



STRATEGIES TO DELAY PROGRESSION OF
ALPHA-1 ANTITRYPSIN DEFICIENCY RELATED
LUNG DISEASE

by

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ABSTRACT

Alpha-1 antitrypsin deficiency (AATD) is a major risk factor for chronic obstructive pulmonary disease (COPD) and specifically development of pulmonary emphysema at an accelerated rate.

The strong emphysema phenotype associated with AATD related lung disease would suggest that interventions which reduce emphysema decline are logical approaches. Such therapies are much needed with a paucity of new treatments in the last three decades. This thesis explores interventions that target two models of emphysema progression; 1) accelerated lung destruction from pulmonary exacerbations and 2) overall basal elastin degradation rate.

As with usual COPD, individuals with AATD often suffer with periodic worsening of symptoms known as exacerbations. Exacerbations contribute to reduced quality of life (QOL) for individual patients and are a significant financial and resource burden. They also accelerate emphysema decline. Most exacerbations present with a combination of breathlessness, increased sputum production and increased sputum purulence but it is not understood which exacerbation phenotypes lead to lung damage. Symptoms alone are unlikely to make this distinction.

The discovery of a specific biomarker that detects the start and recovery from an exacerbation, in combination with symptoms, would not only guide management options but identify pathophysiological pathways with potential discovery of novel drug targets.

The first intervention involves the use of both detailed symptom monitoring and point of care (POC) biomarker tracking for the early detection and treatment of exacerbations.

These interventions were assessed via the 'Elastin degradation in exacerbations of AATD related lung disease' study, an 18-month prospective cohort of 55 AATD patients with frequent exacerbations. The hypothesis was that prompt and appropriate treatment of exacerbations could limit their destructive effects and therefore slow emphysema progression. Since an enhanced monitoring approach is fairly novel, the main aim of this study was to assess feasibility and efficacy of an electronic symptom diary (eDiary) and POC urine lateral flow immune assay alongside identification of a suitable urinary biomarker. Promising results would then prompt assessment of whether emphysema progression is improved with such interventions.

Results showed that the use of an eDiary was feasible and was able to predict exacerbations, though not accurately enough to be used alone. The addition of urinary biomarkers was unable to improve the predictive algorithm. There were mean changes in CRP and TIMP1 across the time course of an exacerbation. The pilot data from the urine device has pioneered alterations to its design to improve its accuracy and reliability.

The second model of emphysema progression, basal elastin degradation rate, already has an established therapy. Alpha-1 antitrypsin (AAT) augmentation therapy has been shown to slow the rate of decline in CT lung density and is currently the only approved disease modifying therapy for AATD patients. The effect of augmentation therapy on more conventional outcomes such as mortality and QOL have not yet been established which has delayed approval of augmentation therapy in the UK.

This thesis explored the efficacy of AAT augmentation therapy via a large prospective study comparing two separate cohorts of AATD patients; one receiving AAT augmentation therapy and one standard COPD care alone. This was designed to investigate conventional

outcomes that have not been addressed through randomised controlled trials. Results showed that the mortality benefit of AAT augmentation therapy remains uncertain, though there appears to be a reduction in rate of decline in QOL in the augmentation group compared to control. The cost effectiveness of augmentation therapy is also discussed.

STATEMENT OF CONTRIBUTION

The body of work that has led to the writing of this thesis was performed by myself.

Though the main bulk of the patient recruitment and data collection was performed prior to the beginning of the COVID-19 pandemic, there was an impact on the sample analysis process, particularly for ELISA. The initial intention was for me to visit Mologic (the company that I collaborated with) for 2 weeks where I would carry out a large portion of ELISA's myself under supervision. Due to UK wide restrictions and the pivotal role that Mologic played in the early development of COVID-19 lateral flow assays, I was unfortunately not permitted to carry out the ELISAs. They were performed without my input by Mologic. Lateral flow data was sent to Mologic and returned with the corresponding concentrations for the recorded optical densities.

Data based work for the entire thesis including data cleaning, data analysis and data visualisation was performed by myself. This included all statistical work.

DEDICATION

For my wife Sarah, and my children, Sophia and Freddie.

You have made me stronger, better and more fulfilled than I could ever have imagined.

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ABBREVIATIONS

6MWT	6-minute walk test
AAT	Alpha-1 antitrypsin
AATD	Alpha-1 antitrypsin deficiency
AECOPD	Acute exacerbations of chronic obstructive pulmonary disease
AES	Advanced encryption standard
BEAT-COPD	Biomarkers to Target Antibiotic and Systemic Corticosteroid Therapy in COPD Exacerbations
BMI	Body mass index
BSA	Bovine serum albumin
CAT	COPD assessment test
CC16	Clara cell protein
CF	Cystic fibrosis
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CRISPER	Clustered regularly interspaced short palindromic repeats
CRP	C-reactive protein
CT	Computed tomography
CXR	Chest radiograph
DLCO	Diffusion capacity of lung for carbon monoxide
EBC	Endobronchial coils
EBV	Endobronchial valves
eDiary	Electronic symptom diary
ELISA	Enzyme Linked Immunosorbent Assay
EMA	European Medicines Agency
EXACT	EXAcerbations of Chronic Pulmonary Disease Tool
FBC	Full blood count
FDA	Federal Drug Administration
FEV1	Forced Expiratory Volume in 1 second
fMLP	N-Formylmethionine-leucyl-phenylalanine
FRC	Functional Residual Capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GUI	Graphical User Interface
GWAS	Genome Wide Association Studies
HHIP	Hedgehog interacting protein
HIPAA	US Health Insurance Portability and Accountability Act
LABA	Long acting B ₂ agonist
LAMA	Long-acting muscarinic antagonist
ICER	Incremental cost-effectiveness ratio
ICS	Inhaled corticosteroid

IL-	Interleukin
IPF	Interstitial pulmonary fibrosis
IQR	Interquartile Range
KCO	Carbon monoxide transfer coefficient
LVR	Lung volume reduction
LVRS	Lung volume reduction surgery
MCID	Minimal clinically important difference
MMA	Marketing Authorisation Application
MMP	Matrix metalloproteinase
mMRC	Modified Medical Research Council
MPO	Myeloperoxidase
NE	Neutrophil elastase
NGAL	Neutrophil gelatinase associated lipocalin
NHS	National Health Service
NIHR	National Institute for Health Research
NIV	Non-invasive ventilation
NPV	Negative Predictive Value
OR	Odds ratio
PACE	Point of care testing to target antibiotics for chronic obstructive pulmonary disease exacerbations
PBS	Phosphate buffering saline
PBST	Phosphate buffered saline, 0.1% Tween® 20 Detergent
Pi	Protease inhibitor
PIN	Personal identification number
pNPP	p-Nitrophenyl Phosphate
POC	Point of care
PPV	Positive Predictive Value
QALY	Quality adjusted life years
RAAV1	Recombinant adeno-associated virus type 1
RAPID	Intravenous augmentation treatment and lung density in severe alpha-1 antitrypsin deficiency
RBAC	Role Based Access Control
RBP4	Retinol binding protein 4
RCT	Randomised controlled trial
RNASE3	Ribonuclease A Family Member 3
ROC	Receiver operator characteristic
RR	Relative risk
SD	Standard Deviation
SERPINA1	Serine protease inhibitor family A member 1
SGRQ	St Georges Respiratory Questionnaire
SPC	Statistical Process Control
SPIROMICS	Study of COPD Subgroups and Biomarkers
SSL	Secure Sockets Layer

TBST	Tris-Buffered Saline, 0.1% Tween® 20 Detergent
TIMP	Tissue inhibitor of metalloproteinases
TLC	Total lung capacity
UE	Urea and electrolytes
UK	United Kingdom
US	United States
VAS	Visual analogue score
VATS	Video assisted thoracoscopy
WHO	World Health Organisation

1 INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable lung condition that is projected to be the third leading cause of death globally within the next decade.(1) In the UK, COPD accounts for 115,000 emergency admissions per year and 24,000 deaths. Of these deaths, 16,000 are within 90 days of being admitted to hospital.(2) Chronic disease management and the burden of hospital admissions cost the NHS approximately £1.9 billion annually.

