in venesection were experienced and trained in performing this procedure. Consent was sought from the participants for the responsible clinician/consultant to inform the participant's General Practitioner of any incidental abnormalities during routine blood testing.

2.2.4 Inclusion and exclusion criteria

The inclusion criteria for the study were patients aged 35 and above with a confirmed diagnosis of COPD as defined by GOLD criteria (Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease) [GOLD, 2022]. Participants receiving any medical treatment that could affect coagulation (anticoagulant therapy such as Warfarin, Heparin, Rivaroxaban, Dabigatran and Apixaban or any other anticoagulants) were excluded from the study.

2.2.5 Sampling points

SCOPD patients had one blood sampling point because this group were coming to the respiratory clinic for follow up and had ongoing treatment and investigations to keep the disease under control. They were ambulatory and therefore, it was assumed that this group will have a d_f identical to healthy individuals. On the other hand, AECOPD patients presenting to ED are often unwell and require hospital admission for ongoing medical treatment with

nebulisers, steroids, oxygen, antibiotics and occasionally ventilatory support based on the Health Board clinical care pathway (CID 306 COPD-Specialist Management of Acute Exacerbation pathway, 2020). It is anticipated that with appropriate treatment the exacerbation symptoms would subside, and the patient could be discharged back to primary care. However, this depends on disease severity and the response to the treatment and the average hospital length of stay for COPD patients admitted to Morriston Hospital during the period from 1st September 2016 to 31st August 2017 was 7.7 days. It was assumed that AECOPD group has increased d_f that may change with treatment over time. Therefore, it was decided to perform four sampling points at 0 hours, 4-6 hours, 24 hours and 3-7 days. Occasionally, some AECOPD patients dramatically improve with treatment, therefore they were discharged early. These patients were also included in the final analysis.

2.2.6 Blinding

As discussed in Chapter 1.4, the collected unadulterated blood sample was placed into the rheometer and was subjected to oscillations at four frequencies. The gel point (GP) was located at the cross over of four frequencies and from the GP, d_f was determined. The GP analysis was done by two independent rheologists who were blinded to the study and sample. The results were compared and the GP measurement with less than two degrees of difference was used to determine d_f.

2.2.7 Collection of clinical data and confidentiality

Patients' information was collected on a standardised case report form (CRF) from the medical notes requested by the ED secretary and from clinical portals such as SYNAPSE for x-rays, Indigo/ Welsh Clinical Portal for blood investigations and Zylab for ED notes. CRF was filed and stored in a locked cupboard within the HBRU, which has a security door and is accessible only by the research team. WCEMR and HBRU are part of the emergency department and access to these areas are strictly controlled. The data was collected on an excel spreadsheet which is stored only on NHS computers that has security features in-line with the Health Board policy. Patient confidentiality was considered paramount and was adhered to throughout the study. All research staff involved in this study were GCP trained and were aware of their ethical and legal obligations about maintaining the confidentiality of the data collected about the

research subjects. Any changes in the research methodology were communicated to the Research Ethics Committee and Research & Development (R&D) department. Prior consent, costing, study reference and blood test form were obtained from the Morriston Hospital laboratory services. All the data that was stored will be held for five years and then will be destroyed as per WCEMR policy guided by R&D department.

2.3 Blood sampling

Blood samples were collected from a vein using a Vacutainer® Multiple Sample Needle or from an arterial line if the COPD patient was admitted to ICU or occasionally at the time of insertion of intravenous cannula. The first 3-5 mls was discarded to avoid alteration in blood coagulation due to the formation of micro blood clots which was due to activation of coagulation pathway by trauma in case of direct venepuncture or dilution if taken from an arterial line. Blood samples were then collected in multiple plastic tubes. A plain plastic tube that collects 7.0 ml of whole blood was used for rheology, 4.0 ml vacuum sealed K2EDTA (EDTA-Ethylenediaminetetraacetic acid) tube (Greiner Bio-one, Stonehouse, UK) for full blood count (FBC), 2.7 ml vacuum-sealed tubes containing sodium citrate (3.2%) for coagulation profile, d-dimer and FXIII (Factor XIII), 5.0 ml SST II Clot activator & serum gel separator for CRP (C-reactive protein) and Procalcitonin (PCT) and3.0 ml Multiplate® Hirudin Blood Tube (Double-Wall) for platelet aggregation **(Table 2.1)**.

Table 2.1: Different vacutainers tubes, blood tests carried out and the amount of blood required for this study

Vacutainer tubes	Tests	Amount of blood
1×plain 	Rheology	7ml
1×EDTA 	FBC/ cellular components	4ml
3×Citrate 	PT, APTT, Clauss Fibrinogen D-Dimer FXIII Anti-Xa	2.7ml
1×SST II, Clot activator & serum gel separator 	CRP Procalcitonin	5ml
1×Hirudin 	Platelet aggregometry	3ml

If patients had any of the blood tests included in this study as part of their routine care, then that blood sample was not repeated to avoid wastage of blood, duplication of test, patient discomfort and cost-effectiveness. This practice was followed at all sampling points and therefore timing of taking the blood samples coincided with a phlebotomist taking blood samples on the wards. The laboratory samples were sent immediately after collection via pneumatic air tube transport systems. Pneumatic air tube transport system is now a standard feature in every NHS hospital that connect central laboratory to ED, ICU, operation theatres, clinics and wards. This system helps to reduce specimen turnaround times, so that blood tests can be processed quickly which can improve clinical decision making and therefore better patient outcome.

2.3.1 Rheology test

The blood (7mls) for rheology was collected in a 9ml vacutainer without any additive (Griner Bio-One GmbH, Austria, Ref: 455001). The instrument used for rheology testing for this study was AR-G2 Magnetic Bearing Rheometer by TA instruments, New Castle, DE, USA. The temperature was set at 37^{o}C for standardisation of all the tests. Once the rheometer reached the required temperature then gap was zeroed, and the geometry was mapped for three iterations using the standard mapping settings. From the blood sample, 7mls was loaded into the coaxial double concentric cylinder stainless-steel geometry. It was a standardised procedure to use coaxial double concentric cylinder geometry over parallel plate geometry because it gives an increased surface area, therefore more sensitivity and accurate measurement of gel point. As soon as the blood is placed, the test is commenced, and few drops of low viscosity oil was applied to the exposed surface of the double concentric cylinder using a disposable pipette to provide a tight air seal to prevent drying of the sample. Rheology testing is time critical because once the coagulation starts within the sample, then the tests become void. Therefore, the rheology was performed by named researchers who are appropriately trained and experienced in rheometric analysis. A torque of 12μNm was applied at frequencies of 0.20Hz, 0.6Hz, 1.2Hz and 2.00Hz and phase angle and elastic module is measured at each frequency over time. To eliminate bias and enhance accuracy, data was anonymised and analysed independently by two haemorheologists who was blinded to the sample origin.

The point at which phase angle (δ) is independent of frequency is called gel point (GP). The time from the start of the experiment to the GP is called the gel time (T_{GP}). The fractal dimension was calculated as per the formula, $d_f = (d + 2)\ (2\alpha\text{-}d)/\ 2(\alpha\text{-}d)$, where d is the space dimensions (d=3 for blood) and $\alpha=2\delta/\pi$ (Evans et al. 2010a).

2.3.2 Full blood count

The full blood count was assessed in all patients at all sample points. A 4ml aliquot of blood was drawn into plastic, full-draw dipotassium EDTA Vacuettes (Greiner Bio-One, Stonehouse, UK Ref: 454286). The sample was sent to Morriston Hospital Haematology laboratory and were analysed using a Sysmex XE2100 automated haematology analyser within 2 hours of collection. There are standard parameters measured, however those relevant and included in

this study are haemoglobin (Hb), white cell count (WBC), neutrophil count, platelet count (Plt) and Haematocrit (HCT).

2.3.3 Coagulation profile/ kinetic markers

The standard coagulation profile includes prothrombin time (PT), activated thromboplastin time (APTT) and Fibrinogen. Samples were collected in 4.5 ml 3.2% sodium citrate vacutainers (0.109 M, Beckton- Dickenson, Plymouth, UK Ref: 367691). The sodium citrate binds to calcium in the blood thereby preventing blood coagulation. The samples are sent to the haematology laboratory in Morriston hospital where it is centrifuged to separate out the platelet poor plasma which is then used for testing. Thromboplastin and calcium chloride are the reagents used to perform PT, silica or ellagic acid and phospholipids for APTT and to measure fibrinogen 'clauss' method is used 10µL of sample is added to 90µL of Owren buffer followed by addition of 50µL of thrombin. All tests are then analysed using Sysmex CA1500 (Sysmex UK, Milton Keynes, UK) and all reagents are sourced from Siemens Healthcare Diagnostics Products GmbH, Marbyrg, Germany.

2.3.4 Platelet aggregometry

This test is used to assess platelet aggregation in COPD patients. An aliquot of 3 mL of whole blood was collected in hirudin tubes (RocheDiagnostics GmBH, Mannhein, Austria Ref: 06675751) and kept at 37^{o}C for 30 minutes for platelets to regain receptivity. There are multiple channels in Multiplate, so that several tests can be done simultaneously. Multiplate test cells that has silver electrodes are placed which is then connected to electrical channel. 500µL of hirudinised blood is added followed by 500µL of normal saline. This is followed by addition of adenosine diphosphate (ADP) [ADPtest, RocheDiagnostics GmBH, Mannhein, Austria Ref: 06675794] to one of the channels to test P2Y and arachidonic acid (ASPI) [ASPI test, RocheDiagnostics GmBH, Mannhein, Austria Ref: 06675816] to another channel to test COX-1 pathway. The impedance increases with platelets aggregation, which is then captured as a curve, one for each electrode. The difference between the mean from the curve is recorded and the area under curve (AUC) was calculated and reported as arbitrary aggregation units (U). If the difference between the mean is >20%, then test is repeated as recommended by the manufacture.

2.3.5 D-dimer

D-dimer reflects the fibrinolytic activity in the body, and is therefore widely used to detect VTE. The fibrin that is formed in the body is immediately acted upon by plasmin cleaving it into fibrin degradation products. The smallest protein fragment is called D-dimer which has one E and two D domains crosslinked by FXIIIa (Johnson, Schell, & Rodgers, 2019). TriniLIA Auto-D-dimer$^{@}$ is used to detect the D-dimer concentration. This turbidimetric assay utilises monoclonal antibody coated latex particles which binds to D domain and causes aggregation which increases the turbidity and increasing light scattering which is proportional to the concentration of D-dimer in the sample. D-dimer can be added to the same sample for coagulation profile testing.

2.3.6 Inflammatory markers

COPD is an inflammatory process and therefore it is vital to quantify the inflammatory response by checking inflammatory markers such as Procalcitonin (PCT) and C- reactive protein (CRP). Immune-assay testing was carried out using the appropriate ELIZA (enzyme-linked immunosorbent assay) kit assay in the hospital laboratory. Serum aliquots were stored at -80^{o}C in the central laboratory for retrospective batch analysis.

2.3.7 FXIII

Factor XIII is a coagulation factor that cross links fibrin network when activated (FXIIIa) and makes the clot firm. According to Bazzoan et al. 2020, FXIII is upregulated in COPD. FXIII was analysed using the appropriate ELIZA (enzyme-linked immunosorbent assay) kit assay and serum aliquots were stored at -80^{o}C in the central laboratory for retrospective batch analysis.

2.3.8 Anti-Xa

AECOPD patients receive low molecular weight heparin prophylaxis as part of the clinical care pathway. Low molecular weight heparin combines with antithrombin to form antithrombin-heparin complex which then combines with factor-Xa to form anti-Xa. Therefore, heparin

concentration can be monitored by measuring the anti-Xa levels. Anti-Xa assay is a quantitative test performed indirectly by adding excess antithrombin to the patients sample along with a known quantity of FXa.

2.4 Statistical analysis

All statistical analysis was carried out on IBM Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 (Armonk, NY:IBM Corp). Prior to analysis, data normality was assessed using the Shaprio-Wilk test throughout. The values are reported as means ± standard deviation (SD) or median (interquartile ranges [IQR]) where appropriate. Comparisons between two groups was undertaken with two sample t-test (mean ± SD) or Mann-Whitney U [median (IQR)]. To determine the significance between four time points in AECOPD group (A, B, C, D) either one-way ANOVA (mean ± SD) or Kruskal-Wallis test [median (IQR)]. Data was deemed significant when $p < 0.05$.

As mentioned before, each blood sample has different individual tests (for example Coagulation profile has PT/ APTT/ Fibrinogen) that shows a different component of the physiological process of inflammation and coagulation. To enable better understanding of the disease process in COPD, these individual blood tests were arranged in groups based on the pathological process for statistical analysis **(Table 2.2)**.

Table 2.2: Arrangement of individual blood tests in groups to understand the disease process in COPD and for data analysis.

Groups	**Blood tests**
Rheological markers	d_f, T_{GP}
Inflammatory markers	WBC, Neutrophils, CRP, PCT
Markers of primary haemostasis	Platelets, ADP, ASPI
Markers of secondary haemostasis	PT, APTT, Fibrinogen, FXIII
Markers of tertiary haemostasis	D-dimer
Anti-Xa	Anti-Xa
Markers of blood viscosity	Hb, HCT

2.5 Ethical approval

This study obtained full ethical approval from Wales Research Ethics Committee 6 (REC6). The study was conducted in compliance with the principles of the declaration of Helsinki and adhered to all regulatory requirements of the Research and Development (R&D) department of the Swansea Bay University Health Board. An informed written consent was obtained from all participants and those who were not able to consent, a declaration form was obtained from personal or professional representative. The study strictly adhered to confidentiality as set out in the Caldicott guidelines. All information collected was held in confidence within the standardised case report form (CRF) and stored only on password protected NHS computers that has security features in-line with the Health Board policy. A trial master file had all the essential documents and the documents with any identifiable participant data were stored in a locked cupboard within a locked office. All research staff involved in this study were GCP trained and are aware of their ethical and legal obligations about maintaining the confidentiality of the data collected about the research subjects.

Chapter 3: COPD retrospective study

3.1 Introduction

Given the importance of thrombotic events in COPD, this thesis has the overarching aim of determining the level of thrombogenicity in acute exacerbation of COPD (AECOPD) utilising the whole blood biomarker, fractal dimension (d_f). In clinical research, it is necessary and good practice to ensure that the patient group that is enrolled in a study is as much as possible representative of the disease population, thus allowing the results obtained to be generalised (Elfil and Negida, 2017). According to Public Health Wales Observatory report analysing the SAIL (Secure Anonymised Information Linkage) databank, the prevalence of COPD in Swansea population was about 1.4% which equates to approximately 6000 individuals. There were 431 admissions to secondary care per year and approximately 111 patients died with COPD every year which was roughly 25% (Public Health Wales, 2011). This report however did not investigate the prevalence of venous thromboembolism (VTE) among AECOPD patients. In addition, there were no data to suggest the severity of COPD and mortality rate during admission and in one year after an admission with acute exacerbation. Furthermore, there were no other studies that specifically investigated the incidence of VTE in the Swansea population. Therefore, it was decided to undertake a retrospective review of the medical records of all the COPD patients who presented to Morriston Emergency Department (ED) with acute exacerbation for a period of one year. COPD patients who are admitted to the hospital with exacerbations, may remain non-ambulatory for several days and this is dependent upon the severity of the illness and the presence of comorbidities. Hospital acquired thrombosis (HAT) is the term used to account for all VTE that occurs during hospital admission and up to 90 days from the time of hospital discharge. In their study (Heit et al. 2000), found that hospital admission is an independent risk factor for VTE and hospitalisation for acute medical illness has the highest risk of VTE next to major surgery and active cancer (Heit, Spencer & White 2016). Therefore, to prevent HAT, the admitted patients should receive prophylactic thromboprophylaxis as per the national guidelines within 14 hours of admission to hospital (National Institute for Health and Care Excellence, 2018). However, it is to be noted that visits to an Emergency Department are not considered as hospital admission.

The aim of conducting this retrospective study, was to analyse the demographics and comorbidities of COPD patients who present with acute exacerbation to Morriston ED. This study investigated what percentage of patients admitted with acute exacerbation of COPD were investigated for VTE, allowing the determination of the prevalence of VTE in this group. In addition, there were no published data regarding the severity of COPD admissions and mortality during admission and in one year following admission with an acute exacerbation. COPD severity is classified based on FEV1 (Forced Expiratory Volume in 1 second) which is measured using spirometry (GOLD, 2022) and according to Bikov et al. 2020, FEV1 is a better predictor of mortality. Therefore, it was decided to collect clinical data on COPD severity and mortality in this retrospective study. Furthermore, NICE (2018) recommends that all patients should receive thromboprophylaxis within 14 hours of hospital admission. Therefore, this study investigated whether all COPD patients with acute exacerbation received appropriate thromboprophylaxis within 14 hours of admission. As per Chapter 2.2.5, the AECOPD study requires blood samples to be collected for the analysis of d_f and other biomarkers at four sampling points (0 hours, 4-6 hours, 24 hours and 3-7 days). The Haemostasis Biomedical Research Unit (HBRU), the laboratory where the research for this thesis was carried out, opens from 9am-5pm. To factor in the second sampling point at 4-6 hours, it was decided to recruit AECOPD patients during 9am-1pm. Therefore, the retrospective study was also necessary to estimate the number of patients who present during these hours, allowing the exploration of the available number of patients to be recruited to the prospective AECOPD study.

3.1.1 Aims

This retrospective study aimed to investigate:

1. The distribution and the demographics of patients presenting with AECOPD to the Emergency Department
2. The prevalence of VTE in patient presenting with AECOPD
3. COPD severity (FEV1) and mortality during admission and in one year following admission with acute exacerbation
4. To investigate whether AECOPD patients received appropriate thromboprophylaxis once admitted to the hospital
5. To inform the feasibility of carrying out the prospective study by using the data from this retrospective study

3.2 Methodology

This retrospective study was approved as 'non-research' by the Joint Study Review Committee of Swansea Bay University Health Board. This was because this retrospective study was not comparing the current practice with existing standards or answering a hypothesis. However, the study was assessing the quality of the service that is provided, therefore this study was deemed as a service evaluation (Twycross and Shorten 2014). The hospital records identified a total of 534 patients from 1st September 2016 to 31st August 2017. The study collected data that included patient demographics, past medical history/ co-morbidities, clinical observations, routine blood tests that included inflammatory, coagulation markers and radiological investigations. The data was obtained using Zylab®, which is a software programme that contains patient clinical notes from each emergency department admission in PDF format. The blood results were obtained from Indigo (clinical system) and radiology results from Synapse, which is the software used by the radiology department. The data was collected using Microsoft Excel which was stored in Swansea Bay University Health Board secured drives. The Welsh Index of Multiple Deprivation (WIMD, 2019) rank was obtained by inserting the postcode of each patient into the Welsh Government website https://apps.dataunitwales.gov.uk/welshindexofmultipledeprivation/. Each Welsh postcode has got a unique code and is ranked from 1-1909 (1909 being the highest rank). The data collected was analysed using IBM Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 (Armonk, NY:IBM Corp). The data normality was assessed using the Shaprio-Wilk test throughout, prior to analysis. The values are reported as means ± standard deviation (SD) or median (Interquartile range [IQR]) or n (percentage) and the graphs are generated using either SPSS or Microsoft Excel. The significance was analysed using independent samples t-test between two groups or one-way ANOVA if there are more than one group were appropriate.

3.3 Results

A total of 534 patients presented to Morriston Emergency Department (ED) with acute exacerbation of COPD from the 1st September 2016 to 31st August 2017.

3.3.1 COPD attendances by months and season

During the study period of 12 months, December and January witnessed high attendances of COPD patients (**Figure 3.1**). The attendances during various seasons were winter (31%), spring (25%), summer (23%) and autumn (21%). The seasonal variation showed highest attendances during the winter months as expected, however this was not statistically significant (p=0.23) **[Figure 3.2]**.

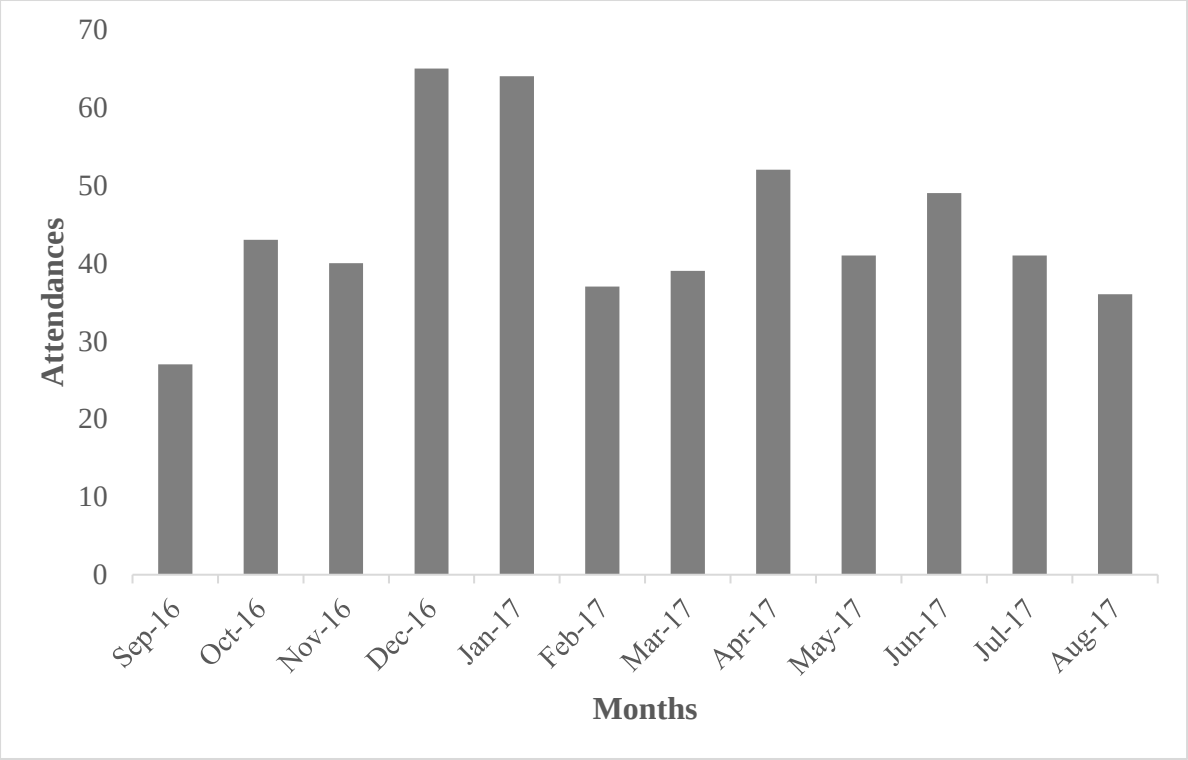


Figure 3.1: Monthly attendances of COPD retrospective group patients to the Emergency Department. There was an increase in the attendances during December and January and lowest during the month of September.

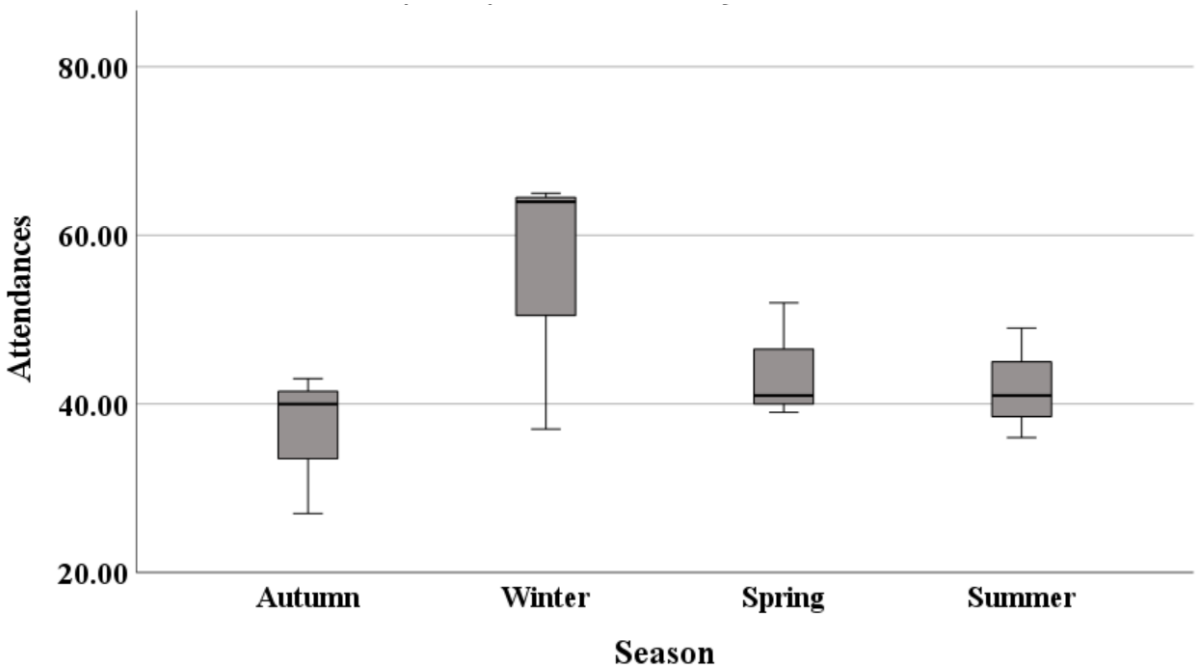


Figure 3.2: Seasonal variation in attendances of COPD patients in the retrospective group to the Emergency Department showing highest attendance during winter months (p=0.23). Significance between the groups was assessed using one-way analysis of variance (ANOVA).

3.3.2 Baseline characteristics and Welsh Index of Multiple Deprivation (WIMD 2019) in COPD patients in retrospective study

3.3.2.1 Baseline characteristics of COPD patients

The details of the baseline characteristics of the patients in the retrospective study is shown in **Table 3.1**. Patients presenting with exacerbations are elderly, more females and 38% of them were current smokers. The most common comorbidities were hypertension (HTN), ischaemic heart disease (IHD) and diabetes. The mortality rate was high with 9% who died on this admission and 35% died within one-year of the presentation to ED. Only 4% of the COPD patients had a history of venous thromboembolism and 1% had venous thromboembolism diagnosed during the admission.

Table 3.1: Baseline characteristics of COPD retrospective group patients presenting to the Emergency Department. Data presented n (percentage) or mean ± standard deviation (SD). N= total number of patients recruited

	N=534
Age (years) (mean ± SD)	70 ± 11
Sex (M (%)/F (%))	242 (45%) /292 (55%)
Current smoker (%)	138/314 (38%)
HTN (%)	182/534 (34%)
IHD (%)	110/534 (20%)
Diabetes (%)	64/534 (12%)
Atrial fibrillation (%)	59/534 (11%)
Cancer (%)	51/534 (10%)

CVA (%)	43/534 (8%)
Heart failure (%)	35/534 (7%)
Hospital admission (%)	337/534 (63%)
ICU admission (%)	35/534 (7%)
VTE history (%)	20/534 (4%)
Investigated for VTE (%)	26/534 (5%)
VTE during admission (%)	5/534 (1%)
Admitted to hospital (%)	337/534 (63%)
Thromboprophylaxis (%)	337/337 (100%)
Died in this admission (%)	47/534 (9%)
Died within 1 year (%)	185/534 (35%)

3.3.2.2 Welsh Index of Multiple Deprivation (WIMD) 2019 rank in COPD patients

The Swansea Bay University Health Board (SBUHB) serves a geographical area that has regions of substantial levels of deprivation. Deprivation is a known factor in the pathophysiology of several diseases including COPD. The Welsh Index of Multiple Deprivation (WIMD) 2019 rank is based on the postcode and was ranked out of 1909 (1909 being the highest rank). COPD patients was grouped into quintiles 1-191, 192-382, 383-573, 574-955 and 956-1909 (The Welsh Index of Multiple Deprivation 2019). The COPD patients in the retrospective study showed that those with WIMD at the scale's extremes had the highest rate of emergency department attendance (**Figure 3.3**). The most deprived are significantly older than the least deprived (73 ± 10 vs 65 ± 12, p<0.0005).

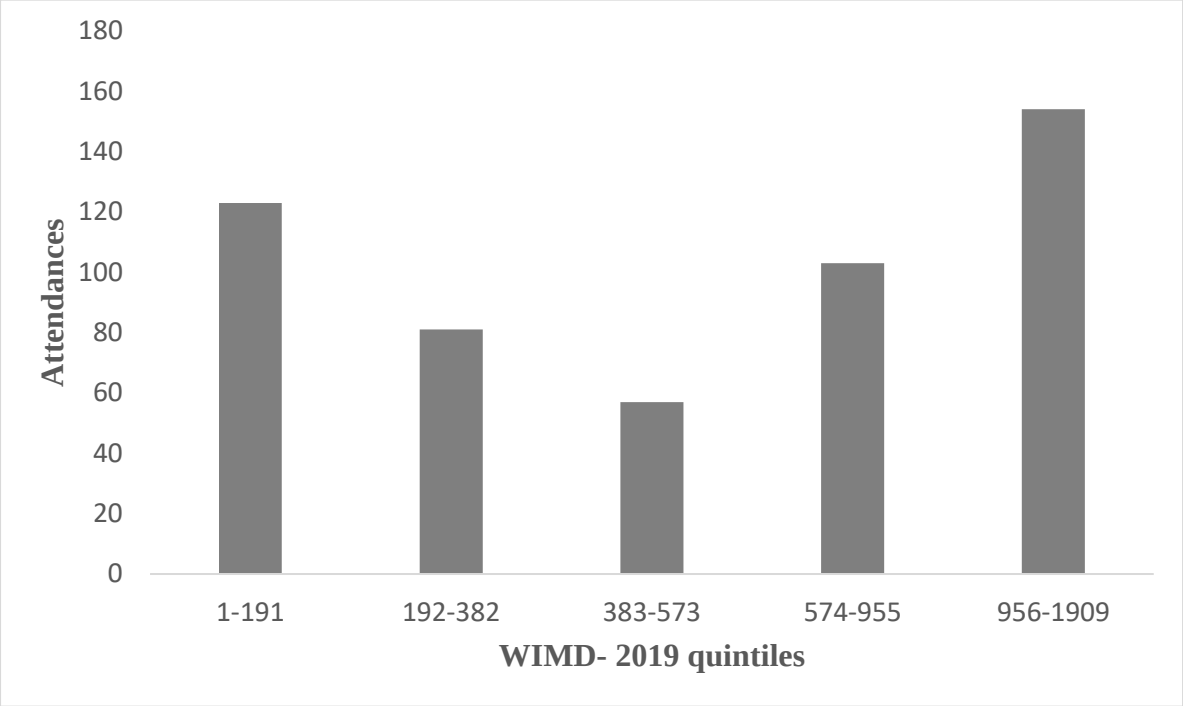


Figure 3.3: Welsh Index of Multiple Deprivation (WIMD 2019) quintiles for the COPD retrospective group patients. Highest number of ED attendances were from the most deprived (1-191) and least deprived (856-1909).

3.3.3 Clinical observations and blood gas analysis recorded in the Emergency Department for the COPD patients in retrospective study

3.3.3.1 Clinical observations in COPD patients

The clinical observations show that COPD patients presenting with exacerbations were tachycardic, hypertensive and in respiratory distress with high respiratory rate requiring oxygen therapy (**Table 3.2**). The National Early Warning Score (NEWS) 2 score is used as an early warning score derived from temperature, pulse rate, systolic blood pressure, respiratory rate, oxygen saturation and level of consciousness. NEWS2 is developed by the Royal College of Physicians (RCP) and helps to identify acutely unwell patients (Royal College of Physicians. 2017).

Table 3.2: Clinical observations for the COPD retrospective group patients and the normal values. Data presented as mean ± SD. * indicates higher than normal values.

	COPD patients (mean ± SD)	**Normal value**
Temperature (^{o}C)	37.1 ± 0.8	<38
Pulse (beats/ minute)	103 ± 20*	60-100
Systolic Blood Pressure (mm of Hg)	137 ± 27*	≤120
Diastolic Blood Pressure (mm of Hg)	77 ± 14	≤80
Respiratory rate (breaths/minute)	25 ± 6*	12-16
Oxygen saturation (%)	92 ± 5*	≥ 94 (on air)
Oxygen requirement (%)	24 ± 7*	21% (air)
NEWS score	7 ± 3*	0-4

3.3.3.2 Blood gas measurements in COPD patients

The blood gas analysis performed at presentation to the Emergency Department showed COPD patients had a normal pH, but pCO2 and HCO3 were higher than the normal values (**Table 3.3**). This is suggestive of the patients being in type 2 respiratory failure.

Table 3.3: Blood gas measurements for the COPD retrospective group patients and normal values. Data presented as mean ± SD. * indicates higher than normal values.

	COPD patients (mean ± SD)	**Normal value**
pH	7.37 ± 0.10	7.35- 7.45
pCO2 (kPa)	6.9 ± 2.7*	4.3-6.4
HCO3 (mmol/L)	26.6 ± 4.2*	21.0-26.0
Lactate (mmol/L)	1.6 ± 1.4	0.5-1.6

3.3.4 Inflammatory and haemostatic biomarkers of the COPD patients

Routine blood tests performed in the Emergency Department showed that COPD patients during exacerbations had levels of inflammatory markers such as white blood cell (WBC), neutrophil count and CRP that were higher than the normal range as were the coagulation markers tested such as the PT and Fibrinogen (**Table 3.4**). The coagulation markers analysed here, PT were associated with anticoagulation therapy and the inflammatory markers were indicative of the pro-inflammatory status of the patients.

Table 3.4: Inflammatory markers, coagulation markers, haemoglobin (Hb) and haematocrit (HCT) in COPD retrospective group patients. Data presented as mean ± SD or median (IQR). * indicates higher than normal values.

	COPD patients	**Normal value**
WBC ($\times 10^9$/L) (mean ± SD)	12.9 ± 7.7*	4.0-11.0
Neutrophils ($\times 10^9$/L) (mean ± SD)	10.2 ± 7.1*	1.7-7.5
CRP (mg/L) median (IQR)	19 (6-65)*	<5
Platelets ($\times 10^9$/L) (mean ± SD)	288 ± 100	150-400
PT (sec) median (IQR)	14.1 (9.7-83.4)	9.0-12.5
APTT (sec) (mean ± SD)	26.4 ± 6.3	22.1-30.9
Fibrinogen (g/L) (mean ± SD)	4.5 ± 1.1*	2.0-4.0
Haemoglobin (g/L) (mean ± SD)	135 ± 35	130-180
Haematocrit (L/L) (mean ± SD)	0.41 ± 0.06	0.40-0.52

3.3.5 COPD severity and mortality

Spirometry is a type of lung function test that is used to assess disease severity in COPD patients. About 45% (239/534) of the patients had documented FEV1 (Forced Expiratory Volume in one second). Out of this, the majority of patients who presented to the ED with exacerbations had moderate-severe disease (FEV1 49 ± 22 [mean ± SD]) [**Figure 3.4**].

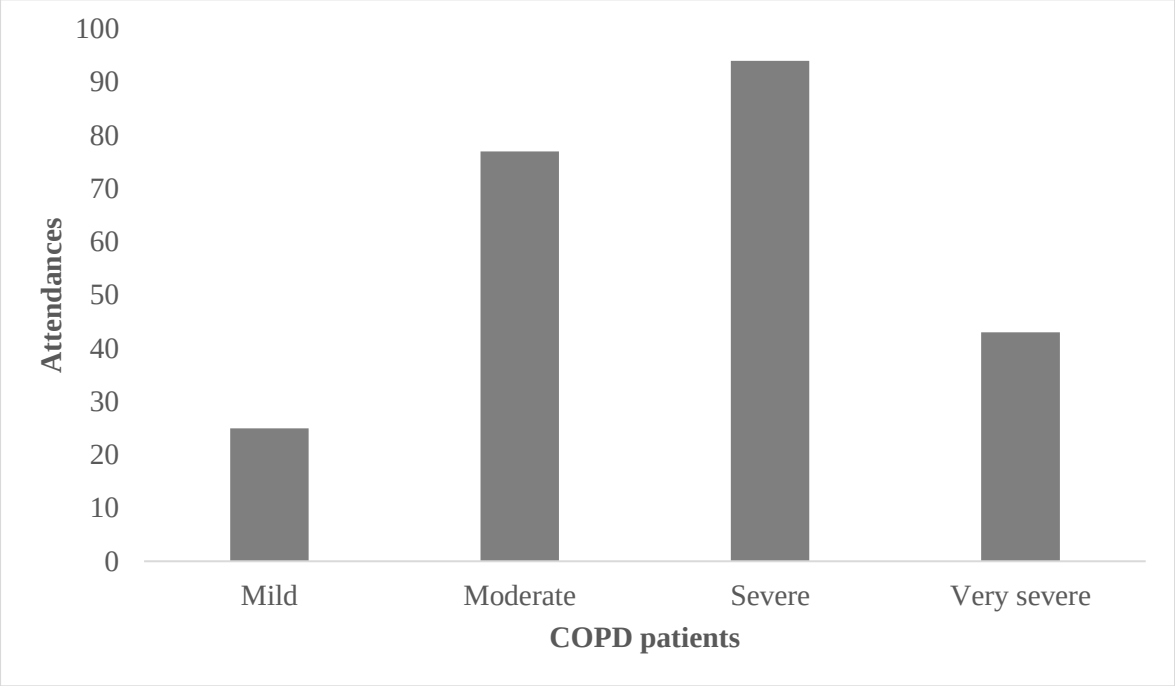


Figure 3.4: COPD severity based on FEV1 (Forced Expiratory Volume in one second) and number of attendances to the Emergency Department. Most of the patients who presented with acute exacerbation had moderate-severe disease in the retrospective group.

Out of 45% (239/534) patients had a documented FEV1, patients who died during admission and at one-year had lower when compared to those survived which was expected and those patients who died in one-year had significantly lower FEV1 when compared to those who survived (**Table 3.5**).

Table 3.5: Disease severity based on FEV1 in patients who died and survived during the admission and in one-year. Data presented as mean ± SD and the significance was assessed using independent samples t-test. * indicates significant value (p <0.05).

FEV1	**Died**	**Survived**	***p* value**
On admission (mean ± SD)	42 ± 19	49 ± 22	0.32
One-year (mean ± SD)	43 ± 22	50 ± 22	0.02*

3.4 Discussion

One of the aims of this retrospective data collection was to understand the demographics and comorbidities of COPD patients presenting to an ED with exacerbation. COPD is a disease of the respiratory system and respiratory illnesses often increases during winter months (Wise et al. 2018). According to the British Lung Foundation, there were 80% more lung disease admissions during winter months and 54% of those admissions were people aged 65 and above (British Lung Foundation, 2017). This retrospective study agrees with these findings of attendances highest during winter months and patients were elderly. The presentations with acute exacerbations in this study were increased during afternoons and during night hours. The study by Goyal, Goel, Bhattacharya, Verma, and Tiwari (2019) showed reduced spirometry during night hours and increased spirometry during day hours. This study showed more female attendances compared to males. According to Han et al. (2007), women with COPD are hospitalised more than men. This might be due to several reasons such as increase in the tobacco use; women are more likely to seek medical help and increased longevity. There were significant number of smokers (38%) in this retrospective study, that reflects similar trends in the United States (38%) according to Wheaton, Cunningham, Ford, and Croft (2015) and in the UK (34.9%) as per Shahab, Jarvis, Britton, and West (2006). The most common co-morbidity among the COPD group was cardiovascular diseases of which hypertension was observed most frequently, followed by ischemic heart disease in this study. These findings were similar to those found in a large study by Chetty et al. (2017) in a Scottish study, and similarly, diabetes, cancer and cerebrovascular accidents were also important comorbidities in their group. Approximately 63% of COPD patients were admitted to the hospital, which was slightly higher to what was reported (García-Sanz et al., 2012).