COPD is characterised by persistent respiratory symptoms and airflow obstruction and in some cases recurrent episodes of worsening symptoms more commonly known as exacerbations. It is highly heterogenous in its presentation and progression. There is a close association with developing COPD with exposure to noxious environmental particles or gases including cigarette smoke and biomass fuel.(1) Lung development is also thought to be implicated.(3) Genetics plays an important role in the development of COPD with between 37% and 50% of COPD accounted for by heritability.(4) A recent study has developed a polygenic risk score for COPD using GWAS data from a large sample of population study's.(4) One well documented example of a genetic risk for COPD development is the serum protease inhibitor alpha-1 antitrypsin (AAT).

In 1964, Laurell and Erikson noticed an associated between pulmonary emphysema and the electrophoretic behaviour of AAT in a case series of 5 patients.(5) This discovery was one of the main starting points for the protease-antiprotease hypothesis which led the way in our initial understanding of pulmonary emphysema. Alpha-1 antitrypsin deficiency (AATD) has been the subject of a great deal of research since it's discovery nearly 60 years ago but there is still much more to uncover.

There is some debate as to why the Z and S alleles are prevalent and whether they confer a selection advantage, similar to the heterozygous advantage exhibited in sickle cell trait from enhanced resistance to plasmodium falciparum.(6) Lomas argues that enhanced inflammatory response to pulmonary infections in the pre-antibiotic era is one possibility. In modern times with longer life expectancy, antibiotics and the advent of smoking this advantage may no longer be relevant.(7) Others suggest that a positive selection bias relates to enhanced fertility and increased rate of twins in mothers with PiMZ or PiMS genotypes.(8-10) It may be that there is neither a negative or positive selection advantage and the current prevalence of AAT mutations is as a result of genetic drift with an initially rare allele becoming stable in the population by chance.(11)

1.1 Pathophysiology of AATD

Alpha 1-antitrypsin (AAT) is a 52 kDa glycoprotein consisting of 394 amino acids encoded on chromosome 14q31-32.(12) It is a highly polymorphic gene locus(13) with more than 300 variations identified.(14) Its primary function is to inactivate neutrophil elastase and other potent serine proteases such as cathepsin G and proteinase 3. It also has a secondary role as an acute phase protein with anti-inflammatory effect.(15) The terminology used to describe the different genetic variations of the AAT allele derives from the migration of glycoprotein on isoelectric gels. Protease inhibitor M (PiM) describes homozygotes for the allele resulting in normal levels and functioning of AAT. Other letters are used depending on the comparative speed of migration; S = slow, Z = very slow, F = fast.(16) The most common deficiency alleles are S (Glu264Val) and Z (Glu342Lys)) with a higher incidence in Northern and Western Europe (1 in 500-5000).(17) Homozygotes for the S allele (PiS) have only modest reductions in circulating levels of AAT (approximately 60% of PiM individuals) and therefore are not at increased risk of emphysema. In comparison, those who are

homozygous for the Z allele (PiZ) have significant reductions in circulating AAT (approximately 15%). There is a proposed protective threshold for development of emphysema of 11 μ M/L (Figure 1.1).(18) This is still debated and there is ongoing research as to whether the threshold is higher which has significance for dosing of AAT augmentation therapy.(19)

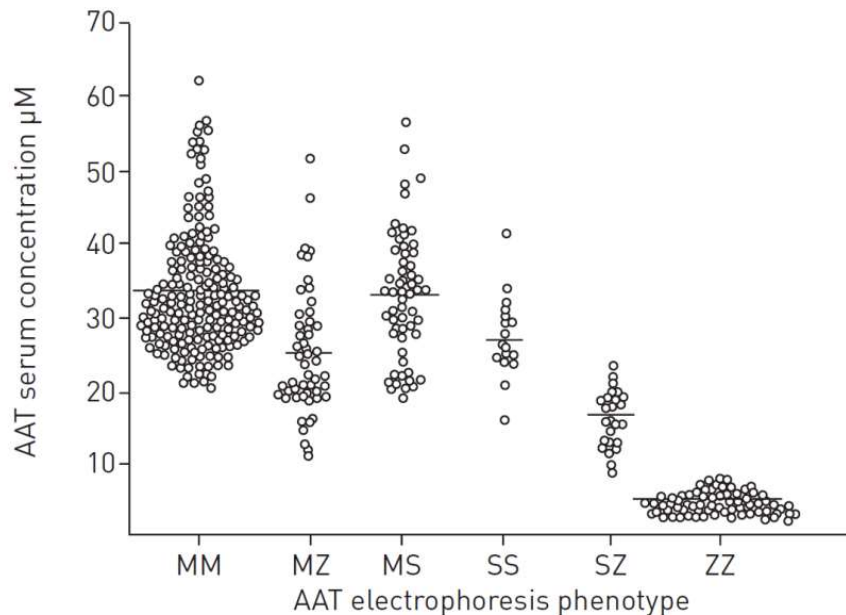


Figure 1.1: Serum AAT levels of 443 individuals with heterozygous and homozygous genotypes for the S and Z alleles. Horizontal bars are the mean AAT concentration for each AAT phenotype. Adapted from Brantly et al.(20)

Other rarer variants in the AAT gene result in an absence of circulating AAT, the so-called null variants. Patients who possess a null allele alongside a Z allele (PiZnull) have a considerably higher risk of developing emphysema compared with their PiZ counterparts.(21)

Deficiency of AAT has consequences for the structure and function of alveolar tissue, clinically manifested as emphysema. The mechanisms in which AAT inactivates neutrophil elastase (NE) is intricate. A mobile reactive loop within the AAT structure using a 'mouse

trap' like action upon engagement with NE.(22) Though key to the function of the enzyme, the complexity of the mechanism of the mobile reactive loop makes it a sensitive target for genetic mutation. Single point mutations coding for this region of the genome results in conformational changes in the glycoprotein structure having dramatic consequences for its function as a serine protease inhibitor.(21) This reduction in function characterises AATD.

AAT is synthesised almost exclusively by hepatocytes, although small quantities are also produced by intestinal and bronchial epithelial tissues and macrophages.(23, 24) Normal AAT, once synthesised within the endoplasmic reticulum of the hepatocyte, is released into the systemic circulation. It concentrates in alveolar tissue within the lungs where it is able to limit the destructive properties of NE and other proteases secreted by alveolar neutrophils.

The genetic single point mutation at position 342 that substitutes a positively charged lysine for a negatively charged glutamic acid causes a conformational change in enzyme structure leading to a tendency for AAT to form polymers; large chains of AAT molecules. Histologically, polymerised AATD form inclusion bodies that are periodic acid Schiff-positive and diastase-resistant.(25) The formation of AATD inclusion bodies within hepatocytes has two main consequences; 1) failed release of functional AAT into systemic circulation leading to deficiency in alveolar tissue and 2) a toxic effect on hepatocyte function, though this mechanism is poorly understood.(26) Interestingly, patients homozygous for a null variant of AATD are thought not to progress to liver cirrhosis due to the null variant truncated glycoprotein structures not forming insoluble aggregates in hepatocytes.(27)

There are multiple factors contributing to the development of emphysema in AAT patients. Firstly, as with usual emphysema, cigarette smoking is often the catalyst for development of emphysema in AATD, albeit up to 10-20 years earlier than in individuals with normal levels of AAT.(28) Those diagnosed in adulthood may have already consumed a significant number of cigarettes during their lifetime and are only diagnosed with AATD once they develop symptoms of emphysema. Others are identified through screening following diagnosis of a relative or during investigation for the onset of liver disease during the neonatal period. Early smoking cessation may limit disease progression. Emphysema can develop in non-smoking AATD PiZ patients, possibly due to exposure to other environmental agents such as air pollutants or respiratory pathogens, though not to the degree of cigarette smoke exposure.(29)

The presence of cigarette smoke within airways causes increased influx of inflammatory cells including neutrophils, macrophages and lymphocytes.(29) Release of proteolytic enzymes, such as NE, from azurophilic granules within neutrophils promotes the destruction of elastin in alveolar walls. In healthy individuals, AAT inactivates NE and other proteolytic enzymes limiting the destruction of alveolar architecture. The combination of cigarette smoke exposure and reduced concentrations of AAT in the alveolar space accelerates the emphysematous process since proteolytic activity is uninhibited. Campbell et al(30) found the area of activity of proteases from azurophil granules was inverse and non-linear, with a steep rise in proteolytic activity at AAT levels below 11 μ M. This indicates that at low concentrations, as in PiZ AATD, small reductions in AAT levels cause large increases in activity of proteases. Inversely, at higher concentrations of AAT, as in PiSZ or PiS AATD, larger reductions in AAT levels do not have the same magnitude of effect on protease activity.

In addition, PiZ AAT is up to five times less effective at inactivating NE than PiM AAT due to its abnormal structure.(21) Furthermore, as in hepatocytes, PiZ AAT polymerises within alveolar tissue.(31) Polymerisation of AAT inactivates the protease neutralising properties and acts as a chemoattractant recruiting further inflammatory cells to the alveolar milieu. Cigarette smoke also creates a more acidic environment within the lung which has been proven to favour the formation of AAT polymers.(31) Finally, cigarette smoke and oxygen free species secreted from leukocytes have been found to inactivate AAT via oxidation of P1 methionine residues, further driving lung parenchymal inflammation.(22)

It is worth mentioning that there is incomplete penetrance of both pulmonary and hepatic disease in AATD, the reasons of which are currently poorly understood. In pulmonary disease, the influence of intensity of cigarette smoking and late presentation of patients with lung disease (almost exclusively in adults over the age of 30) makes understanding this phenomenon challenging. Other non-SERPINA1 genetic modifiers may contribute to the overall penetrance of the AATD that have yet to be established.

1.2 Clinical features

AATD is characterised by the early development of pulmonary emphysema which is typically pan acinar and lower zone predominant (Figure 1.2 and 1.3), although this is not always the case. Further progression of the disease leads to gas trapping with subsequent increase in residual volumes and total lung capacity. There can be a decrease in gas transfer before an obstructive spirometry pattern develops that represents emphysema.(32, 33) Patient presentation is similar to that of usual COPD, although typically patients develop symptoms at a younger age and have a more modest smoking history. Asthma is sometimes incorrectly identified as being the cause which can delay the diagnosis and has led to guidance from the World Health Organisation (WHO) that all patients with a diagnosis of

COPD or adult-onset asthma should be tested for AATD.(34) Symptoms include progressive breathlessness, increased sputum production and pulmonary exacerbations though the pattern of presentation varies widely. There is a wide range of reported prevalence of bronchiectasis in AATD related lung disease with one study reporting 27% of patients with clinically relevant bronchiectasis.(35) Another study found only 0.5% of patients in AATD patients and therefore AAT testing is not routinely recommended.(36)

A personal history of liver cirrhosis or a family history of both pulmonary and hepatic disease may give a clue to the diagnosis of AATD.



Figure 1.2: Bibasal pan-lobular emphysema in a patient with AATD (with permission from Professor Robert Stockley)

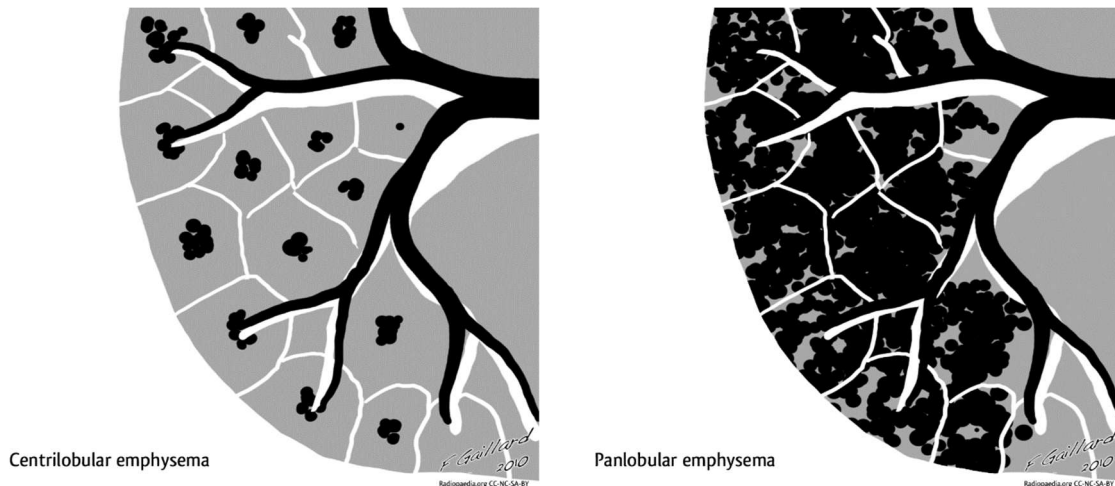


Figure 1.3: Comparison of radiological appearance of centrilobular and panlobular emphysema.(37)

1.3 Non-specific therapies for AATD related lung disease

There is a paucity of high-level evidence such as randomised controlled trials (RCTs) and large prospective cohort studies investigating the medical management of AATD related lung disease, aside from studies of specific therapy, in the form of AAT augmentation. This is mainly because most RCTs for inhaled and oral pharmacological therapies in COPD either exclude AATD patients from eligibility criteria or there are too few patients with AATD for any meaningful sub analysis to be generated. Since the pathogenesis and symptoms of AATD related lung disease and usual COPD are similar, the standard care for AATD related lung disease is usually aligned with treatment for usual COPD, with the exception of AAT augmentation therapy.

Prevention of development of lung disease is preferential and therefore once a patient has been identified as having a severe deficiency of AATD their risk factors for emphysema progression should be addressed. Smokers should be strongly encouraged to stop as rate of lung function decline correlates with cigarette consumption in AATD(38) and declines more rapidly than usual COPD.(39) Use of recreational drugs such as cannabis should be elicited,

as this would further amplify the risk of lung function decline. The risk of occupational exposures through inhalation of noxious particles should also be assessed with action towards minimising the impact of any exposures e.g., masks, adequate ventilation, change of occupation if necessary. Occupational exposure has been shown to relate to lung function decline in AATD, with different effects according to sex.(40)

Use of inhaled therapies including short and long-acting B₂-agonists, long-acting muscarinic antagonists, and inhaled corticosteroids should be prescribed dependent on degree of breathlessness, FEV₁% predicted and exacerbation frequency as outlined by Global Obstructive Lung Disease(GOLD).(41) One specific study of PiZ AATD patients reported a reduction in FEV₁ decline in patients with persistent eosinophil levels above $0.2 \times 10^9/L$ when using an inhaled corticosteroid (ICS).(42) Those with low levels of eosinophils had no change in rate of FEV₁ decline if on an ICS, implying that blood eosinophils might act as a biomarker to select patients for this treatment, similar to in usual COPD. Long term macrolide antibiotics or roflumilast should be considered for frequent exacerbators with optimised medical management.(43) Those with tenacious sputum or comorbid bronchiectasis should be offered a trial of mucolytics.(44) Pulmonary rehabilitation in patients with AATD related lung disease has shown similar benefits in six-minute walk test (6MWT) improvement compared with usual COPD(45) and is recommended for those with mMRC dyspnoea score ≥ 2 . There is no obvious reason why AATD patients would benefit less from rehabilitation, although we might speculate that age-related deconditioning is less relevant AATD patients who present early. All patients with AATD related lung disease should be offered pneumococcal and annual influenza vaccination, and have been shown to be of benefit in a US study.(46)

1.4 Exacerbations of COPD in AATD

As with usual COPD, an important feature of AATD related lung disease are episodes of acute worsening of respiratory symptoms, more commonly known as exacerbations. Such episodes often require additional therapy in an attempt to reduce their impact, duration and limit the rate of emphysema decline. Similar to the long-term treatment of AATD, with the exception of AAT augmentation therapy, treatment of exacerbations in AATD related lung disease is in line with guidelines for usual COPD.(47) There is some debate as to whether patients with AATD require a longer course of steroids and antibiotics during exacerbations due to the increase risk of emphysema decline though there is no evidence for this currently. For this reason, this section will include discussion about usual COPD exacerbations unless exacerbations in AATD related lung disease are specified.

In AATD, exacerbations are associated with increased mortality,(48) worse quality of life(49) and more rapid lung function decline.(50) Acute exacerbations of COPD (AECOPD) are responsible for a considerable proportion of the burden of COPD management, a proportion of which will have AATD. In the UK, over 140,000 hospital admissions were due to AECOPD in 2011, accounting for over 1 million bed-days.(51)

Despite this, there has been little change to the repertoire of therapies available to AECOPD in the last 30 years.(52) The mainstay of treatment remains oral glucocorticoids and inhaled bronchodilators plus antibiotics if a bacterial infection is suspected.(1) In severe cases non-invasive ventilation (NIV) can be used but this is reserved only for those hospitalised with respiratory failure. Patients that require NIV represent just a small subset of AECOPD as the majority of cases are managed in primary care. Here, without the additional insight from blood tests, arterial blood gases and chest radiographs, treatment of COPD exacerbations is mainly based on symptoms alone. Since the symptoms of COPD

exacerbations are the result of numerous possible disease processes (infection, environmental, psychological) with little in the way of features that can predict the exact cause, most are treated with a 'one size fits all' approach.