Relatively, high numbers of the patients attending, were from the most deprived areas and supports a recent study (Collins, Stratton, Kurukulaaratchy, and Elia, 2018), confirming that deprivation is associated with hospital admission and mortality for COPD. However, high level of admission was also observed in the subjects from the least deprived areas of the study population. It is unclear why this should be the case as low deprivation is almost universally a risk factor for disease. An additional analysis of these two groups showed that the patients from the most deprived area were significantly older than the least deprived area. The study by Stone et al. (2012) found that elderly COPD patients are less likely to attend hospitals, and this may

be due to decreased knowledge about the disease, failure of recognition of exacerbation symptoms and reduced access to health care. The in-hospital mortality rate was 9% which was slightly lower than a meta-analysis that reported 11.4%-19% (Hoogenddorn, Hoogenveen, Rutten-van Mölken, Vestbo, & Feenstra, 2011). A high percentage of COPD patients died within one year (35%) which in this case agrees with other studies (Soler-Cataluña et al., 2005). All these findings suggest that the demographics and comorbidities of COPD patients presenting with exacerbation to the hospital in Swansea were similar to what has been reported in the literature.

Only 45% of the patients had a documented FEV1, which was one of the limitations of this study. Based on the FEV1, the patients were classified into mild, moderate, severe and very severe disease. The study by Tsai, Griswold, Clark, & Camargo (2007) found that one of the factors affecting the frequency of admissions to the ED was severe COPD and this study confirms this finding that the greatest number of patients presenting to ED had severe COPD (39%). Patients who died during the admission and at one-year had low FEV1 meaning they had increased severity of disease. Another interesting finding was that patients who survived at one year had significantly higher FEV1 when compared to those who died which agrees with a UK based study (Ching et al. 2019).

COPD is characterised by chronic inflammation of the airways and lungs which is frequently associated and caused by smoking and other risk factors mentioned earlier in this thesis (Chapter 1). This retrospective study showed that the prevalence of smoking was high in this COPD population (38%). As described before, toxic chemicals in cigarette smoke induces inflammation followed by infiltration of inflammatory cells resulting in the chronic airway obstruction of COPD. The clinical observations in this group were abnormal which is expected during acute exacerbation. The inflammatory markers investigated such as WBC, Neutrophils and CRP were above the expected range that confirms the activation of the inflammatory process. As COPD progresses, there is impairment of gas exchange, and this worsens during exacerbation leading to type 2 respiratory failure characterised by low oxygen (hypoxia) and high carbon-dioxide (hypercapnia). This is why COPD patients in this retrospective study were tachypnoeic, tachycardic and had carbon-dioxide retention and hypoxic requiring supplementary oxygen. When there is carbon dioxide retention, physiologically the homeostatic systems neutralise the carbonic acid formed, by increasing renal bicarbonate

reabsorption as explained in detail in Chapter 4.3. This was the reason for high bicarbonate in the blood gas with a normal pH which is termed compensated respiratory acidosis.

In this study, only 4% of COPD patients had a history of venous thromboembolism. All COPD patients admitted to the hospital received thromboprophylaxis and screening for VTE is not routine among COPD patients. This was because the symptoms of COPD exacerbation and PE such as shortness of breath, tachycardia and hypoxia overlap. However, wheezing is characteristic of COPD and all patients with exacerbation have a wheeze. Therefore, in clinical practice, VTE will only be investigated in those patients with 'out of proportional' hypoxia and tachycardia. The study shows that 18/534 COPD patients had D-dimer checked, of which 9 were positive with the CTPA performed only on two patients who both tested negative. Alternatively, CTPA was performed in 22 patients who did not have a D-dimer undertaken due to clinical observation, of these 4 were positive (18%) which given the inflammatory and VTE risk of the disease was not unexpected. In addition, doppler study was performed on 6 patients and one patient was positive for DVT. Collectively, only 1% of patients were diagnosed to have VTE in this study. It appears that because of the overlap of symptoms, less COPD patients are investigated for VTE which possibly might have resulted in low number of detected VTE. According to Børvik et al. (2016) severe COPD patients had 1.6-fold increase in VTE. In this study there were only five patients who developed VTE and only three of them had a documented FEV1, therefore it was difficult to perform an analysis and come with a meaningful interpretation. Therefore, further study is required to assess the thrombogenicity in COPD patients who present with exacerbation.

As described before (Chapter 3.1), one of the reasons for undertaking this retrospective study was to explore whether adequate number of patients can be recruited to the AECOPD study. There were 95/534 (18%) patients who attended the ED during 9am-1pm, therefore, it was feasible to undertake the prospective AECOPD study requiring the recruitment of a minimum of 85 patients over a period of three years. This retrospective study has several limitations, firstly this was a single centre study representing the population of Swansea Bay University Health Board area, therefore the results are not generalisable to whole of Wales or the UK. There are no similar local retrospective studies to compare the data other than the epidemiology data mentioned before. Secondly this study involved retrospective data collection. To avoid selection bias, the patient attendance data was requested through the ED reception team and all patients presented to ED over one year was included. Thirdly, the main limitations in

interpreting the severity analysis were because only 45% of the patients had a documented FEV1. This was because spirometry will not be undertaken during acute exacerbation because it will not give an accurate measurement. Therefore, patients are brought back to clinic to perform spirometry. Lastly, this was a service evaluation specifically looking into the number of patients who had VTE investigations.

3.5 Conclusion

The study demonstrates that the population studied had demographics, comorbidities and mortality rates comparable to published data of COPD patients in the literature. The admission numbers were adequate to carry out the prospective study. Patients who died during admission and in one year had severe form of the disease. All COPD patients admitted to hospital received appropriate thromboprophylaxis. The overall incidence of VTE was very low which might be due to the fact that COPD patients presenting with exacerbation are not often investigated for VTE because of the overlap of symptoms. Further studies are required to demonstrate the thrombogenicity in COPD patients during exacerbation.

Chapter 4: Clot microstructure (d_f) as a marker of thrombogenicity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)

4.1 Introduction

COPD is often associated with coagulation abnormalities (Liu, Hu, Jiang, & Mei, 2021). Venous thromboembolism (VTE) occurs when blood clots are formed in the deep veins (deep vein thrombosis-DVT) which then get dislodged and travel to the lungs causing pulmonary embolism (PE). However, approximately 50% of pulmonary embolisms are not caused by DVTs (Marongiu, Mameli, Grandone, and Barcellona, 2019). The study by Castellana et al. (2021) showed that only 33% of COPD patients with PE had DVT. Therefore, there must be certain pathologies occurring locally within the lungs that causes PE's. As mentioned in Chapter 1.2.3, there are several risk factors that can cause COPD. The most important one is cigarette smoking. As per the Office of National Statistics (2023), 23.8% of people living in most deprived area are current smokers when compared to 6.8% in the least deprived area. Therefore, it is important to obtain deprivation data using Welsh Index of Multiple Deprivation (WIMD 2019). Exposure to these risk factors can trigger local inflammation leading to the release of various cytokines. In addition, local tissue and endothelial damage releases tissue factor (TF) which then activates the coagulation pathways. Subsequently, this leads to increased fibrin production and increased fibrin in the blood can trigger VTE. The cytokines such as IL-6 and TNF-α released as a consequence of local inflammation can then trigger systemic inflammation (Dadvand et al. 2014). Furthermore, systemic inflammation triggers coagulation pathways and adds to more fibrin production. Therefore, all these factors make COPD patients thrombogenic. The use of conventional coagulation markers such as prothrombin time (PT) and activated partial thromboplastin time (APTT) has got several limitations. Firstly, PT and APTT investigates specific coagulation pathways (extrinsic and intrinsic respectively) by adding activators. PT uses high concentration of tissue factor negating the activity of other coagulation markers. In addition, PT and APTT are undertaken on platelet poor plasma, therefore this ignores the effect of platelets on blood coagulation (Curry and Pearce 2007). The limitations mentioned above makes these tests not suitable to detect

coagulation abnormalities especially the thrombogenicity (Meybohm, Zacharowski, and Weber, 2013). Blood tests such as D-dimer help to identify increased fibrinolysis as in VTE, however D-dimer can be elevated in infection and inflammation.

The biomarker, fractal dimension (d_f) is a mechanistic test that is performed in whole blood without adding any reagents. The transition of the blood from liquid to solid is possible only after the formation of enough fibrin polymers which then branches and interlinks forming the fibrin mesh/ clot microstructure. This fibrin mesh that is arranged three dimensionally apply resistance to the frequencies applied by rheometer which then is mapped into a graph that gives the d_f (Evans et al. 2010a). If the clot microstructure is denser and tighter (hypercoagulable), then it applies more resistance, therefore higher d_f. Similarly, if the clot microstructure is looser and weaker (hypocoagulable), then it applies less resistance, therefore low d_f (Davies et al. 2016). Patients with denser and tighter clot microstructure are therefore thrombogenic. D_f is studied extensively in different acute illnesses and quantifies clot microstructure thereby able to demonstrate thrombogenicity. Studies have shown that anticoagulation (Evans et al. 2010b) and haemodilution (Lawrence et al. 2014) produces loose and weaker clots as evidenced by low d_f. In their study, Davies et al. 2015 found that advanced lung cancer patients had tighter and denser clots as evidenced by high d_f. Patient with acute ischaemic stroke had high d_f (Stanford et al. 2015) and in sepsis, patients with sepsis and severe sepsis had high d_f and in septic shock, d_f was low (Davies et al. 2016). However, there have not been studies to date assessing the thrombogenicity in COPD patients. Therefore, it was hypothesised that patients with acute exacerbation of COPD (AECOPD) have tighter and denser clot microstructure. The aim of this study was to assess the thrombogenicity in AECOPD patients using d_f. In addition, to understand the pathophysiology, several inflammatory and haemostatic biomarkers have also been studied.

4.1.1 Aims

1. To investigate whether AECOPD group are more thrombogenic when compared to SCOPD group by comparing the d_f between both groups
2. To compare the difference between biomarkers of inflammation and hemostasis between SCOPD and AECOPD group

3. To investigate the change in d_f with treatment in the AECOPD group across four time points
4. To investigate the incidence of VTE in both groups
5. To investigate the effect of COPD severity in d_f

4.2 Methodology

As explained in detail in Chapter 2, this prospective study was undertaken in the Welsh Centre for Emergency Medicine Research (WCEMR) which is based in the Emergency Department, Morriston Hospital, Swansea. The analysis of blood samples was carried out in the Haemostasis Biomedical Research Unit (HBRU) which is part of the WCEMR and the main laboratory at Morriston Hospital. HBRU is a well-equipped laboratory that can undertake rheology, platelet aggregation and thromboelastometry. The inclusion criteria for the AECOPD study were patients aged 35 and above with a confirmed diagnosis of COPD (GOLD, 2022). All those COPD patients who were on anticoagulants was excluded. This study recruited two group of patients (N=115). The first group was stable COPD (SCOPD) patients (N=30) recruited from the chest clinic. This group of patients had a stable COPD disease and was attending the chest clinic for follow up. All SCOPD patients had capacity, therefore after obtaining consent, approximately 30mls of blood was taken to perform rheological, inflammatory and haemostatic markers. Therefore, in the SCOPD group, only one sampling point was undertaken. The second group was COPD patients with acute exacerbation (AECOPD) who presented to the Emergency Department (N=85). Consent form was obtained from those patients who had capacity and a consultee declaration was obtained from the next-of-kin or the admitting team in those patients who lacked capacity. Approximately 30 mls of blood was obtained at the time of recruitment (0 hours), 4-6 hours, 24 hours and 3-7 days. Therefore, in AECOPD patients there were four sampling points and the reason for this was to analyse the effect of treatment on clot microstructure. The rheological markers that are investigated are d_f (fractal dimension) and T_{GP} (time to gel point). This test was undertaken by placing 7 mls of whole blood into the rheometer. Other than platelet aggregometry and thromboelastometry all other haemostatic and inflammatory biomarkers were sent to the Haematology and Biochemistry laboratory in Morriston Hospital. Welsh Index of Multiple Deprivation (WIMD 2019) scores were obtained online from https://www.data.cymru/wimd by individually inserting the postcodes. The score ranges from 1 to 1909 with 1 being the most deprived and 1909 is the least deprived area.

4.3 Results

4.3.1 Patient recruitment

From 2016 to 2021, the study recruited a total of 115 patients in two groups (**Figure 4.1**). The stable group (SCOPD) was recruited from the Chest Clinic and Pulmonary Rehabilitation programme at Morriston Hospital (n=30). The acute exacerbation group (AECOPD) was recruited from Morriston Hospital's Emergency Department (n=85). Some of the AECOPD patients improved substantially with medical treatment and were therefore discharged from the ED after recruitment. In addition, those patients who improved significantly were discharged from the hospital by the admitting team within 24 hours or over the next few days. SCOPD patients had one sampling point after being recruited into the study and AECOPD patients had four sampling points: at the time of admission (0 hours) [AECOPD-A], 4-6hours (AECOPD-B), 24 hours (AECOPD-C) and 3-7 days (AECOPD-D). All the patients recruited were included in the analysis.

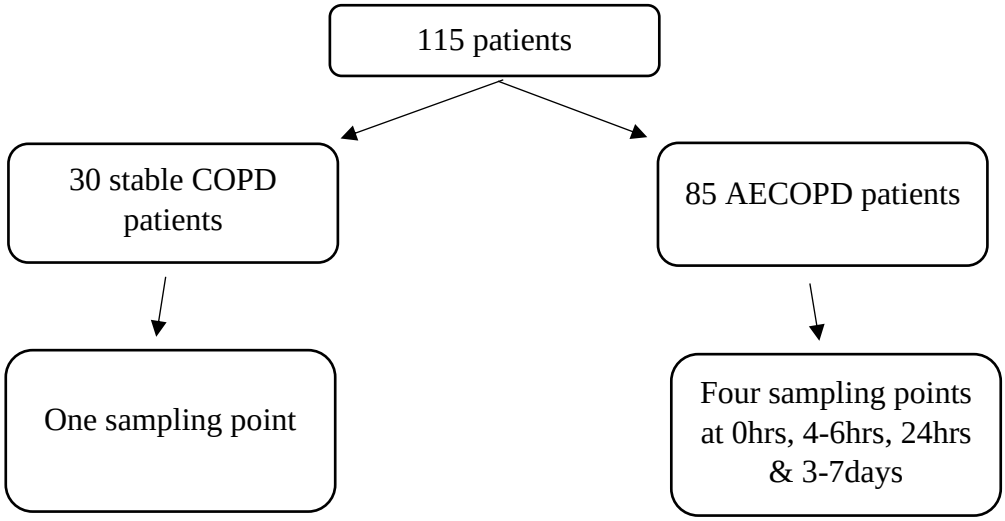


Figure 4.1: Flow diagram illustrating total patient recruitment and blood sampling strategy for stable COPD and AECOPD patient groups.

4.3.2 Baseline characteristics and Welsh Index of Multiple Deprivation (WIMD 2019) of SCOPD and AECOPD patients

The baseline characteristics of the patients included is shown in **Table 4.1**. Between the SCOPD and AECOPD group, patients were well matched for age, sex, body mass index (BMI) and comorbidities except hypertension that was significantly higher in the AECOPD group. There was a significantly higher number of patients who died within one year in the AECOPD group. Even though 9% of the AECOPD patients were investigated for venous thromboembolism, only 1% were diagnosed to have venous thromboembolism.

Table 4.1: Baseline characteristics of the SCOPD and AECOPD patients. Values were presented as mean ± SD or n (percentage) or median (IQR). The significance between the groups was assessed using independent samples t-test or Chi-square test or Kruskal-Wallis test. * indicates significant result (p<0.05).

	SCOPD (n=30)	**AECOPD (n=85)**	***p value***
Age (years) [mean ± SD]	67 ± 10	70 ± 10	0.19
Sex (M:F)	15:15	41:44	0.87
BMI (mean ± SD)	27.5 ± 7.7	29.2 ± 8	0.46
Current smoker (%)	9 (30%)	36 (44%)	0.43
HTN (%)	5 (17%)	30 (35%)	0.02*
Diabetes (%)	6 (20%)	18 (21%)	0.89
IHD (%)	4 (13%)	2 (2%)	0.12
CVA (%)	1 (3%)	8 (9%)	0.10
Heart failure (%)	2 (7%)	5 (6%)	0.88
Previous VTE (%)	2 (7%)	1(1%)	0.26
Cancer (%)	2 (7%)	7 (8%)	0.40
Hospital length of stay (days)	-	6 (2-14)	-

median (IQR)			
ICU length of stay (days) median (IQR)	-	11 (3-31)	-
ICU admission (%)	-	9 (11%)	-
Died in this admission (%)	-	10 (12%)	-
Died within 1 year (%)	4 (13%)	25 (30%)	0.02*
Investigated for VTE (%)	-	8 (9%)	
VTE during admissions (%)	-	1 (1%)	-

Welsh Index of Multiple Deprivation (WIMD) 2019 data indicated that majority of the SCOPD patients who attended the respiratory clinic were from the least deprived area. In contrast, in the AECOPD group the majority of the patients admitted with COPD exacerbation to the Emergency Department was from the most deprived area (**Figure 4.2**).

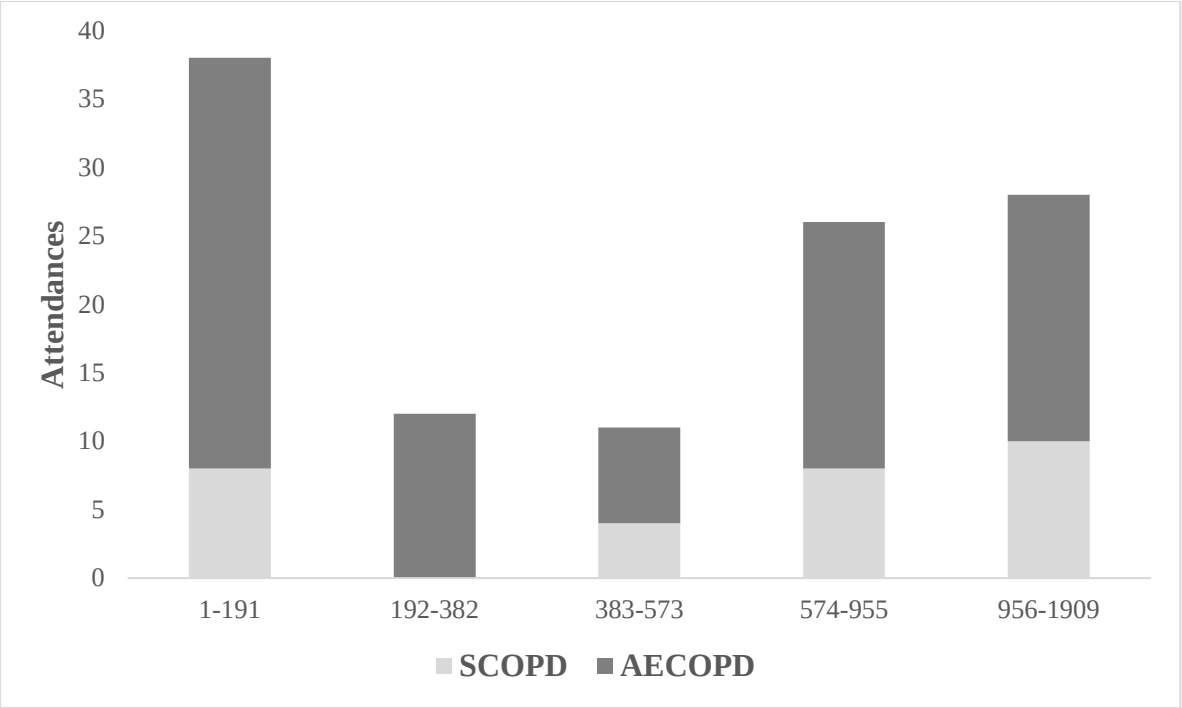


Figure 4.2: Welsh Index of Multiple Deprivation (WIMD 2019) for both SCOPD and AECOPD group. WIMD 2019 is a measure of deprivation with 1 being most deprived and 1909 being least deprived which is equally split into 5 parts or quintiles. SCOPD patients attending the respiratory clinic were from least deprived areas and AECOPD patients who attended the Emergency Department were from the most deprived areas.

4.3.3 Clinical observations and blood gas analysis in AECOPD patients at the time of admission

At the time of admission AECOPD patients were tachycardic with a high respiratory rate and low oxygen saturation and requiring a mean oxygen of 30% (air has 21% oxygen). It is not a clinical practice to perform observations in SCOPD patients unless they are clinically unwell (**Table 4.2**).

Table 4.2: Clinical observations in AECOPD patients and normal values. Data presented as mean ± SD. * indicates higher than normal values.

	AECOPD (mean ± SD)	**Normal value**
Temperature (^{o}C)	36.8 ± 0.8	<38
Pulse (beats/ minute)	104 ± 22*	60-100
Systolic Blood Pressure (mm of Hg)	136 ± 33*	≤120
Diastolic Blood Pressure (mm of Hg)	75 ± 18	≤80
Respiratory rate (breaths/minute)	26 ± 8*	12-16
Oxygen saturation (%)	92 ± 7*	≥ 94 (on air)
Oxygen requirement (%)	30 ± 17*	21% (air)
NEWS score	8 ± 3*	0-4

The mean blood gas analysis of the AECOPD patients (**Table 4.3**) showed that most were in severe type 2 respiratory failure which is characterised by low blood oxygen and high carbon dioxide leading to respiratory acidosis (pH≤7.35). As previously stated, it is not a normal clinical practice to perform blood gas analysis for SCOPD patients.

Table 4.3: Blood gas measurements in AECOPD group and normal values. Data presented as mean ± SD. * indicates lower than normal values, **indicates higher than normal values.

	AECOPD (mean ± SD)	**Normal value**
pH	7.31 ± 0.11*	7.35- 7.45
pCO2 (kPa)	8.2 ± 3.5**	4.3-6.4
HCO3 (mmol/L)	27.0 ± 6.7**	21.0-26.0
Lactate (mmol/L)	1.8 ± 1.3**	0.5-1.6

4.3.4 Rheological markers in SCOPD and AECOPD patients

4.3.4.1 Rheological markers in SCOPD and AECOPD patients

The use of rheological markers of haemostasis has not been applied to COPD to date. The two biomarkers investigated in these patients include fractal dimension (d_f) and the time to gel point (T_{GP}). Both these biomarkers are indicators of changes in clot structure and of hyper and hypocoagulability. The d_f was significantly higher in AECOPD group at presentation to the emergency department when compared to the SCOPD group and is suggestive of the fibrin clot structure in these patients being denser with enhanced cross-linking. In contrast, the T_{GP} was significantly lower in AECOPD group at presentation to the ED indicating that the time to blood clot formation was reduced (**Table 4.4**).

Table 4.4: Blood rheological markers in SCOPD and AECOPD group. Data presented as mean ± SD, significance between the groups was assessed using independent samples t-test. * indicates significant result (p<0.05).

	SCOPD	**AECOPD**	***p value***
d_f (mean ± SD)	1.69 ± 0.05	1.71 ± 0.06	0.03*
T_{GP} (mean ± SD)	316 ± 101	275 ± 73	0.004*

4.3.4.1 Rheological markers in AECOPD patients at four time points

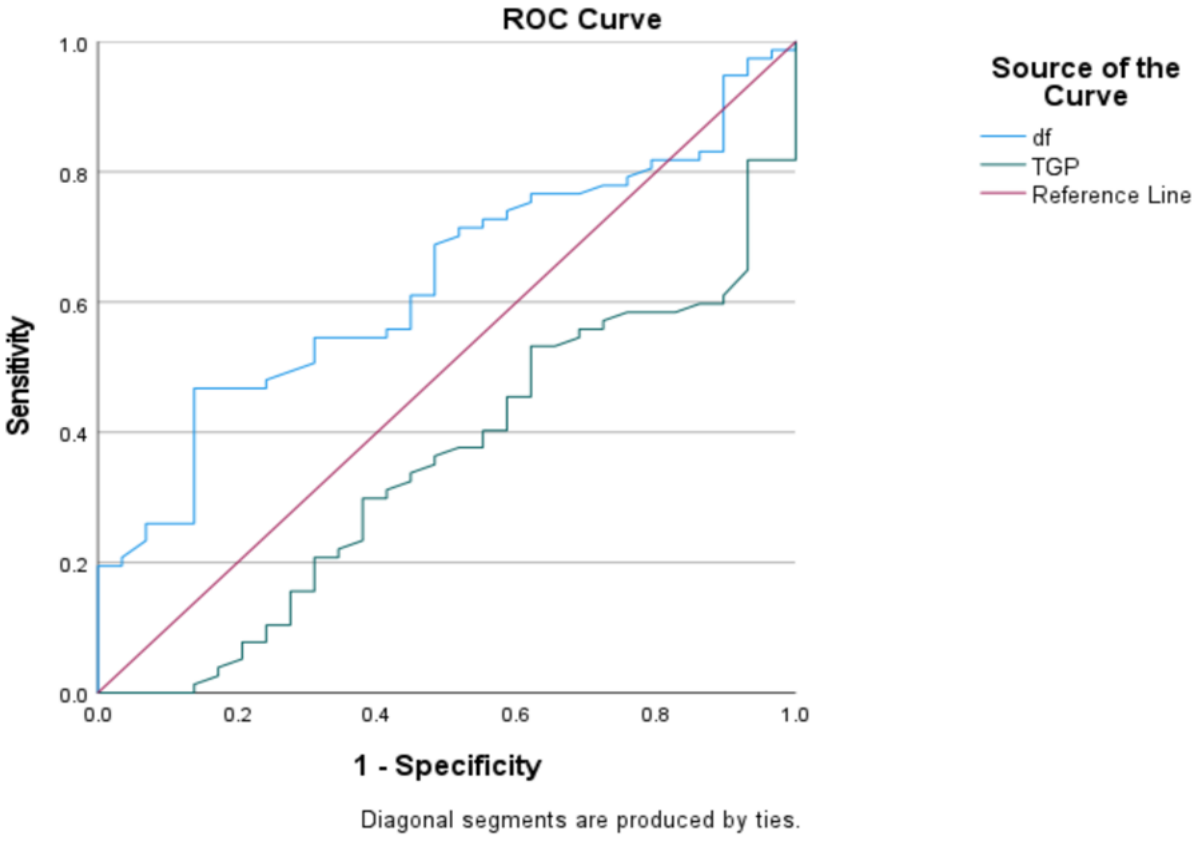
The analysis demonstrated that there were no statistically significant changes in d_f and T_{GP} in response to therapeutic intervention over the four-time points sampled in the AECOPD group (**Table 4.5**).

Table 4.5: Changes in the rheological markers within the four AECOPD group. Data presented as mean ± SD and significance between the four time points (A, B, C, D) was assessed using one-way analysis of variance (ANOVA).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	***p value***
d_f (mean ± SD)	1.71 ± 0.06	1.70 ± 0.52	1.70 ± 0.07	1.71 ± 0.07	0.275
T_{GP} (mean ± SD)	275 ± 73	256 ± 84	291 ± 142	308 ± 170	0.114

4.3.4.3 Receiver operating curve for rheological markers between SCOPD and AECOPD group

Receiver operating characteristics (ROC) curve can be used to plot the sensitivity (true positive) and specificity (true negative) and therefore can be used to evaluate biomarker utility in clinical diagnosis of a disease. The area under the ROC curve which is between 0 and 1 indicates the predictive capability of a biomarker. Therefore, higher the area (near to 1) indicates higher predictive capability of a biomarker (Nahm, 2022). ROC curve shows that d_f and T_{GP} demonstrated an ability to act as significant discriminators between AECOPD and SCOPD patients, however d_f had greater discrimination when compared to T_{GP} (**Figure 4.3, Table 4.6**).



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
d_f	.630	.056	.040*	.521	.739
T_{GP}	.353	.058	.020*	.240	.466

Figure 4.3: Receiver operating characteristics curve shows that d_f and TGP demonstrated an ability to act as significant discriminators between AECOPD and SCOPD patients, however d_f had greater discrimination when compared to T_{GP}. *indicates asymptomatic significance <0.05.

Table 4.6: Receiver operating characteristics for rheological markers for the discrimination between AECOPD and SCOPD patients showing no significance. *indicates asymptomatic significance p<0.05.

4.3.5 Inflammatory markers in SCOPD and AECOPD patients

4.3.5.1 Inflammatory markers in SCOPD and AECOPD group

The inflammatory markers WBC, Neutrophils, CRP and PCT were all significantly higher in AECOPD patients at presentation to ED as compared to SCOPD as expected (**Table 4.7**).

Table 4.7: Inflammatory markers in SCOPD and AECOPD group. Data presented as mean ± SD or median (IQR) and the significance between the groups was assessed using independent samples t-test or Mann-Whitney U test. *indicates significant result (p<0.05).

	SCOPD	**AECOPD**	***p value***
WBC ($\times 10^9$/L) (mean ± SD)	9.5 ± 3.5	15.1 ± 8.1	<0.001*
Neutrophils ($\times 10^9$/L) (mean ± SD)	6.9 ± 3.8	12.6 ± 7.5	<0.001*
CRP (mg/L) [median (IQR)]	0 (0-6)	38 (12-75)	<0.001*
PCT (ug/L) [median (IQR)]	0.04 (0.02-0.05)	0.11 (0.05-0.57)	<0.001*

4.3.5.2 Inflammatory markers in AECOPD group at four time points

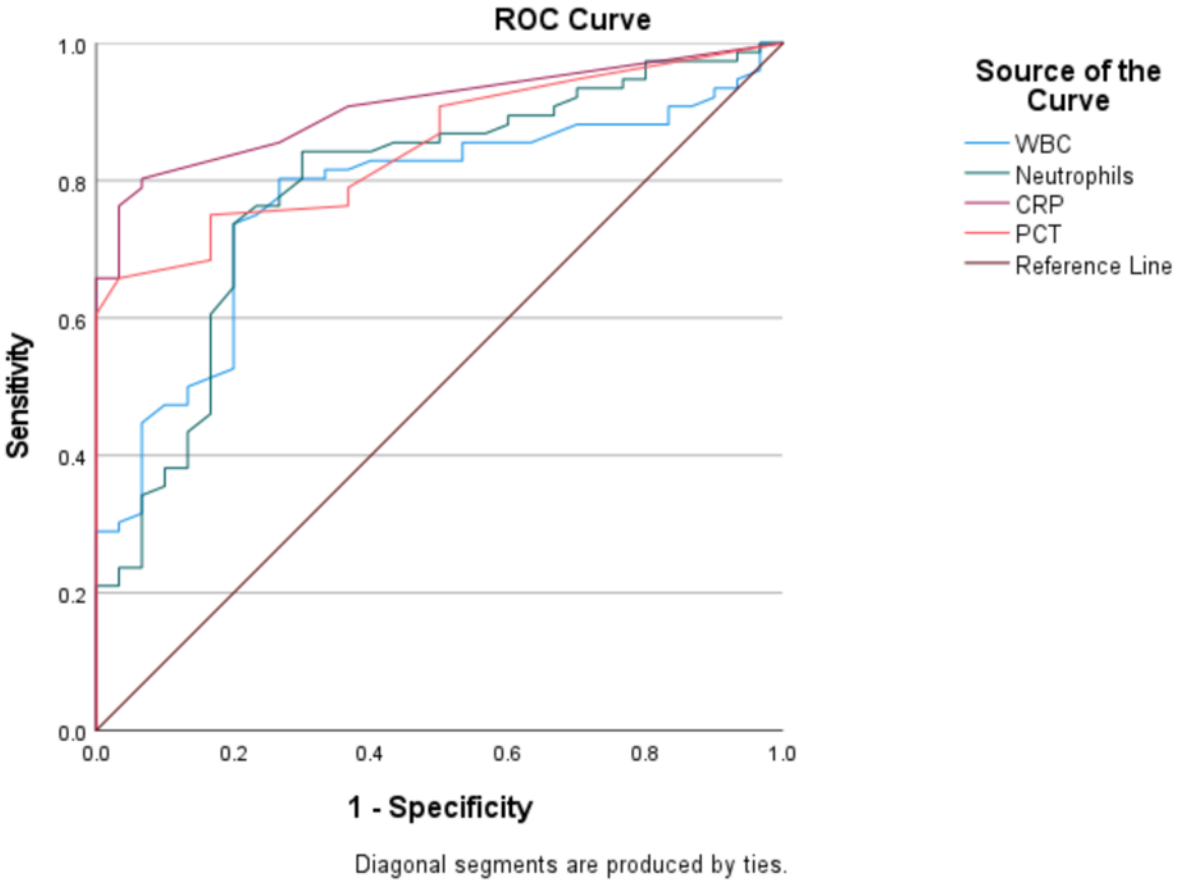
The neutrophil count and both CRP and PCT are commonly used as biomarkers of the inflammatory status of a patient. PCT in particular is significantly associated with bacterial infections. There was significant difference in WBC, Neutrophils, CRP and PCT between the four sampling points in AECOPD group. The WBC and Neutrophil improves with treatment. However, CRP and PCT increased in the second sampling point (AECOPD-B) and with treatment it significantly decreased **(Table 4.8)**.

Table 4.8: Inflammatory markers at the four sampling points. Data presented as mean ± SD or median (IQR). The significance between the four AECOPD groups (A, B, C, D) was assessed using either one-way ANOVA or Kruskal-Wallis test. * indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	***p value***
WBC (mean ± SD)	15.1 ± 8.1	12.9 ± 6.2	11.6 ± 4.4	10.7 ± 3.9	0.001*
Neutrophils (mean ± SD)	12.6 ± 7.5	11.6 ± 5.9	9.7 ± 4.0	8.8 ± 3.7	0.005*
CRP [median (IQR)]	38 (12-75)	47 (21-111)	44 (23-134)	15 (7-51)	0.004*
PCT [median (IQR)]	0.11 (0.05-0.57)	0.24 (0.10-2.30)	0.35 (0.08-1.77)	0.15 (0.06-0.76)	0.01*

4.3.5.3 Receiver operating curve for inflammatory markers between SCOPD and AECOPD group

The ROC curve analysis demonstrates that all the inflammatory markers acted significantly in their ability to discriminate AECOPD from SCOPD, however CRP had the greatest level of discrimination (**Figure 4.4, Table 4.9**).



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
WBC	.772	.048	<0.0005*	.679	.866
Neutrophils	.788	.049	<0.0005*	.691	.885
CRP	.906	.028	<0.0005*	.851	.961
PCT	.850	.036	<0.0005*	.780	.920

Figure 4.4: Receiver operating characteristics for inflammatory markers for the discrimination between AECOPD and SCOPD patients. CRP had the greatest level of discrimination than WBC, Neutrophils or PCT. **Table 4.9**: Receiver operating characteristics for inflammatory markers for discrimination between AECOPD and SCOPD patients. WBC, Neutrophils, CRP and PCT were all significant discriminators. *indicates asymptomatic significance <0.05.

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4.3.6 Markers of haemostasis in SCOPD and AECOPD patients

4.3.6.1 Markers of haemostasis between SCOPD and AECOPD group

There was no significant difference in the markers of primary haemostasis such as platelet count and platelet aggregation between the two groups. There was no significant difference between PT and APTT. The fibrinogen levels were significantly higher in the AECOPD group and interestingly Factor XIII was significantly lower in the AECOPD group. In addition, the D-dimer was significantly higher in AECOPD compared to SCOPD patients **(Table 4.10)**.

Table 4.10: Markers of primary haemostasis between SCOPD and AECOPD group. Data presented as mean ± SD or median (IQR) and the significance between the groups was assessed using independent samples t-test or Mann-Whitney U test. * indicates significant result (p<0.05).

	SCOPD	**AECOPD**	***p value***
Markers of primary haemostasis			
Platelets ($\times 10^9$/L) (mean ± SD)	265 ± 63	301 ± 117	0.36
ADP (mean ± SD)	51 ± 29	55 ± 33	0.74
ASPI (mean ± SD)	72 ± 35	81 ± 45	0.25
Markers of secondary haemostasis			
PT (sec) (mean ± SD)	10.8 ± 1.0	11.1 ± 1.2	0.22
APTT (sec) (mean ± SD)	23.4 ± 1.9	24.6 ± 4.3	0.13
Fibrinogen (g/L) (mean ± SD)	3.4 ± 1.0	4.6 ± 1.2	0.001*
FXIII (IU/dL) (mean ± SD)	138 ± 21	132 ± 65	0.02*
Markers of tertiary haemostasis			
D-dimer [median (IQR)] (<500 ug/L)	445 (323-726)	870 (393-1980)	0.003*

4.3.6.2 Markers of haemostasis in AECOPD patients at four time points

There was no significant difference in the primary, secondary and tertiary markers of coagulation **(Table 4.11)**.

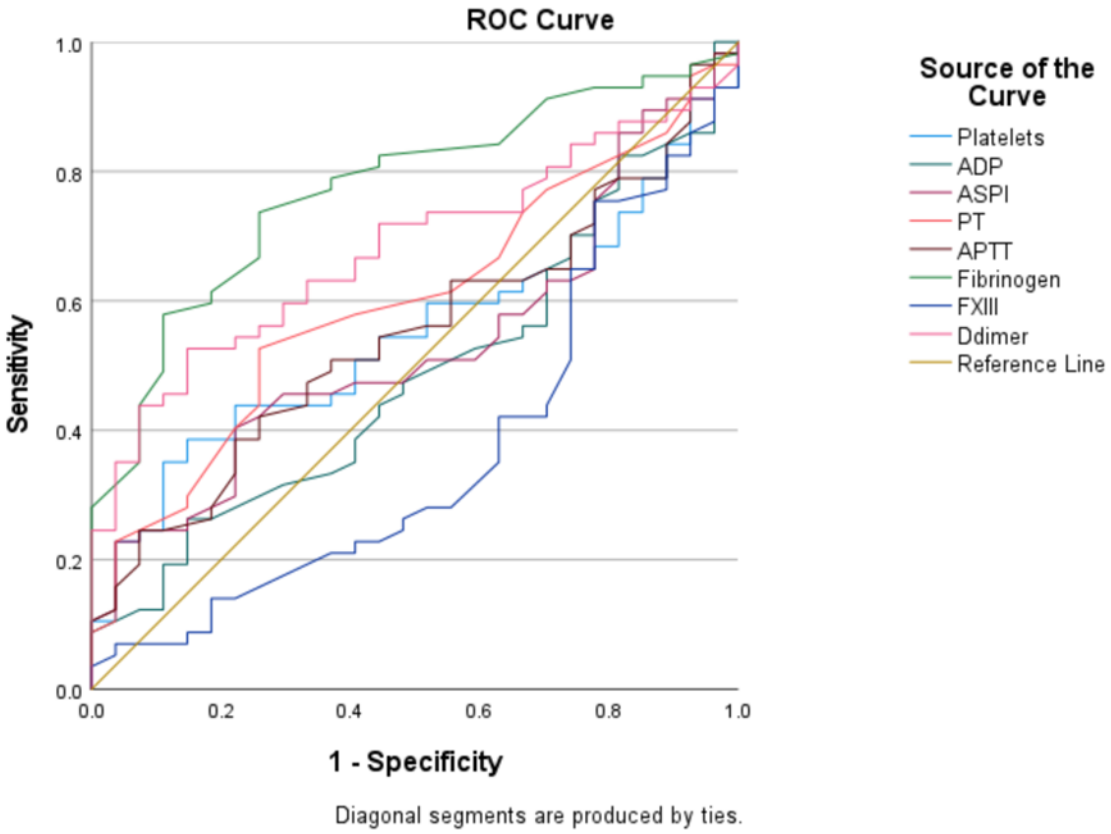
Table 4.11: Markers of primary haemostasis between four sampling points of AECOPD group (A, B, C D). Data presented as mean ± SD or median (IQR), significance between the groups was assessed using one-way ANOVA test or Kruskal-Wallis test. * indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	***p value***
Markers of primary haemostasis					
Platelets ($\times 10^9$/L) (mean ± SD)	301 ± 117	261 ± 94	257 ± 93	268 ± 96	0.05
ADP (mean ± SD)	55 ± 33	64 ± 31	51 ± 25	52 ± 29	0.15
ASPI (mean ± SD)	81 ± 45	95 ± 42	76 ± 44	68 ± 39	0.05
Markers of secondary haemostasis					
PT (sec) (mean ± SD)	11.1 ± 1.2	11.1 ± 1.9	11.3 ± 1.2	10.9 ± 0.9	0.71
APTT (sec) (mean ± SD)	24.6 ± 4.3	25.1 ± 2.5	25.1 ± 3.4	23.3 ± 3.9	0.12
Fibrinogen (g/L) (mean ± SD)	4.6 ± 1.2	4.6 ± 1.1	4.6 ± 1.1	4.2 ± 1.0	0.36
FXIII (IU/dL) (mean ± SD)	132 ± 65	126 ± 25	117 ± 30	116 ± 23	0.19
Markers of tertiary haemostasis					
D-dimer [median (IQR)]	870 (393-1980)	661 (361-1755)	688 (442-1851)	970 (414-1483)	0.675

(<500 ug/L)					

4.3.6.3 Receiver operating curve for haemostatic markers between SCOPD and AECOPD group

ROC curve shows that Fibrinogen and D-dimer were the two significant discriminators between AECOPD and SCOPD. Fibrinogen was more discriminator in diagnosing AECOPD (**Figure 4.5; Table 4.12**).



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
Platelets	.546	.062	.496	.424	.668
ADP	.486	.065	.837	.358	.614
ASPI	.533	.064	.629	.407	.659
PT	.600	.063	.140	.477	.723

APTT	.549	.064	.470	.424	.674
Fibrinogen	.773	.052	<0.0005*	.672	.874
FXIII	.367	.066	.050	.239	.496
D-dimer	.681	.057	.008*	.568	.793

Figure 4.5: Receiver operating characteristics for coagulation markers for the discrimination between AECOPD and stable patients. Fibrinogen demonstrated greatest discrimination between AECOPD and SCOPD. **Table 4.12**: Receiver operating characteristics for coagulation markers for the discrimination between AECOPD and SCOPD patients showing no significance. Both Fibrinogen and D-dimer was found to be significant. * indicates asymptomatic significance (p<0.05).

4.3.7 Heparin prophylaxis in AECOPD patients

As mentioned before, anti-Xa is a surrogate marker of heparin concentration and the normal prophylactic range of anti-Xa is 0.02-0.3 IU/ml. Anti-Xa increased significantly on subsequent sampling points indicating that patients who are admitted to the hospital were receiving daily prophylactic dose of low molecular weight heparin. At 24 hours the anti-Xa level peaked and then it stayed static because the patients receiving same dose, once daily dose of prophylactic Tinzaparin **(Table 4.13)**.

Table 4.13: Anti-Xa data between the four sampling points of AECOPD patients (A, B, C, D). Data presented as median (IQR) and the significance between the groups was assessed using Kruskal-Wallis test. * indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	***p value***
Anti-Xa (units/mL) [median (IQR)]	0.01 (0-0.04)	0.03 (0.01-0.05)	0.08 (0.02-0.16)	0.08 (0-0.10)	<0.001*

4.3.8 Haemoglobin and haematocrit in SCOPD and AECOPD group

No statistical difference was observed in the Hb and HCT between SCOPD and AECOPD group. As explained before haemoglobin and haematocrit are the surrogate markers for blood viscosity **(Table 4.14)**.

Table 4.14: Haemoglobin and haematocrit between SCOPD and AECOPD patients showing no significance. Data presented as mean ± SD and the significance between the groups was assessed using independent samples t-test.

	SCOPD	**AECOPD**	***p value***
Haemoglobin (g/L) (mean ± SD)	143 ± 15	137 ± 22	0.25
Haematocrit (L/L) (mean ± SD)	0.43 ± 0.04	0.43 ± 0.06	0.80

Both Hb and HCT significantly decreased on successive sampling points in the AECOPD group, potentially due to haemodilution **(Table 4.15)**.

Table 4.15: Haemoglobin and haematocrit between AECOPD patients at four time points (A, B, C, D). Data presented as mean ± SD, significance between the groups was assessed using one-way ANOVA test. * indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	***p value***
Hb (g/L) (mean ± SD)	137 ± 22	130 ± 23	123 ± 25	129 ± 19	0.005*
HCT (L/L) (mean ± SD)	0.43 ± 0.06	0.40 ± 0.07	0.39 ± .06	0.40 ± .06	0.003*

4.3.9 Correlations between d_f with other biomarkers

To analyse whether there is an association between d_f and other biomarkers in SCOPD and AECOPD patient, correlation analysis was carried out. d_f was significantly correlated with NEWS2 score in AECOPD patients and PT in SCOPD patients, otherwise there was no significant correlation observed with any of other biomarkers investigated **(Table 4.16)**.

Table 4.16: Correlations between d_f and NEWS2, blood gas, inflammatory markers, markers of primary, secondary and tertiary haemostasis, anti-Xa and haemoglobin and haematocrit. Analysis performed using Pearson's correlation. * indicates significant result (p<0.05).

	SCOPD **Pearson Correlation** **Sig. (2 tailed) *P<0.05***	**AECOPD** **Pearson Correlation** **Sig. (2 tailed) *P<0.05***
Clinical biomarker		
NEWS2	- 	0.245 0.04*
Blood gas		
pH	- 	0.098 0.398
pCO2 (kPa)	- 	0.044 0.708
HCO3 (mmol/L)	- 	0.211 0.067
Lactate (mmol/L)	- 	0.097 0.408
Inflammatory markers		
WBC ($\times 10^9$/L)	0.310 0.101	0.143 0.207
Neutrophils ($\times 10^9$/L)	0.338 0.073	0.080 0.478
CRP (mg/L)	0.114 0.556	0.179 0.114

PCT (ug/L)	0.101 0.604	0.084 0.467
Primary haemostasis		
Platelets ($\times 10^9$/L)	0.033 0.865	0.108 0.341
ADP	0.193 0.316	0.042 0.728
ASPI	0.087 0.652	0.075 0.530
Secondary haemostasis		
PT (sec)	0.439 0.017*	0.208 0.080
APTT (sec)	0.205 0.286	0.164 0.169
Fibrinogen (g/L)	0.265 0.165	0.149 0.212
FXIII (IU/dL)	0.051 0.792	0.163 0.171
Tertiary haemostasis		
D-dimer (<500 ug/L)	0.227 0.236	0.002 0.985
Other biomarkers		
Anti-Xa (units/mL)	-	0.256 0.097
Hb (g/L)	0.086 0.659	0.197 0.079
HCT (L/L)	0.246 0.198	0.206 0.066

Interestingly a weak association between d_f and NEWS2 score was observed in AECOPD **(Figure 4.6)**.

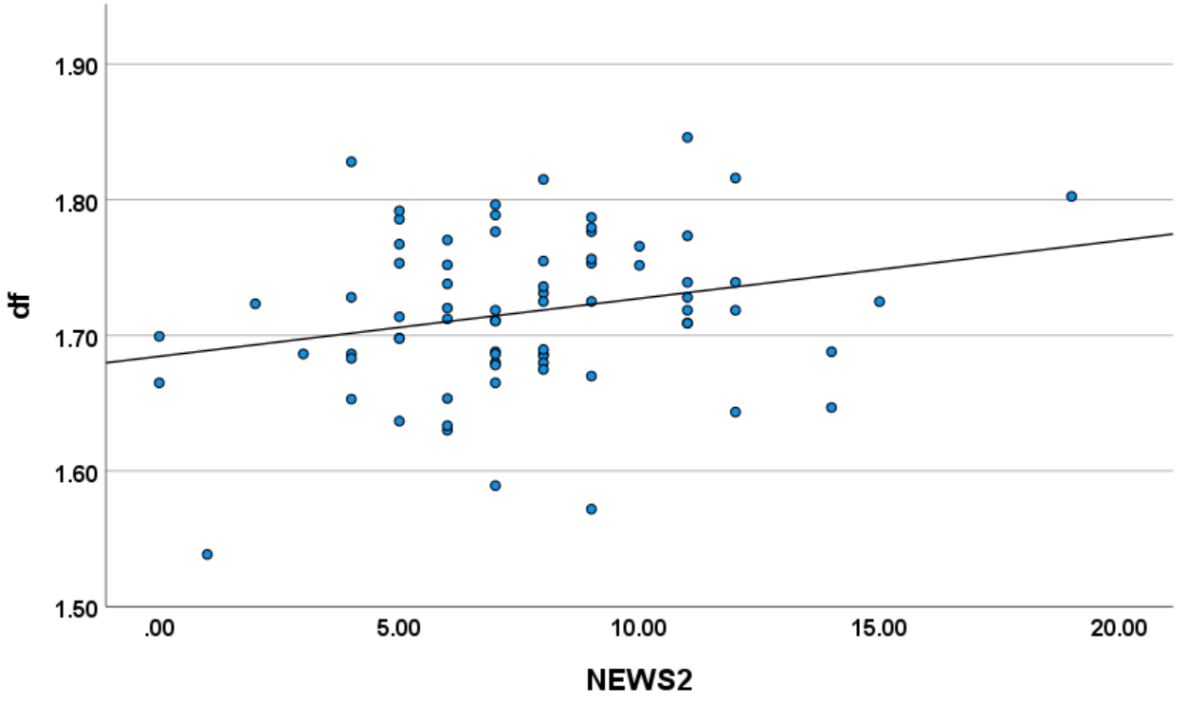


Figure 4.6: Pearson's correlation between d_f and NEWS2 in AECOPD patients (r=0.245; *p*=0.04), with the best fit regression line demonstrating a weak association.

4.3.10 COPD severity and rheological markers in SCOPD and AECOPD

Based on FEV1 (Forced Expiratory Volume in 1 second), the severity of COPD is classified into mild (FEV1 ≥ 80% predicted), moderate (50% ≤ FEV1 < 80% predicted), severe (30% ≤ FEV1 <50%) predicted and very severe (FEV1 <30% predicted) [GOLD, 2022]. FEV1 is part of the spirometry (lung function test assessment) done in the chest clinic and all 30 SCOPD patients had the measurement, however only 33 AECOPD patients had a recorded baseline spirometry performed. It is not a routine practice to perform spirometry during exacerbations and is only performed when the disease is stable. The severity was moderate in most of the patients in both groups **(Figure 4.7)**.

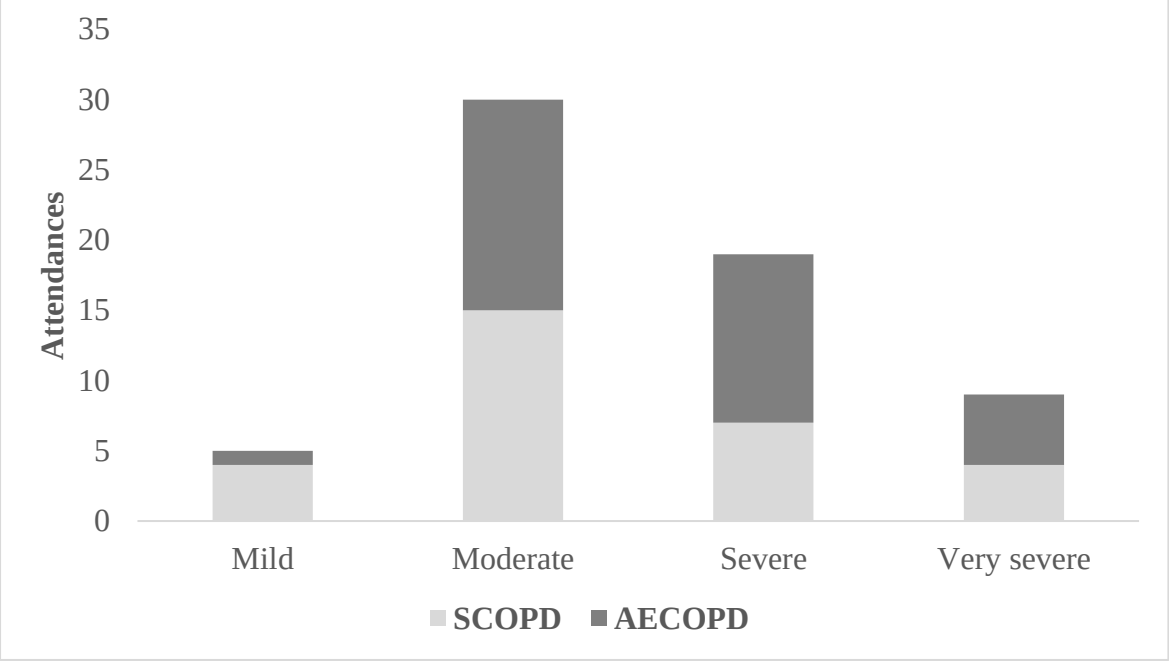


Figure 4.7: COPD severity based on FEV1 (Forced Expiratory Volume in one second) and Emergency Department attendances. Most of the SCOPD patients presenting to the respiratory clinic and AECOPD patients presenting to the Emergency Department has moderate-severe form of the disease.

Linear regression analysis shows that with decreasing severity as indicated by increase in FEV1, the d_f decreases in both SCOPD (p=0.24) and AECOPD (p=0.24), however this was not statistically significant (**Figure 4.8**).

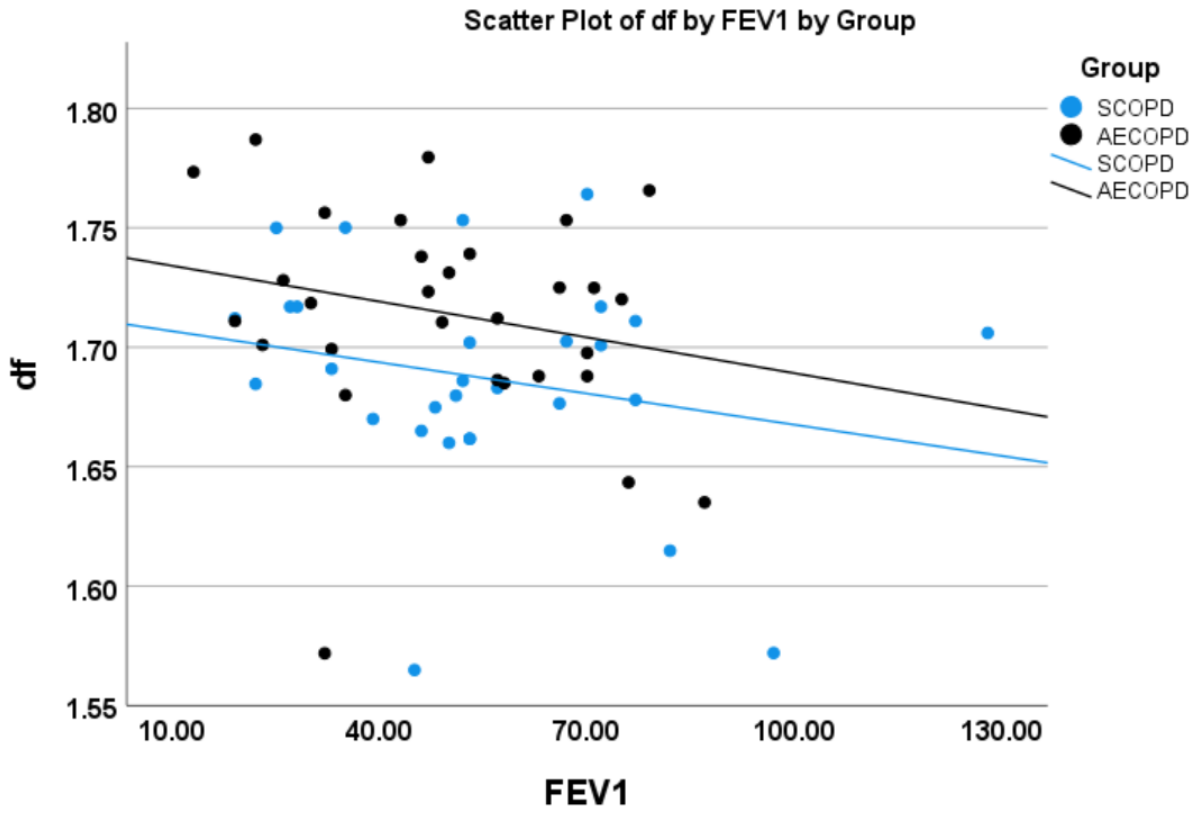


Figure 4.8: Linear regression analysis of d_f and FEV1 in SCOPD and AECOPD patients. d_f decreases with decrease in disease severity.

The linear regression analysis shows that with decreasing disease severity there was increase in the T_{GP}, however there was no association between T_{GP} and FEV1 in SCOPD (p= 0.54) and AECOPD (P=0.08) [**Figure 4.9**].

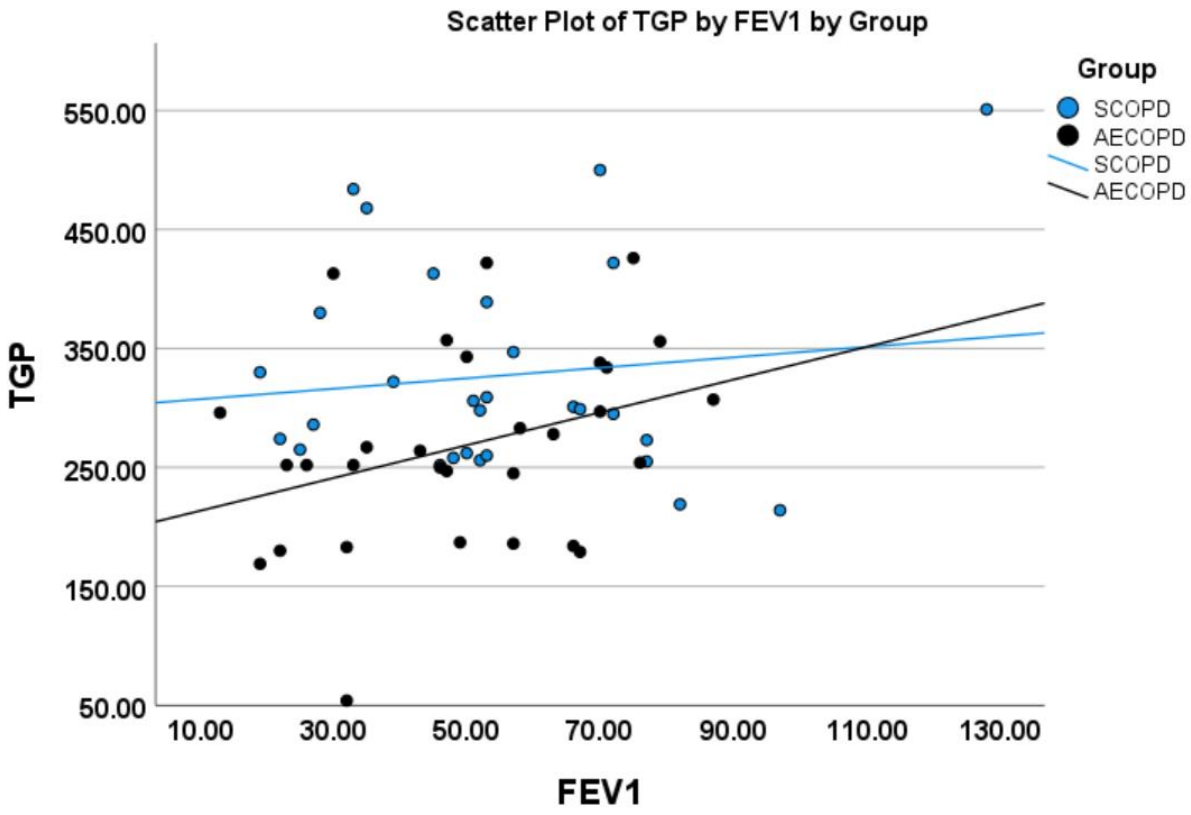


Figure 4.9: Linear regression analysis of time to gel point (T_{GP}) and Force Expiratory Volume in one second (FEV1) in SCOPD and AECOPD patients showing no association.

FEV1 were lower in patients who died during admission and was significantly lower in those who died in one year (**Table 4.17**).

Table 4.17: Force Expiratory Volume in one second (FEV1) of died and survived patients in AECOPD group. Data presented as mean ± SD and the significance between the groups was assessed using independent samples t-test. * indicates significant result ($p<0.05$).

FEV1	**Died**	**Survived**	***p* value**
This admission	31 ± 1.4	52 ± 20	0.15
One-year	39 ± 16	55 ± 20	0.03*

4.4 Discussion

This prospective observational study investigated thrombogenicity in COPD patients using the biomarker of clot microstructure namely fractal dimension (d_f). The literature review suggests there was higher incidence of VTE during COPD exacerbations. The hypothesis for this study was that COPD patients during exacerbations have denser and tighter clot microstructure (higher d_f), i.e. they are thrombogenic. This study therefore recruited patients with stable COPD (SCOPD) from the chest clinic as controls and patients with exacerbation of COPD symptoms (AECOPD) from the emergency department. The results demonstrated that both the groups were matched for demographics such as age, sex and body mass index. As mentioned previously, COPD remains the disease of the elderly and the AECOPD group was older compared to the SCOPD group. The study by Stone et al. 2012 reviewing the 2008 UK Audit demonstrated that elderly COPD patients had less knowledge about COPD. Because of the lack of understanding of the disease, the elderly COPD patients may fail to recognise the exacerbation symptoms which then leads to worsening of the clinical condition and finally ending up in the emergency department. This might explain the finding why AECOPD group were older. The Cochrane review showed that self-management of COPD was associated with improved quality of life and reduced hospital admissions with exacerbation (Schrijver et al. 2022). Furthermore, elderly patients might not be attending the follow-up clinics because of several factors such as ignorance, lack of transportation and frailty. Therefore, age is a risk factor in the management of COPD (Stone et al. 2012). There were more females who presented with exacerbation to the emergency department. DeMeo et al. (2018) in their study found that the severity of COPD is greater in women, and they are prone to severe exacerbations requiring hospital admissions. Similar findings were found by Kilic, Kokturk, Sari, and Cakır (2015) and Stolz et al. (2019). Both the study groups in this study were overweight with highest in AECOPD, however this was not statistically significant. These findings agree with the study of Vanfleteren et al. (2016) on a large group of patients. Smoking prevalence in both groups were high with more smokers in the AECOPD group which agrees with the data from UK (Shahab, Jarvis, Britton, & West, 2006). This demonstrates that despite being diagnosed with COPD, patients continue to smoke which then accelerates the inflammatory process leading to frequent exacerbations. To conclude, the demographic findings in this study shows that the population recruited reflects the COPD patients across the UK.

The inclusion and exclusion criteria for both SCOPD and AECOPD group were the same, therefore the comorbidities should have been similar in the group. Comorbidities such as diabetes, heart failure, and cancer was similar in both groups. Hypertension was the most common comorbidity in both groups which agrees with other studies, however the occurrence of hypertension was significantly higher in the AECOPD group. This might be due to several reasons, firstly, the AECOPD group had the highest number of smokers and there is a strong association between smoking and hypertension. Smoking initiates pathophysiology process damaging both cardiovascular system and respiratory system (Virdis, Giannarelli, Neves, Taddei, and Ghiadoni, 2010). Secondly, COPD patients use medications such as beta-2 agonist (salbutamol) and steroids which can cause hypertension. Thirdly, as the disease progresses, COPD patients become more sedentary which itself is a risk factor for hypertension (Sohn et al., 2014). According to Holst, Jensen, and Prescott (2010), hypertension itself is not a risk factor for VTE. Therefore, even though AECOPD group has significantly higher incidence of hypertension, this alone could not have increased the incidence of VTE. The study by Rothnie et al. (2018) showed that the risk of myocardial infarction and CVA increases in 91 days, but more within 28 days of COPD exacerbation. This might explain why CVA was more common in the AECOPD group. COPD patients are often referred to chest clinic after being admitted with an exacerbation for spirometry and follow up when the disease is stable. Another observation that was found was a non-significant difference in VTE between both groups, which might be due to smaller sample size in the SCOPD group. The study showed that the AECOPD group was mostly from the most deprived areas. It is a known fact that people from more deprived areas have less education, low socio-economic status and reduced access to health facilities. AECOPD patients were tachycardic, hypertensive, were in respiratory distress requiring oxygen supplement which is expected. This study showed 11% of patients were admitted to Intensive Care Unit and the mortality rate was 12%. The high one-year mortality (30%) in the AECOPD group was mirroring what other studies have found (Hoogendoorn, Hoogenveen, Rutten-van Mölken, Vestbo, & Feenstra, 2011). This was significantly high when compared to the stable group (13%), which shows that exacerbations itself increase the disease process.

When compared to SCOPD group, there was statistically significant increase of d_f in the AECOPD group at admission. This demonstrates that the AECOPD group had a denser and tighter clot microstructure when compared to SCOPD. This might be due to several reasons. Firstly, COPD is a chronic inflammatory condition and exacerbation is nothing but flare up of

this inflammation. The evidence from previous studies demonstrate that acute inflammation causes tighter and denser clot microstructure (Davies et al., 2016; Stanford et al., 2015). The background inflammation in COPD was not pronounced to be detected by the biomarkers used in this study. This was evidenced by the fact that all inflammatory biomarkers in SCOPD were within normal limits. However, during exacerbation/ flare up, all the inflammatory markers were significantly elevated in the AECOPD group. The blood drawing procedure was kept standard; therefore, this might not have impacted on the heterogeneity of the data. Similarly, all AECOPD patients received steroids. Secondly, as previously mentioned enhanced inflammation activates the coagulation pathways. Therefore, in SCOPD there was no activation of the coagulation pathway which was reflected by the fact that the biomarkers of primary (no activation of platelets), secondary (normal activation of coagulation pathways and fibrin production) and tertiary haemostasis (no elevated fibrinolysis) were all within normal limits. The FXIII which forms FXIIIa to crosslink the fibrin networks were also within normal limits indicating that there was no utilisation for fibrin crosslinks in SCOPD group. Because of all the above, when blood in SCOPD patient's clots, it forms a normal clot microstructure (normal d_f). COPD patients who attend the respiratory follow up clinic are generally compliant with the treatment, and this might be the reason why inflammatory process is under control in SCOPD group. During the clinic consultation, if there are any indications of worsening of the inflammatory process by assessing the symptoms and using spirometry, then the treatments are modified. As expected, the number of stable COPD patients who died in one year was also less compared to the AECOPD group which shows that adhering to appropriate treatment and rehabilitation programme improves the mortality.

Due to airway remodelling in COPD, the natural defence mechanisms against infection and irritants are diminished. Therefore, exposure to infection or irritants can enhance the background inflammatory process leading to flare up of the symptoms which is called exacerbation. All inflammatory markers (WBC count, neutrophil count, CRP and PCT) were significantly high in AECOPD patients compared to stable group as expected. With effective treatment, at 3-7 days, the inflammation settles down, reflected by near normalisation of these inflammatory markers. The treatment for AECOPD includes nebulisers, steroids and antibiotics (if indicated). It is known that WBC and Neutrophil count can significantly increase with steroid treatment (Shoenfeld, Gurewich, Gallant, and Pinkhas, 1981). Therefore, these markers have very low sensitivity in guiding treatment and initiation of antibiotics. The haemoglobin (Hb%) and haematocrit (HCT) were within normal limits in both SCOPD and

AECOPD groups which demonstrates that the blood viscosity was not different between both groups. Pillai et al. 2021 argued that one of the reasons for patients presenting with DKA having denser and tighter clot microstructure was because of high viscosity due to severe dehydration. Chronic hypoxia can lead to an increase in red blood cells (polycythaemia), which is a physiological response of the body to increase the oxygen carrying capacity which is seen in people living in high altitudes. COPD patients, therefore, are at risk of developing polycythaemia and there by high blood viscosity (Zhang et al. 2021). Recent SPIROMICS study found that secondary polycythaemia is associated with reduced severe COPD exacerbations (Fawzy et al. 2021). However, anaemia is found to be more common (7.5-33%) in COPD (Sarkar, Rajta, and Khatana, 2015). In the AECOPD group, both Hb% and HCT was significantly reduced on consecutive sampling points which indicates that the blood viscosity was significantly reduced. This might be due to haemodilution from fluid therapy or might be because patients start to eat and drink when the symptoms get better. Furthermore, breathing is one of the physiological processes where the body loses water and in COPD patients once they receive treatment the respiratory effort gets better and therefore the body loses less water.

In the AECOPD group, the markers of haemostasis were higher when compared to the SCOPD group. There is activation of platelets indicated by an increase in platelet count and aggregation. This is followed by activation of coagulation pathways. Even though, the tests that reflect extrinsic (PT) and intrinsic pathway (APTT) remain normal, they were higher than the SCOPD group indicating that there was some degree of activation of the coagulation pathways. This was reflected by a significant increase in the fibrinogen concentration in the AECOPD group. Fibrinogen is also an acute phase protein that increases during inflammation (Jain, Gautam, & Naseem, 2011). The polymerisation and branching of the fibrin form a three-dimensional mesh which then is crosslinked by Factor XIIIa which then holds the red blood cell to form the blood clot. The AECOPD group had significantly low FXIII which might be due to the increased utilisation for the formation of the cross links. This might be the reason that AECOPD patients had significantly high d_f when compared to SCOPD patients. However, as explained before, there is no significant activation and aggregation of platelets and no significant activation of coagulation pathways to form enough fibrin to make the clot microstructure denser and tighter. This is reflected by the fact that d_f even though significantly higher in AECOPD when compared to SCOPD was within the reference range in healthy individuals (Evans et al. 2010a). There is enhanced fibrinolysis as indicated by high D-dimer in the AECOPD group when compared to the stable group. This shows that the enhanced inflammation does activate the

coagulation pathways minimally to produce enough fibrin network to activate fibrinolysis. With appropriate treatment over the days with oxygen, nebulisers, steroids and antibiotics (if indicated), the inflammatory and haemostatic markers gradually got better and were normalised at 3-7 days. The AECOPD group received prophylactic LMWH within 14 hours and anti-Xa values shows that at 24 hours and 3-7 days they were at prophylactic dose range (0.02-0.3 IU/ml). This prophylactic dosing of heparin might also contribute to low normal d_f. Evans et al. (2010b) showed that progressive increase in heparin reduces the d_f.

There were only 9% of the AECOPD patients investigated for VTE of which 7% had CTPA and 2% had doppler and only one patient was positive for VTE (PE). The low incidence of VTE in this study might be due to several factors. Firstly, all patients who were on anticoagulation therapy were excluded. The incidence of VTE was only 1% in the AECOPD group and other studies have shown that only 1.5% of the patients with VTE who were on anticoagulation developed recurrent VTE (Douketis, Kearon, Bates, Duku, and Ginsberg, 1998). COPD patients have high incidence of atrial fibrillation and AF is a known risk factor for VTE (Hornestam et al., 2021). Secondly, COPD patients can present with symptoms similar to PE, therefore there could be an underdiagnosis of PE. Thirdly, all AECOPD patients received a prophylactic dose of tinzaparin, therefore non-life-threatening PE getting worse was minimal and might have been missed. And lastly, all patients were given appropriate treatment as per the Health Board guidelines which directly halts the flare up/ inflammatory process and thereby putting a stop to the activation of the coagulation pathways.

As mentioned in Chapter 1, the severity of COPD increases with age and is accelerated by ongoing exposure to risk factors such as smoking and exposure to noxious particles. This is due to chronic inflammation leading to significant damage to the lungs and subsequently causing chronic airflow obstruction and impaired gas exchange. As the severity increases the frequency of exacerbation also increases. In addition, the exercise tolerance also substantially reduces, making COPD patients more vulnerable to VTE. This analysis was undertaken to assess the relationship between COPD severity and d_f. In both the SCOPD and AECOPD groups, d_f increases with severity, but this does not attain a statistical significance. This might be due to a several factors, the first being the number of patients in both groups. This study was not powered to specifically answer this question. In the AECOPD group only 33/85 had recorded spirometry. This is because it is not a routine practice to perform spirometry in AECOPD patients while admitted to hospital, as it will not give a true reflection of the disease

process and is always performed on a later date when the disease is stable. Secondly there were a significant number of patients who are current smokers that accelerates the disease process. It is not a normal practice to perform clinical observations or blood gas analysis in SCOPD patients. Therefore, it was difficult to compare these parameters that was obtained in AECOPD group and was one of the limitations of this study. The blood sampling was kept standard and all AECOPD patients received the same standard treatment including nebuliser, steroids and anticoagulation, therefore there was no impact on the heterogeneity of the data which was the strength of this study.

4.5 Conclusion

This study demonstrated that the AECOPD group were more thrombogenic than the SCOPD group at admission. Exacerbation causes enhancement of the inflammatory process which then activated the coagulation pathways leading to high fibrin formation. This led to significantly reduced time in the initiation of clot formation (T_{GP}) and significantly denser and tighter clot microstructure (d_f) in the AECOPD group at presentation. With appropriate treatment and prophylactic anticoagulation, there was no further increase in d_f at four time points. In addition, the VTE incidence was very minimal. With severity, d_f increases, however this was not statistically significant. Therefore, the study concludes that d_f is a useful biomarker that measures thrombogenicity and the effect of treatment in AECOPD patients.

Chapter 5: The effect of infection and inflammation on fractal dimension in AECOPD patients

5.1 Introduction

In this subgroup analysis the effect of infection on d_f in AECOPD patients was evaluated. Infection means entrance and development of a microorganism in the human body causing a disease (Barreto, Teixeira, & Carmo, 2006). Systemic inflammatory response syndrome (SIRS) is characterised when there are changes in two or more of the clinical signs such as heart rate (>90 beats/ minute), respiratory rate (>20 breaths per minute), body temperature (<36^{o}C or >38^{o}C) or white blood cells (<4000mm^{-3} or >12000mm^{-3}). If there is SIRS in the presence of an infection, it is called sepsis and sepsis in the presence of end organ damage is termed severe sepsis and severe sepsis with refractory hypotension is septic shock (Hotchkiss et al., 2016). All these different pathological processes are collectively known as sepsis spectrum (Bone et al., 1992). The interaction between the infectious agent and host immune response leads to widespread inflammation, therefore sepsis is an inflammatory disorder (Nedeva, Menassa, & Puthalakath, 2019). However, local infections such as upper or lower respiratory tract infection on occasions may not cause a systemic response. As explained in detail in Chapter 1 of this thesis, the activation of various inflammatory pathways, in turn activates the coagulation system. Therefore, patients with sepsis develop several coagulation disorders that could range from abnormalities in biomarkers to disseminated intravascular coagulation (DIC). DIC is characterised by bleeding caused by widespread thrombosis and thereby consumption of coagulation factors (consumptive coagulopathy). According to Inghammar et al. (2014), COPD patients have a 2.5-fold increased risk of presence of bacteria in blood (bacteraemia).

The most common cause for COPD exacerbation is infection. Papi et al. (2006) in their study detected bacterial or viruses in sputum samples in 78% of the COPD patients during exacerbation. Therefore, whenever COPD patients present to the hospital with exacerbation, in addition to nebulisers and steroids, they are treated with antibiotics. However, the decision to give antibiotics is made clinically along with inflammatory markers and radiological findings. Inflammatory markers such as WBC and Neutrophils can increase in steroid therapy, therefore it is not a reliable biomarker. Similarly, CRP increases in certain inflammatory disorders.

According to Cochrane meta-analysis and systematic review procalcitonin (PCT) can be used as a reliable biomarker to differentiate bacterial infection (Schuetz et al., 2017). The changes in clot microstructure have been studied extensively across the sepsis spectrum. When compared to healthy individuals, the d_f was high in sepsis and severe sepsis patients, however in septic shock, the d_f was lower. This demonstrates that in sepsis and severe sepsis, the clot microstructure was denser and tighter indicating hypercoagulable state and in septic shock weaker and denser and clot microstructure indicating hypocoagulable state (Davies et al., 2016). The study by Davies et al. (2016) had several COPD patients included in the study, however the changes in d_f in those patients were not analysed separately.

This study recruited 85 AECOPD patients from the Emergency Department. Blood samples were drawn at presentation (0 hours), 4-6 hours, 24 hours and 3-7 days. The blood tests that were carried out included rheological markers, inflammatory markers, markers of primary, secondary and tertiary haemostasis and markers for viscosity.

5.1.1 Aims

This sub analysis aims to,

1. To analyse whether there was any significant difference in d_f between infective and non-infective group
2. To analyse whether there was any difference in d_f between stable compared to infective and non-infective group
3. To compare the inflammatory and haemostatic biomarkers between infective and non-infective group. In addition to compare stable group with infective and non-infective group
4. The effect of infection in mortality in AECOPD group

5.2 Results

In this sub analysis, AECOPD patients were divided into those with (infective exacerbation) or without infection (non-infective exacerbation). This was based on procalcitonin (PCT) level at admission and those who did not have PCT result were excluded (n=8).

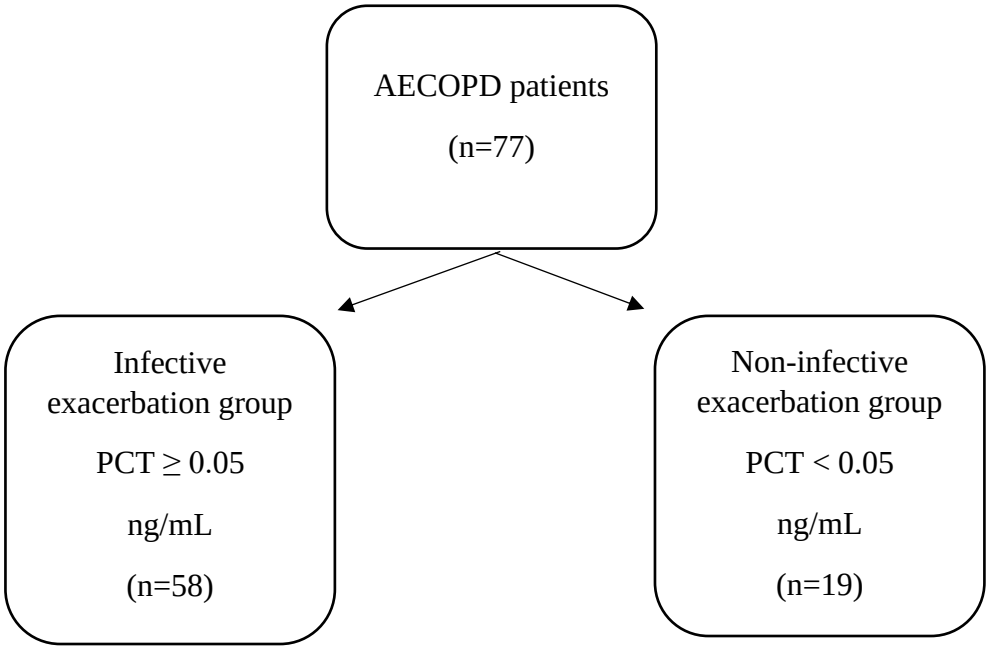


Figure 5.1: Flow diagram illustrating AECOPD group with infection and without infection.