Even in AATD related lung disease, the use of AAT augmentation therapy has not been shown to improve severity, frequency or length of symptoms for AECOPD. In RAPID,(53) the annual frequency of exacerbations and relative duration of exacerbations was actually lower in the placebo group compared to AAT augmentation therapy, though the magnitude of effect was small and not statistically significant. Similarly, in a trial of inhaled AAT augmentation therapy the primary outcome of time to first exacerbation was longer in patients on placebo though again the results did not meet statistical significance.(54)

An example of the complex nature of exacerbations can be illustrated with the common symptom of breathlessness. It can be caused by increased sputum in the small airways or bronchoconstriction obstructing flow in response to bacteria or viruses, increased tissue oedema from allergy or cardiogenic factors,(55) increased perception of breathlessness(56) and a host of other factors (Figure 1.4).

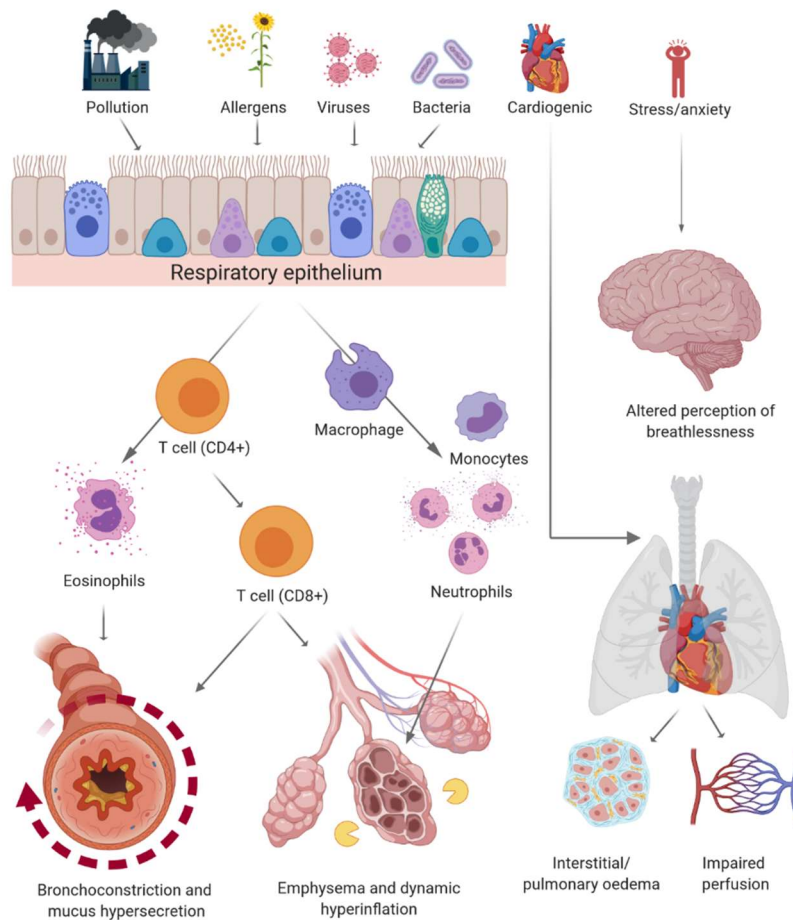


Figure 1.4: Aetiology and pathophysiology associated with AECOPD.

Bacteria, viruses and environmental factors induce inflammatory changes in the airway epithelium. Eosinophilic and T cell derived inflammatory cytokines induce bronchoconstriction and mucus hypersecretion which contribute to dyspnoea and sputum production. Proteases secreted by neutrophils also drive inflammation and development of emphysema and subsequent dynamic hyperinflation. Cardiac dysfunction may cause interstitial and pulmonary oedema with impaired perfusion. Stress and anxiety alter breathlessness perception.

AATD/COPD patients also suffer with marked variation in day-to-day symptoms including breathlessness, sputum production, sputum colour, fatigue and levels of stress and anxiety.(57) This presents a challenge for physicians to accurately treat exacerbations. How can a distinction be made between the usual daily variation in symptoms and an emerging exacerbation? Delays in treatment are frequent and could result in either unwarranted hospital admission or worse quality of life (QOL) for the patient. Equally, over treatment

could lead to reliance on antibiotics and steroids for mere fluctuations in a chronic disease(58) which are themselves associated with side effects.(59, 60)

Currently the tool most used by primary care doctors to diagnose COPD exacerbations remains clinical history. The most commonly used symptom-based definition was published in 1987(20); Anthonisen classified exacerbations depending on the presence of dyspnoea, increased sputum production or increased sputum purulence. Type I exacerbations feature all three symptoms mentioned and had a larger response to antibiotics than the other groups.(61) This data is supported by the observation that presence of sputum purulence correlates with bacterial load.(62)

Treatment of acute exacerbations often includes use of a short course of oral steroids and/or antibiotics. Despite their wide spread use, evidence remains limited for the treatment of COPD exacerbations, particularly for mild to moderate episodes. In 7 randomised control trials (RCTs) of antibiotics vs placebo between 1987 and 2017 (antibiotic arm 605, placebo 586) there was a reduction in treatment failure for mild to moderate exacerbations between 7 days and one month after treatment initiation (RR 0.72, 95% CI 0.56 to 0.94, absolute reduction in treatment failure 295 to 212 per 100 treated participants, 95% CI 165 to 227) though evidence quality was deemed low.(63) There were similar reductions in treatment failure for hospitalised patients (n= 803) across 5 studies though results were not statistically significant (RR 0.76, 95% CI 0.58 to 1.00). Despite the reported benefit of antibiotics vs placebo in both community based and hospital-based care, there are a lack of studies that assess primary end points such as mortality or health related QOL scores. One study showed no difference in overall mortality in 301 patients treated for a moderate exacerbation of COPD with doxycycline against placebo (treatment group;

10/150, placebo; 8/151, OR 1.27, 95% CI 0.49 to 3.3), though overall patient numbers were relatively low and the study was underpowered to detect an event of low prevalence such as mortality. Though these results demonstrate some reduction in treatment failure, often exacerbations get better without antibiotics. It may be that only a subset of the patients studied benefited from antimicrobial therapy.

One strategy to unravelling the complexity of exacerbations is understanding the immune response and how inflammation develops from an initial triggering event. The BEAT-COPD study clustered exacerbations into four distinct phenotypes; bacterial, viral, eosinophilic and pauci-inflammatory, based on profiling sputum and serum biomarkers of exacerbations.(64) This approach could lead to identifying novel drug targets to treat specific exacerbation phenotypes rather than blanket treatment with steroids and antibiotics, though more research is needed for it to be applied clinically.

It is clear that more sophisticated and personalised approaches are required to improve accuracy of treating COPD exacerbations and crucially early identification of moderate or severe exacerbations to enable prompt treatment. Precision medicine has been defined as ‘treatments targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations.’(65) The ultimate aim of personalised medicine is to improve clinical outcomes for patients whilst avoiding side effects for those who are unlikely to benefit from therapy.(65) In the context of treating exacerbations this means diagnosing and treating patients based on factors that separate exacerbations into unique phenotypes. This is likely to involve a two-fold approach: 1) detailed characterisation of patient symptoms in real time and 2) measurement of biological samples (blood, urine or

sputum) during the early phase of an exacerbation and potentially by monitoring at regular intervals to detect when an exacerbation is developing. Such advanced near patient testing is on the horizon with the development of electronic symptom diaries and more reliable and cost-effective methods of measuring patient biomarkers in a primary care setting.(66) In the context of AATD, the underlying pathophysiological footprint for AECOPD may differ from usual COPD due to increased activity of proteases and therefore may require separate investigation. Crucially, given the strong emphysema phenotype associated with AATD related lung disease, enhanced monitoring of patients could encourage early treatment of exacerbations and reduce overall emphysema decline. Use of symptoms diaries and the detection such biomarkers in AATD related lung disease to reduce emphysema decline will be explored later in the introduction and forms part of the hypotheses and aims for this thesis.