5.2.1 Patient demographics and WIMD 2019 score in infective and non-infective exacerbation of COPD

5.2.1.1 Patient demographics between infective and non-infective group

The patient demographics were well matched for age, sex, BMI and smoking status for both the infective and non-infective group. There was no significant difference between co-morbidities, however the infective group were more hypertensive and had a greater number of cancer occurrences, while the non-infective group had higher incidence of diabetes. There was no difference in the length of hospital stay, ICU admission rate nor ICU length of stay. Significant number of infective groups died during the admission and within 1-year of exacerbation. There was no difference in patients investigated for VTE and who developed VTE during this admission.

Table 5.1: Patient demographics in infective and non-infective exacerbation of COPD. The values were presented as mean ± SD or n (percentage) or median (IQR). Significance between the groups were assessed using independent samples t-test or Chi-square test of Kruskal-Wallis test. *indicates significant result (p<0.05).

	Infective (n=58)	**Non-infective (n=19)**	***p value***
Age (mean ± SD)	69 ± 10	68 ± 11	0.67
Sex (M:F)	26:32	10:9	0.56
BMI (mean ± SD)	29.9 ± 8.2	26.7 ± 7.5	0.50
Current smoker (%)	29 (50%)	8 (42%)	0.64
HTN (%)	21 (36%)	3 (16%)	0.07
Diabetes (%)	10 (17%)	5 (26%)	0.37
IHD (%)	1 (2%)	1 (5%)	0.33

CVA (%)	6 (10%)	2 (11%)	0.77
Heart failure (%)	3 (5%)	1 (5%)	0.36
Previous VTE (%)	1 (2%)	0 (0%)	0.29
Cancer (%)	6 (10%)	1 (5%)	0.26
Hospital length of stay (days) [median (IQR)]	7 (2-15)	5 (2-13)	0.35
ICU length of stay (days) [median (IQR)]	11 (3-24)	26 (4-26)	0.53
ICU admission (%)	8 (14%)	2 (11%)	0.72
Died in this admission (%)	8 (14%)	1 (5%)	0.23
Died within 1 year (%)	23 (40%)	1 (5%)	<0.001*
Investigated for VTE (%)	6 (10%)	2 (11%)	0.98
VTE during admissions (%)	1 (2%)	0 (0%)	0.60

5.2.1.2 Welsh Index of Multiple Deprivation (WIMD) 2019 score for infective and non-infective group

WIMD 2019 score shows that infective group were from the most deprived areas.

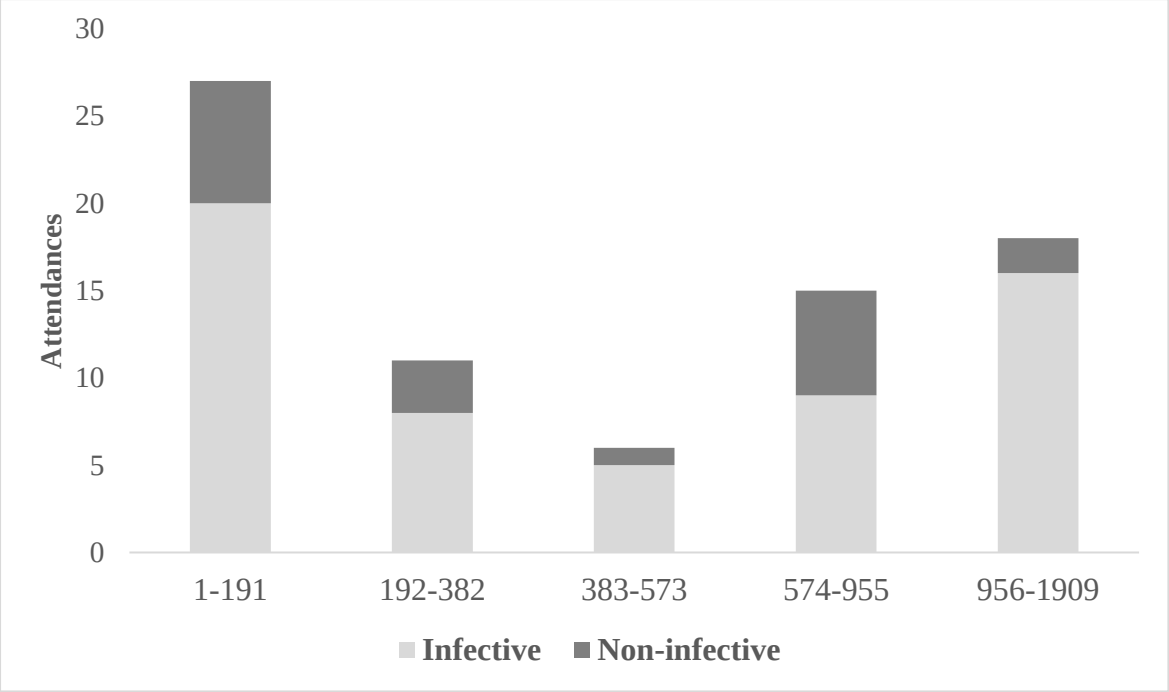


Figure 5.2: Welsh Index of Multiple Deprivation (WIMD) 2019 score for infective and non-infective AECOPD groups. The areas are ranked from 1 which is the most deprived to 1909 which is the least deprived. The scores are arranged in four equal parts or quintiles. The infective group were from the most deprived areas.

5.2.2 Clinical observations and blood gas analysis in infective and non-infective AECOPD group

Infection in COPD is a major cause of morbidity. There was no significant difference in clinical difference between the infective and non-infective except the NEWS2 score which was significantly high in patients with infective exacerbation.

Table 5.2: Clinical observations between infective and non-infective group. The values were presented as mean ± SD and significance was assessed using independent samples t-test. *indicates significant results (p<0.05).

	Infective (mean ± SD)	**Non-infective** (mean ± SD)	***p value***
Temperature (^{o}C)	36.8 ± 0.8	36.6 ± 0.6	0.25
Pulse (beats/ minute)	104 ± 23	106 ± 19	0.84
Systolic Blood Pressure (mm of Hg)	134 ± 31	142 ± 35	0.39
Diastolic Blood Pressure (mm of Hg)	73 ± 19	76 ± 20	0.58
Respiratory rate (breaths/minute)	27 ± 8	26 ± 9	0.86
Oxygen saturation (%)	91 ± 8	93 ± 4	0.35
Oxygen requirement (%)	30 ± 17	31 ± 23	0.77
NEWS2 score	8 ± 3	6 ± 3	0.02*

The infective group were more acidotic with significantly higher pCO2. Even though the HCO3 was higher in the infective group it was not statistically significant as is the pH.

Table 5.3: Blood gas analysis between the infective and non-infective group. Data presented as mean ± standard deviation, significance between the groups was assessed using independent samples t-test.* indicates significant result ($p<0.05$).

	Infective (mean ± SD)	**Non-infective** (mean ± SD)	***p* value**
pH	7.30 ± 0.11	7.34 ± 0.13	0.17
pCO2 (kPa)	9 ± 4	7 ± 2	0.005*
HCO3 (mmol/L)	28 ± 7	25 ± 6	0.15
Lactate (mmol/L)	1.7 ± 1.0	2.4 ± 2.0	0.16

5.2.3 Rheological markers between infective and non-infective group

5.2.3.1 Rheological markers between the infective and non-infective group in AECOPD patients

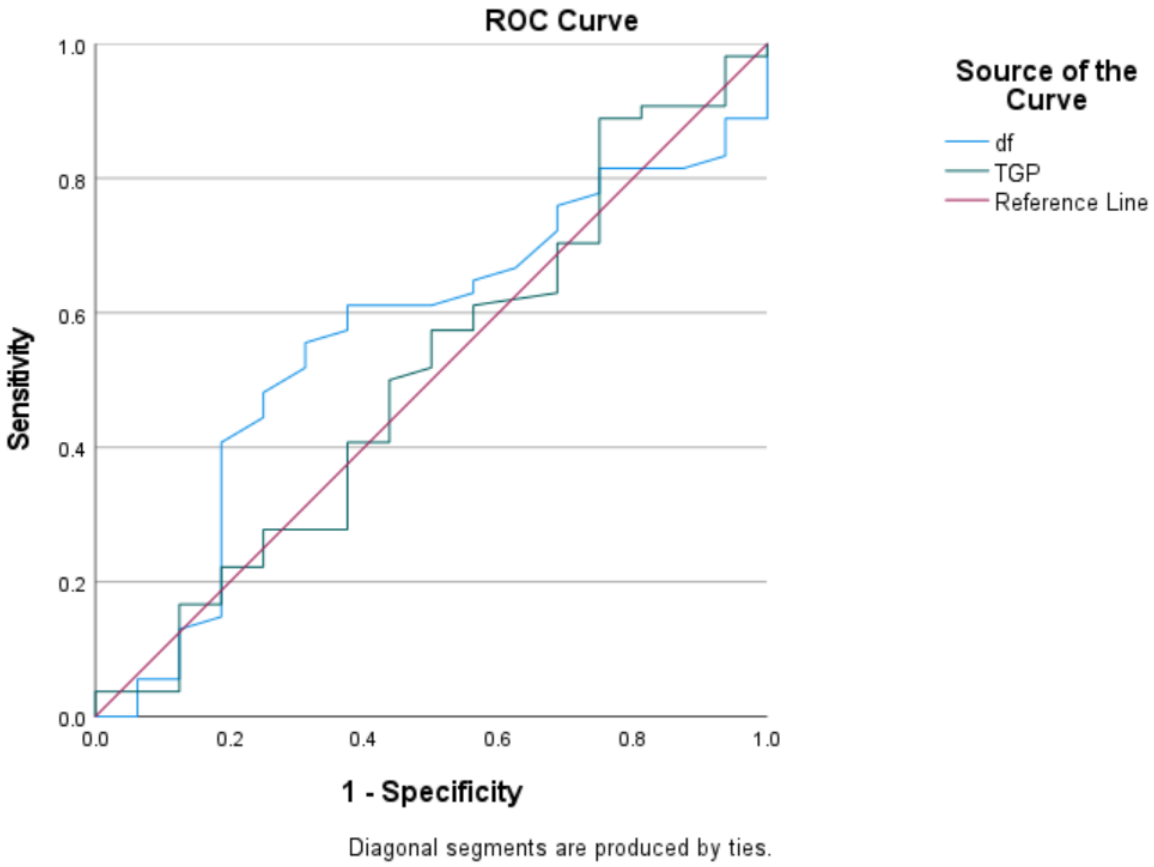
There was no statistical difference in the rheological markers between infective and non-infective group at each time points A, B, C, D. Similarly, there was no statistical difference between four time points in either infective or non-infective group.

Table 5.4: Rheological markers between the infective and non-infective group at four time points (A, B, C, D). Data presented as mean ± standard deviation, significance between the infective and non-infective groups was assessed using independent samples t-test. The significance between for time points is assessed using one-way ANOVA test.

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (*p* value) between 4 time points**
d_f (infective) [mean ± SD]	1.72 ± 0.06	1.71 ± 0.06	1.70 ± 0.06	1.71 ± 0.07	0.69
d_f (non-infective) [mean ± SD]	1.71 ± 0.06	1.72 ± 0.05	1.70 ± 0.08	1.72 ± 0.07	0.81
d_f (infective vs non-infective, *p* value)	0.53	0.56	0.87	0.65	
T_{GP} (infective) [mean ± SD]	275 ± 69	261 ± 91	294 ± 161	313 ± 195	0.37
T_{GP} (non-infective) [mean ± SD]	270 ± 83	241 ± 59	293 ± 46	290 ± 92	0.50
T_{GP} (infective vs non-infective, *p* value)	0.81	0.56	0.99	0.75	

5.2.3.2 Receiver operating curve for rheological markers between the infective and non-infective group in AECOPD patients

Rheological markers were not significant discriminators between the infective and non-infective group.



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
d_f	.562	.081	.454	.404	.720
T_{GP}	.509	.086	.916	.340	.677

Figure 5.3: Receiver operating characteristics for rheological markers for the discrimination between infective and non-infective group AECOPD patient group. None of the rheological markers were significant discriminators. **Table 5.5**: Receiver operating characteristics for rheological markers for the discrimination between infective and non-infective group

AECOPD patient group that showed both of them were not significant discriminators between infective and non-infective.

5.2.4 Inflammatory markers between infective and non-infective group

5.2.4.1 Inflammatory markers between the infective and non-infective group in AECOPD patients

There was no statistical difference in the inflammatory markers between the infective and non-infective group at each time points (A, B, C, D). However, WBC, Neutrophils, CRP and PCT showed significant at four time points in the infective group reduction. In non-infective group, only WBC and PCT showed significant reduction at four time points.

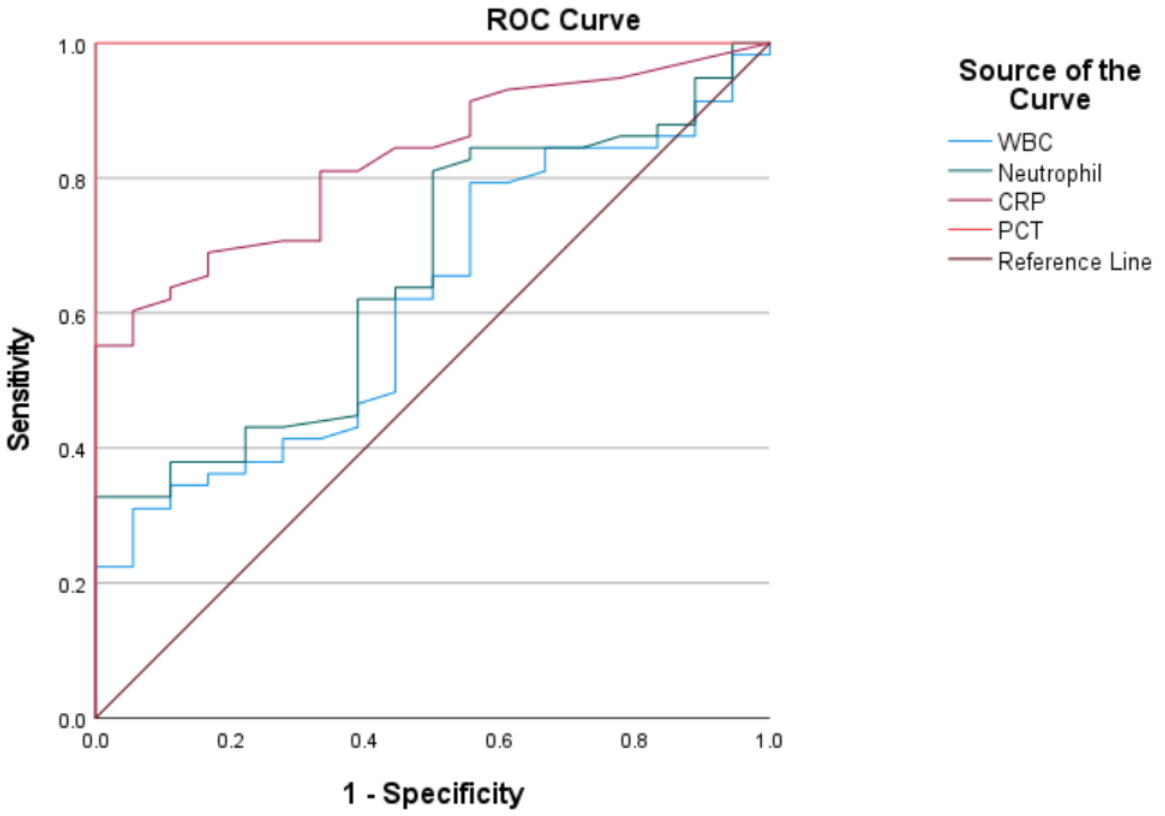
Table 5.6: Inflammatory markers between the infective and non-infective group at four time points (A, B, C, D). Data presented as mean ± SD or median (IQR). The significance between the infective and non-infective group was assessed using independent samples t-test or Mann-Whitney U test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA or Kruskal Wallis test. *indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
WBC ($\times 10^9$/L) (infective) [mean ± SD]	16.2 ± 9.2	13.2 ± 6.6	11.6 ± 4.6	11.1 ± 4.2	0.003*
WBC ($\times 10^9$/L) (non-infective) [mean ± SD]	12.6 ± 4.3	11.9 ± 4.8	12.0 ± 4.3	9.3 ± 2.7	0.04*
WBC (infective vs non-infective, p value)	0.11	0.54	0.78	0.27	
Neutrophils (infective) [mean ± SD]	13.7 ± 8.4	12.0 ± 6.0	9.9 ± 4.1	9.2 ± 4.0	0.008*
Neutrophils (non-infective) [mean ± SD]	9.5 ± 3.7	10.0 ± 5.0	9.8 ± 3.9	7.5 ± 2.2	0.41

Neutrophils (infective vs non-infective, *p* value)	0.04	0.44	0.98	0.25	
CRP (mg/L) (infective) [median (IQR)]	44 (18-102)	56 (33-149)	67 (30-148)	18 (7-51)	<0.001*
CRP (mg/L) (non-infective) [median (IQR)]	11 (4-26)	21 (8-35)	23 (12-35)	11 (7-18)	0.32
CRP (infective vs non-infective, *p* value)	<0.001	<0.001	0.006	0.71	
PCT (ug/L) (infective) [median (IQR)]	0.16 (0.10-1.40)	0.48 (0.15-3.47)	0.66 (0.17-2.20)	6.85 (0.11-78.35	0.04*
PCT (non-infective) [median (IQR)]	0.04 (0.02-0.04)	0.05 (0.04-0.06)	0.06 (0.05-0.49)	0.15 (0.06-0.66)	0.003*
PCT (ug/L) (infective vs non-infective, *p* value)	<0.001	<0.001	0.004	0.01	

5.2.4.2 Receiver operating curve for inflammatory markers between the infective and non-infective group in AECOPD patients

Neutrophils, CRP and PCT were significant discriminators between infective and non-infective group, however PCT was the highest.



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
WBC	.616	.071	.138	.477	.756
Neutrophil	.660	.069	.041*	.525	.795
CRP	.827	.047	.000*	.734	.920
PCT	1.000	.000	.000*	1.000	1.000

Figure 5.4: Receiver operating characteristics for inflammatory markers for the discrimination between infective and non-infective AECOPD patient group. CRP demonstrated the greatest discrimination between infective and non-infective groups. **Table 5.7**: Receiver operating characteristics for inflammatory markers for the discrimination between infective and non-infective AECOPD patient group. *indicates asymptomatic significance (p<0.05).

5.2.4.3 Relationship between Procalcitonin (PCT) and d_f in AECOPD patients

Based on PCT values, the AECOPD patients were divided into five groups. The highest number was those with ≥ 0.05 to <0.5ng/mL (51.95%).

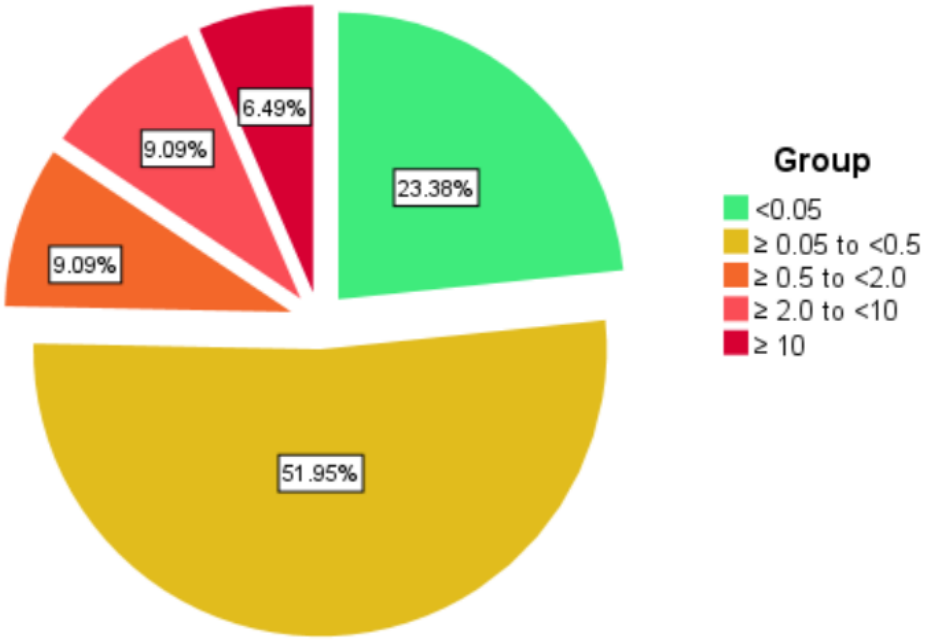


Figure 5.5: The number of AECOPD patients and PCT values (ng/mL). As per NICE (2014), the interpretation of PCT levels were no SIRS (<0.05ng/mL), SIRS or local infection (≥ 0.05 to <0.5ng/mL), sepsis (≥ 0.5 to <2.0ng/mL), severe sepsis (≥ 2.0 to <10ng/mL) and septic shock (≥ 10ng/mL). The highest number was those with ≥ 0.05 to <0.5ng/mL (51.95%).

d_f was highest when the PCT level was ≥0.5 to <2.0 and d_f significantly correlated with PCT at this level (p=0.04) **[Figure5.6]**.

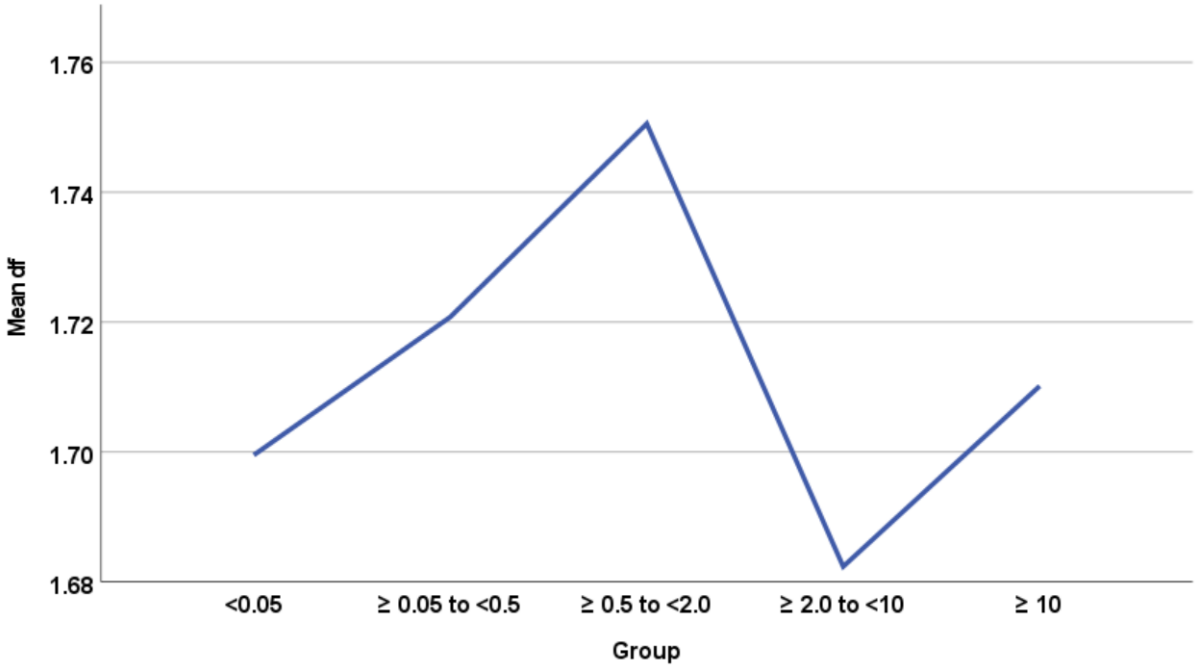


Figure 5.6: Relationship between d_f and PCT (ng/mL) in AECOPD patients. Correlation done using Pearson Correlation. At PCT level of ≥0.5 to <2.0, d_f was high demonstrating significant correlation (p=0.04).

5.2.5 Markers of haemostasis between infective and non-infective group

5.2.5.1 Markers of haemostasis between the infective and non-infective group in AECOPD patients

None of the biomarkers of haemostasis showed significant difference between both the infective and non-infective group at each time points (A, B, C, D). Similarly there was no significant changes of each haemostatic biomarkers at four time points for either infective or non-infective groups.

Table 5.8: Markers of primary haemostasis between the infective and non-infective group at four time points (A, B, C, D). Data presented as mean ± SD or median (IQR). The significance between the infective and non-infective group was assessed using independent samples t-test or Mann-Whitney U test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA or Kruskal Wallis test. *indicates significant result (p<0.05).

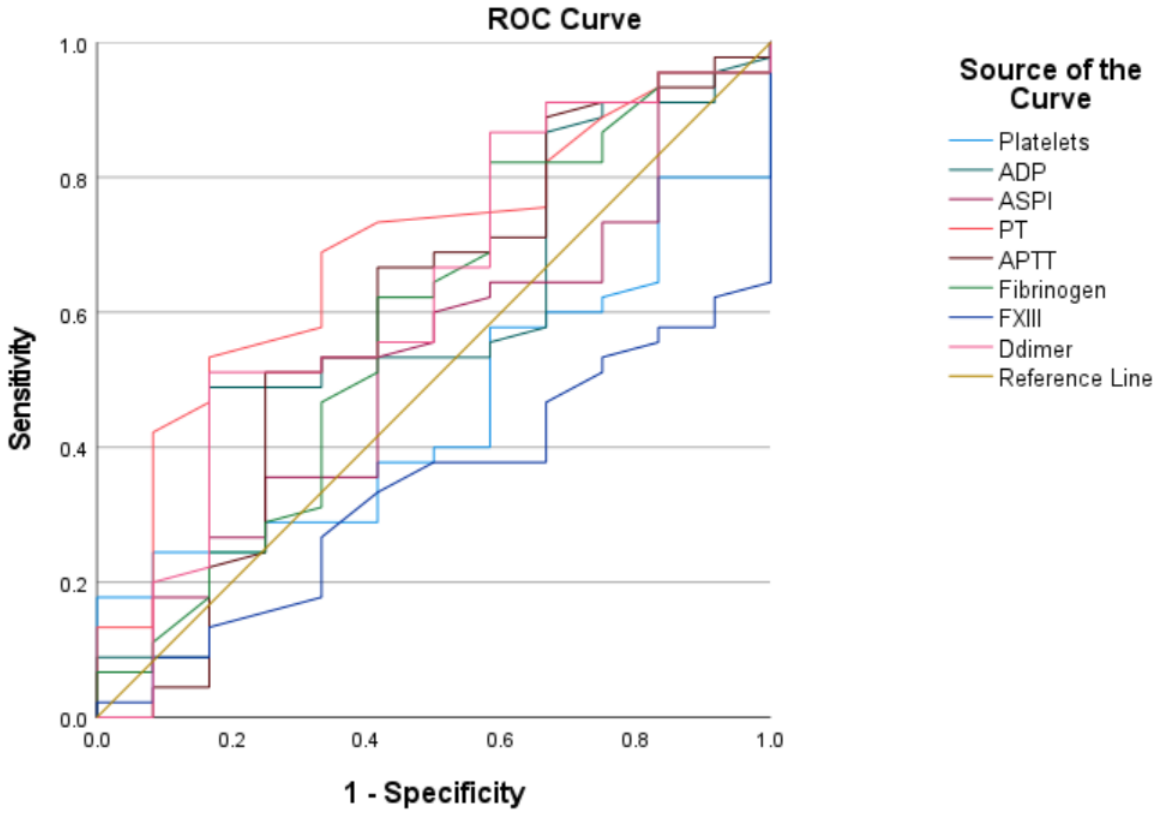
	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
Primary haemostasis					
Platelets (infective) [mean ± SD]	307 ± 132	263 ± 104	255 ± 100	282 ± 98	0.11
Platelets (non-infective) [mean ± SD]	291 ± 69	252 ± 45	245 ± 51	226 ± 81	0.07
Platelets (infective vs non-infective, p value)	0.50	0.61	0.80	0.15	
ADP (infective) [mean ± SD]	60 ± 32	61 ± 36	49 ± 27	50 ± 31	0.26
ADP (non-infective) [mean ± SD]	48 ± 30	50 ± 34	41 ± 27	54 ± 27	0.81
ADP (infective vs non-infective, p value)	0.21	0.37	0.38	0.74	

ASPI (infective) [mean ± SD]	85 ± 46	91 ± 47	73 ± 47	66 ± 40	0.12
ASPI (non-infective) [mean ± SD]	76 ± 42	69 ± 50	66 ± 44	66 ± 41	0.93
ASPI (infective vs non-infective, *p* value)	0.46	0.17	0.67	0.99	
Secondary haemostasis					
PT (infective) [mean ± SD]	11.3 ± 1.3	11.1 ± 2.1	11.2 ± 1.2	10.8 ± 0.8	0.45
PT (non-infective) [mean ± SD]	10.6 ± 0.7	11.0 ± 0.8	11.4 ± 1.2	11.3 ± 1.4	0.19
PT (infective vs non-infective, *p* value)	0.04	0.85	0.64	0.17	
APTT (infective) [mean ± SD]	24.4 ± 2.7	25.4 ± 2.6	25.4 ± 3.6	23.0 ± 3.6	0.008
APTT (non-infective) [mean ± SD]	25.2 ± 8.1	23.9 ± 1.5	24.0 ± 2.3	24.4 ± 4.8	0.93
APTT (infective vs non-infective, *p* value)	0.72	0.08	0.28	0.38	
Fibrinogen (infective) [mean ± SD]	4.6 ± 1.2	4.6 ± 1.0	4.7 ± 1.2	4.2 ± 1.0	0.28
Fibrinogen (non-infective) [mean ± SD]	4.3 ± 1.2	4.3 ± 1.2	4.3 ± 0.9	4.4 ± 1.1	0.99
Fibrinogen (infective vs non-infective, *p* value)	0.38	0.30	0.38	0.62	
FXIII (infective) [mean ± SD]	132 ± 74	122 ± 27	114 ± 30	113 ± 23	0.26
FXIII (non-infective) [mean ± SD]	134 ± 22	136 ± 16	128 ± 31	125 ± 23	0.69
FXIII (infective vs non-infective, *p* value)	0.92	0.13	0.19	0.21	
Tertiary haemostasis					

D-dimer (infective) [median (IQR)]	1009 (461-2182)	735 (431-1754)	713 (535-1888)	1008 (517-1483)	0.77
D-dimer (non-infective) [median (IQR)]	727 (286-1456)	312 (212-9290)	531 (204-2571)	496 (219-1552)	0.90
D-dimer (infective vs non-infective, *p* value)	0.16	0.32	0.26	0.35	

5.2.5.2 Receiver operating curve for haemostatic markers between infective and non-infective group

Fibrinogen and D-dimer was the significant discriminator between infective and non-infective group with prothrombin time the highest **(Figure 5.7; Table 5.9)**.



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
Platelets	.453	.082	.618	.292	.614

ADP	.582	.094	.384	.398	.767
ASPI	.526	.092	.784	.345	.707
PT	.693	.081	.042*	.534	.851
APTT	.593	.102	.328	.393	.792
Fibrinogen	.582	.099	.384	.389	.776
FXIII	.340	.076	.090	.191	.489
D-dimer	.632	.095	.162	.447	.818

Figure 5.7: Receiver operating characteristics for coagulation markers for the discrimination between infective and non-infective AECOPD patient group. Prothrombin time (PT) was the greatest discriminator among the haemostatic markers. **Table 5.9**: Receiver operating characteristics for coagulation markers for the discrimination between infective and non-infective AECOPD patient group showing Prothrombin time (PT), the significant discriminator.

5.2.6 Haemoglobin and haematocrit between infective and non-infective group

There were no significant changes in haemoglobin and HCT between the infective and non-infective group at each time points (A, B, C, D). However, in the infective group, there was significant reduction in haemoglobin and HCT at four time points **(Table 5.10)**.

Table 5.10: Haemoglobin and haematocrit between infective and non-infective AECOPD patients at four time points (A, B, C, D). Data presented as mean ± standard deviation. The significance between the infective and non-infective group was assessed using independent samples t-test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA. *indicates significant result (P<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (*p* value) between 4 time points**
Haemoglobin g/L (infective) [mean ± SD]	137 ± 23	128 ± 25	121 ± 27	126 ± 20	0.01*
Haemoglobin g/L (non-infective) [mean ± SD]	145 ± 13	137 ± 14	135 ± 12	139 ± 9	0.15
Haemoglobin g/L (infective vs non-infective, *p* value)	0.08	0.24	0.12	0.09	
Haematocrit L/L (infective) [mean ± SD]	0.43 ± 0.07	0.40 ± 0.07	0.39 ± 0.07	0.39 ± 0.06	0.01*
Haematocrit L/L (non-infective) [mean ± SD]	0.45 ± 0.03	0.42 ± 0.04	0.41 ± 0.04	0.43 ± 0.03	0.05
Haematocrit L/L (infective vs non-infective, *p* value)	0.19	0.26	0.42	0.04	

5.2.7 Comparing biomarkers of stable COPD group with infective and non-infective group

Infective group had significantly higher d_f when compared to stable group, however T_{GP} was significantly lower in both infective and non-infective when compared to stable group. All inflammatory markers were significantly elevated in infective group when compared to stable group **(Table 5.11)**.

Table 5.11: Comparing biomarkers of stable COPD group with infective and non-infective group. Data presented as mean ± SD or median (IQR) and the significance between the groups was assessed using independent samples t-test or Mann-Whitney U test. *indicates significant result (p<0.05).

	Stable & infective group ***p value***	**Stable & non-infective group** ***p value***
d_f (mean ± SD)	1.69 ± 0.05 vs 1.72 ± 0.06 (0.02)*	1.69 ± 0.05 vs 1.71 ± 0.05 (0.19)
T_{GP} (mean ± SD)	327 ± 88 vs 275 ± 69 (0.004)*	327 ± 88 vs 270 ± 83 (0.04)*
WBC (mean ± SD)	9.5 ± 3.5 vs 16.2 ± 9.2 (<0.001)*	9.5 ± 3.5 vs 12.6 ± 4.3 (0.009)*
Neutrophils (mean ± SD)	6.9 ± 3.8 vs 16.2 ± 9.2 (<0.001)*	6.9 ± 3.8 vs 9.5 ± 3.7 (0.02)*
CRP [median (IQR)]	0 (0-6) vs 44 (0-407) (<0.001)*	0 (0-6) vs 11 (0-40) (<0.001)*
PCT [median (IQR)]	0.04 (0.02-0.05) vs 0.16 (0.05-97.32), (<0.001)*	0.04 (0.02-0.05) vs 0.04 (0.02- 0.05) (0.39)
Platelets (mean ± SD)	265 ± 63 vs 307 ± 132 (0.04)*	265 ± 63 vs 291 ± 69 (0.18)
PT (mean ± SD)	10.8 ± 0.61 vs 11.3 ± 1.3 (0.01)*	10.8 ± 0.61 vs 10.6 ± 0.69 (0.34)
APTT (mean ± SD)	23.4 ± 1.9 vs 24.4 ± 2.7 (0.04)*	23.4 ± 1.9 vs 25.2 ± 8.1 (0.39)

Fibrinogen (mean ± SD)	3.4 ± 0.8 vs 4.6 ± 1.2 (<0.001)*	3.4 ± 0.8 vs 4.3 ± 1.2 (0.01)*
FXIII (mean ± SD)	138 ± 21 vs 132 ± 74 (0.64)	138 ± 21 vs 134 ± 22 (0.48)
D-dimer [median (IQR)]	445 (323-726) vs 1009 (148-18077), (<0.001)*	445 (323-726) vs 727 (190-19999) (0.31)
Hb (mean ± SD)	142.6 ± 15.1 vs 137.1 ± 27.4 (0.25)	142.6 ± 15.1 vs 145.2 ± 13.3 (0.54)
HCT (mean ± SD)	0.43 ± 0.04 vs 0.43 ± 0.07 (0.94)	0.43 ± 0.04 vs 0.45 ± 0.03 (0.19)

5.3 Discussion

Differentiating between infection and inflammation in many diseases can be very difficult and this is the case with acute exacerbation of COPD. The most common reason for COPD exacerbation is known to be infection which was reflected in this study. It was thought that bacterial infection was the main cause for exacerbations, however evidence demonstrates that nearly half of the infections are caused by viruses (Linden et al., 2018). Inflammatory markers are often elevated in infection, however most of them are not reliable. Procalcitonin is known to be a reliable marker of infection. NICE (2014) published guidelines on the interpretation of PCT levels as no SIRS (<0.05ng/mL), SIRS or local infection (≥ 0.05 to <0.5ng/mL), sepsis (≥ 0.5 to <2.0ng/mL), severe sepsis (≥ 2.0 to <10ng/mL) and septic shock (≥ 10ng/mL). In this study, about 67% of AECOPD patients were clinically diagnosed as having infection and were commenced on antibiotics. However, when PCT levels are taken into consideration, 75% of the AECOPD group had infection. Furthermore, in the infective exacerbation group 22% were clinically diagnosed as not having infection and in the non-infective group 32% were treated as having infection. This demonstrates huge discrepancy in the management of AECOPD patients and the limitation of the application of clinical diagnosis. One of the reasons might be that clinicians often rely on change in colour of sputum as a guide to commence antibiotics. The change in colour (yellow or green) of the sputum is often interpreted as having an infection. However, this is not a reliable sign of infection as studies have shown that only 12% of COPD patients with coloured phlegm had proven infection (Altiner et al., 2009).

The age was well matched between both the groups; however, there were more females in the infective group. It is a known fact that females have more severe exacerbations than males (Kilic, Kokturk, Sari, & Cakır, 2015). The BMI was higher in the infective group. Cigarette smoking in COPD is a substantial risk factor in developing invasive bacterial and viral infections (Arcavi & Benowitz, 2004). There were more smokers in the infective group in this study. There was no significant difference in the co-morbidities, however there were more hypertensives in the infective group and more diabetics in the non-infective group. Most of the infective and non-infective group were from the most deprived areas. The ICU admission rate, hospital and ICU length of stay was not significant. This demonstrates that regardless of the cause, COPD exacerbation has significant morbidity. A significant number of patients in the infective group died in this admission and within one year of exacerbation. As discussed before

COPD exacerbation is associated with increased mortality and morbidity. However, when there is associated infection, then the in-hospital mortality and one year mortality is significantly high. The clinical observations were not significantly different between the two groups, however the infective group had significantly high NEWS2 score. Mellhammar et al. (2019) found that NEWS2 scores were superior in detecting sepsis. Another important finding to note is that there was no significant difference in the body temperature between both groups and the body temperature was within normal limits in the infective group. It is to be noted that not all infections are associated with high body temperature (El-Radhi, 2019). The blood gas analysis demonstrates that the infective group had severe type 2 respiratory failure.

The changes in clot microstructure across sepsis spectrum was studied previously. In their study, Davies et al. (2016) found that when compared to the healthy group, d_f in sepsis and severe sepsis groups was significantly higher compared to the healthy group indicating that these groups had tighter and denser clot microstructure. This was because the profound inflammatory response in sepsis activated the coagulation pathway which increases the fibrin production, thereby formation of a denser clot. When compared to stable group, patients with infective exacerbation had significantly higher d_f. In addition, there was significant increase in inflammatory, haemostatic and fibrinolytic markers. This clearly demonstrates that infection activates coagulation pathways and forms tighter and denser clot microstructure. However, when compared to stable group, the non-infective group did not have significant increase in d_f. This was because there was no activation of the coagulation pathway as indicated by non-significant haemostatic and fibrinolytic markers. The finding in this therefore mirrors what was found by Davies et al. (2016) that infection activates coagulation pathways causing increasing in d_f. In addition, the d_f was highest and significantly correlated when PCT levels were consistent with sepsis.

There was no significant difference in d_f between the infective and non-infective group at each time points. This was a surprising finding because when compared to stable group, patients in infective group had significantly elevated d_f than non-infective group. The reason might be that this sub-analysis was not powered specifically to investigate this which is a limitation and there were less of number participants in non-infective group. In addition, d_f did not change significantly between four time points in either infective or non-infective group. This might be due to several factors. Firstly, all patients received standard treatment for exacerbation that included nebulisation and steroids. Secondly, all patients admitted to hospital received the

standard prophylactic anticoagulation. What this shows is that standard treatment is effective in the management of COPD exacerbation regardless of the cause, i.e infective or non-infective. The inflammatory markers reduced significantly at four time points in infective group demonstrating that the standard treatment had pronounced effect in infective group rather than non-infective group. In addition, the haemodilution was more in infective group as indicated by haemoglobin and haematocrit which mirrors the study of Davies et al. (2016). Patients investigated for VTE was similar in both groups and occurrence of VTE was very minimal in both groups. Because there was not power calculation performed for this sub analysis, any conclusions should be therefore carefully considered.

5.4 Conclusion

The acute exacerbation of COPD caused by infection has significantly higher denser and tighter clot microstructure when compared to the stable COPD group. There was no significant changes in clot microstructure between the infective and non-infective group at each time points. Similarly, clot microstructure did not significantly change between four time points. The infective group had severe form of the illness and may explain why more patients died during admission. The infective group had significant high mortality in one year when compared to non-infective group. This sub analysis, therefore, concludes that patients presenting with infective exacerbation of COPD were more thrombogenic than the stable group, but not when compared with non-infective group. There is a requirement of adequately powered larger study to investigate this further.

Chapter 6: The effect of pH on fractal dimension in AECOPD patients

6.1 Introduction

Acid-base haemostasis is vital for the normal function of the body. The optimal pH at which body functions is from 7.35 to 7.45. Changes in pH can lead to impairment of cellular metabolism, denaturation of enzymes and impairment of membrane transport systems at cellular level (Hamm, Nakhoul & Hering-Smith, 2015). Therefore, changes in pH can alter homeostasis which is detrimental for the normal body physiology. The pH below 7.35 is acidosis and more than 7.45 is termed alkalosis. The factors that regulate pH of the blood are carbon-dioxide, relative electrolyte concentration and total weak acid concentration (Kellum, 2000). Carbon-dioxide (CO2) is a waste product that is formed in the cell during glucose metabolism. It dissolves in blood with water (H2O) to form carbonic acid (H2CO3) which further dissociates into hydrogen ion (H^+) and $HCO3^-$ (bicarbonate) and excreted via the lungs ($CO2 + H2O \leftrightarrow H2CO3 \leftrightarrow H^+ + HCO3^-$). The hydrogen ion ($H^+$) that is formed is the most important determinant of blood pH (Patel, Miao & Yetiskul, 2022). Therefore, high carbon-dioxide concentration in the blood causes high H^+ concentration which then leads to acidosis (pH <7.35) and this is called respiratory acidosis. When there is acute retention of CO2, the increased H^+ ion that is formed is buffered by blood proteins such as haemoglobin ($H2CO3 + {}^-Hb \rightarrow HHb + HCO3^-$) and other intracellular non-bicarbonate buffers. However, this process is limited and therefore the body starts to excrete H^+ ion and increases the reabsorption of $HCO3^-$ through the kidneys (Bruno & Valenti, 2012) which increases the $HCO3^-$ concentration in the blood. One of the complications from COPD is that due to the destruction of alveoli, the normal gas exchange is affected leading to poor oxygenation and carbon dioxide retention (type 2 respiratory failure). The type 2 respiratory failure in COPD patients is therefore chronic and because of this these patients have high blood $HCO3^-$ and normal pH. This is called compensated respiratory acidosis. Hypoxia itself increases the respiratory drive and the resulting - high concentration of oxygen reduces this drive causing hypoventilation, hypercapnia, severe respiratory acidosis and ultimately death. The randomised controlled trial by Austin, Wills, Blizzard, Walters, and Wood-Baker (2010) found that titrated oxygen significantly improved mortality in COPD patients. This is because when a COPD patient is

given high flow oxygen, it reduces hypoxic respiratory drive which then causes cardon-dioxide retention. This is the reason why in COPD patients, the oxygen is titrated to target oxygen saturation of 88-92%. The treatment for respiratory acidosis (pH < 7.35) in AECOPD is to give controlled oxygen, nebulisers, steroids and if this fails, then to offer non-invasive ventilation that helps to clear CO2 retention (Plant & Elliot, 2003).

If there is an increased acid load in the blood, the body compensates for this by reducing the CO2 in blood by hyperventilation. However, if the acid load is very high, then the compensatory mechanisms fail, and this leads to metabolic acidosis. As discussed above, H^+ that is formed in the body is excreted by the kidneys and in kidney failure there is increased acid load leading to metabolic acidosis. Chronic metabolic acidosis is known to cause progression of chronic kidney diseases (Chen & Abramowitz, 2014). Studies from animal models showed that acidosis causes reduced cardiac contractibility (Stengl et al. 2013). There is evidence to suggest that acidosis may lead to several coagulation problems. According to Martini & Holcomb (2007), severe metabolic acidosis caused impairment of blood coagulation with increased fibrinogen consumption in animal models. In vitro studies have demonstrated that artificially lowering blood pH in healthy individuals leads to impairment of blood coagulation (Engström et al., 2006; Gissel et al., 2016). However, in their in-vitro study, White, Bird, Sosnowski, and Jones (2016) found that there was no association between acidosis and coagulation. The lethal triad in trauma such as acidosis, hypothermia and coagulability leads to high mortality and acidosis, inhibits propagation phase of thrombin generation and accelerates fibrinogen degradation (Martini, 2009).

The effect of pH on clot microstructure had not been extensively studied. According to Pillai et al. (2021), patients presenting with diabetic ketoacidosis (DKA) had significantly high d_f. This indicates that these DKA patients had denser and tighter clot microstructure that changes significantly within 24 hours of treatment with fluids and insulin. This may be due to the combined effect of severe dehydration and metabolic acidosis. There is no study to date that investigated the effect of respiratory acidosis on clot microstructure. Therefore, this sub analysis aimed to study the effect of respiratory acidosis on clot microstructure.

6.1.1 Aims

This sub analysis aims to,

1. To analyse whether there was any significant difference in d_f between acidotic and non-acidotic group
2. To compare the inflammatory and haemostatic biomarkers between acidotic and non-acidotic group.
3. To compare stable group (SCOPD) with acidotic and non-acidotic group
4. The effect of acidosis in mortality in AECOPD group

6.2 Results

For this sub-analysis, AECOPD patients who had blood gases performed during their initial presentation were included (n=80) and others excluded **(Figure 6.1)**. Patients were acidotic if pH was <7.35. There were 47 patients in the acidotic group and 33 patients in the non-acidotic group. Patients with acidosis stayed in hospital more when compared to the non-acidotic group [9 (3-16) vs 6 (2-9), *p*=0.11].

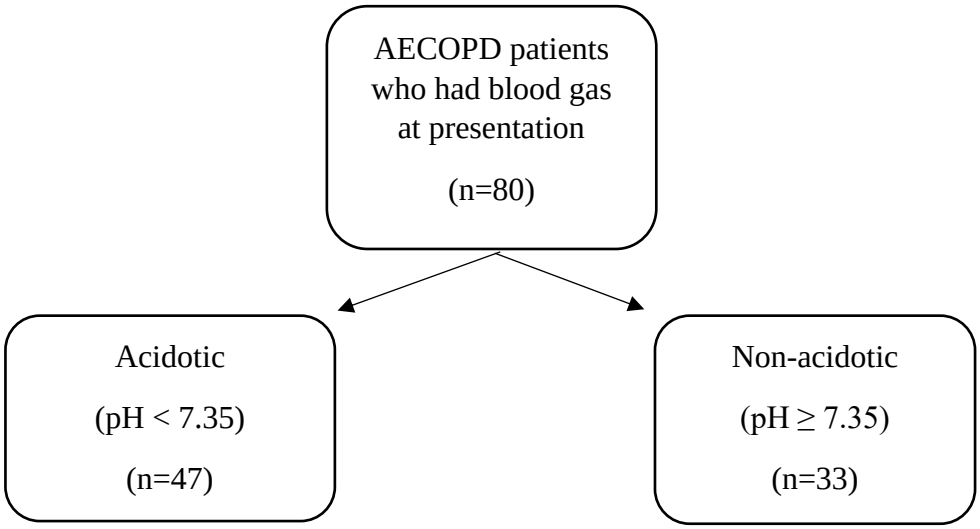


Figure 6.1: Flow diagram showing the number of patients in acidotic and non-acidotic group.

6.2.1 Demographics and WIMD 19 score of patients in acidotic and non-acidotic group

The patients in the acidotic and non-acidotic group were well matched for age, sex, BMI, smoking. All the co-morbidities except cancer was higher in the acidotic group but was significantly different. A significant number of acidotic patients were admitted to ICU and died during admission. The 1-year mortality was also significantly higher in the acidotic group. More patients in the non-acidotic group were investigated for VTE and one patient had VTE **(Table 6.1)**.

Table 6.1: Patient demographics in acidotic and non-acidotic group. Values were presented as mean ± SD or n (percentage), significance between the groups were assessed using independent samples t-test or Chi-square test. *indicates significant result (p<0.05).

	Acidotic (n=47)	**Non-acidotic (n=33)**	***p value***
Age (years) [mean ± SD]	69 ± 10	70 ± 10	0.46
Sex (M/F)	24:23	17:16	0.97
BMI (mean ± SD)	29.1 ± 8.6	27.4 ± 5.4	0.71
Current smoker (%)	20/47 (43%)	12/33 (36%)	0.64
HTN (%)	18/47 (38%)	9/33 (27%)	0.13
Diabetes (%)	12/47 (26%)	4/33 (12%)	0.37
IHD (%)	1/47 (2%)	0/33 (0%)	0.80
CVA (%)	7/47 (15%)	1/33 (3%)	0.06
Heart failure (%)	3/47 (6%)	1/33 (3%)	0.51
Previous VTE (%)	1/47 (2%)	0/33 (0%)	0.41

Cancer (%)	2/47 (4%)	4/33 (12%)	0.19
Hospital length of stay (days)	9 (3-16)	6 (2-9)	0.35
ICU length of stay (days)	15 (3-37)	6 (6-6)	0.53
ICU admission (%)	9/47 (19%)	1/33 (3%)	0.02*
Died in this admission (%)	8/47 (17%)	1/33 (3%)	0.03*
Died within 1 year (%)	18/47 (38%)	6/33 (18%)	0.02*
Investigated for VTE (%)	2/47 (4%)	5/33 (15%)	0.06
VTE during admissions (%)	0/47 (0%)	1/33 (3%)	0.16
FEV1 (mean ± SD)	51 ± 22	48 ± 17	0.70

WIMD 2019 score shows that a greater number of individuals in the acidotic group are from the areas defined as the most deprived **(Figure 6.2)**.

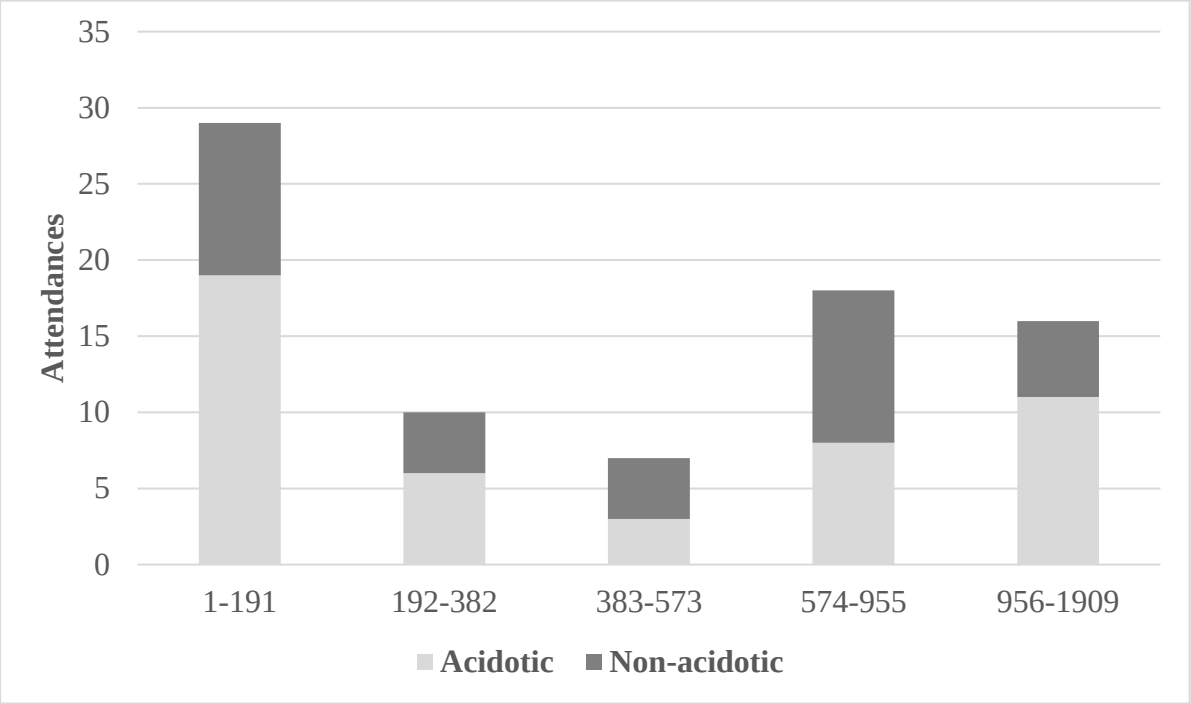


Figure 6.2: Welsh Index of Multiple Deprivation (WIMD) 2019 score for acidotic and non-acidotic patients which is scored from 1 (most deprived) to 1909 (least deprived) areas which is then divided into equal parts or quintiles. Most patients in acidotic group is from the most deprived areas.

6.2.2 Clinical observations and blood gas analysis in acidotic and non-acidotic AECOPD group

Clinical observations of the AECOPD group showed that no significant difference existed between both groups apart from the NEWS2 score. All patients were tachycardic, hypertensive, had high respiratory failure with low oxygen saturation requiring oxygen therapy **(Table 6.2)**.

Table 6.2: Clinical observations between acidotic and non-acidotic group. Values presented as mean ± SD, significance was assessed using independent t-test. *indicates significant result (p<0.05).

	Acidotic (mean ± SD)	**Non-acidotic** (mean ± SD)	***p* value**
Temperature (^{o}C)	36.6 ± 0.9	36.9 ± 0.8	0.24
Pulse (beats/ minute)	103 ± 21	105 ± 24	0.75
Systolic Blood Pressure (mm of Hg)	140 ± 33	130 ± 34	0.23
Diastolic Blood Pressure (mm of Hg)	75 ± 19	75 ± 18	0.92
Respiratory rate (breaths/minute)	28 ± 9	25 ± 7	0.27
Oxygen saturation (%)	92 ± 8	91 ± 5	0.37
Oxygen requirement (%)	31 ± 18	27 ± 15	0.38
NEWS score	8 ± 3	7 ± 3	0.04*

As expected, the acidotic group had significantly low pH and significantly high pCO2 when compared to the non-acidotic group **(Table 6.3)**.

Table 6.3: Blood gas measurements between the acidotic and non-acidotic group. Data presented as mean ± standard deviation, significance between the groups was assessed using independent samples t-test. * indicates significant result ($p<0.05$).

	Acidotic (mean ± SD)	**Non-acidotic** (mean ± SD)	***p* value**
pH	7.24 ± 0.09	7.41 ± 0.04	< 0.001*
pCO2 (kPa)	9.8 ± 3.7	5.9 ± 1.5	< 0.001*
HCO3 (mmol/L)	27 ± 8	27 ± 4	0.66
Lactate (mmol/L)	2.1 ± 1.6	1.5 ± 0.8	0.05

6.2.3 Rheological markers between the acidotic and non-acidotic group

6.2.3.1 Rheological markers between the acidotic and non-acidotic group in AECOPD patients

Except for sample point D, there was no significant difference between d_f at sample points A, B and C between the acidotic and non-acidotic group. There was no significant difference in rheological markers between four points in acidotic and non-acidotic groups **(Table 6.4)**.

Table 6.4: Table showing rheological markers for the acidotic and non-acidotic groups at four time points (A, B, C, D). Data presented as mean ± standard deviation, significance between the acidotic and non-acidotic groups were assessed using independent samples t-test. The significance between for time points were assessed using one-way ANOVA test. *indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
d_f (acidotic)	1.72 ± 0.06	1.71 ± 0.05	1.69 ± 0.08	1.69 ± 0.06	0.13
d_f (non-acidotic)	1.71 ± 0.05	1.71 ± 0.05	1.73 ± 0.06	1.76 ± 0.06	0.07
d_f (acidotic vs non-acidotic, p value)	0.36	0.76	0.16	0.005*	
T_{GP} (acidotic)	273 ± 75	259 ± 96	284 ± 173	331 ± 195	0.28
T_{GP} (non-acidotic)	284 ± 74	259 ± 68	299 ± 64	264 ± 102	0.32
T_{GP} (acidotic vs non-acidotic, p value)	0.51	0.98	0.76	0.30	

6.2.3.2 D_f in patients who died and survived in the acidotic group

In the acidotic group 17% of patients died compared to 3% in the non-acidotic group. The mean d_f of those died was significantly higher when compared to those who survived ((1.76 ± 0.03 vs 1.71 ± 0.06, p=0.04) **[Figure 6.3]**.

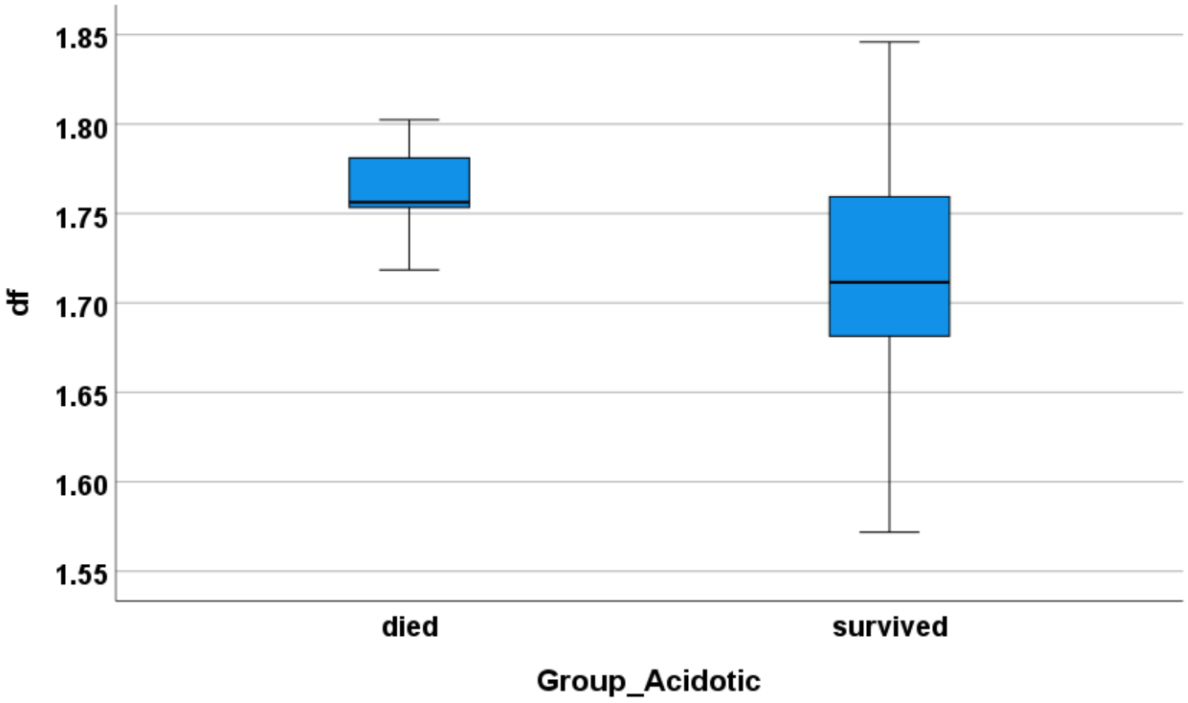
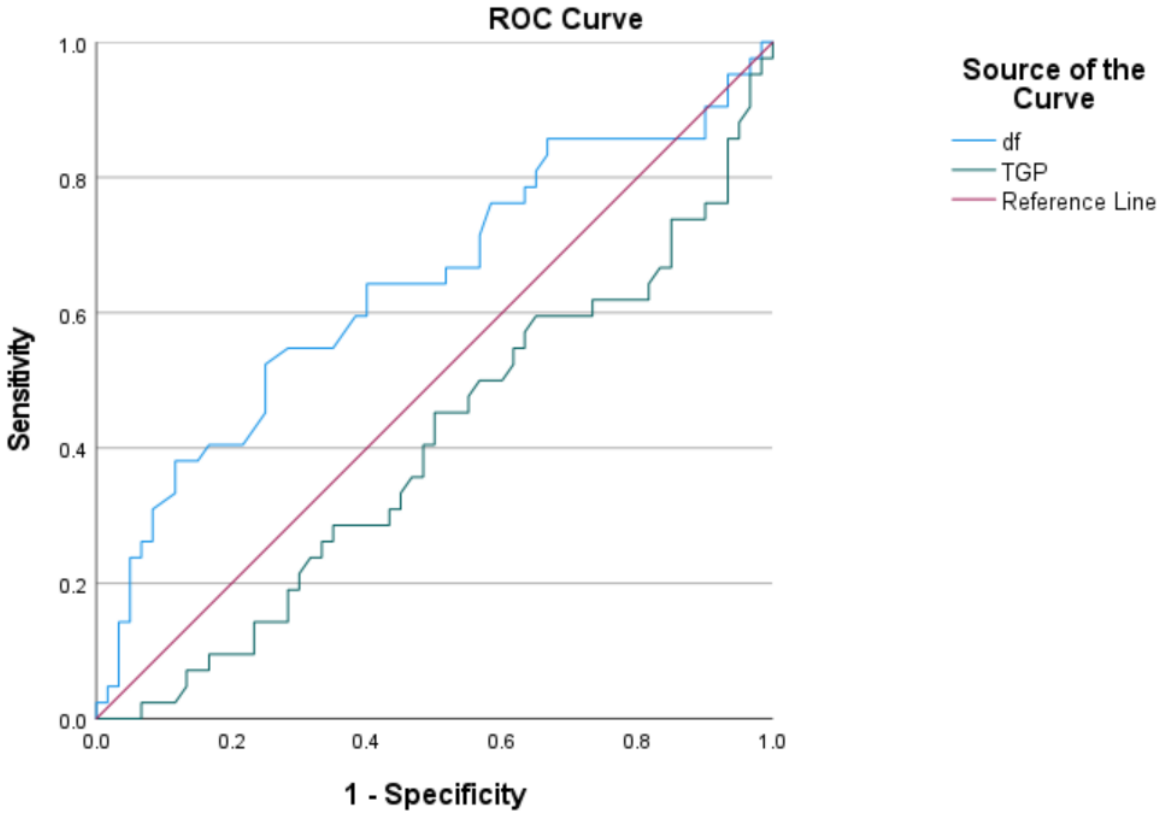


Figure: 6.3: d_f is significantly raised in patients who died in the acidotic group. Significance between the group was assessed using independent samples t-test.

6.2.3.3 Receiver operating curve for rheological markers between the acidotic and non-acidotic group in AECOPD patients

ROC analysis shows that d_f acts as a significant discriminator between acidotic and non-acidotic group **(Figure 6.4; Table 6.5)**.



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
d_f	.640	.058	.016*	.527	.753
T_{GP}	.402	.057	.093	.290	.514

Figure 6.4: Receiver operating characteristics for rheological markers for the discrimination between acidotic and non-acidotic group. Df was significant discriminator between acidotic and non-acidotic groups. **Table 6.5**: Receiver operating characteristics for rheological markers for the discrimination between acidotic and non-acidotic group. *indicates asymptomatic significance (<0.05).

6.2.4 Inflammatory markers between acidotic and non-acidotic group

6.2.4.1 Inflammatory markers between the acidotic and non-acidotic group in AECOPD patients

WBC, Neutrophil and CPR significantly reduced between four time points in acidotic group. Neutrophil count significantly reduced at sample point A between the acidotic and non-acidotic group. PCT significantly reduced at all time points (A, B, C, D) between the acidotic and non-acidotic groups **(Table 6.6)**.

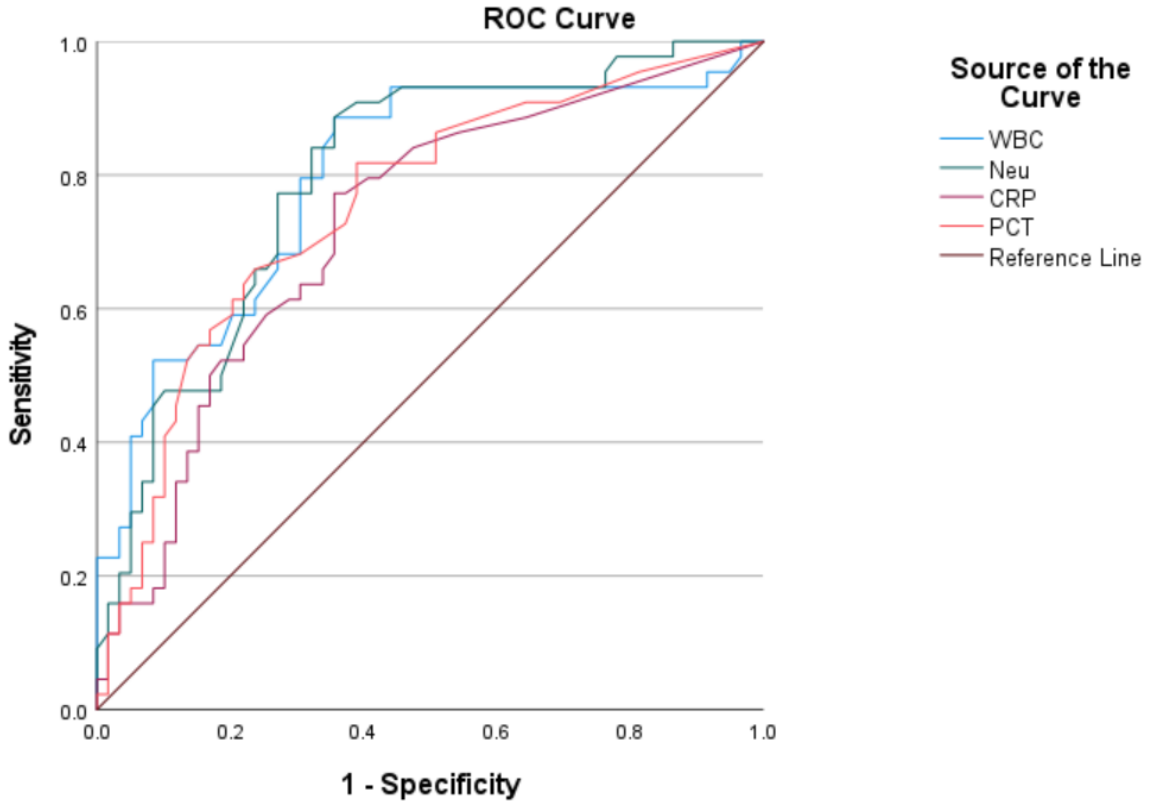
Table 6.6: Inflammatory markers in the acidotic and non-acidotic groups at four time points (A, B, C, D). Data presented as mean ± SD or median (IQR). The significance between the acidotic and non-acidotic group was assessed using independent samples t-test or Mann-Whitney U test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA or Kruskal Wallis test. *indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
WBC ($\times 10^9$/L) (acidotic) [mean ± SD]	17.4 ± 9.4	14.0 ± 6.4	12.5 ± 5.0	11.3 ± 4.5	0.003*
WBC ($\times 10^9$/L) (non-acidotic) [mean ± SD]	12.3 ± 4.7	12.1 ± 5.8	10.4 ± 2.8	9.5 ± 2.4	0.18
WBC (acidotic vs non-acidotic, p value)	0.005*	0.30	0.07	0.22	
Neutrophils (acidotic) [mean ± SD]	14.6 ± 8.7	12.6 ± 5.9	10.5 ± 4.4	9.4 ± 4.1	0.007*
Neutrophils (non-acidotic) [mean ± SD]	10.2 ± 4.8	10.8 ± 5.8	8.6 ± 2.9	7.8 ± 2.6	0.20
Neutrophils (acidotic vs non-acidotic, p value)	0.01*	0.31	0.08	0.26	

CRP (mg/L) (acidotic) [median (IQR)]	39 (12-82)	47 (21-167)	63 (29-143)	16 (9-68)	0.03*
CRP (mg/L) (non-acidotic) [median (IQR)]	31 (11-72)	37 (24-77)	32 (14-84)	14 (6-43)	0.22
CRP (acidotic vs non-acidotic, *p* value)	0.57	0.51	0.13	0.44	
PCT (ug/L) (acidotic) [median (IQR)]	0.14 (0.06-0.92)	0.72 (0.16-5.79)	1.13 (0.21-3.18)	0.32 (0.10-0.94)	0.62
PCT (non-acidotic) [median (IQR)]	0.08 (0.04-0.16)	0.12 (0.06-0.28)	0.08 (0.06-0.38)	0.10 (0.04-0.15)	0.21
PCT (acidotic vs non-acidotic, *p* value)	0.10	0.007*	0.001*	0.006*	

6.2.4.2 Receiver operating curve for inflammatory markers between the acidotic and non-acidotic group in AECOPD patients

All the inflammatory markers were significant discriminators between acidotic and non-acidotic group **(Figure 6.5; Table 6.7)**.



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
WBC	.797	.046	<0.0005*	.707	.886
Neutrophils	.798	.044	<0.0005*	.711	.885
CRP	.725	.051	<0.0005*	.625	.824
PCT	.755	.049	<0.0005*	.659	.850

Figure 6.5: Receiver operating characteristics for inflammatory markers for the discrimination between acidotic and non-acidotic AECOPD patient group. All inflammatory markers were significant discriminators between acidotic and non-acidotic group. **Table 6.7**: Receiver operating characteristics for inflammatory markers for the discrimination between acidotic and non-acidotic AECOPD patient group. *indicates asymptomatic significance (<0.05).

6.2.5 Markers of haemostasis between acidotic and non-acidotic group

6.2.5.1 Markers of haemostasis between the acidotic and non-acidotic group in AECOPD patients

D-dimer significantly lowered in non-acidotic group compared to acidotic group in all four time points (A, B, C, D). No statistically significant differences occurred in the haemostatic biomarkers between four time points in both acidotic and non-acidotic group **(Table 6.8)**.

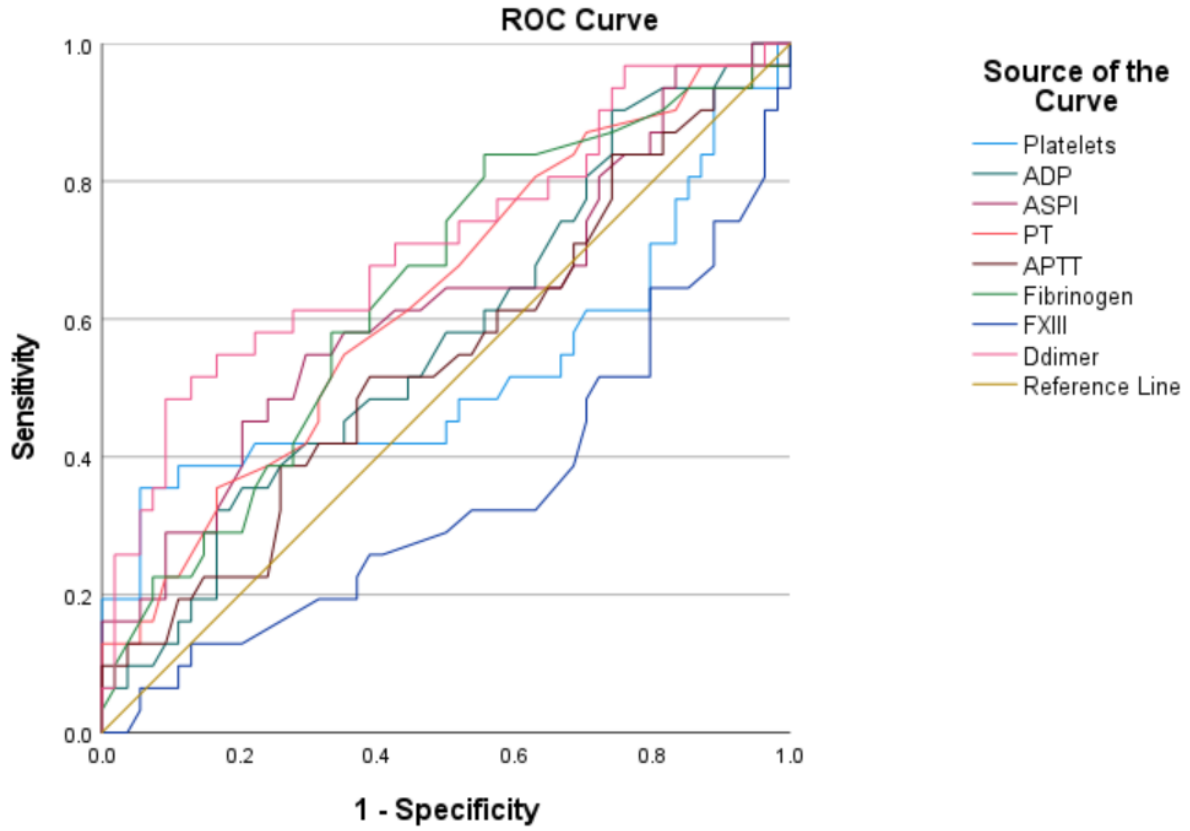
Table 6.8: Markers of primary haemostasis between acidotic and non-acidotic at four time points (A, B, C, D). Data presented as mean ± SD or median (IQR). The significance between the infective and non-infective groups was assessed using independent samples t-test or Mann-Whitney U test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA or Kruskal Wallis test. *indicates significant result (p<0.05).

	AECOPD-A	AECOPD-B	AECOPD-C	AECOPD-D	Significance (*p* value) between 4 time points
Primary haemostasis					
Platelets (acidotic) [mean ± SD]	323 ± 136	276 ± 108	268 ± 107	272 ± 107	0.14
Platelets (non-acidotic) [mean ± SD]	275 ± 83	242 ± 70	241 ± 62	261 ± 77	0.32
Platelets (acidotic vs non-acidotic, *p* value)	0.06	0.24	0.27	0.76	
ADP (acidotic) [mean ± SD]	61 ± 32	66 ± 34	48 ± 26	47 ± 33	0.47
ADP (non-acidotic) [mean ± SD]	51 ± 33	63 ± 28	54 ± 24	61 ± 20	0.56
ADP (acidotic vs non-acidotic, *p* value)	0.22	0.82	0.48	0.20	
ASPI (acidotic) [mean ± SD]	89 ± 48	103 ± 44	77 ± 45	61 ± 42	0.77
ASPI (non-acidotic) [mean ± SD]	73 ± 40	84 ± 38	73 ± 43	79 ± 32	0.57
ASPI (acidotic vs non-acidotic, *p* value)	0.16	0.16	0.77	0.18	
PT (acidotic) [mean ± SD]	11.3 ± 1.4	11.0 ± 2.3	11.2 ± 1.0	10.8 ± 1.0	0.63
PT (non-acidotic) [mean ± SD]	10.9 ± 0.8	11.2 ± 1.0	11.4 ± 1.5	11.1 ± 0.8	0.44
PT (acidotic vs non-acidotic, *p* value)	0.19	0.65	0.59	0.33	
APTT (acidotic) [mean ± SD]	25.1 ± 5.5	24.9 ± 2.6	25.2 ± 3.6	23.4 ± 4.4	0.46
APTT (non-acidotic)	24.0 ± 2.4	25.2 ± 2.3	24.9 ± 3.4	23.0 ± 2.8	0.13

[mean ± SD]					
APTT (acidotic vs non-acidotic, *p* value)	0.32	0.75	0.83	0.77	
Fibrinogen (acidotic) [mean ± SD]	4.6 ± 1.2	4.6 ± 1.1	4.6 ± 1.3	4.3 ± 1.2	0.78
Fibrinogen (non-acidotic) [mean ± SD]	4.6 ± 1.1	4.6 ± 1.0	4.7 ± 0.8	4.1 ± 0.6	0.35
Fibrinogen (acidotic vs non-acidotic, *p* value)	0.96	0.89	0.75	0.45	
FXIII (acidotic) [mean ± SD]	120 ± 30	125 ± 22	116 ± 28	111 ± 24	0.31
FXIII (non-acidotic) [mean ± SD]	149 ± 98	125 ± 30	115 ± 34	124 ± 20	0.34
FXIII (acidotic vs non-acidotic, *p* value)	0.08	0.96	0.94	0.14	
Tertiary haemostasis					
D-dimer (acidotic) [median [IQR)])	1290 (502-2389)	1053 (440-2534)	774 (583-3445)	1037 (469-1799)	0.90
D-dimer (non-acidotic) [median [IQR)])	640 (253-1060)	495 (253-919)	656 (229-1411)	620 (349-1222)	0.90
D-dimer (acidotic vs non-acidotic, *p* value)	<0.001*	0.007*	0.01*	0.02*	

6.2.5.2 Receiver operating curve for haemostatic markers between acidotic and non-acidotic group

ROC analysis shows that D-dimer, FXIII and Fibrinogen are significant discriminator between acidotic and non-acidotic with D-dimer the highest **(Figure 6.6; Table 6.9)**.



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
Platelets	.527	.074	.678	.383	.671
ADP	.575	.064	.252	.449	.701
ASPI	.620	.066	.067	.491	.749
PT	.631	.063	.046	.507	.754
APTT	.545	.066	.496	.415	.674
Fibrinogen	.641	.062	.031*	.519	.764
FXIII	.345	.064	.018*	.220	.471

D-dimer	.709	.061	.001*	.590	.828

Figure 6.6: Receiver operating characteristics for coagulation markers for the discrimination between acidotic and non-acidotic AECOPD patient group. Both Fibrinogen, FXIII and D-dimer were significant discriminators between the two groups. **Table 6.9**: Receiver operating characteristics for coagulation markers for the discrimination between acidotic and non-acidotic AECOPD patient group. *indicates asymptomatic significance (<0.05).

6.2.6 Haemoglobin and haematocrit between acidotic and non-acidotic group

Both haemoglobin and HCT decreased significantly between four time points in acidotic group **(Table 6.10)**.

Table 6.10: Haemoglobin and haematocrit between acidotic and non-acidotic at four time points (A, B, C, D). Data presented as mean ± standard deviation. The significance between the acidotic and non-acidotic group was assessed using independent samples t-test. The significance between four time points (A, B, C, D). was assessed using one-way ANOVA test. *indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
Haemoglobin g/L (acidotic) [mean ± SD]	139 ± 25	130 ± 26	118 ± 28	127 ± 22	0.007*
Haemoglobin g/L (non-acidotic) [mean ± SD]	138 ± 17	130 ± 19	132 ± 15	132 ± 13	0.66
Haemoglobin g/L (acidotic vs non-acidotic, p value)	0.80	0.95	0.07	0.49	
Haematocrit L/L (acidotic) [mean ± SD]	0.44 ± 0.07	0.41 ± 0.07	0.39 ± 0.06	0.39 ± 0.06	0.002*
Haematocrit L/L (non-acidotic) [mean ± SD]	0.43 ± 0.05	0.41 ± 0.06	0.41 ± .05	0.41 ± 0.04	050
Haematocrit L/L (acidotic vs non-acidotic, p value)	0.29	0.94	0.17	0.37	

6.2.7 Comparing biomarkers of stable COPD group with acidotic and non-acidotic group

The acidotic group had significantly higher d_f when compared to the stable group, however T_{GP} was significantly lower in both acidotic and non-acidotic groups when compared to the stable group. All inflammatory markers were significantly elevated in infective group when compared to stable group. Fibrinogen was significantly higher in both groups, however FXIII was significantly lower, and D-dimer was significantly higher in acidotic group when compared to stable group **(Table 6.11)**.