1.4.1 Personalised approaches to treatment of acute exacerbations

Exacerbations in both usual COPD and AATD related COPD have numerous causes, often with similar presentations, namely breathlessness and sputum production. Similar symptoms do not infer similar underlying pathophysiology. For example, a bacterial exacerbation may respond well to antibiotics but may be worsened by the use of steroids due to immunosuppression. Equally, exacerbations with an allergic or environmental trigger may have little or no response to antibiotics and expose the patient to side effects and potential antibiotic resistance needlessly. It is difficult to be sure which types of exacerbations lead to lung destruction, if at all. Causes of exacerbations that mimic symptoms of 'true' AECOPD, such as those triggered by cardiogenic factors or by stress and anxiety, may not be associated with underlying damage to lung tissue or disease progression. Discovery of exacerbation phenotypes; traits that are observed clinically or

functionally, or even endotypes; cellular and molecular pathways involved in the pathogenesis of the disease, would enable more personalised treatment of acute episodes.(67) This is likely to include use of symptoms, physiological measurements or biomarkers to stratify exacerbations.

The acute phase protein C-reactive protein (CRP) is a widely used clinical biomarker of inflammation and infection (68) and several studies have looked at its usefulness as a biomarker to select patients who would benefit from antibiotics during COPD exacerbations.

One study examined the use of POC CRP testing to guide antibiotic management of COPD exacerbations.(66) There was a reduction in antibiotic use rate in the CRP group (47.7% vs 60.7%, OR 0.31 0.21 to 0.45) at initial consultation with no difference in COPD related health status. Similar results were found in a study of 220 hospitalised AECOPD patients with no difference in treatment failure at 30 days between CRP guided therapy and standard therapy (44.5% in CRP group; 45.5% in standard therapy group, adjusted OR 1.146 95% 0.649 to 1.187).(69) There was no difference in symptoms, QOL score or serious adverse events.

CRP is usually raised in both viral and bacterial infections and cannot easily distinguish between the two. This potentially limits its use as a precision medicine tool to guide antibiotic use. Clark et al (70) found that CRP was typically higher in viral infections compared to bacterial infections and was associated with a higher temperature. This does not necessarily mean that antibiotics should be avoided when a viral cause is suspected. The interplay between viral infections and bacterial colonisation remains unclear. Studies have shown higher bacterial loads in association with concomitant viral infections(71, 72)

and there may still be a place for antibiotics in this setting. Only the PACE study (66) has carried out an RCT of COPD patients in a primary care setting and results of other studies may not be generalisable to the majority of COPD exacerbations that are treated in the community.

Another potential biomarker of bacterial infection is procalcitonin. A metanalysis of 5 clinical trials of hospitalised patients randomised to either antibiotics or placebo showed reduction in antibiotic use and overall antibiotic exposure.(73) There was also less treatment failure (RR 0.821, 95% CI 0.62 to 1.06), though a mortality benefit was not seen. Procalcitonin tests are currently limited by expense and availability in routine clinical care.

As with predicting future risk using eosinophils, acute treatment of exacerbations with oral glucocorticoids in those patients with higher eosinophil counts was found to be non-inferior to standard treatment in hospitalised patients(74) and further adds merit to the theory of the eosinophilic exacerbation phenotype.

It is worth a special mention of how exacerbations are defined in clinical trials of drugs used to treat exacerbations and how this may have impeded research progress. Most clinical trials employ a treatment-based definition of exacerbations which for reasons already discussed is problematic. Patients may self-treat or seek medical help for reasons that may not directly relate to the underlying process of an exacerbation. Additionally, use of the Anthonisen criteria to help guide patients with timing of treatment is potentially a crude barometer of underlying pathophysiology. Subsequently, it is difficult to be sure if exacerbations in separate arms of randomised control trials are comparable. Several patient reported outcome (PRO) measures of exacerbations and symptom diaries have been designed and tested with variable results.(75)

Despite their promise, use of CRP and procalcitonin to guide antibiotic treatment and eosinophils to guide use of oral glucocorticoids may be seen as a backward step. Instead of pioneering new treatments for exacerbations it is merely rationalising old ones. To truly move forward into the age of precision medicine we must identify specific exacerbation endotypes with the hope of identifying novel drug targets. In order to achieve this, we must deepen our understanding of the pathophysiological process's involved in the early and established stages of the exacerbation period.

An alternative to measuring direct markers of inflammation is to identify biomarkers that represent organ or tissue damage. One well established example is troponin which has revolutionised triage of patients with myocardial infarction.(76) It has been proposed that COPD exacerbations could be considered 'lung attacks' with each episode furthering damage to the lungs with consequences for future morbidity and mortality.(77) Markers of elastin degradation have been considered by many to be the lung equivalent biomarker of lung tissue destruction since loss of alveolar elastic tissue within the lungs is the main contributor to pulmonary emphysema, a process driven by an imbalance of proteases and anti-proteases.(27) This process is enhanced in AATD related lung disease due to serum deficiency of AAT. This has led to particular interest into elastin degradation products as biomarkers of emphysema progression(78) and pulmonary exacerbations of COPD and in particular AATD.(79)

Several studies have aimed to validate desmosine as a surrogate marker of lung tissue destruction to define treatment outcomes in drugs aimed at reducing emphysema decline or impact of exacerbations. Despite early promise,(80) it has been difficult to validate clinically. Issues of analytical validity with wide variability in reported overall accuracy of

assays have been noted.(81) Secondly, studies were not adequately powered to predict clinical outcomes for patients, including exacerbations, partly because of confounding by disease activity and severity i.e., frequent exacerbators, rapid progression of emphysema exhibiting higher turnover of desmosine. In advanced pulmonary emphysema the baseline desmosine level may be lower since there is less lung tissue left to be broken down, independent of disease activity.(82) Elastin is also found in blood vessels(83) and therefore 'non-lung' elastin may introduce additional noise to the data. This is backed by results from the SUMMIT study which found desmosine correlated poorly with emphysema progression but was associated with cardiovascular disease.(84) More longitudinal studies and more reliable and repeatable measurement techniques are required to further assess desmosine as a biomarker of exacerbations. Table 1.1 summarises the evidence for endotypic biomarkers in AECOPD.

The latter part of this introduction in Section 1.8 will further explore the role of biomarkers in detecting exacerbations and lung damage further and set out hypotheses and aims to address this (Sections 1.9 and 1.10).

Table 1.1: Summary of evidence for precision biomarkers for AECOPD.

Biomarker	Study	Design	Threshold	Primary Outcomes	Results
C-Reactive Protein (CRP)	Butler et al 2019 (66)	RCT N = 653 COPD patients in primary care	CRP $\geq 50\text{mg/L}$	Patient reported use of Abx within 4 weeks, COPD related health status at 2 weeks	↓Abx use in CRP group 57.0% vs 77.4% (OR 0.31, 95% CI 0.20 to 0.47)
	Prins et al 2019 (69)	RCT N = 220 Hospitalised COPD patients	CRP $\geq 50\text{mg/L}$	Rate of Abx prescription, 30-day treatment failure	↓Abx treatment in CRP group 31.7% vs 46.2% (adjusted OR 0.178, 95% CI 0.077 to 0.411) NSD treatment failure
Procalcitonin	Corti et al 2016 (85)	RCT N = 120 Hospitalised COPD patients	PCT $>0.25\text{ ng/mL}$	Rate of Abx use ≥ 5 days	↓Abx exposure ≥ 5 days in PCT group (41.9% vs 67.2%, RR 0.62, 95% CI 0.44 to 0.88) NSD readmission rates/ITU admission/mortality
	Verduri et al 2015 (86)	RCT N = 184 Hospitalised COPD patients – Anthonisen I or respiratory failure only	PCT $>0.25\text{ ng/mL}$ or $>0.1\text{ng/mL}$ + severe disease	Proportion of patients with at least 1 AECOPD in 6 months	20.5% vs standard therapy 13.3% (Risk difference 7.12, 95% CI -3.85 to 18.09) NSD adverse events
	Stolz et al 2007 (87)	RCT N = 208 Hospitalised COPD patients	PCT $>0.25\text{ ng/mL}$	Abx exposure rate at index AECOPD, Abx requirement AECOPD in following 6 months	Reduction in index Abx use in PCT group 40% vs 72% (RR 0.56, 95% CI 0.43 to 0.73), ↓Abx exposure following 6 months (RR 0.76, 95% CI 0.64 to 0.92)