Table 6.11: Comparing biomarkers of stable COPD group with acidotic and non-acidotic group. Data presented as mean ± SD or median (IQR) and the significance between the groups was assessed using independent samples t-test or Mann-Whitney U test. *indicates significant result (p<0.05).

	Stable & acidotic group ***P value***	**Stable & non-acidotic group** ***P value***
d_f (mean ± SD)	1.69 ± 0.05 vs 1.72 ± 0.06 (0.02)*	1.69 ± 0.05 vs 1.71 ± 0.05 (0.19)
T_{GP} (mean ± SD)	327 ± 88 vs 275 ± 69 (0.004)*	327 ± 88 vs 270 ± 83 (0.04)*
WBC (mean ± SD)	9.5 ± 3.5 vs 17.4 ± 9.4 (<0.001)*	9.5 ± 3.5 vs 12.3 ± 4.7 (0.009)*
Neutrophils (mean ± SD)	6.9 ± 3.8 vs 14.6 ± 8.7 (<0.001)*	6.9 ± 3.8 vs 10.2 ± 4.8 (0.003)*
CRP [median (IQR)]	0 (0-6) vs 39 (0-407) (<0.001)*	0 (0-6) vs 31 (0-322) (<0.001)*
PCT [median (IQR)]	0.04 (0.02-0.05) vs 0.14 (0.02-97.32), (<0.001)*	0.04 (0.02-0.05) vs 0.08 (0.02-23.95), (<0.001)*
Platelets (mean ± SD)	265 ± 63 vs 323 ± 136 (0.007)*	265 ± 63 vs 275 ± 83 (0.59)
PT (mean ± SD)	10.8 ± 0.61 vs 11.3 ± 1.4 (0.07)	10.8 ± 0.61 vs 10.9 ± 0.8 (0.61)

APTT (mean ± SD)	23.4 ± 1.9 vs 25.1 ± 5.5 (0.11)	23.4 ± 1.9 vs 24.0 ± 2.4 (0.26)
Fibrinogen (mean ± SD)	3.4 ± 0.8 vs 4.6 ± 1.2 (<0.001)*	3.4 ± 0.8 vs 4.6 ± 1.1 (<0.001)*
FXIII (mean ± SD)	138 ± 21 vs 120 ± 30 (0.005)*	138 ± 21 vs 149 ± 98 (0.57)
D-dimer [median (IQR)]	445 (323-726) vs 1290 (152-19999), (<0.001)*	445 (323-726) vs 640 (148-6389) (0.34)
Hb (mean ± SD)	142.6 ± 15.1 vs 139.0 ± 25.0 (0.44)	142.6 ± 15.1 vs 137.8 ± 16.7 (0.24)
HCT (mean ± SD)	0.43 ± 0.04 vs 0.44 ± 0.07 (0.54)	0.43 ± 0.04 vs 0.43 ± 0.04 (0.58)

6.3 Discussion

COPD is a progressive disease that affects the airways and lungs leading to chronic airflow obstruction and impairment in gas exchange. The changes in the lungs are irreversible and there is a steady decline in FEV1 (Trzaska-Sobczak, Brożek, Farnik, & Pierzchała, 2013). Smoking cessation is the one intervention that can significantly improve the progression (Løkke, Lange, Scharling, Fabricius, & Vestbo, 2006). As the disease progresses, COPD patients suffer from frequent exacerbations requiring hospital admissions. The patients can be acidotic or non-acidotic depending upon the severity of the exacerbation. In this sub-analysis, the majority of the patients were acidotic (59%). As previously mentioned in Chapter 4.4 the majority of the AECOPD patients had moderate-severe COPD disease. The age and sex were matched for both groups. Even though not statistically significant, the acidotic group had higher BMI, more smokers and increase in co-morbidities except cancer. This indicates that COPD patients with higher BMI and comorbidities have a higher chance of getting acidotic during exacerbation. In clinical practice, if AECOPD patients are acidotic, then treatment is optimised initially by giving controlled oxygen, nebulisers, and steroids. However, if treatment optimisation fails to correct acidosis, then patients will be offered non-invasive ventilation (NIV). NIV is offered to conscious patients and without evidence of pneumonia in the lung. If patients are very unwell, then they will be referred to ICU for mechanical ventilation if appropriate, otherwise they will be admitted to the ward with NIV as the ceiling of care. This might be the reason why in this study significant number of patients in the acidotic group were admitted to ICU. The hospital and ICU length of stay was higher in the acidotic group. This was not surprising because the acidotic group tend to be more unwell than the non-acidotic group. Less number of patients were investigated for VTE in the acidotic group. This might be due to overlap of the COPD symptoms and depending upon the disease severity, further investigations or treatment was deemed to be futile. Significant number of patients in the acidotic group died during admission and in one-year. According to Gungor et al. (2018), a severely acidotic COPD patient has poorer short term and long-term prognosis. No difference in the disease severity as per FEV1. Both acidotic and non-acidotic groups were from the most deprived areas as expected. Clinical observations were worse and NEWS score was significantly higher in the acidotic group. This demonstrates that the acidotic group was more clinically unwell. The acidotic group had severe respiratory acidosis because of the markedly raised carbon-dioxide. This is because this group

of patients has a chronic compensated respiratory acidosis and during exacerbation, this worsens.

The rheology markers performed showed that when compared to stable patients, acidotic group had significantly higher d_f indicating that they got denser and tighter clot microstructure. This was because, in acidotic group there was activation of the coagulation pathway. This was evidenced by significantly higher inflammatory, haemostatic and fibrinolytic markers. This indicates that respiratory acidosis may impair coagulation. Another interesting finding was that clot microstructure in the acidotic group who died was significantly denser and tighter when compared to those who survived indicated by high d_f. The disease severity as indicated by FEV1 was significantly low in patients who died indicating that acidotic patients who died had the severe form of disease. There was no significant difference in d_f in patients who died and survived in one-year. d_f was a significant discriminator in the acidotic group. The analysis of the inflammatory markers in both the acidotic and non-acidotic group showed that they were raised above the normal levels. The WBC and Neutrophils were significantly higher in the acidotic group in sample A. The PCT was significantly high in B, C, D sample in the acidotic group. All these demonstrate that the inflammatory process in the acidotic group was worse when compared to the non-acidotic group. This is further evidenced by the analyses that inflammatory markers were significant discriminators between both groups. The markers of primary and secondary haemostasis did not demonstrate a significant change, however D-dimer was significantly elevated at all sampling points in the acidotic group indicating that there was enhanced fibrinolysis. When compared to the SCOPD group the AECOPD group had significantly high fibrinogen and significantly low FXIII which was again reflected in the acidotic and non-acidotic groups. This demonstrates that there was activation of the coagulation pathway and increase in the formation of fibrin which then underwent fibrinolysis indicated by high D-dimer. This was further evidenced by the analysis that Fibrinogen, FXIII and D-dimer were significant discriminators of acidotic from non-acidotic group. All these findings point out that, despite activation of the coagulation pathway, the clot microstructure becomes weaker in the acidotic group at four sampling points. The possible explanation for this is the fact that acidosis impairs coagulation as discussed above. The limitation of this sub-analysis was that there was no power calculation undertaken. Therefore, any conclusions should be drawn cautiously.

6.4 Conclusion

There was no difference between the clot microstructure of acidotic and non-acidotic group, however there the clot microstructure was significantly denser and tighter in the acidotic group when compared to stable group. Acidotic group was critically unwell and significant number of patients died during admission and in one year. The clot microstructure on those patients who died in the acidotic group was significantly denser and tighter. The clot microstructure in the acidotic group gets progressively weaker when compared to the non-acidotic group. There is a need to perform an adequately powered study to confirm this finding.

Chapter 7: Does clot microstructure (d_f) predict mortality in COPD?

7.1 Introduction

COPD is the third most common cause of death worldwide after ischaemic heart disease and stroke (Lazano et al., 2012). After lung cancer, COPD deaths (26%) are the most common deaths among lung diseases in the UK. In the UK, approximately 30,000 people and in Wales approximately 1500 die each year with COPD. Several risk factors such as smoking and exposure to irritants leads to chronic inflammation and destruction of the airways and lung alveoli. Subsequently, COPD patients develop chronic airway obstruction and impairment in gas exchange. The airway obstruction as measured by FEV1 and the impairment of gas exchange which is hypoxia and hypercapnia (type 2 respiratory failure) progressively deteriorates. These changes are irreversible that ultimately lead to death. The study done by Hansell, Walk, and Soriano (2003) found that nearly 60% of COPD deaths are caused by underlying respiratory failure. Other causes of death in COPD patients were ischaemic heart disease, lung cancer and pneumonia. In moderate COPD disease, FEV1 was found to be a stronger predictor of mortality (Bikov et al., 2020) and COPD patients with higher FEV1 had low risk of cardiovascular and all-cause mortality (Ching et al., 2019). In addition, FEV1 was found to be a predictor of cardiovascular disease and hospitalisation (Duong et al., 2019). COPD patients develop several co-morbidities and cardiovascular diseases are the commonest of them. Other significant comorbidities include diabetes, lung cancer and VTE. Smoking cessation is a single intervention that can prevent the acceleration of this inflammatory process. However, studies report that there is no association between smoking prevalence and COPD mortality rates. This was evidenced from low-income countries where the smoking prevalence is low, however there are high COPD mortality rates. Therefore, the possible cause might be a link between poverty COPD mortality (Burney et al., 2014). Several studies found that poverty is a risk factor for COPD (Raju et al., 2018; Lee et al., 2019).

The functional biomarker of clot microstructure, d_f was investigated in different disease conditions. The study by Davies et al. (2015) showed that the d_f was significantly higher in lung cancer patients compared to healthy individuals and those with extensive disease when

compared to limited disease. The study found that the 12-month mortality was significantly higher in those patients who have extensive disease. However, no further analysis was carried out to see whether d_f predicts mortality. The study by Davies et al. (2016) found that d_f was a significant predictor of mortality in patients with sepsis at 28-days. COPD is a disease where there is high in-patient and one-year mortality rate (Connors et al., 1996). In this thesis, it was found that the study group in fact had a high in-hospital and one-year mortality. Therefore, it would be sensible to undertake an analysis to see whether the d_f was significantly higher in patients who died during the admission and at one-year. Because this was a sub-analysis no power calculation was undertaken.

7.1.1 Aims

The aims of this sub-analysis were to,

1. To compare d_f between the AECOPD patients who died and survived this admission and at one-year
2. To analyse inflammatory and haemostatic markers between AECOPD patients who died and survived this admission and at one-year
3. To evaluate whether d_f predicts mortality in AECOPD patients

7.2 Results

This sub analysis was conducted to see whether clot microstructure predicts mortality in COPD patients. The in-patient mortality rate among AECOPD patients was 12% (10/85). The one-year mortality among SCOPD patients was 13% (4/30) and for AECOPD it was 30% (25/85) which was significantly high **(Figure 7.1)**.

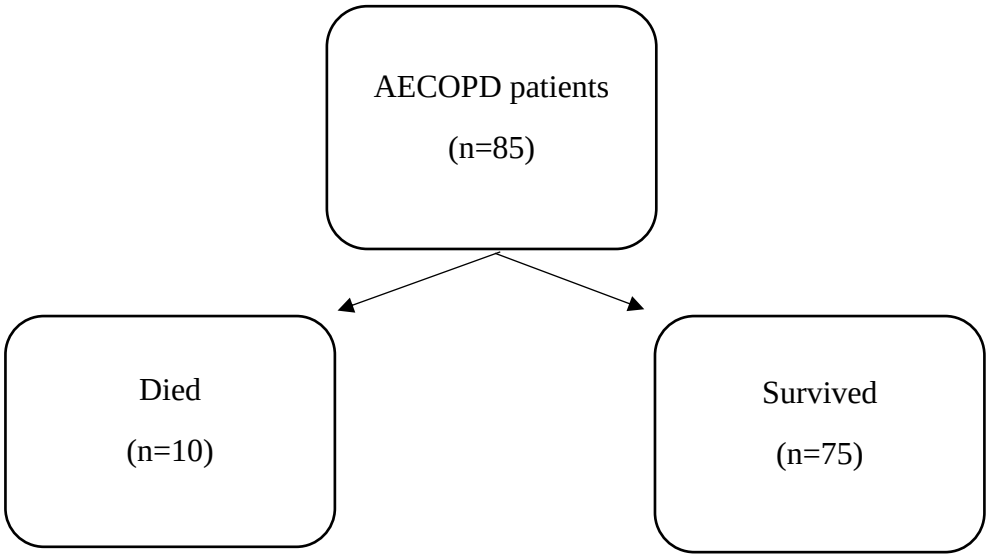


Figure 7.1: Flow diagram illustrating AECOPD patients who died and who survived.

7.2.1 Patient demographics and WIMD 2019 score for patients who died and survived in AECOPD group

7.2.1.1 Patient demographics between AECOPD patients who died and survived

Patient demographics shows that there was no significant difference between the patients who died and survived expect for hypertension which was significantly high in those who survived (**Table 7.1**).

Table 7.1: Patient demographics between the patient who died and survived in the AECOPD group. The values were presented as mean ± SD or n (percentage). Significance between the groups were assessed using independent samples t-test or Chi-square test. *indicates significant result (p<0.05).

	Died (n=10)	**Survived (n=75)**	***p value***
Age (mean ± SD)	70 ± 6	70 ± 11	0.83
Sex (M/F)	4:6	37:38	0.58
BMI (mean ± SD)	25.6 ± 8.6	29.6 ± 8.1	0.26
Current smoker (%)	5/10 (50%)	31/75 (41%)	0.48
Hypertension (%)	1/10 (10%)	29/75 (39%)	0.009*
Diabetes (%)	2/10 (20%)	16/75 (21%)	0.88
IHD (%)	0/10 (0%)	2/75 (3%)	0.60
CVA (%)	2/10 (10%)	6/75 (8%)	0.42
Heart failure (%)	1/10 (10%)	4/75 (5%)	0.59
Previous VTE (%)	1/10 (10%)	0/75 (0%)	0.34

Cancer (%)	2/10 (20%)	5/75 (7%)	0.36
Hospital length of stay (days)	10 (3-17)	6 (2-13)	0.58
ICU length of stay (days)	0	11 (3-31)	0.22
Investigated for VTE (%)	0/10 (0%)	8/75 (11%)	0.98
VTE during admissions (%)	0/10 (0%)	1/75 (1%)	0.60

7.2.1.2 WIMD 2019 score for AECOPD patients who died and survived

Patients who survived were more from the most deprived areas and those who died were from both the most and least deprived areas (**Figure 7.2**).

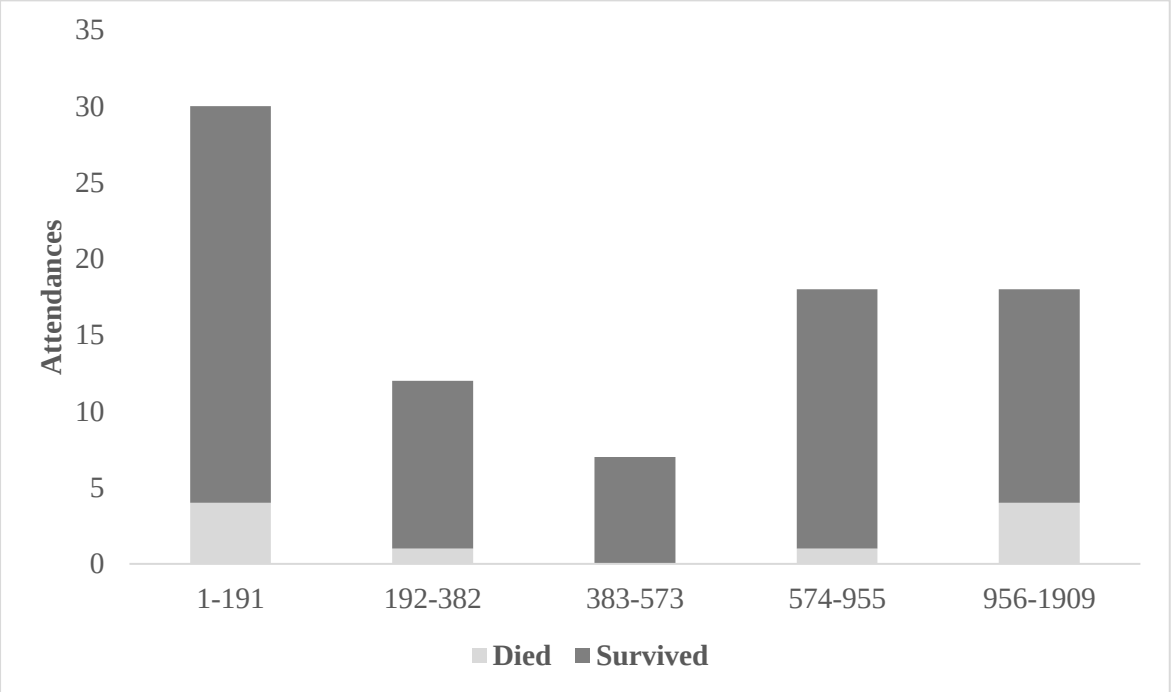


Figure 7.2: WIMD 2019 score AECOPD patients who died and survived. The areas are ranked from 1 which is the most deprived to 1909 which is the least deprived. The scores are arranged in four equal parts or quintiles. Patients who survived were from the most deprived areas.

7.2.1.3 Disease severity in died and survived group

Patients who died had lower but non-significant FEV1 as compared to those who survived (p=0.15) [**Figure 7.3**].

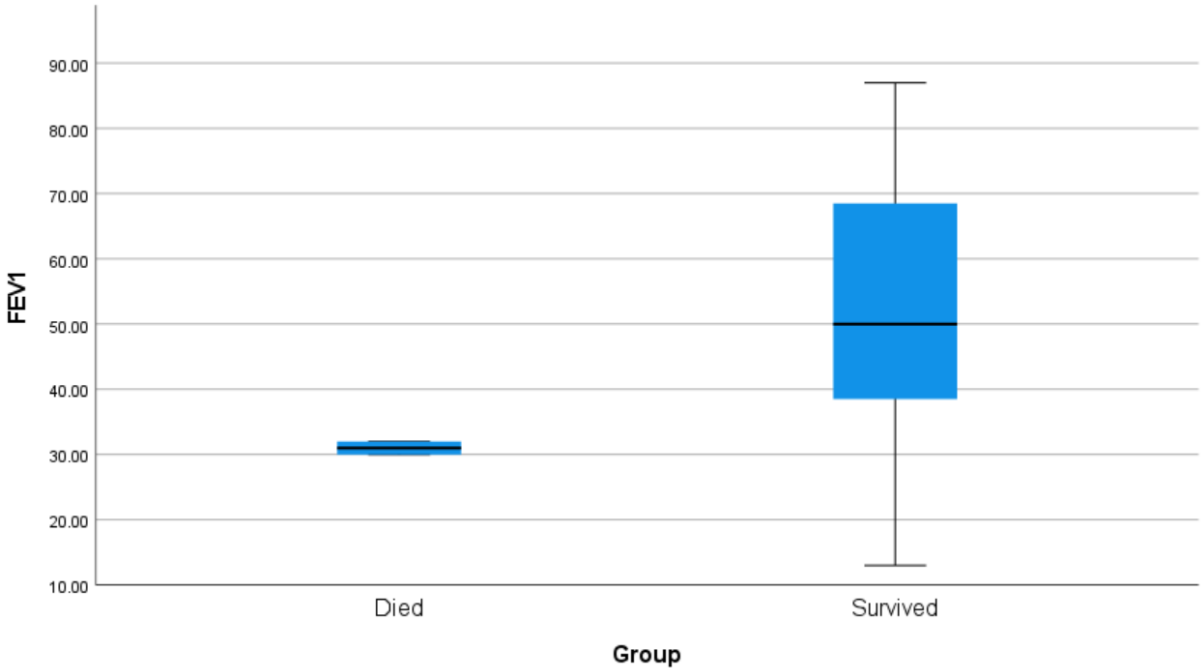


Figure 7.3: FEV1 was lower in patients who died when compared to those who survived, however there was no statistical significance (p=0.15). The significance was assessed using independent samples t-test

7.2.2 Clinical observations and blood gas analysis for the AECOPD patients who died and survived

7.2.2.1 Clinical observations between the patients who died and survived

There was no significant difference in the clinical observations between the patients who died and survived. The patients who died had increased respiratory rate, required more oxygen compared to those who survived (**Table 7.2**).

Table 7.2: Clinical observations in AECOPD patients who died and survived. The values are presented as mean ± SD and the significance between both groups are assessed using independent t-test. *Indicates significant results (p<0.05)

	Died (mean ± SD)	**Survived** (mean ± SD)	***p* value**
Temperature (^{o}C)	37.7 ± 1.0	36.8 ± 0.8	0.70
Pulse (beats/ minute)	99 ± 21	104 ± 22	0.49
Systolic Blood Pressure (mm of Hg)	142 ± 34	136 ± 33	0.56
Diastolic Blood Pressure (mm of Hg)	72 ± 19	75 ± 18	0.71
Respiratory rate (breaths/minute)	29 ± 16	26 ± 6	0.69
Oxygen saturation (%)	95 ± 5	92 ± 7	0.23
Oxygen requirement (%)	55 ± 36	27 ± 12	0.11
NEWS2 score	9 ± 4	7 ± 3	0.25

7.2.2.2 Blood gas measurements between patients who died and survived

Patients who died were more acidotic with a significantly high pCO2. HCO3 and lactate was increased but not significant in those patients who died (**Table 7.3**).

Table 7.3: Blood gas analysis between the died and survived group. Data presented as mean ± standard deviation, significance between the groups was assessed using independent samples t-test. *indicates significant result (p<0.05).

	Died (mean ± SD)	**Survived** (mean ± SD)	***p* value**
pH	7.25 ± 0.08	7.32 ± 0.11	0.09
pCO2 (kPa)	12 ± 4	8 ± 3	<0.001*
HCO3 (mmol/L)	35 ± 12	26 ± 5	0.07
Lactate (mmol/L)	2.2 ± 2.3	1.9 ± 1.1	0.62

7.2.3 Rheological markers between died and survived group

7.2.3.1 Rheological markers between the died and survived AECOPD patients

D_f was significantly higher in the group who died when compared to AECOPD patients who survived at admission (sample A). Binary regression analysis showed that d_f was a significant predictor of mortality (p=0.024). There was no significant difference in the d_f between died and survived at time points B, C, D. In addition, there was no significant difference in the rheological markers between the four time points (A, B, C, D) in died or survived (**Table 7.4; Figure 7.4**).

Table 7.4: Rheological markers between the died and survived group at four time points (A, B, C, D). Data presented as mean ± standard deviation, significance between the died and survived groups was assessed using independent samples t-test. The significance between for time points is assessed using one-way ANOVA test. *indicates a significance value of p<0.05

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
d_f (died) [mean ± SD]	1.76 ± 0.03	1.73 ± 0.07	1.67 ± 0.08	1.73 ± 0.01	0.10
d_f (survived) [mean ± SD]	1.71 ± 0.06	1.70 ± 0.05	1.71 ± 0.07	1.71 ± 0.07	0.94
d_f (died vs survived, p value)	0.02*	0.29	0.29	0.75	
T_{GP} (died) [mean ± SD]	273 ± 114	254 ± 95	260 ± 60	300 ± 79	0.91
T_{GP} (survived) [mean ± SD]	277 ± 67	256 ± 84	295 ± 150	309 ± 178	0.21
T_{GP} (died vs survived, p value)	0.91	0.96	0.60	0.94	

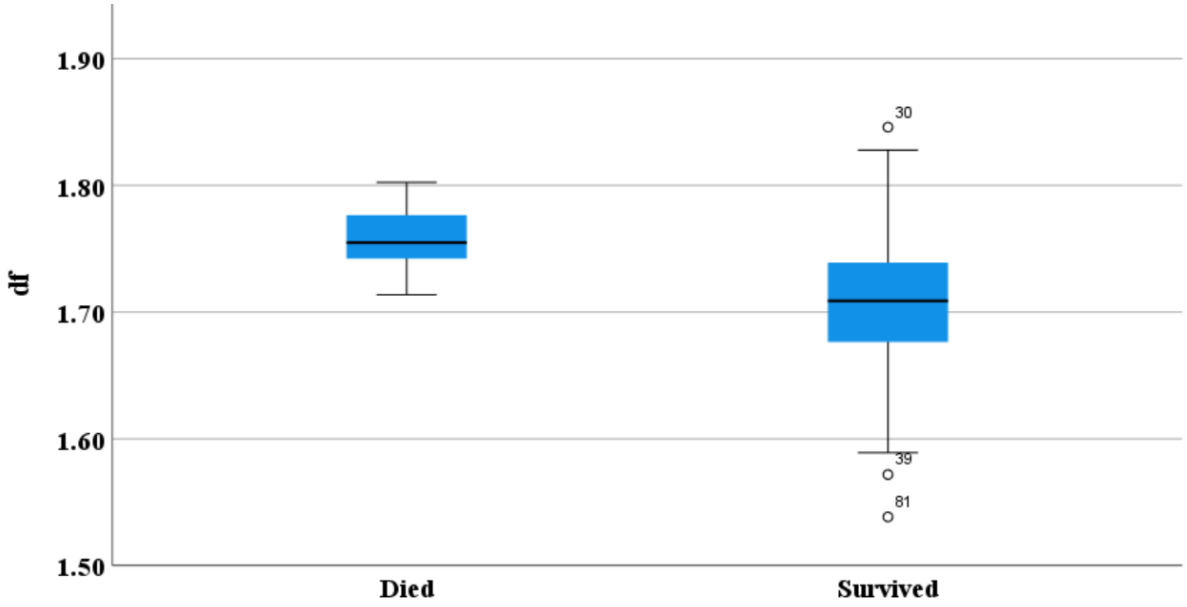
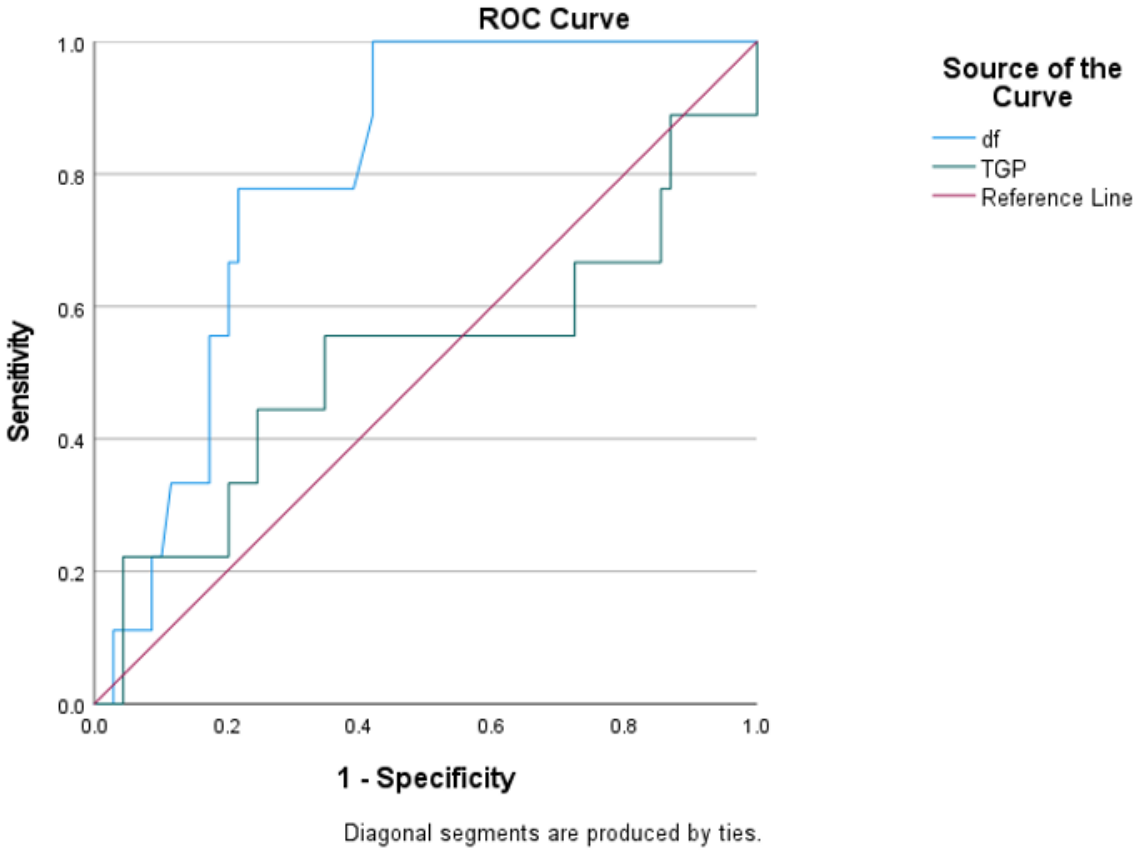


Figure 7.4: D_f in AECOPD patients who died was significantly higher when compared to those who survived at admission.

7.2.3.2 Receiver operating curve for inflammatory markers between the infective and non-infective group in AECOPD patients

ROC analysis showed that d_f was a significant discriminator between the patients who died (**Figure 7.5; Table 7.5**).



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
d_f	.798	.057	.004*	.687	.909
T_{GP}	.519	.123	.857	.278	.759

Figure 7.5: Receiver operating characteristics for rheological markers for the discrimination between died and survived AECOPD patient group that showed d_f to be a significant discriminator. **Table 7.5**: Receiver operating characteristics for rheological markers for the

discrimination between acidotic and non-acidotic AECOPD patient group. *indicates asymptomatic significance (p<0.05).

7.2.3.3 Rheological markers between died and survived patients at one-year

There was no significant difference between the d_f of SCOPD patients who died (1.72 ± 0.02) and survived (1.68 ± 0.05) at one-year (p=0.10) in SCOPD group (**Figure 7.6**).

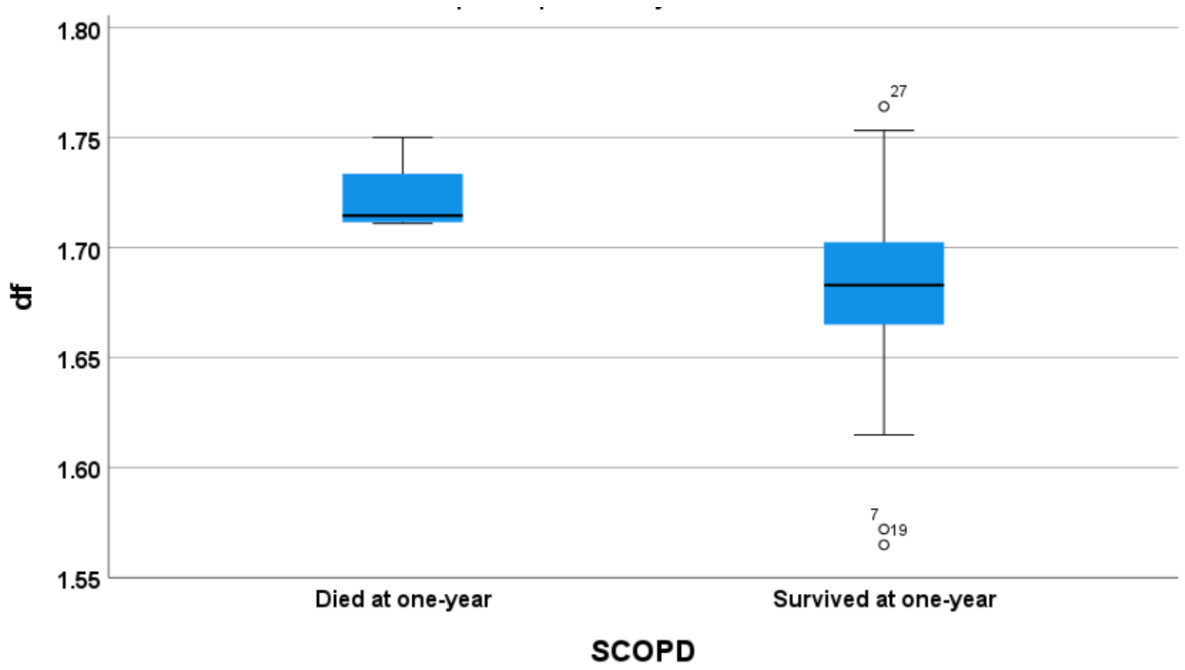


Figure 7.6: Clot microstructure between the died and survived SCOPD patients at one-year showing no significant difference. Means compared using independent t-test.

There was no statistical difference in the d_f of patients who died (1.72 ± 0.05) and survived (1.71 ± 0.06) at one-year (*P*=0.31) in AECOPD group (**Figure 7.7**).

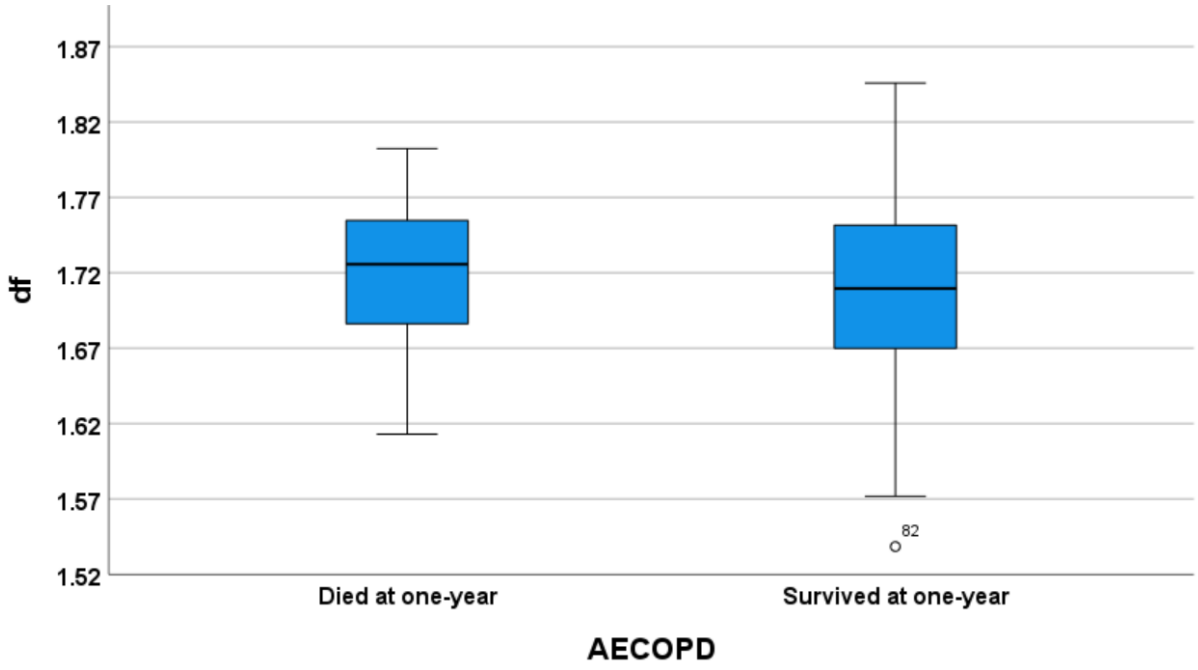


Figure 7.7: Clot microstructure between died and survived AECOPD patients at one year showing no significant difference. Means compared using independent t-test.

7.2.4 Inflammatory markers between died and survived group

7.2.4.1 Inflammatory markers between the infective and non-infective group in AECOPD patients

None of the inflammatory markers were significantly different between the died and the survived group at each time points (A, B, C, D). The inflammatory markers WBC, Neutrophils and PCT significantly reduced between four time points (A, B, C, D) in the survived group (**Table 7.6**).

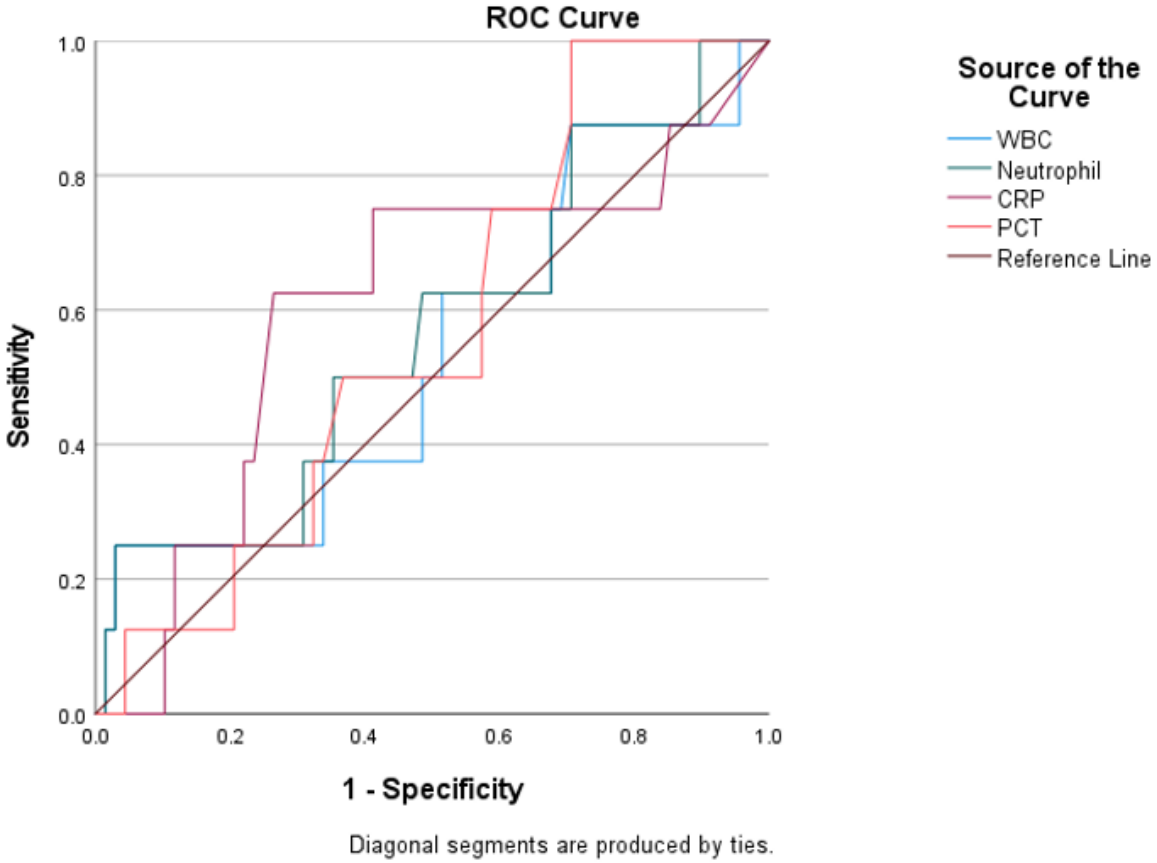
Table 7.6: Inflammatory markers between the died and survived group at four time points (A, B, C, D). Data presented as mean ± SD or median (IQR). The significance between the infective and non-infective group was assessed using independent samples t-test or Mann-Whitney U test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA or Kruskal Wallis test. *indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
WBC ($\times 10^9$/L) (died) [mean ± SD]	15.6 ± 9.0	11.7 ± 6.3	9.5 ± 2.5	8.8 ± 2.7	0.27
WBC ($\times 10^9$/L) (survived) [mean ± SD]	15.1 ± 8.1	13.0 ± 6.3	11.8 ± 4.6	10.9 ± 4.0	0.008*
WBC (died vs survived, p value)	0.85	0.69	0.28	0.31	
Neutrophils (died) [mean ± SD]	13.2 ± 8.4	10.9 ± 6.4	8.0 ± 2.1	7.2 ± 2.5	0.35
Neutrophils (survived) [mean ± SD]	12.5 ± 7.4	11.7 ± 6.0	9.9 ± 4.2	9.0 ± 3.8	0.02*
Neutrophils (died vs survived, p value)	0.78	0.79	0.36	0.36	

CRP (mg/L) (died) [median (IQR)]	57 (23-115)	56 (33-118)	6 (0-7)	29 (4-435)	0.54
CRP (mg/L) (survived) [median (IQR)]	34 (12-71)	43 (21-116)	28 (0-88)	14 (7-51)	0.06
CRP (died vs survived, *p* value)	0.28	0.62	0.96	0.59	
PCT (ug/L) (died) [median (IQR)]	0.10 (0.06-0.77)	0.47 (0.26-17.26)	0.04 (0.02-0.05)	6.85 (0.11-78.35	0.67
PCT (survived) [median (IQR)]	0.16 (0.04-0.64)	0.20 (0.09-2.11)	0.09 (0.04-1.09)	0.15 (0.06-0.66)	0.007*
PCT (died vs survived, *p* value)	0.96	0.15	0.30	0.17	

7.2.4.2 Receiver operating curve for inflammatory markers between the infective and non-infective group in AECOPD patients

None of the inflammatory markers are significant discriminator between died and survivors (**Figure 7.8; Table 7.7**).



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
WBC	.536	.114	.741	.312	.760
Neutrophils	.567	.111	.537	.349	.785
CRP	.606	.114	.330	.382	.830
PCT	.565	.091	.548	.388	.743

Figure 7.8: Receiver operating characteristics for inflammatory markers for the discrimination between died and survived AECOPD patient group showing no significant discriminators. **Table 7.7**: Receiver operating characteristics for inflammatory markers for the discrimination

between died and survived AECOPD patient group. *indicates asymptomatic significance (p<0.05).

7.2.5 Markers of haemostasis between died and survived group

7.2.5.1 Markers of haemostasis between the infective and non-infective group in AECOPD patients

Fibrinogen increased significantly at sample point AECOPD-C and FXIII increased significantly at sample point AECOPD-D in survived group. There was no significant difference in the markers of haemostasis between four time points (A, B, C, D) in the died or survived group (**Table 7.8**).

Table 7.8: Markers of primary haemostasis between the died and survived group at four time points (A, B, C, D). Data presented as mean ± SD or median (IQR). The significance between the died and survived group was assessed using independent samples t-test or Mann-Whitney U test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA or Kruskal Wallis test. *indicates significant result (p<0.05).

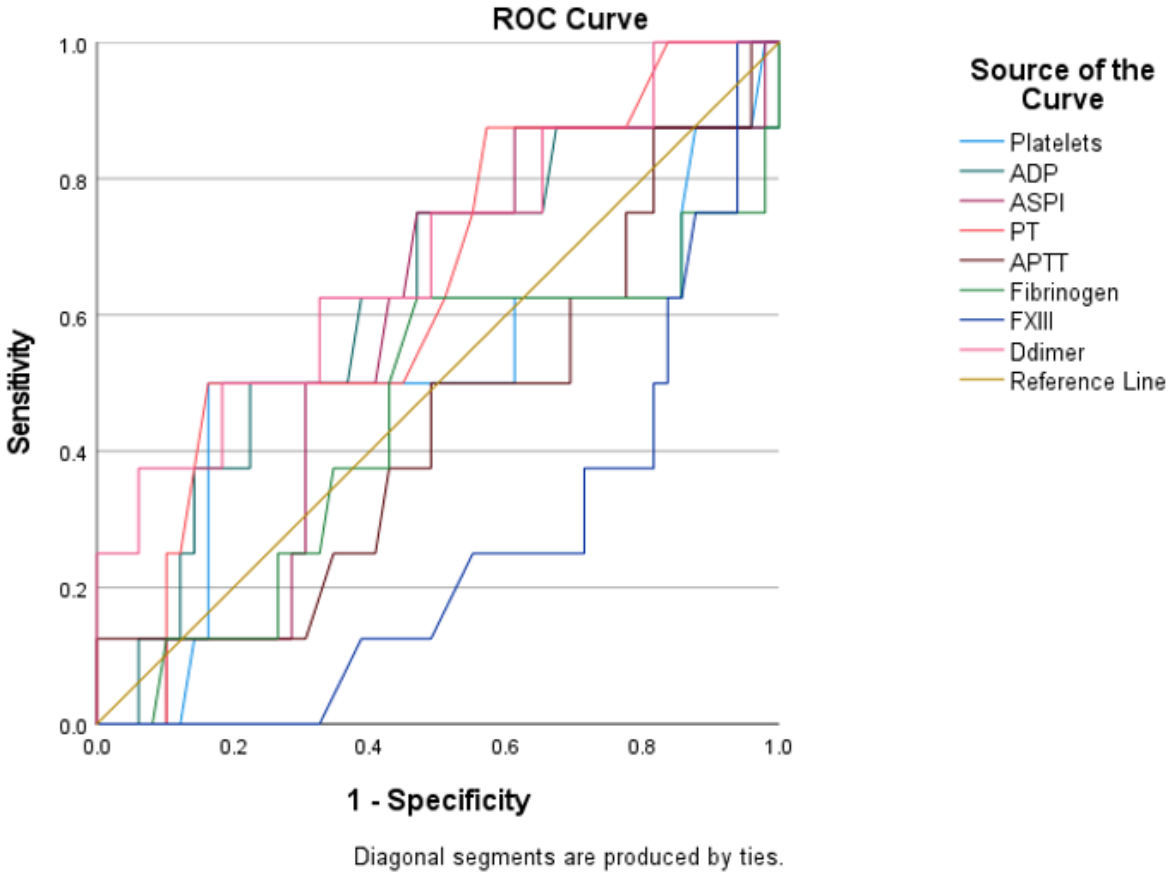
	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
Primary haemostasis					
Platelets (died) [mean ± SD]	319 ± 145	333 ± 88	255 ± 133	221 ± 142	0.54
Platelets (survived) [mean ± SD]	298 ± 113	254 ± 93	257 ± 89	275 ± 90	0.06
Platelets (died vs survived, p value)	0.61	0.11	0.97	0.30	
ADP (died) [mean ± SD]	62 ± 40	76 ± 37	55 ± 31	42 ± 44	0.56
ADP (survived) [mean ± SD]	54 ± 32	63 ± 31	50 ± 25	54 ± 27	0.29
ADP (died vs survived, p value)	0.52	0.34	0.70	0.42	

ASPI (died) [mean ± SD]	81 ± 45	98 ± 38	85 ± 34	61 ± 30	0.57
ASPI (survived) [mean ± SD]	81 ± 46	94 ± 43	75 ± 45	69 ± 40	0.10
ASPI (died vs survived, *p* value)	0.98	0.87	0.65	0.71	
Secondary haemostasis					
PT (died) [mean ± SD]	11.4 ± 0.8	9.5 ± 4.7	11.4 ± 1.7	11.8 ± 1.9	0.49
PT (survived) [mean ± SD]	11.1 ± 1.2	11.3 ± 1.2	11.2 ± 1.1	10.8 ± 0.7	0.25
PT (died vs survived, *p* value)	0.44	0.46	0.79	0.36	
APTT (died) [mean ± SD]	26.6 ± 10.6	24.1 ± 1.3	25.0 ± 2.9	25.3 ± 7.0	0.94
APTT (survived) [mean ± SD]	24.3 ± 2.6	25.2 ± 2.6	25.1 ± 3.5	23.0 ± 3.4	0.01*
APTT (died vs survived, *p* value)	0.54	0.34	0.94	0.56	
Fibrinogen (died) [mean ± SD]	4.1 ± 1.5	4.1 ± 1.3	3.5 ± 1.3	3.6 ± 1.8	0.86
Fibrinogen (survived) [mean ± SD]	4.7 ± 1.1	4.6 ± 1.0	4.8 ± 1.1	4.3 ± 0.8	0.33
Fibrinogen (died vs survived, *p* value)	0.18	0.31	0.02*	0.49	
FXIII (died) [mean ± SD]	110 ± 24	105 ± 33	106 ± 30	93 ± 20	0.77
FXIII (survived) [mean ± SD]	135 ± 68	128 ± 23	118 ± 31	119 ± 22	0.23
FXIII (died vs survived, *p* value)	0.30	0.05	0.39	0.03*	
Tertiary haemostasis					

D-dimer (died) [median (IQR)]	1271 (439-11683)	1021 (452-15364)	738 (465-13671)	2254 (798-10380)	0.96
D-dimer (survived)	856 (392-1810)	661 (330-1755)	687 (426-1741)	793 (366-1433)	0.82
D-dimer (died vs survived, *p* value)	0.25	0.47	0.45	0.11	

7.2.5.2 Receiver operating curve for haemostatic markers between died and survived group

FXIII was the only significant discriminator in between died and survived patients (**Figure 7.9; Table 7.9**).



	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower bound	Upper bound
Platelets	.509	.129	.936	.256	.762
ADP	.617	.114	.291	.394	.840
ASPI	.566	.101	.550	.368	.765
PT	.642	.100	.202	.445	.838
APTT	.440	.112	.589	.220	.660
Fibrinogen	.449	.121	.646	.211	.687
FXIII	.251	.082	.025*	.091	.411
D-dimer	.684	.109	.098	.470	.897

Figure 7.9: Receiver operating characteristics for coagulation markers for the discrimination between died and survived AECOPD patient group demonstrating that FXIII was the only discriminator. **Table 7.9**: Receiver operating characteristics for coagulation markers for the discrimination between died and survived AECOPD patient group.*indicates significance value of (p<0.05).

7.2.6 Haemoglobin and haematocrit between died and survived group

There was no significant difference in haemoglobin and haematocrit between died and survived group at each time point (A, B, C, D). Haematocrit reduced significantly in the survived group between the four time points (A, B, C, D) [**Table 7.10**].

Table 7.10: Haemoglobin and haematocrit between died and survived AECOPD patients at four time points (A, B, C, D). Data presented as mean ± standard deviation. The significance between the died and survived group was assessed using independent samples t-test. The significance between four time points (A, B, C, D) was assessed using one-way ANOVA. *indicates significant result (p<0.05).

	AECOPD-A	**AECOPD-B**	**AECOPD-C**	**AECOPD-D**	**Significance (p value) between 4 time points**
Haemoglobin g/L (died) [mean ± SD]	133 ± 16	125 ± 25	102 ± 50	132 ± 13	0.25
Haemoglobin g/L (survived) [mean ± SD]	138 ± 23	130 ± 23	126 ± 20	129 ± 20	0.12
Haemoglobin g/L (died vs survived, p value)	0.48	0.69	0.34	0.78	
Haematocrit L/L (died) [mean ± SD]	0.43 ± 0.05	0.40 ± 0.08	0.40 ± .04	0.41 ± 0.04	0.67
Haematocrit L/L (survived) [mean ± SD]	0.43 ± 0.06	0.40 ± 0.07	0.39 ± .06	0.41 ± 0.06	0.007*
Haematocrit L/L (died vs survived, p value)	0.79	0.82	0.90	0.82	

7.3 Discussion

In this sub analysis, comparison was made between the d_f in those AECOPD patients who died and survived during the admission. In addition, comparison of d_f was performed between the patients who died and survived at one-year in both SCOPD and AECOPD groups. The in-patient mortality in AECOPD patients in this study was about 12%. The European COPD audit of more than sixteen thousand patients showed the inpatient mortality to be 10.8% (Hartl et al., 2016). The patient demographics (age and sex) between those who died and survived did not show significant difference. However, the BMI among those patients who died was lower when compared to those survived and this agrees with current studies (Wada et al., 2021). Another study by Guo et al. (2016) showed that patients with a lower BMI had increased risk of death. There were more smokers in the group who died which demonstrates that smoking is one of the significant risk factors that worsens disease progression and smoking cessation improves survival (Godtfredsen et al., 2008). Recent studies showed that comorbidities were an independent risk factor of mortality in COPD (Kim, Kim, Kang, & Cho, 2021). Hypertension was significantly higher in those who survived, and even though not significant CVA, heart failure, previous VTE and cancer was higher in the patients who died. No patients were investigated for VTE in patients who died, none of the patients were admitted to ICU, but the patients who died stayed in the hospital for longer. Another interesting fact was that there was a higher number of patients who survived from the most deprived areas. Patients who died were from both the most and least deprived areas. The FEV1 that indicates severity was lower in the patients who died. Bikov et al. (2020) showed that FEV1 is a strong predictor of mortality in COPD patients with moderate disease. The clinical observations did not show any significant difference between the groups. The patients who died were more acidotic and had significantly high pCO2, HCO3 and lactate. This shows that patients who died were critically unwell. Lactate was also high in the group that died however, it can be raised because of the use of salbutamol.

It is to be noted that d_f was significantly raised in the patients who died at admission when compared to those who survived. This was because COPD patients who died had more severe form of the disease as indicated by lower FEV1 and more acidotic blood gas. There was no significant difference between the d_f at four time points in both died and survived group. This shows that with appropriate treatment and administration of prophylactic anticoagulation, the

d_f was not increasing. As explained before none of the patients who died had VTE, nor were admitted to ICU. This shows that the patients who died had very severe COPD with a non-reversable respiratory failure. In clinical practice, patients with very severe COPD will be offered non-invasive ventilation (NIV) as the ceiling of care that can be offered on an NIV ward and will not be admitted to ICU because offering mechanical ventilation was not in their best interest. There was no difference in the T_{GP} and out of the rheological markers d_f was the significant discriminator between the died and survived group. The study showed that, there was no significant difference between the d_f of patients who died and survived in one-year in both SCOPD and AECOPD groups. The inflammatory markers were raised from baseline, however there was no significant difference, and this was the same with markers of haemostasis between the groups. The inflammatory markers such as WBC, Neutrophils and CRP was non-significantly higher in the patients who died which shows that the background inflammation was increased. Conversely, WBC, Neutrophils and PCT decreased significantly in survived group at four time points indicting that the treatment was more effective in those who survived. The platelet number and aggregation were more in the patients who died which shows that there was activation of the platelets. In addition, the extrinsic and intrinsic pathway were activated more in the patient group who died as evidenced by higher PT and APTT. Therefore, there should have been an increase in the fibrinogen levels in patients who died, however this was not the case. That said, in both groups, the fibrinogen levels were above the normal levels. FXIII was non-significantly low in the died group demonstrating increased utilisation for the formation of crosslinks. D-dimer was high in patients who died that shows that there is increased activation of thrombolytic pathways, due to increase in the formation of fibrin. Among all these biomarkers, FXIII was the significant discriminator in patients who died.

To date, there was only one study that investigated d_f as a predictor of mortality. Davies et al. (2016) found that d_f was a significant predictor of mortality in septic patients at 28-days. In this study the d_f was significantly higher in patients who died at admission and regression analysis demonstrates that d_f was a significant predictor of mortality. d_f therefore can be used to predict mortality in AECOPD patients. One of the limitations was that no power calculation was undertaken because this was a sub analysis, therefore the conclusions drawn should be interpreted with caution.

7.4 Conclusion

AECOPD patients who died were critically unwell with severe respiratory failure. They had significantly denser and tighter clot microstructure at admission. With treatment, the clot microstructure remained stable. There was no difference in the clot microstructure of patients who died or survived at one year. Similarly, there was no significant difference in the clot microstructure of patients with SCOPD who died in one year. Therefore, d_f may be used as a biomarker to predict mortality in AECOPD patients. This study however is not an outcome study; therefore, a larger study is required to specifically investigate this finding.

Chapter 8: Final discussion and conclusion

8.1 Discussion

This was the first study that assessed changes in clot microstructure in COPD patients utilising the functional biomarker, fractal dimension (d_f). COPD is a chronic inflammatory disease of the airways characterised by airway obstruction and impairment of gas exchange. In COPD, there is abnormal inflammatory response to smoking and exposure to other noxious particles. It is important to note that only 20% of all smokers develop COPD (Terzikhan et al. 2016). However, 85-90% of patients with confirmed diagnosis of COPD had a history of smoking and approximately 35-40% of COPD patients continue to smoke (Shahab, Jarvis, Britton, & West, 2006). Chronic inflammation leads to damage of the lung tissues releasing several cytokines which then triggers the coagulation pathways. This leads to increased production of fibrin thereby increasing the risk for developing venous thromboembolism (VTE). COPD patients often develop exacerbations where there is flare up of the background inflammatory process triggered by either infection or exposure to smoke or other noxious particles. Studies have shown that the incidence of VTE in COPD are particularly high during exacerbation (Børvik et al. 2016). Therefore, exacerbations can make COPD patients thrombogenic.

Fractal dimension (d_f) quantifies the clot microstructure by analysing the gel point which is the point at which blood changes from liquid to solid (gel) form (Evans et al. 2010a). Gelation occurs once there are enough fibrin polymers that branches three dimensionally and interlinked with FXIII enabling it to hold red blood cells to form a blood clot. The conventional biomarkers measure specific pathways of blood coagulation and use anticoagulated blood (Meybohm, Zacharowski, & Weber, 2013). The measurement of d_f is performed using a rheometer with whole unadulterated blood, therefore this mechanistic test enables quantitative measurement of fibrin formation in real time. d_f can be obtained within 15 minutes, therefore there is a potential for this test to be used as a point of care test which enables the clinician to make appropriate clinical decision. There were several studies that investigated the changes in d_f in different physiological and pathophysiological conditions. Davies et al. (2016), found that d_f significantly increases after vigorous exercise and returns to normal levels at 60 minutes. In their study, Lawrence et al. (2015) found that in anticoagulated patients with history of VTE, the clot microstructure was abnormal when compared to non-VTE group. Therefore, d_f can be

used as an effective biomarker to detect abnormalities in clot microstructure which other biomarkers fail to detect. In patients with obstructive sleep apnoea, there was a diurnal variation in clot microstructure where the d_f was significantly higher during the early hours (D'Silva et al. 2016). Previous studies demonstrated that in acute inflammatory conditions, the clot microstructure was tighter and denser as evidenced by high d_f. In patients presenting with stroke the mean d_f was 1.76 (Stanford et al. 2015) and in sepsis it was 1.78 and in severe sepsis 1.80 (Davies et al. 2016). In certain disease conditions such as septic shock (mean d_f of 1.66), the clot microstructure becomes looser and weaker (low d_f) [Davies et al. 2016]. It was therefore hypothesised that during acute exacerbation, COPD patients have tighter and denser clot microstructure (high d_f).

To test the hypothesis, a prospective observational study was designed to recruit 30 stable COPD (SCOPD) patients from chest clinic as controls and 85 acute exacerbation of COPD (AECOPD) patients from the Emergency Department. To better understand the demographics and comorbidities of the population studied, a retrospective study was undertaken that included all COPD patients who presented to the Emergency Department with an exacerbation over a period of one year. The population group was elderly [mean age 70 (±11, SD)], there was more attendances during winter months and the most common comorbidity was cardiovascular diseases including hypertension, ischaemic heart disease, heart failure and atrial fibrillation. The demographic and comorbidities were similar to what was published showing that the population group that was studied was not different. About 63% of patients were admitted to the hospital with an in-hospital mortality of 9% and one-year mortality of 35%. Only 4% had a history of VTE and very few of them were diagnosed to have VTE during the admission (1%). This finding however was not agreeing with what was published showing a high prevalence of VTE in acute exacerbation of COPD. The investigation for VTE was low (5%) and the reason might be that these groups of patients may be under investigated. Therefore, it was vital to understand whether acute exacerbation increases the risk of thrombogenicity in COPD patients.

To investigate this, a prospective observational study was undertaken to evaluate the thrombogenicity in acute exacerbation of COPD (AECOPD) utilising the functional biomarker of clot microstructure- fractal dimension (d_f). In addition, to better understand the pathologic process involved several biomarkers of inflammation and haemostasis were analysed. The stable (SCOPD) patients had only one blood sample taken after recruitment and AECOPD

patients had four blood samples taken at 0 hours, 4-6 hours, 24 hours, and 3-7 days. The reason for taking blood samples at four time points in the AECOPD group was to evaluate whether treatment makes any difference to clot microstructure over time. SCOPD patients had a stable disease which was evidenced by normal inflammatory markers. There were no activation of the coagulation pathway and therefore the fibrinogen concentration remained within normal limits. Because of this, there was no increased fibrin production or polymerisation and consequently d_f in SCOPD patients was within normal limits (mean d_f of 1.69). This was further evidenced by a normal fibrinolytic process (D-dimer).

When compared to SCOPD patients, the AECOPD group had a profound inflammatory response as evidenced by significantly raised inflammatory markers. Because of this there was activation of the coagulation pathway as evidenced by non-significantly raised haemostatic markers. There was significantly high fibrinogen in the AECOPD group demonstrating the activation of coagulation pathway which led to increased fibrin polymerisation. This was the reason why the AECOPD group on admission had a significantly higher d_f when compared to the SCOPD group (mean d_f of 1.71 and 1.69 respectively). In addition, it took less time for the initiation of blood clot formation (significantly low T_{GP} in AECOPD group). The fibrinolytic activity was significantly higher in the AECOPD group that shows that there was increased fibrin polymerisation activity in the blood. This thesis therefore demonstrates that during acute exacerbation, COPD patients were more thrombogenic than the stable group. With appropriate treatments for AECOPD and thromboprophylaxis, the thrombogenicity did not increase. This was evidenced by no significant changes in d_f over the four time points (3-7days). The inflammatory markers according to Wageck, Cox and Holland (2019) take about two weeks to normalise in COPD patients after exacerbation. Therefore, the current practice of providing thromboprophylaxis to AECOPD patients who are admitted to the hospital appears to be appropriate.

Davies et al. (2016) argued that infection can cause changes in clot microstructure. d_f increased in sepsis and severe sepsis significantly (mean d_f of 1.78 and 1.80 respectively), while in septic shock it significantly reduced (1.66). The reason for this was that infection triggers an inflammatory response, which then activates the coagulation pathway causing dense and tight clot microstructure. It is estimated that 60-70% of the COPD exacerbation is caused by an infection (bacterial or viral). Therefore, it was hypothesised that in AECOPD patients with an

infection, d_f should be high. This was the reason for conducting a sub-analysis, where procalcitonin (PCT) was used to differentiate between infective and non-infective exacerbation of COPD. The d_f was significantly increased in the infective group when compared to the stable group (mean d_f of 1.72 and 1.71), however there was no significant difference between the infective and non-infective group (mean d_f of 1.72 and 1.71 respectively). The inflammatory markers such as CRP and PCT were significantly high in the infective group. This was further evidenced by higher (non-significant) fibrinolytic activity as shown by increased level of D-dimer in the infective group. The changes in d_f in the infective group was similar to the finding by Davies et al. (2016) in their sepsis study. However, with appropriate treatment and thromboprophylaxis, there was no further increase of d_f. Furthermore, the incidence of VTE was low in both infective and non-infective group. This demonstrates that regardless to the cause of exacerbation (infective or non-infective), the current management was appropriate.

Pillai et al. (2021) reported that in DKA patients the d_f on admission was significantly higher when compared to the matched healthy group (mean d_f of 1.78 and 1.74 respectively). With appropriate treatment, the mean d_f significantly dropped down to 1.66 at 24 hours. The possible reasons for high d_f were severe metabolic acidosis and dehydration. However, it is known that acidosis impairs coagulation. Therefore, a sub-analysis was performed to analyse the effect of respiratory acidosis in AECOPD group. The acidotic group had a mean d_f of 1.72 which was not statistically significant to the non-acidotic group (mean d_f 1.71). However, there was an interesting result where at 3-7 days the d_f in the acidotic group was significantly lower than the non-acidotic group (mean d_f of 1.69 vs 1.76 respectively). There was a non-significant drop of mean d_f from sample A to D (1.72 to 1.69) which agrees with the fact that acidosis possibly impairs coagulation. However, when compared to stable group, the acidotic group had significantly high df. This might be due to severe respiratory failure that causes severe dehydration by loss of fluids as moisture in breath and patients not able to eat or drink. Similar observations were made by Pillai et al. (2021). Furthermore, there was significant reduction in viscosity as measured by haemoglobin and haematocrit in the acidotic group at four time points. This was the first time where an analysis was undertaken to investigate the effect of respiratory acidosis on clot microstructure.

In both the retrospective and the prospective study, COPD patients had high in-hospital (9% and 12% respectively) mortality rate and one-year mortality rate (35% and 30% respectively).

This shows that exacerbation itself is a significant contributing factor to mortality and after each exacerbation this risk increases. The reason for this is that exacerbation becomes more frequent when the severity of disease increases. FEV1 is used to predict mortality and this study found that patients who died had lower FEV1 when compared to those who survived. In AECOPD group, those patients who died in one year had significantly lower FEV1. The study done by Davies et al. (2016) showed that d_f was a significant predictor of mortality in sepsis. This thesis showed that d_f in patients who died was significantly high d_f (mean d_f 1.76) when compared to those who survived (mean d_f 1.71) on admission. However, there was no significant difference in d_f in both SCOPD and AECOPD groups in those patients who died and survived in one-year. In addition, binary regression analysis showed that d_f was a significant predictor of mortality (p=0.024). None of the patients who died were either investigated for or had VTE and none of them were admitted to ICU. This shows that because of the severity, these patients have been placed on limitations with NIV being the ceiling of care. Therefore, it was difficult to ascertain whether these patients had VTE.

This study had several limitations. This was a prospective single centre study, therefore the results from this study might not be generalisable. However, what retrospective and the prospective study showed was that the demographics and comorbidities of the population being studied agreed with what is being published locally, nationally, and internationally. The study was initially planned to start in 2016, however due to GDPR (General Data protection Regulation) issues, it was delayed for two years. The study started to recruit AECOPD patients from 2018, however 2020/2021 was challenging due to the COVID-19 pandemic, with most of the non-COVID-19 studies suspended for nearly 6-8 months. Careful consideration was taken not to skew the results; therefore, no COVID-19 positive COPD patients were recruited in this study and the recruitment was completed in 2021. This study was mechanistic in design and not powered for a clinical outcome. This thesis undertook several sub analyses that demonstrated interesting findings; however, this study was not adequately powered to validate those findings. Therefore, the conclusions from sub-analyses in Chapter 5, 6 and 7 should be interpreted cautiously. It was not possible to compare clinical observations and blood gas analysis in between SCOPD and AECOPD group. This was because it is not a routine practice to check clinical observations and perform blood gas analysis in patients who present to the respiratory clinic with stable COPD disease.

The d_f test requires 7mls of whole unadulterated blood that should be transferred to the rheometer within a couple of minutes before the coagulation starts, otherwise it is not possible to get a d_f reading. It was therefore challenging to do the test when the COPD patients get admitted to different areas of the hospital. This issue was mitigated by having three people, one to take blood samples from the patient, one to convey samples to HBRU and one to do the test. Conventional blood tests such as coagulation markers (PT/ APTT and Fibrinogen) samples are anticoagulated and then activated by adding reagents. This was the same for platelet aggregation and rotational thromboelastometry testing. Therefore, further research is required in this field to see whether anticoagulated blood can be used to perform rheological tests. As mentioned before the amount of blood required to do the rheological tests was 7ml, which is comparatively large when compared to other blood tests. Therefore, further research into reducing the size of the geometry should be considered. The stainless-steel geometry used was re-usable and require thorough cleaning under strict aseptic conditions. This was challenging during the pandemic; however, this issue was mitigated using PPE (Personal protective equipment) and additional infection control measures. Therefore, the development of a disposable geometry should be considered to reduce the risk of blood contamination to the operators.

The d_f was measured by setting the geometry at a standard temperature of 37^{o}C. It is a known fact that the coagulation pathways are very sensitive to changes in temperature. However, Lawrence et al. (2016) reported that there were significant changes to d_f only at temperature $\leq$32^{o}C and the mean temperature in the AECOPD group was 36.8^{o}C. Therefore, this might not have had any influence in the d_f measurements. To determine d_f, gel point was first interpreted from the graph. There is a possibility of an interpretation bias, which was mitigated by using two independent rheologists who were blinded to the study. Now-a-days most of the blood tests are automated to avoid this bias, therefore developing a fully automated machine should be considered to avoid human errors. Finally, the whole process requires substantial training and for d_f to be used widely and effectively in the future, there is a requirement to develop user-friendly interface. In addition, this will help to conduct a multi-centre study.

8.2 Conclusions and future studies

- COPD is a chronic inflammatory disease with progressive deterioration of airflow and impairment of gas exchange. Stable COPD patients have normal inflammatory and haemostasis markers. However, in the AECOPD group, there was significant increase in the inflammatory process leading to activation of coagulation pathways and thereby significant increase in fibrinogen.

- AECOPD patients had significantly high d_f at presentation to the Emergency Department when compared to SCOPD. This indicates that acute exacerbation increases thrombogenicity in COPD patients.

- There was no significant increase in d_f at subsequent blood sampling at 4-6 hours, 24 hours and 3-7days. This indicates that with appropriate treatment and thromboprophylaxis, there was no further increase in the thrombogenicity in the AECOPD group. This is evidenced by low incidences of VTE in the AECOPD group.

- Infection increases thrombogenicity in COPD patients. This was evidenced by significantly high d_f in the infective group when compared to the stable group. However, with appropriate treatment and thromboprophylaxis, there was no further increase in thrombogenicity evidenced by low incidence of VTE in the infective exacerbation group.

- Respiratory acidosis increases the thrombogenicity in COPD patients. This was evidenced by significantly high d_f in the acidotic group when compared to the stable group. However, appropriate treatment and thromboprophylaxis reduced further increase in thrombogenicity and the acidotic group had low incidence of VTE.

- AECOPD patients who died in the hospital had significantly high d_f when compared to those who survived indicating that these patients were thrombogenic. Even though not significant, VTE incidences were higher in patients who died. Binary regression analysis showed d_f as a significant predictor of mortality, however this study was not powered for outcome. Further studies are required to validate this finding.

- COPD patients admitted with exacerbation had significantly higher one-year mortality rate when compared to SCOPD (30% vs 13%). The in-hospital mortality rate with exacerbation was high (12%). This was further evidenced in the retrospective study of the same population which showed that one-year mortality was 35% and in-hospital mortality was 9%. This demonstrates that COPD patients should adhere to treatment avoiding exacerbations.

- There was increase in d_f with severity measured by FEV1 in both SCOPD and AECOPD, therefore d_f could be used as a biomarker for COPD severity. A further adequately powered study is required to validate this finding.

- There is a potential to undertake a large multicentre adequately powered study recruiting AECOPD patients to answer some of the questions raised in the above conclusion.

Appendices

Appendix A

Courses attended as part of the PhD

Date	Course	Organiser
22/11/2017	Introduction to Statistics Using SPSS	Imperial College London
09/01/2018 - 10/01/2018	Data Management & Statistical Analysis Using SPSS	Imperial College London
27/02/2018- 28/02/2018	Basic Rheology Course	TA Instruments
03/04/2019	How to write a research paper	Swansea University
18/08/2021	Submission to award meeting	Swansea University

Appendix B

Thesis abstracts and publications

Abstracts in conferences

1. 2023: '*Does clot microstructure (d_f) predict mortality in acute exacerbation of mortality in Covid-19 patients'*. Abstract accepted as poster, International Symposium in Intensive Care and Emergency Medicine, Brussels- 2023

2. 2023: '*Low FEV1 is associated with increased mortality in acute exacerbation of COPD (AECOPD) in one year'*. Abstract accepted as poster, International Symposium in Intensive Care and Emergency Medicine, Brussels- 2023

3. 2020: '*Fractal Dimension as a Biomarker of Thrombogenicity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)*'. Swansea University Medical School PGR Conference. **Abstract winner** for 2nd year.

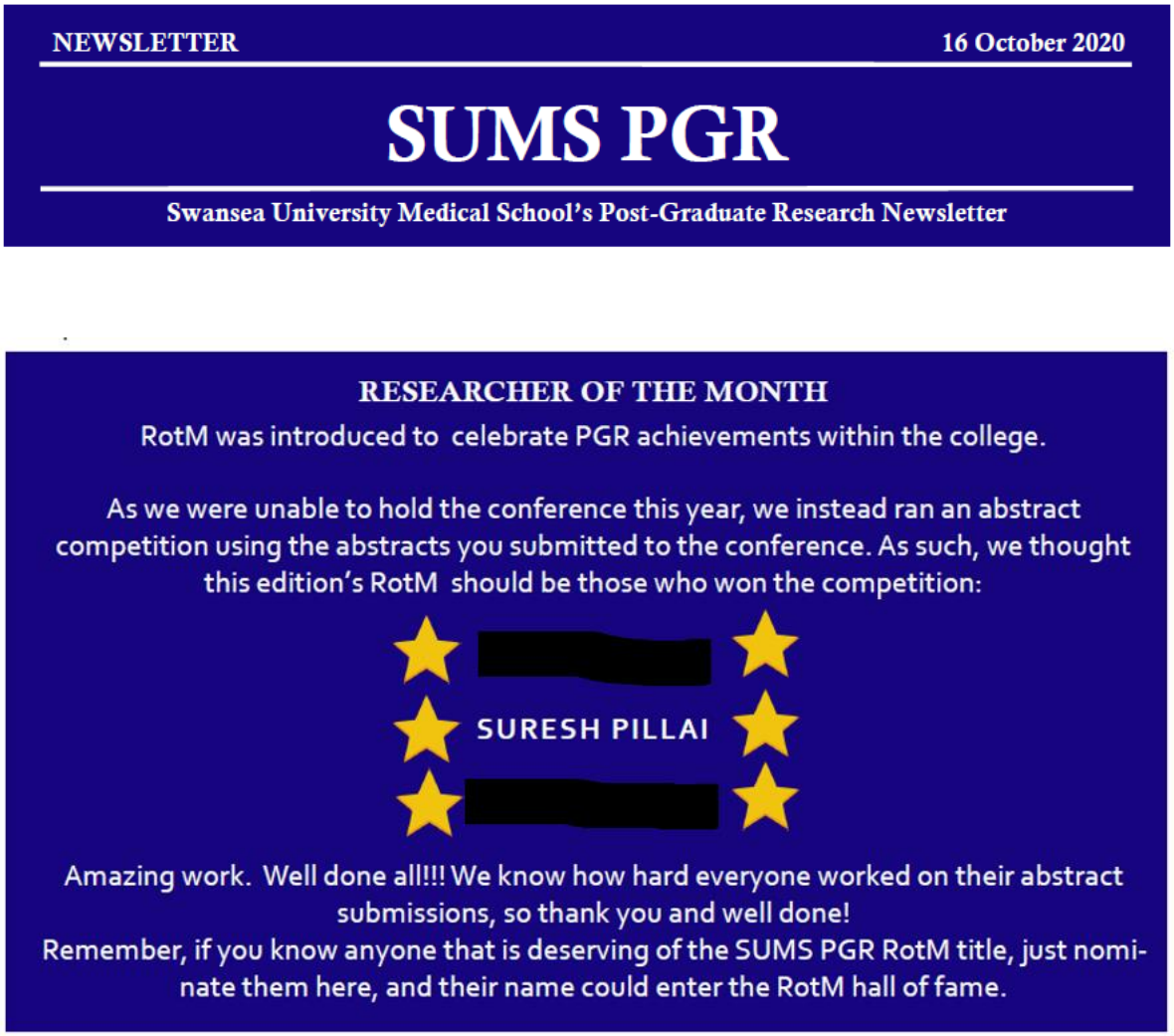
4. 2019: '*Fractal Dimension as a Biomarker of Thrombogenicity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)*'. Swansea University Medical School PGR Conference. Presented the to-date findings of the study.

International published proceedings:

1. **Pillai, S.**, Lawrence, M., Zaldua, J. C., Whitley, J., Watson, O., Howard, M., Harrison, K., Hawkins, K., Morris, K., Evans, P. A. (2022). Relationship between the procalcitonin levels and clot microstructure in acute exacerbation of chronic obstructive pulmonary disease (AECOPD). *Critical Care*, 26(Suppl 1): P067

2. **Pillai, S.**, Zaldua, J. C., Lawrence, M., Whitley, T., Howard, M., Watson, O., Harrison, K., Hawkins, K., Morris, K., Evans, P. A. (2022). Clot microstructure (df) as a biomarker and measurement of thrombogenicity in acute exacerbation of chronic obstructive pulmonary disease (AECOPD). *Critical Care*, 26(Suppl 1): P072

3. **Pillai, S.**, Lawrence, M., Whitley, J., Harrison, K., Evans, E., Mughal, A., Hilldrup, S., Williams, P. R., Morris, K., Evans, P. A. (2020). The underlying inflammatory effect of stable COPD (chronic obstructive pulmonary disease) patient on thrombogenicity and clot microstructure. *Critical Care*, 24(Suppl 1): P121



NEWSLETTER 16 October 2020

SUMS PGR

Swansea University Medical School's Post-Graduate Research Newsletter

RESEARCHER OF THE MONTH

RotM was introduced to celebrate PGR achievements within the college.

As we were unable to hold the conference this year, we instead ran an abstract competition using the abstracts you submitted to the conference. As such, we thought this edition's RotM should be those who won the competition:

[redacted]

SURESH PILLAI

[redacted]

Amazing work. Well done all!!! We know how hard everyone worked on their abstract submissions, so thank you and well done!

Remember, if you know anyone that is deserving of the SUMS PGR RotM title, just nominate them here, and their name could enter the RotM hall of fame.

Appendix C

Retrospective study approval from Swansea Bay University Health Board

Suresh Kumar Gopala Pillai (Swansea Bay UHB - Emergency & Intensive Care Medicine)

From: Anne-Claire Owen (ABM ULHB - Research & Development Department)
Sent: 12 November 2018 16:03
To: Suresh Kumar Gopala Pillai (ABM ULHB - Emergency & Intensive Care Medicine)
Subject: Project: Venous thromboembolism in acute exacerbation of chronic obstructive pulmonary disease (AECOPD)

Project: Venous thromboembolism in acute exacerbation of chronic obstructive pulmonary disease (AECOPD)

Dear Suresh

The above project was reviewed at the Joint Study Review Committee (JSRC) that met on 7 November 2018, under the agenda item for projects requesting ratification as a 'non-research', not requiring a NHS REC or NHS R&D application. Based on the information provided the committee agreed that the project could be classed as 'non-research'/service evaluation, and agreed that neither a NHS REC nor NHS R&D application is required.

Please ensure you obtain appropriate directorate permissions and in publishing your results, please confirm that the project was ratified as 'non-research'/ service evaluation.

Good luck with your project.

Best wishes
Anne-Claire

Anne-Claire Owen
Assistant Manager | Research & Development | ABMU Health Board
Rheolwr Cynorthwyol | Ymchwil a Datblygu | Bwrdd Iechyd PABM

Abertawe Bro Morgannwg University Health Board / Bwrdd Iechyd Prifysgol Abertawe Bro Morgannwg
[redacted] / Athrofa Gwyddor Bywyd 2
Swansea University / Prifysgol Abertawe
Singleton Park / Parc Singleton
SA2 8PP

[redacted]

From Monday 16 April 2018, the submission process for studies and amendments in Wales is changing. Read more here.