Eosinophils	Sivapalan et al 2019 (74)	RCT – Eosinophil guided therapy vs prednisolone 37.5mg N = 318 Hospitalised COPD patients	Eosinophils $\geq 0.3 \times 10^9$	Number of days alive and out of hospital within 14 days	NSD in days alive and out of hospital within 14 days: mean EOS guided 8.9 days (95% CI 8.3 to 9.6), standard care 9.3 days (8.7 to 9.9), No difference in treatment failure after 30 days.
	Bafadhel et al 2012 (88)	RCT Eosinophil guided therapy vs prednisolone 30mg (14 days) N = 166 exacerbations in 109 patients. Outpatient COPD patients	Eosinophils $> 2\%$	Non-inferiority of health status, treatment failure rate	CRQ change: EOS 0.8 vs standard care 1.1: (mean difference 0.3, 95% CI 0.0 to 0.6), Treatment failure: EOS 5% vs standard care 13% (95% CI -1 to 16)
Desmosine	Huang et al 2012 (80)	Prospective study – no intervention N = 47 50 exacerbations (urine) 102 exacerbations (blood)	N/A	N/A	Increased uDES and bDES in stable COPD compared to stable asthma and healthy controls. Increased uDES during COPD exacerbations compared to stable COPD.

Abbreviations: CRP; C-Reactive protein, PCT; procalcitonin, RCT; randomised control trial, N; number of study participants, Abx; antibiotics, EOS; eosinophils, ↓; reduced, ↑; increased, NSD; no significant difference; CRQ; Chronic respiratory questionnaire, uDES; urinary desmosine, bDES; blood (serum) desmosine, N/A; not applicable, AECOPD; acute exacerbation of COPD, RR; relative risk, CI; confidence interval.

1.4.2 Predicting frequent exacerbators of COPD

Assessing individual risk of having pulmonary exacerbations forms a key part in choosing initial and subsequent therapy. Stratifying COPD exacerbation risk continues to be a challenge, particularly when a COPD patient is first diagnosed. Analysis of data from the SPIROMICS cohort in 2017 found marked variation in annual frequency of moderate to severe exacerbations requiring hospitalisation.(89) The most consistent group during 3 years of follow up were those not experiencing exacerbations at all. There was also considerable year to year variation in those with exacerbations; 41.3% of patients had an inconsistent pattern (both years with and without exacerbations) during the study.

Some interventions can be used in all patients to help reduce the risk of future exacerbations including smoking cessation,(90) vaccinations against influenza and pneumococcus(91) and pulmonary rehabilitation.(92) However, no one AATD patient is the same and therefore a more personalised approach is warranted.

Spirometry is a useful tool to assess severity in COPD/AATD, and those with an FEV₁ <50% predicted are at higher risk of suffering with exacerbations. Although GOLD severity level correlates with risk of COPD exacerbations, FEV₁ alone varies too widely to be a reliable predictor. It is, however, useful in conjunction with other patient factors.(48) Deteriorating airflow obstruction is associated with increasing prevalence of exacerbations and hospitalisations.(50) An important predictor of future exacerbations is a previous history of pulmonary exacerbations(93) and should be asked about in all patients with COPD.

The most commonly used therapy to prevent exacerbations is inhaled corticosteroids (ICS), usually in combination with inhaled long acting B₂-agonist (LABA) and long-acting

muscarinic antagonists (LAMA). The decision to include ICS as part of a patient's treatment requires careful consideration.

In recent years there has been research interest in the role of eosinophils in precision prevention of exacerbations of COPD. A recent systematic review (94) examined the results of 11 post-hoc analyses of RCTs (n = 25,881) and 5 retrospective observational studies (n = 109,704) of the effect of ICS on COPD exacerbation rate. They found a treatment effect at both a cut off of $\geq 2\%$ (RR, 0.80, 95% CI, 0.74 – 0.85) and ≥ 150 cells/ μL blood eosinophil count (RR, 0.65, 0.52 – 0.79), though there was no observed effect in 4 of the 5 observational studies. Seven of the eleven RCTs did not exclude patients with significant airway reversibility whereas 4 out of 5 observational studies excluded patients with a history of asthma. The only observational study to include a previous diagnosis of asthma found a positive association at higher blood eosinophil levels and concomitant ICS therapy. The most recent GOLD guidelines have now incorporated eosinophil count to be considered as a factor for starting an ICS in those with a higher eosinophil count in combination with clinical assessment of exacerbation risk.(1) Blood eosinophils are a good example of precision medicine in action today and its use as a biomarker for future exacerbations may mean that patients with a low eosinophil count will avoid unnecessary side effects such as osteoporosis(60) who would have ordinarily received an ICS as part of routine care.

There are other factors that may influence a decision to use ICS in patients with COPD/AATD. In those with a previous history of asthma or significant bronchodilator reversibility it may be beneficial to add in ICS therapy. Risk of pneumonia should always be thought about too, especially in the elderly, those with a low BMI and patients with

bronchiectasis.(95) These may be considered ‘treatable traits’ and could be a flexible alternative to splitting patients into rigid phenotypes.(67)

High eosinophils are also an example of a COPD phenotype in which a patient characteristic is associated with a clinically meaningful outcome, namely exacerbations. The underlying mechanism for eosinophilic exacerbations remains poorly understood but clinical trials that target inflammatory pathways that involve eosinophils are promising. Mepolizumab, an anti-IL-5 monoclonal antibody, has been shown to reduce annual exacerbation frequency of moderate or severe exacerbations vs placebo in eosinophilic COPD. An initial RCT of Benralizumab,(96) an IL-5 receptor alpha-directed cytolytic monoclonal antibody that induces rapid eosinophil depletion, showed no improvement in annual frequency of exacerbation, however post-hoc analysis identified those with >3 exacerbations per year treated with the higher 100mg dose had a reduced exacerbation frequency compared to placebo(97) and a trial is now ongoing which focuses on this population (NCT04053634).

Other biomarkers of exacerbation risk have been explored via large cohort studies but have been unsuccessful so far. Comparison of SPIROMICS and COPDGene data (n = 2146 patients in total) showed no biomarker candidates that can predict retrospective self-reported exacerbations or prospective exacerbations as defined by health care use.(98)

Though the genetic susceptibility to emphysema is clearly influenced by the SERPINA1 gene, the relationship of individual genetic factors to exacerbations remains poor. Since COPD is a highly heterogenous condition, it seems reasonable to infer that other genes contribute to an individual’s exacerbation susceptibility. Similarly, the incomplete penetrance of AATD related lung disease independent of smoking history suggests other genetic influencers contribute to the overall phenotype. To date, there has been little

reported specifically about genetic factors and exacerbations. The ECLIPSE study (99) identified an association between Hedgehog interacting protein (HHIP) with both retrospective and prospective exacerbations. A similar association was seen in COPDGene though it was more closely related to severe emphysema.(100) HHIP participates in a series of signalling pathways that include pulmonary organogenesis and the response to lung injury from cigarette smoke and the cytokines downstream from this pathway are biologically plausible drug targets. Meta-analysis of NETT Genetics, ICGN, ECLIPSE and COPDGene(101) confirmed associations of moderate to severe COPD with HHIP alongside two other gene loci though exacerbations did not feature as part of their analysis. A further whole genome sequencing study of COPD had similar findings though again did not comment on the relationship between exacerbations and HHIP.(102) More studies are therefore required to explore genetic influences upon exacerbations in order to discover novel drug targets.

1.4.3 Future directions for treatment of exacerbations

Most of the biomarkers discussed so far have been cross sectional, either during the early stages of an exacerbation or often sampled during stable periods which do not capture the dynamic nature of exacerbations. Within a hospital setting this can be achieved from admission through to discharge quite easily since patients remain in hospital and often have regular blood and sputum tests. It does not, however, account for baseline biomarkers and changes that occur on the lead up to the treatment of an exacerbation. It is possible that the pathophysiological process responsible for an exacerbation occur over several days with a preceding subclinical period, though symptoms can escalate rapidly.(103) By the time a patient's symptoms have developed sufficiently to warrant admission to hospital the underlying inflammatory cascade may already be well established and the catalyst for the

exacerbation may be difficult to delineate. Early changes in the inflammatory profile of an exacerbation may be key to understanding the downstream effects. It is well known that several inflammatory cells have similar end points in activating the immune system.(52) If these early changes could be found then it may shed more light on the cause of an exacerbation. This is not easily done in a hospital setting but could be achieved in clinical trials with the use of home biomarker monitoring paired with symptom diaries. This would capture subclinical episodes and daily fluctuations that would otherwise be missed when using the healthcare utilisation criteria for exacerbations. It must be noted that even if a suitable biomarker is found, acceptable and applicable methods would need to be available and cost effective in order to make it clinically useful.

Four potential candidates for groups of biomarker based on underlying pathophysiological pathways are as follows; 1) elastin degradation, 2) inflammation, 3) extracellular matrix turnover and 4) endothelial dysfunction.

1.4.3.1 Elastin degradation biomarkers

Elastin degradation, amongst other extracellular matrix components, is the result of an imbalance of proteinases and anti-proteinases leading to the classical pathological feature of emphysema(104)(Figure 1.5).

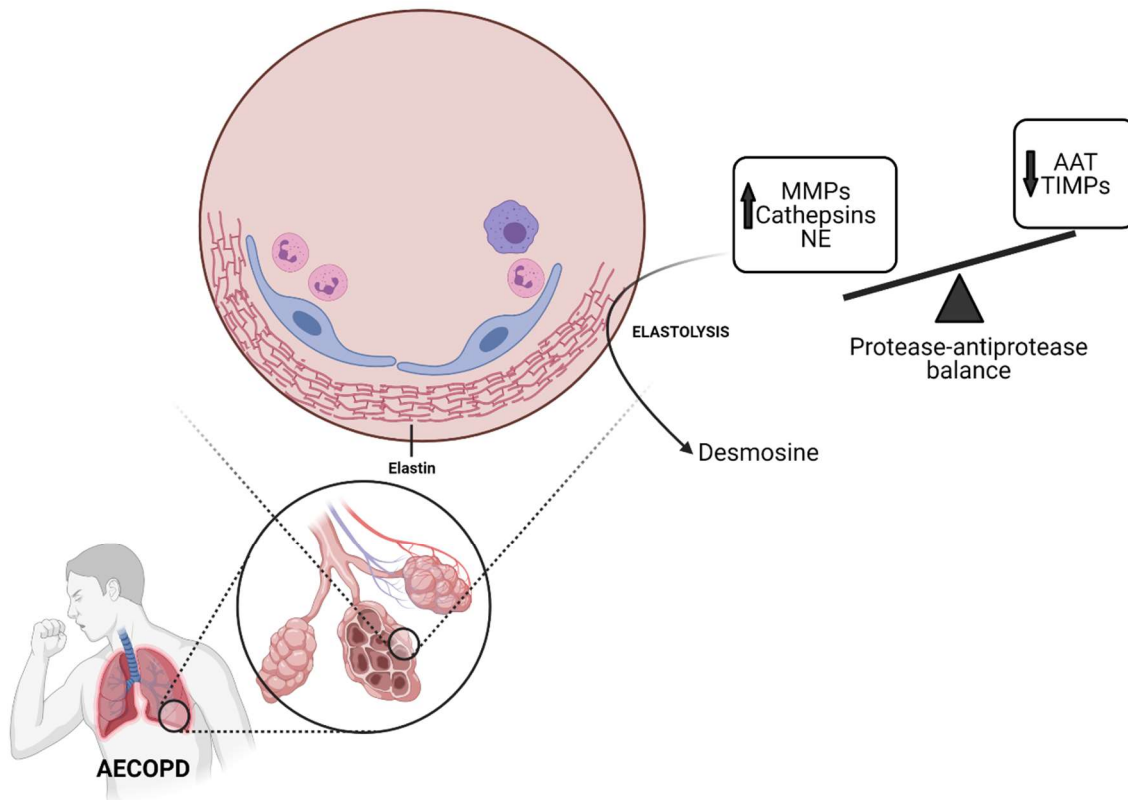


Figure 1.5: Protease-antiprotease imbalance during AECOPD of AATD related lung disease

This process is accelerated in AATD.(62) One proposed marker of elastin breakdown within lung tissue is desmosine. Desmosine is only found in elastin fibres and is not metabolised prior to excretion in urine. Desmosine has been studied in trials of AAT augmentation as a potential biomarker of efficacy in addition to a disease severity marker for emphysema.(19, 105) Other studies have looked to see if urinary desmosine is increased in exacerbating COPD patients compared to the stable state with conflicting results.(80, 106-110) Most were smaller case control studies with significant risk of bias.

Huang et al,(80) the largest of the studies and possibly with the least bias, investigated plasma and urinary desmosine in a cohort of COPD patients with comparisons to 4 control groups; healthy non-smoker, healthy smokers, asthmatic non-smokers and asthmatic

smokers. Plasma desmosine was shown to be raised in stable COPD compared to all control groups (COPD 0.29 ng/ml (0.24 – 0.35), healthy non-smokers 0.21 (0.21 – 0.24), $p < 0.001$). This difference was not found in desmosine adjusted for creatinine except in asthmatic non-smokers. There was a marked increase in desmosine in healthy volunteers compared to COPD patients during an exacerbation with a mean difference of 8 ng/mg creatinine (6 - 12). This comparison used hospitalised COPD exacerbations which by definition are more severe. The authors note in their paper that there was an increase in urinary desmosine with increasing age. It should be noted that there was no age or sex matching of participants and healthy volunteers were on average 57 years younger than exacerbating COPD patients.

Other biomarkers that could signify elastin degradation include NE, a potent protease released during neutrophilic inflammation, and A α -Val³⁶⁰, a fibrinogen cleavage product and surrogate marker of NE activity(111).

1.4.3.2 Inflammatory biomarkers

A key feature of pulmonary exacerbations in AATD is inflammation.(112) There are several inflammatory markers that may be useful in the precision diagnosis of AECOPD, including CRP, eosinophils, and procalcitonin which were discussed above in Section 1.4.1.

1.4.3.3 Extracellular matrix turnover biomarkers

In addition to desmosine there are several other markers associated with increased extracellular matrix turnover in lung tissue. Proteases including cathepsins and MMPs, alongside serine proteases such as NE, are capable of tissue destruction via both direct damage and cleavage of anti-inflammatory molecules.(113) This is particularly relevant in AATD related lung disease since increased NE has been shown to upregulate both cathepsins and MMPs.(113)

There are at least 15 naturally occurring MMPs. Initially it was thought that each separate MMP has a specific target substrate. The early nomenclature of MMPs reflected this with names such as neutrophil collagenase and 92kDa gelatinase. More recent research as discovered considerable substrate overlap between MMPs leading to the renaming of MMPs using numbers to avoid confusion. For example, MMP9, formally known as gelatinase, is capable of degrading elastin(114, 115) and AAT(116) in addition to numerous collagens. MMP8 is also able to degrade AAT.(117) MMPs and other proteases, such as cathepsins, are potent pro-inflammatory molecules, hence their selection for measurement. Results from this study have shown increased levels of Cathepsin B in stable AATD compared to controls though there was no difference in MMP8 or MMP9.

Molecules that counteract the inflammatory drive of proteases are innate inhibitors of proteases. TIMP1 and TIMP2 both act to inhibit MMPs(118) with AAT also having some activity against MMPs and cathepsins. The balance between proteases and antiproteases is thought to influence the level of inflammation and in the context of COPD/AATD, elastin degradation and emphysema progression.

Another protein, Chitinase-3-like protein, has been shown to be upregulated in COPD where it may sustain the production of proinflammatory molecules and MMPs by alveolar macrophages.(119-121).

1.4.3.4 Endothelial dysfunction biomarkers

In addition to direct effects in the lung, it has been proposed that during AECOPD and in stable COPD patients there is endothelial dysfunction.(122-124) This is relevant as increased endothelial permeability at the level of the glomerulus may affect urinary excretion of biomarkers, in addition to the systemic inflammatory effects of AECOPD and

COPD in the stable state. Though unlikely to be useful alone, addition of biomarkers of endothelial dysfunction to a panel may help distinguish between stable AATD and those with AECOPD.