R&D RESEARCH & DEVELOPMENT Abertawe Bro Morgannwg University Health Board

Ymchwil Iechyd a Gofal Cymru Health and Care Research Wales

We constantly strive to improve our services and value your feedback. We'd really like to hear from you and your responses will, of course, remain confidential and you won't be identified in any results. Please click on this link to leave your feedback: www.healthandcareresearch.gov.wales/your-views/

Appendix D

Protocol for the prospective study- version 5

STUDY PROTOCOL

Version 5 (12/07/2017)

Title of the study	**Fractal Dimension as a Biomarker of Thrombogenicity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)**
Name of candidate	**Dr Suresh Kumar Gopala Pillai** MBBS, FRCP, FCEM, EDIC, FFICM PgDip Medical Toxicology, MSc Critical Care Consultant in Emergency & Intensive Care Medicine Morriston Hospital, Swansea
Name of supervisors	**Professor Phillip A Evans** MBBS, MD, FRCS, FFAEM Professor of Emergency Medicine and Haemostasis **Dr Kim Harrison** Associate Professor in Diffuse Parenchymal Lung Disease Morriston Hospital
Student number	**885116- 6 year part time PhD** 1st October 2016 to 30th September 2022

STUDY PROTOCOL

Version 5 (12/07/2017)

Summary/Abstract:

Chronic obstructive pulmonary disease (COPD) is characterised by non-reversible airflow obstruction to the lungs caused by chronic bronchitis and emphysema. About one million people suffer from COPD in the United Kingdom and there are approximately 25,000 deaths every year as a consequence. The predominant cause of COPD is cigarettes smoking although up to 15% of cases are caused by occupational exposure to industrial dusts and fumes. COPD costs National Health Service (NHS) nearly £800 million annually. People who suffer from COPD have two to four times increased mortality from ischaemic heart disease and one fourth of patients develop blood clot in the lungs (pulmonary embolism) particularly during acute exacerbations. This may be due to an increased systemic inflammatory response, dehydration and immobility. Conventional biomarkers of coagulation have been proved to be ineffective in quantifying the thrombotic risk in a number of diseases such as cancer, stroke and myocardial infarction. Fractal dimension (d_f) is a new biomarker of coagulation that has been shown to quantify the global pathological effects of abnormalities of coagulation and thrombolysis on clot microstructure. The aim of the study is to demonstrate whether the tendency of blood clot formation differs between patients with stable COPD and patients with acute exacerbation of COPD (AECOPD). Patients aged 35 and above with a confirmed diagnosis of COPD as defined by GOLD criteria (Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease [GOLD] 2016) will be identified by the direct care respiratory team and referred to the research team. The study will be conducted in Haemostasis Biomedical Research Unit (HBRU), Morriston hospital. The study aims to recruite approximately 230 patients. Blood samples will be taken from the participants and the thrombogenicity will be assessed using the conventional biomarkers and the new biomarker fractal dimension (d_f).

STUDY PROTOCOL

Version 5 (12/07/2017)

Background:

Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by inflammation in the airways (chronic bronchitis) and destruction of the alveolar walls with resultant loss of elastin (emphysema) which normally maintains airway patency particularly during expiration. About one million people suffer from COPD in the United Kingdom and there are approximately 25,000 deaths every year as a consequence[1]. The predominant cause of COPD is cigarettes smoking although up to 15% of cases are caused by occupational exposure to industrial dusts and fumes[1]. Chronic obstructive pulmonary disease also causes significant burden in terms of disability and impaired quality of life and has an annual cost to the NHS nearly £800 million[2].

People who suffer from COPD have two to four times increased mortality from ischaemic heart disease[3] and one fourth of patients develop pulmonary embolism particularly during exacerbations[4]. In the later stages of disease, people with COPD increasingly present to the Emergency Department (ED) with acute exacerbations that can be infective or non-infective. Factors which might contribute to the increased athero-thrombotic state in COPD include systemic inflammation, platelet activation, oxidative stress[8] and abnormal coagulation. Indeed there is evidence for increased levels of tissue factor- procoagulant activity[5] fibrinogen and factor XIII activity[6]. Furthermore, it is known that fibrin clots in people with COPD are less permeable, more compact and less susceptible to lysis[7]. This thrombotic state is likely increased during acute exacerbations because both dehydration and increased systemic inflammatory response will result in increased viscosity and alterations of blood flow. However this enhanced thrombogenicity and its relationship to thrombotic events is difficult to assess and quantify objectively using conventional biomarkers[9] and many thromboembolic events go undetected at the time of presentation to the Emergency Department. Consequently, the precise rate and risk of thromboembolic events people with AECOPD is uncertain.

Previous research has attempted to assess the level of thromboembolic risk by determining standard markers of coagulation and relating this to images of clot gained from optical techniques such as scanning electron microscopy (SEM)[10]. However, conventional coagulation tests such as the prothrombin time (PT) and activated partial thromboplastin time (APTT) examine only the coagulation factors (intrinsic and extrinsic pathways) but do not take account of abnormalities of platelet number of function. Furthermore, optical methods of visualising clot structure rely on static techniques such as SEM which provide only semi-quantitative measures of clot formed *in vitro*. They are also expensive, time consuming and cannot be undertaken at the bedside. Thus, whilst studies have attempted to relate the kinetics of change in clotting pathways to clot structure, they have limited clinical application and the results are at best of theoretical scientific interest[11]. There is therefore a pressing clinical need to develop a simple and rapid method of assessing thrombogenicity in AECOPD close to the bedside in order to identify those most at risk initiate appropriate treatment.

Abnormal clot development and microstructure are thought to be important pathophysiological features of acute inflammatory vascular disease such as myocardial infarction and stroke. The observation that a new biomarker which uses the microstructure of incipient clot *in vitro*, to assess global risk of thrombogenicity *in vivo*, suggests it may be of clinical value in identifying people with COPD who are at greatest risk of thromboembolism [13,14,15]. Recent studies using optical and turbidity measures have also observed a change in the fibrin fibre length and density which is altered in different pathological conditions[11].

Background to the new biomarker.

Fractal dimension- d_f is a new biomarker of coagulation that has been shown to quantify the global pathological effects of abnormalities of coagulation and thrombolysis on clot microstructure. This new biomarker utilises the point at which blood changes state from behaving as a viscoelastic liquid to a solid known as the Gel Point (GP). The GP is composed of two parameters; Gel Time (Tgel) and Fractal Dimension (d_f). The gel time is the kinetic aspect of pathway change and has been

shown to directly relate to the structural component of clot formation, namely fibrin polymerisation and assembly[12,13,14,15].
As 80% of the structural properties of fibrin clot are determined by its branching fractal network, a biomarker which quantifies this component might give valuable new information which might help predict risk of future thromboembolic events[16].

Aims

Given the evidence presented herein, the aim of this study is to answer the following specific questions:

1. To investigate clot microstructure in AECOPD compared to stable COPD: We hypothesise that AECOPD will have an increase in d_f compared to stable COPD. We will recruit 30 AECOPD and compare with 30 stable COPD patients. This is based on the power calculation below in statistical analysis (a).

2. Investigate effect of treatment and disease progression in AECOPD: We aim to investigate the effect of treatment and progression of disease in AECOPD. We will continue to recruit patients past the initial 30. The power calculation [see below in statistical analysis (b)] suggest to undertake a multiple comparison with the expected difference over four time points would necessitate a minimum of 51 subject to satisfy this aim.

3. Relationship between d_f and other standard markers of thrombogenicity: We will investigate the relationship between d_f and other standard markers (atleast 12) using correlation and multi-regression analysis. Hence although our power calculation suggests a minimum of 51 for this part of the study, we will in fact recruit a much larger numbers of a minimum of 150 to 200 subjects allowing as to undertake a more detailed analysis detailed below namely multi regression and correlation. Please see statistical analysis (c).

By answering these questions it is hoped that we can provide evidence that d_f might act as a biomarker for thrombogenic risk in patients with AECOPD.

Inclusion criteria

1. Patients aged 35 and above with a confirmed diagnosis of COPD as defined by GOLD criteria (Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016)[17]

Exclusion criteria

1. Patients receiving any medical treatment that could affect coagulation (anticoagulant therapy such as Warfarin, Heparin, Rivaroxaban, Dabigatran and Apixaban or any other anticoagulants)
2. Any disease process known to alter coagulation (i.e. liver disease, kidney disease, genetic disorders, cancer)
3. Previous vascular events

Materials and methods:

Ethical considerations

This study aims to recruit two groups of patients: firstly a group of patients with COPD who are clinically stable (Stable COPD group) and secondly, patients undergoing an acute exacerbation of COPD (AECOPD group).

a. The stable COPD patients will be recruited from the Chest Clinic and Pulmonary Rehabilitation programme at Morriston Hospital. Those who appears suitable will be identified by the Consultant Chest Physicians. They will then refer these patients to the Research Team who will be present in the Chest Clinic. The Research Team will offer the patients with participant information sheet (PIS) and allow them as much time

as they wish to ask questions (minimum of 24 hours) and decide if they wish to participate. If they agree, they will be asked to sign their consent, which will be witnessed by an independent Respiratory Nurse Specialist. Once they have given the consent, a single blood sample (up to a maximum of 30ml) will be obtained from this group. Patients will be given an option to opt out at any stage during the study.

b. The AECOPD patient group will be recruited from the populations who present to the Morriston Hospital Emergency Department requiring emergency treatment. About 455 patients attended Morriston Emergency Department with AECOPD in 2016. We hypothesise that this group has got increased tendency for blood clot formation during exacerbations. Therefore 24- hour period required for consenting cannot be applied. These patients will be identified by Consultants in Emergency Medicine who will refer these patients to the Research Team. The Research Team will then approach the patients, enough time will be given to read the PIS and then consent will be obtained. Four blood samples (up to maximum of 30mls each time) will be then collected at 0 hours, 4-6 hours, 24-36 hours and 3-7days.There may be occasions when patients present with confusion due to low oxygen and high carbon dioxide (type 2 respiratory failure). Furthermore, some may present with severe respiratory failure requiring anaesthesia and mechanical ventilation. Under these circumstances, consent cannot be obtained and an assent (presumed consent) will be obtained from the direct care team or family. When the patients recover from the acute illness, then an appropriate consent will be obtained. If they don't recover or lacks capacity, then assent stays unless there is an objection from the direct care team or family. There might be a possibility that some of the patients may die of the illness and if four blood samples at four time points are obtained then these patients will remain in the study otherwise will be excluded.

c. Consent and capacity: Patients should be able to understand information about the decision, remember that information, use that information to make a decision, communicate their decision by talking, using sign language or by any other means. If patients cannot do any of the above, then they lack capacity.

d. Justification of including adults lacking capacity in the study: We hypothesise that the new biomarker *dr* increases with increase in severity of COPD. Therefore patients who are most unwell are likely to have the biggest change in abnormal clot microstructure due to the severity of the inflammatory response. The patients with the most severe form of disease are more likely to lack capacity. We would therefore wish to obtain the full spectrum of the disease. If these patients are excluded then it will negatively impact on the spectrum of intensity of the inflammatory response of participants in the study.

e. Consent and freedom to participate: All patients will be provided with a Participant Information Sheet (PIS) and a copy of the consent from explaining that they can opt out at any time after they consent. The main clinician providing the care will not be involved in obtaining consent to avoid any conflict of interests between providing patient care and research purposes.

f. The study is observational and will not influence the patient's routine care, and only involves taking extra blood and therefore is of very low risk. It is hoped, wherever possible, that blood sample for the study will be taken at the same time as blood required for clinical purposes to minimise the number of venepunctures. Participants are therefore consenting to an additional amount of blood being taken, which is a routine low risk procedure. Venesection can cause some discomfort. All members of the research team involved in venesection are experienced and trained in performing this procedure.

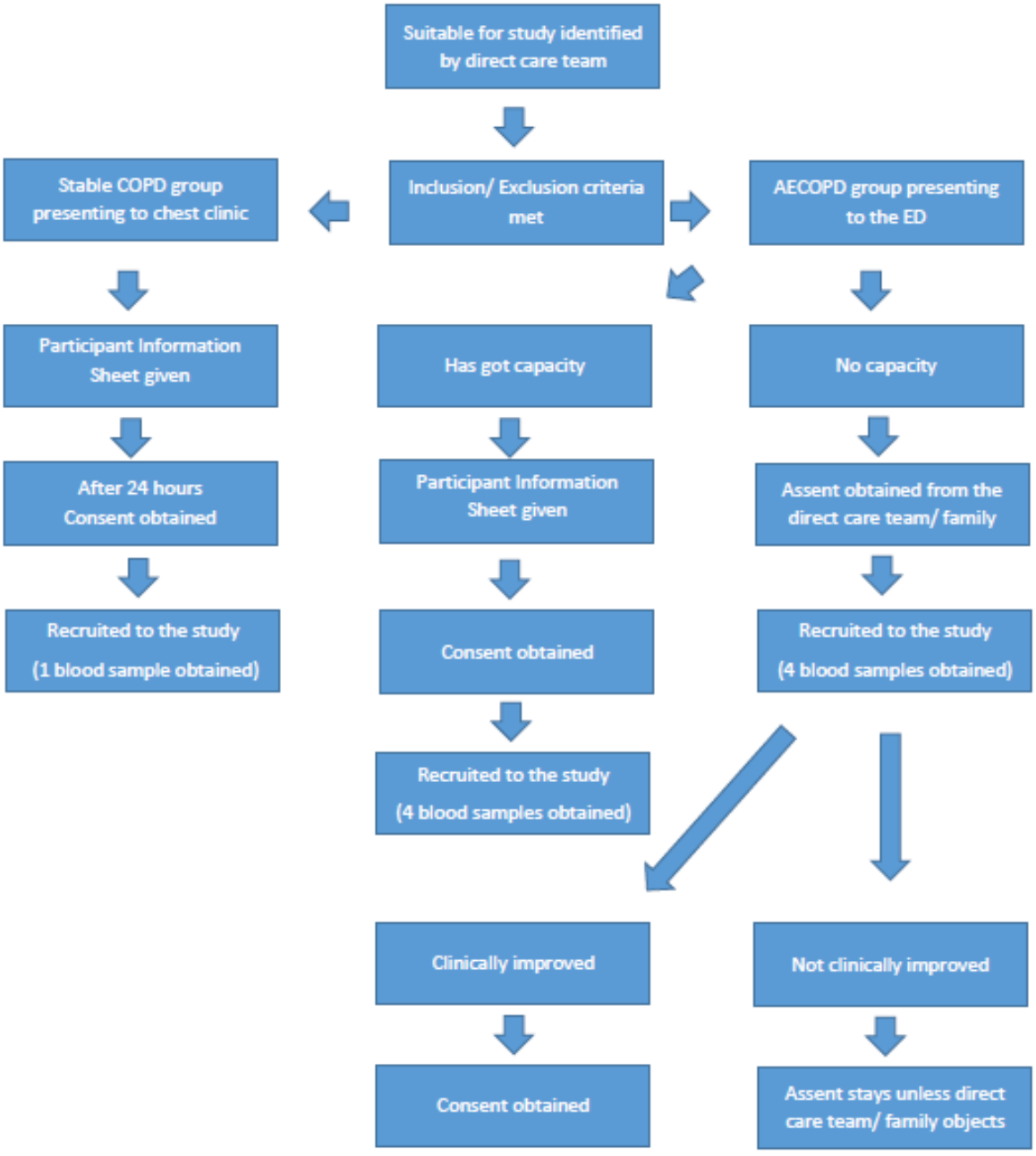
g. Confidentiality: All information collected will be held in confidence within the standardised case report form (CRF) and stored only on NHS computers that has security features in-line with the Hospital policy. All research staff involved in this study are GCP trained and are aware of their ethical and legal obligations about maintaining the confidentiality of the data collected about the research subjects.

g. Abnormal blood results in patients with stable COPD: clinically relevant anomalous results (e.g. anaemia) will be communicated with their GP. This is explained in the

Patient Information Sheet and consent will be sought to inform their GP if there are any incidental abnormalities of routine blood testing.

h. The ultimate aim of the study is to apply the findings to clinical settings. This work will need a relatively large volume of blood (30ml). Hence we will continue to collaborate with our colleagues from the College of Engineering to optimise the technique and reduce the amount of blood needed.

Consent policy



Methods

This is a prospective observation study conducted in the Hemostasis and Biomedical Research Unit (HBRU), Morriston Hospital, Swansea. The site has been awarded a grant for research by the National Institute for Social Care and Health Research (NISCHR) on the basis of its well-structured integrated research care pathway. It will be undertaken in collaboration with the Respiratory Unit, Morriston Hospital.

We intend to recruit patients in two groups.

1. Stable COPD patients

Screening and Informed Consent procedures:

The stable COPD patients will be recruited from the Chest Clinic and Pulmonary Rehabilitation programme at Morriston Hospital. Those who appears suitable will be identified by the Consultant Chest Physicians. They will then refer these patients to the Research Team who will be present in the Chest Clinic. The Research Team will offer the patients with participant information sheet and allow them as much time as they wish to ask questions and decide if they wish to participate. If they agree, they will be asked to sign their consent, which will be witnessed by an independent Respiratory Nurse Specialist. Once they have given the consent, a single blood sample (up to a maximum of 30ml) will be obtained from this group. Patients will be given an option to opt out at any stage during the study.

Data collection and blood sampling:

Once informed written consent has been obtained, one blood sample (up to maximum of 30 ml) will be collected. At the same time demographic and clinical data will be recorded on a standardized case report form (CRF).

2. Acute exacerbation of COPD (AECOPD) patients

Screening and Informed Consent procedures:

The AECOPD patient group will be recruited from the populations who present to the Morriston Hospital Emergency Department requiring emergency treatment. We hypothesise that this group has got increased tendency for blood clot formation during exacerbations. Therefore 24- hour period required for consenting cannot be applied. These patients will be identified by Consultants in Emergency Medicine who will refer these patients to the Research Team. The Research Team will then approach the patients, enough time will be given to read the PIS and then consent will be obtained. Four blood samples (up to maximum of 30mls each time) will be collected at 0 hours, 4-6 hours, 24-36 hours and 3-7days.There may be occasions when patients present with confusion due to low oxygen and high carbon dioxide (type 2 respiratory failure). Furthermore, some may present with severe respiratory failure requiring anaesthesia and mechanical ventilation. Under these circumstances, consent cannot be obtained and an assent (presumed consent) will be obtained from the direct care team (Emergency Physicians) or the family. When the patients recover from the acute illness, then an appropriate consent will be obtained. If they don't recover or lacks capacity, then assent stays unless there is an objection from the direct care came or the family. There might be a possibility that some of the patients may die of the illness and if four blood samples at four time points are obtained then these patients will remain in the study otherwise will be excluded.

Data collection and blood sampling:

Once informed written consent or presumed consent (assent) has been obtained, one blood sample (up to maximum of 30 ml) will be collected from each patient. At the same time demographic and clinical data will be recorded on a standardised case report form (CRF). These patients will be admitted either to a ward bed or the Intensive Care Unit (ICU) at Morriston Hospital. A further 3 samples (up to maximum of 30mls) will then be taken at 4-6 hours, 24-36 hours and 3-7 days will be collected.

Blood will be sampled from each patient using a Vacutainer® Multiple Sample Needle. The first 3-5 mls will be discarded to avoid alteration in blood coagulation due to the formation of micro blood clots or dilution. Whole venous blood will be transferred immediately for rheometric analysis and further samples subsequently transferred into a 3.5 ml vacuum-sealed tube containing sodium citrate (3.2%) and a 4.0 ml vacuum sealed K2EDTA tube (Greiner Bio-one, Stonehouse, UK) and the following tests carried out:

Rheometric analysis (7ml): The biomarkers (TGP, G`GP & df) will be determined from the measurement of viscoelastic properties at the GP of clotting blood as previously described12 ,13, 14, 15. A 6.6 ml sample of whole venous blood will be loaded into a double-gap concentric cylinder measuring geometry of a controlled-stress rheometer (TA Instruments, New Castle, DE, USA) at 37°C ± 0.1°C in a near-patient setting. Rheometric sampling will be carried out by named researchers who are appropriately trained and experienced in rheometric analysis. To eliminate bias and enhance accuracy, data will be anonymised and analysed independently by three haemorheologists blinded to the sample origin.

Scanning Electron and Confocal Microscopy: To relate changes in df of the incipient clot to microstructure of the mature clot, microscopy analysis will be carried out by colleagues in the Perelman School of Medicine, University of Pennsylvania who have extensive experience in microscopy of blood and plasma samples. 12µl of whole blood will be allowed to clot for at least 15 minutes at 37oC prior to washing with cacodylate buffer and fixing with gluteraldehyde. Point critical dehydration with ethanol (30-100%) and hexamethyldisilazane (Sigma Aldrich, UK) will then be carried out prior to coating of samples with gold palladium and imaging on a Hitachi Ultra-high resolution FE-SEM S-4800. Fibrinogen fibre widths will then be calculated using a previously published method. Citrated plasma aliquots will also be stored and transported appropriately to the University of Pennsylvania where it will be re-activated by addition of CaCl2 and thrombin to form plasma fibrin clots and analysed by confocal microscopy as previously described.

Laboratory Markers (15.5ml): PT, APTT and Clauss fibrinogen will be determined using a Sysmex CA1500 analyser within 2 hrs of collection. A full blood count will also be determined using a Sysmex XE2100 automated haematology analyser, within 2 hours of collection. All reagents will be obtained from Siemens, (Frimley, UK) and the analyzer will be calibrated according to manufacturer's instructions. Plasma aliquots will be stored at -80oC for retrospective analysis of PAI-1 and Tissue Factor Microparticles by ELISA (Hyphen Biomed, Quadratech, Epsom, UK). D-Dimer analysis will be carried out using Latex immunoturbidimetric assay (Instrumentation Laboratory, Warrington, UK). Inflammatory markers Procalcitonin and C- reactive protein (CRP), TNF-α, IL6, IL8 and e-Selectin will be determined using the appropriate ELIZA kit assay. Coagulation factors such as Factor VIII and Factor XIII will also be determined.

Factor VIII will be determined by an APTT based one-stage assay using appropriate factor deficient plasma and Actin FS APTT reagent (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany).

STATISTICAL ANALYSIS

This study is based on the hypothesis that d_f may act as a biomarker of thrombogenicity in AECOPD.

a. We will determine if a significant difference in d_f and other markers of coagulation and inflammation exists between patients with AECOPD on admission to the Emergency Department as compared with those with stable COPD who presents to the chest clinic. From data collected from a sepsis and acute inflammatory study and from a range of other studies involving acute exacerbation of inflammatory diseases, we would expect AECOPD patients to have a mean d_f of 1.79 (±SD 0.06). Assuming that stable COPD patients have *df* similar to healthy individuals which we know is 1.73 (±SD 0.04). Using a 2-sample t-test to detect a difference in these two groups with α= 0.05, and a power of 0.8, the mean difference of 0.05 and a combined SD 0.06, the number needed to recruit will be a minimum of 25 and recruitment will stop with 30

subjects in each group to undertake the comparison. At this juncture recruitment of stable COPD will stop, but we will continue recruiting AECOPD patients to satisfy Aim 2.

b. We will determine if a significant difference in d_f occurs in patients with AECOPD during standard treatment and if changes in d_f relate to standard markers of haemostasis and inflammation collected at the same time. Primarily we aim to determine if significant changes in d_f occurs at the 4 time-points in this study (on diagnosis, 4-6 hours where the patient had received treatment, 24 hours and 3-7 days) using one-way ANOVA, as d_f is a normally distributed variable. Given our previous data on the response of d_f to a wide-range of therapeutic interventions and resuscitation methods, that d_f will change by 0.04 with a SD of 0.06. Using $\alpha = 0.05$, power of 0.8 and one-way ANOVA to detect within group differences at four levels, a minimum of 51 subjects would be required for this part of the study. A Bonferroni correction will be used to determine where true significance arises during these multiple comparisons. Given the need to satisfy the aim 3, a much larger number than this will be required for this part of the study.

c. We will also wish to investigate the changes in d_f are related to other markers of haemostasis, thrombogenicity and microstructure such as PT, APTT, Fibrinogen, D-dimer, Multiplate, CRP, Procalcitonin, Microparticles, Factor VIII, Factor XIII and FBC (in total 12 markers) all four time points. We will investigate for significant associations and relationships between d_f and these markers of haemostasis and inflammation using multiple-regression and correlation analysis. Some of these parameters e.g D-dimer are non-normally distributed and either data transformation (e.g. logarithmic) will be used or in the case of correlation analysis, Spearman's method will be utilised. Given the detailed multi-regression and correlation analysis we wish to undertake in AECOPD subjects and the availability of large numbers of AECOPD patients during the time of this study, we will recruit a minimum of 150 but no more than 200 patients that will be used to satisfy aim c on the basis of atleast 10 individuals per variable. Throughout this regression analysis we will confirm the presence of multicollinearity using the Variance Inflation Method.

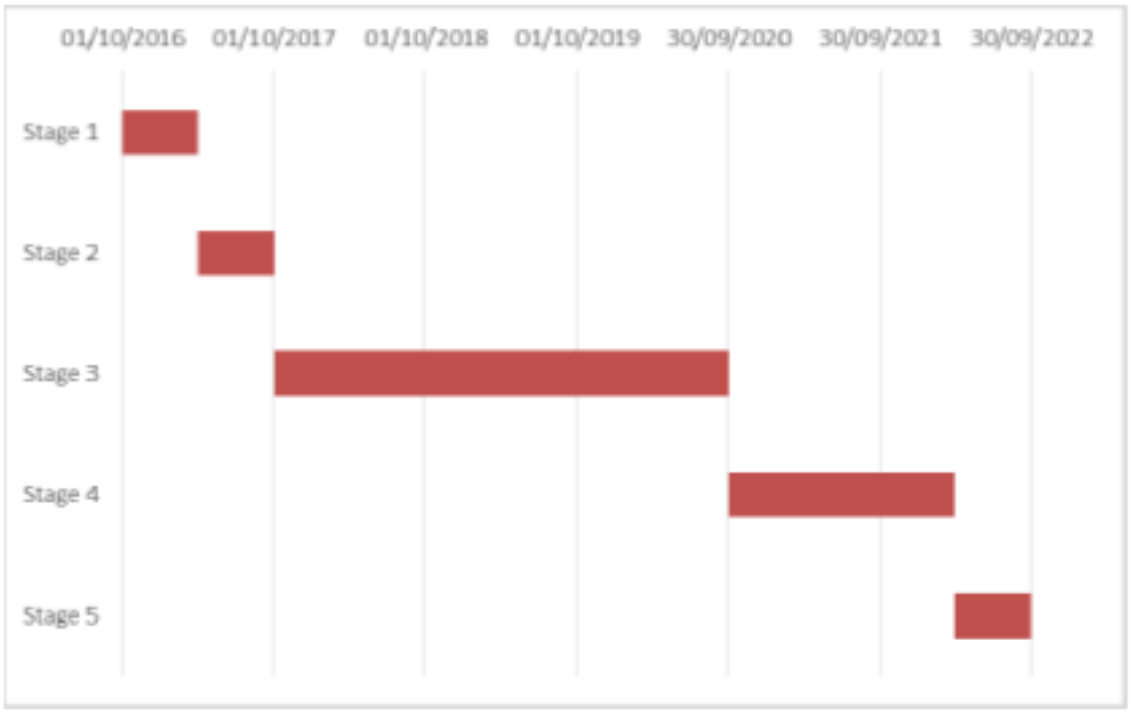
Impact:

There is no direct benefit to the research participants. By answering these questions it is hoped that we can provide evidence that df might act as a biomarker for thrombogenic risk in patients with AECOPD which may potentially improve outcomes and care.

Research Plan:

Stage 1 (6 months)	Ethical application, co-ordinating the research, informing the staff in Emergency Department (ED) and displaying the notice, liaising with the Chest Clinic
Stage 2 (6 months)	Learning rheological techniques, haemostatic techniques, rotem, multiplate, light transmission aggregometry
Stage 3 (36 months)	Recruiting the 2 group of patients, stable COPD patients presenting to the chest clinic (n=30) and AECOPD patients from the Emergency Department (n~170)
Stage 4 (18 months)	Writing up the thesis
Stage 5 (6 months)	Submission of the thesis

GANTT chart


01/10/2016
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01/10/2019
30/09/2020
30/09/2021
30/09/2022
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5

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Appendix E
Consent form

Centre Number:
Study Number:
Participant Identification Number for this study:

Consent Form

Title of Project: Fractal Dimension as a Biomarker of Thrombogenecity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)

Name of Researcher: Prof. Phillip Adrian Evans

Please initial box

1. I confirm that I have read and understand the Participant Information Sheet dated 23/06/2017 (version 5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

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

3. I understand that relevant sections of any of my medical notes and data collected during the study, maybe looked at by responsible individuals from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study and any abnormal blood results of clinical significance to be communicated to my GP. ☐

5. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

Appendix F

Participant Information sheet

HAEMOSTASIS BIOMEDICAL RESEARCH UNIT

MORRISTON HOSPITAL

STUDY TITLE: FRACTAL DIMENSION AS A BIOMARKER OF THROMBOGENECITY IN ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (AECOPD)

PARTICIPANT INFORMATION SHEET

We are currently developing a new method of measuring blood clotting. We have applied the latest techniques from the engineering field to medicine to understand the basics of blood coagulation. The study has already indicated that this new method can produce more sensitive, accurate and reproducible measurements of how normal blood clots, as compared to conventional methods used. This new method should help us to detect certain illness earlier and more accurately. However to validate our findings we need blood samples from a range of individuals.

Who has reviewed this study?
This study was reviewed by the Research Ethics Committee, Wales REC 6 (formerly South West Wales Research Ethics Committee)

What will be involved in taking part in the study?

If you are willing to take part in the study we will take 30 mls of blood (2 tablespoons) from you on upto four different occasions. This volume of blood is so small that it will not affect the overall system. Although the majority of participants would undergo only a single venepuncture, some may require upto to four samples taken at different time points. A senior experienced doctor, who takes blood routinely, will take the sample. The needle will make a small scratch on the arm.

What will my blood be used for?

We intend to recruit patients with different degrees of chest disease. This blood will be rapidly placed in our machine, which we hope will give us new information on how blood clots.
Some of the blood will be sent to the haematology laboratory for independent routine clotting tests. The results from this routine testing will be used to compare the new technique against conventional tests.

Medical History

You may want to know why we are asking certain questions about the person's medical, social and family history. The reason for this is that there is generally some natural variation in how the blood clots. We know that many factors such as ethnic origin, diet, smoking etc can affect clotting slightly. If applicable this information would be useful to help us interpret our findings.

Confidentiality

All the information will be anonymous and only identifiable by a number in order to provide volunteer confidentiality. We will comply strictly with the Government guidelines regarding confidentiality.

What if I am harmed by the study?

The study should cause no harm whatsoever from the study as the bloods given and the investigation carried out are normally carried out regularly in the NHS in this country. If harm is caused due to someone's negligence, then there may be grounds for legal action.

What if there is any abnormality found in the blood?

If we find any abnormality from our tests we will discuss the findings with yourself in confidence, contact your GP with the results and have it referred to a specialist. As stated in the research protocol all results will be treated in the strictest confidence.

Can I withdraw from the study?

Should you wish to withdraw any of your study details or pull out of the study totally, at any time, you are free to do so.

Additional Information

Although you will have read the study details and patient information sheet you are welcome to ask the doctor any additional questions.

Independent contact for complaints procedure

Abertawe Bro Morgannwg Community Health Council
First Floor
Cimla Hospital
Neath SA11 3SU
Tel: 01639 683490

Contact Details

Should you have any further queries please do not hesitate to contact us on [redacted]

Version 5 23/06/2017

Appendix G

Assent form/ Consultee declaration form

Centre Number:
Study Number:
Participant Identification Number for this study:

Assent Form

Title of Project: Fractal Dimension as a Biomarker of Thrombogenecity in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD)

Name of Researcher: Prof. Phillip Adrian Evans

Please initial box

1. I ..... (clinician/ relative name) have been consulted about .....(patient name) participation in this research project. I have had the opportunity to ask questions about the study and understand what is involved. ☐

2. In my opinion he/she would have no objection to taking part in the above study. ☐

3. I understand that I can request he/she is withdrawn from the study at any time, without giving any reason and without his/her care or legal rights being affected. ☐

4. I understand that relevant sections of his/her care record and data collected during the study may be looked at by responsible individuals or from regulatory authorities, where it is relevant to their taking part in this research. ☐

5. I agree to their GP or other care professional being informed of their participation in the study. ☐

Name of Consultee	Date	Signature
Relationship to the Patient		
Name of person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

Appendix H

Consultee Information Sheet

HAEMOSTASIS BIOMEDICAL RESEARCH UNIT

MORRISTON HOSPITAL

STUDY TITLE: FRACTAL DIMENSION AS A BIOMARKER OF THROMBOGENECITY IN ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (AECOPD)

CONSULTEE INFORMATION SHEET

Introduction

We feel your relative/friend/patient is unable to decide for himself/herself whether to participate in this research.

To help decide if he/she should join the study, we would like to ask your opinion whether or not they would want to be involved. We would ask you to consider what you know of their wishes and feelings, and to consider their interests. Please let us know of any advance decisions they may have made about participating in research. These should take precedence.

If you decide your relative/friend/patient would have no objection to taking part we will ask you to read and sign the 'Assent Form'. We'll then give you a copy to keep. We will keep you fully informed during the study so you can let us know if you have any concerns or you think your relative/friend/patient should be withdrawn.

If you decide that your friend/relative/patient would not wish to take part it will not affect the standard of care they receive in any way.

If you are unsure about taking the role of consultee you may seek independent advice.

We will understand if you do not want to take on this responsibility.

The following information is the same as would have been provided to your relative/friend.

The Study

We are currently developing a new method of measuring blood clotting. We have applied the latest techniques from the Engineering field to Medicine to understand the basics of blood coagulation. The study has already indicated that this new method can produce more sensitive, accurate and reproducible measurements of how normal blood clots, as compared to conventional methods used. This new method should help us to detect certain illness earlier

and more accurately. However to validate our findings we need blood samples from a range of individuals.

Who has reviewed this study?

This study was reviewed by the Research Ethics Committee, Wales REC 6 (formerly South West Wales Research Ethics Committee)

What will be involved in taking part in the study?

If your relative/friend/patient is taking part in the study we will take 30 mls of blood (2 tablespoons) from him/her on upto four different occasions. This volume of blood is so small that it will not affect the overall system. Although the majority of participants would undergo only a single venepuncture, some may require upto to four samples taken at four different time points.

A senior experienced doctor, who takes blood routinely, will take the sample. The needle will make a small scratch on the arm.

What will the blood be used for?

We intend to recruit patients with different degrees of chest disease. This blood will be rapidly placed in our machine, which we hope will give us new information on how blood clots.

Some of the blood will be sent to the haematology laboratory for independent routine clotting tests. The results from this routine testing will be used to compare the new technique against conventional tests.

Medical History

You may want to know why we are asking certain questions about the person's medical, social and family history. The reason for this is that there is generally some natural variation in how the blood clots. We know that many factors such as ethnic origin, diet, smoking etc can affect clotting slightly. If applicable this information would be useful to help us interpret our findings.

Confidentiality

All the information will be anonymous and only identifiable by a number in order to provide volunteer confidentiality. We will comply strictly with the Government guidelines regarding confidentiality.

What if my friend/relative/patient harmed by the study?

The study should cause no harm whatsoever from the study as the bloods given and the investigation carried out are normally carried out regularly in the NHS in this country. If harm is caused due to someone's negligence, then there may be grounds for legal action.

What if there is any abnormality found in the blood?

If we find any abnormality from our tests we will discuss the findings with yourself in confidence, contact your GP with the results and have it referred to a specialist. As stated in the research protocol all results will be treated in the strictest confidence.

Can he/her withdraw from the study?

Should you wish to withdraw any of your study details or pull out of the study totally, at any time, you are free to do so.

Additional Information

Although you will have read the study details and patient information sheet you are welcome to ask the doctor any additional questions.

Independent contact for complaints procedure

Abertawe Bro Morgannwg Community Health Council
First Floor
Cimla Hospital
Neath SA11 3SU
Tel: 01639 683490

Contact Details

Should you have any further queries please do not hesitate to contact us on [redacted].

Appendix I

Permission email from Clinical Hemorheology and Microcirculation Journal to use figures in the thesis

Suresh Kumar Gopala Pillai (Swansea Bay UHB - Emergency & Intensive Care Medicine)

From: Suresh Kumar Gopala Pillai (Swansea Bay UHB - Emergency & Intensive Care Medicine)
Sent: 29 June 2022 11:07
To: Carry Koolbergen
Cc: Phillip Evans (Swansea Bay UHB - A & E)
Subject: RE: Question for the Journal Department

Thank you so much Carry. Much appreciate your help.

From: Carry Koolbergen <[redacted]>
Sent: 27 June 2022 12:02
To: Suresh Kumar Gopala Pillai (Swansea Bay UHB - Emergency & Intensive Care Medicine) <[redacted]>
Subject: RE: Question for the Journal Department

You don't often get email from c.koolbergen@iospress.nl. Learn why this is important

WARNING: This email originated from outside of NHS Wales. Do not open links or attachments unless you know the content is safe.

DOI: 10.3233/CH-201030
Citation: Clinical Hemorheology and Microcirculation, vol. 80, no. 2, pp. 139-151, 2022

Citation: Clinical Hemorheology and Microcirculation, vol. 38, no. 4, pp. 267-277, 2008
https://content.iospress.com/articles/clinical-hemorheology-and-microcirculation/ch1057

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Yours sincerely

Carry Koolbergen (Mrs.)
Contracts, Rights & Permissions Coordinator
Not in the office on Wednesdays

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From: Suresh Kumar Gopala Pillai (Swansea Bay UHB - Emergency & Intensive Care Medicine)
[mailto:[redacted]]
Sent: maandag 27 juni 2022 11:21
To: Carry Koolbergen <[redacted]
Cc: Phillip Evans (Swansea Bay UHB - A & E) <[redacted]
Subject: RE: Question for the Journal Department

Dear Carry,
Thanks for the email. I am undertaking PhD with Professor P A Evans. I am also Deputy Director for the Welsh Centre for Emergency Medicine research.

I would like to use the following figures please,

1. Evans VJ, Lawrence M, Whitley J, Johns C, **Pillai S**, Hawkins K, Power K, Morris K, Williams PR, Evans PA. The treatment effect of rivaroxaban on clot characteristics in patients who present acutely with first time deep vein thrombosis. Clin Hemorheol Microcirc. 2022;80(2):139-151. Would like to use Figure 1A and 1B on page number 142 please.
2. Evans PA, Hawkins K, Lawrence M, Barrow MS, Williams PR, Williams RL. Studies of whole blood coagulation by oscillatory shear, thromboelastography and free oscillation rheometry. Clin Hemorheol Microcirc. 2008;38(4):267-77. PMID: 18334781. I am not an author on this paper, but have asked Professor PA Evans. Would like to use Figure 1 on page number 270 please.

Thanks
Suresh

Dr Suresh Kumar Gopala Pillai
MBBS, FRCP, FCEM, EDIC, FFICM
MSc Critical Care, PgDip Medical Toxicology
Consultant in Emergency & Intensive Care Medicine, Morriston Hospital
Vice President and Academic lead, Royal College of Emergency Medicine, Wales
Deputy Director and Director for Clinical Studies, Welsh Centre for Emergency Medicine Research
Honorary Senior Lecturer, Swansea University

From: Carry Koolbergen <[redacted]>
Sent: 27 June 2022 05:44
To: Suresh Kumar Gopala Pillai (Swansea Bay UHB - Emergency & Intensive Care Medicine)
[redacted]
Subject: RE: Question for the Journal Department

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