The use of specific biomarkers to detect exacerbations and reduce emphysema decline form a major component of this thesis and will be explored further in the introduction in Sections 1.8 to 1.10. We now move focus to the other major concept withing this thesis; therapies that reduce the overall basal emphysema progression rate.

1.5 AAT augmentation therapy

AAT accounts for more than 90% of the antineutrophil elastase capacity in the lower respiratory tract.(15) Its deficiency is the main driving factor for the development of panacinar emphysema that characterises AATD. It logically follows that replenishing serum levels of AAT in deficiency individuals might reduce the basal rate of emphysema progression. This is the rationale for, and aim of, AAT augmentation therapy. AAT augmentation therapy involves the weekly administration of purified AAT that derives from pooled human plasma and is currently the only specific therapy for AATD related lung disease. This intervention is not licensed for the treatment of liver disease since this is caused by the accumulation of mutant AAT polymers within hepatocytes which is independent of serum AAT concentration.(22)

Proof of biochemical efficacy; defined as achieving appropriate serum levels of AAT in deficiency individuals, was first described by Gadek et al in 1981.(125) Five patients with PiZ AATD showed maintained increased serum and lung epithelial levels after weekly infusions of 70mg/dL AAT from pooled human plasma. This was achieved in a study of 21 AATD individuals receiving weekly infusions of AAT at a dose of 60mg/kg for 6 months

with repeated demonstration of increased trough levels in serum and lung. The relative safety and feasibility of augmentation therapy was also shown.(18) Following these studies, somewhat controversially, AAT augmentation therapy was approved by the US Federal Drug Administration (FDA) before clinical efficacy was clarified.

Since then, demonstrating clinical efficacy of augmentation therapy and benefits to survival, decline in physiological markers or QOL measures has proved a far more challenging task than biochemical efficacy. This has led to the unusual situation where augmentation therapy has been approved and prescribed for many decades in numerous countries around the world based on biochemical effect alone and without the body of evidence now expected for lung disease outcomes.(126) Before examining the literature, it is worth briefly discussing the difficulties of designing and implementing a study of efficacy of augmentation therapy.

The progression of emphysema in AATD, although more rapid than in usual COPD, is a slow process taking many years for significant lung disease to occur. Typical physiological tests to assess respiratory outcomes such as FEV₁ decline would require follow up of at least 4 years to detect a significant difference in group outcomes(127) at considerable cost. Couple this with the issue of recruiting adequate numbers of patients with a rare disease and the logistical considerations of administering a weekly intravenous infusion illustrates the pitfalls in establishing efficacy of AAT augmentation therapy. Use of more sensitive surrogate markers of emphysema progression is one potential solution. Another is more careful design of observational studies with up-to-date methods for controlling for confounders to gain more meaningful and robust results.(128)

To date there have been 8 randomised control trials of AAT augmentation therapy; 3 vs placebo(53, 129, 130) and 5 compared against another AAT preparation.(131-135) There are a further 6 observational trials with a control comparator group.(136-141) For brevity, we will review the largest observational control trial and three RCTs against placebo. Table 1.2 provides an overview summary of RCTs and observational studies with controls.

In 1998, eleven years following FDA approval and use of Prolastin® in the USA, the results of the largest observational cohort study with a control group were published. More than 1000 patients with AATD were followed up for between 3.5 and 7 years. Overall mortality was reduced in the augmentation therapy group compared to control (RR 0.64 95% CI 0.43-0.94 p=0.02). The study was limited by intermittent treatment regimens and lack of adjustment for important confounders. Although overall FEV₁ decline was not reduced, patients on AAT augmentation therapy with FEV₁ between 35-49% predicted showed a significantly slower decline in lung function (mean difference 27ml/year, 95% CI: 3 to 51 ml/year, p – 0.03). Further RCTs were needed at this point to provide evidence for efficacy of augmentation therapy.

The first RCT, published the following year in 1999,(129) studied 58 PiZ ex-smokers randomised to receive either 250mg/kg body weight AAT every 4 weeks or placebo (human albumin 625mg/kg body weight). All participants had pulmonary emphysema demonstrated by CT thorax and moderate to severe COPD as defined by an FEV₁ between 30 and 80% predicted. Two patients dropped out due to resumption of cigarette smoking. The primary outcome was FEV₁ decline with secondary outcomes of

Table 1.2: Studies investigating the efficacy of AAT augmentation therapy. Adapted from Miravittles et al(126).

Study Design	Author, year	Intervention	Frequency (weeks)	Control	Outcome measure	Participants	Follow up time	Treatment Effect
RCT vs placebo	Dirksen, 1999(129)	250 mg/kg AAT augmentation	4	625 mg/kg albumin solution	FEV ₁ ↓	Aug 28 Placebo 28	≥3 years	No SD in FEV ₁ ↓ 59.1 vs 78.9ml/year (p =0.25) Reduction in CT lung density decline by 1.07g/L/year ± SE 0.58 (p=0.07)
	Dirksen, 2009(130)	60mg/kg Prolastin®	1	2% albumin solution	CT density↓	Aug 38 Placebo 39	≥2 years	Reduction in CT density↓ 0.8g/L/year (p=0.06)
	Chapman, 2015(53)	60mg/kg Zemaira®/Respreeza®	1	Lyophilised preparation	CT density↓	Aug 93 Placebo 87	≥2 years	Reduction in CT density↓ 1.5 vs 2.2g/L/year p=0.03
Prospective cohort with control	Seersholm, 1997(136)	60 mg/kg Prolastin®	1	No AAT augmentation	FEV ₁ ↓	Aug 198 Control 97	1 year	Reduction in FEV ₁ ↓ 53 vs 75 ml/year p=0.02
	AAT registry group, 1998(137)	60mg/kg Prolastin®	1	No AAT augmentation or reduced frequency	FEV ₁ ↓, survival	For 1°: No Aug 277 Partial Aug 261 Always Aug 389	1 to 7 years	Longer survival in Aug group RR 0.64 (p=0.001), NS difference in FEV ₁ decline overall (p=0.40), less

								FEV ₁ ↓ <i>Aug</i> group if baseline FEV ₁ 35-49% 73 vs 93ml/year (p=0.01)
Wencker, 2001(138)	60mg/kg AAT augmentation	1	Data collected before initiation of <i>Aug</i>	FEV ₁ ↓	96	≥1 year	Reduced FEV ₁ decline in <i>Aug</i> group 53 vs 75ml/year (p=0.02)	
Stoller, 2003(139)	60mg/kg AAT augmentation	1	Usual COPD care	Adverse events	Never <i>Aug</i> 382 Partly <i>Aug</i> 357 Always <i>Aug</i> 390	1 to 7 years	17% had adverse event: mild 16%, moderate 76%, severe 9%*	
Tonelli, 2009(140)	Any dosing regime	Any interval	No <i>Aug</i>	FEV ₁ ↓	164	Average 3.5 years	Reduced FEV ₁ ↓ 37 vs 46 ml/year (p=0.05)	
Barros-Tizon, 2012(141)	60 mg/kg Prolastin® or Trypsone®	Any interval	Data collected before initiation of <i>Aug</i>	Exacerbation rate	127	1.5 years	Reduced annual exacerbation frequency 1.2 vs 1 per year (p<0.01)	

RCT: randomised control trial; AAT: alpha-1 antitrypsin; ↓: decline; FEV₁: forced expiratory volume 1 second; SD: significant difference; CT: computed tomography; SE: standard error; 1°: primary outcome; RR: relative risk ratio; *Aug*: alpha-1 antitrypsin augmentation therapy; COPD: chronic obstructive pulmonary disease. *Rounded values hence do not add up to 100%.

decline in D_{LCO} and CT lung density. Although results for decline in physiological measures such as FEV_1 and D_{LCO} were not significantly different, there was a strong trend towards a reduced CT lung density decline in the treatment arm. The authors acknowledged that the trial was underpowered to detect changes in FEV_1 or CT density but the latter may help in future RCTs as a more sensitive marker of emphysema progression in AATD.

The EXacerbations and Computed Tomography scan as Lung End-points (EXACTLE) trial further explored the use of CT densitometry and pulmonary exacerbations for the assessment of the therapeutic effect of augmentation therapy with results published in 2009.(130) Seventy-seven participants, all with PiZ AATD, were randomised to either weekly infusions of human AAT (Prolastin® 60mg/kg body weight) or placebo for between 2 and 2.5 years. The primary end point of change in CT densitometry was assessed by four different methods in order to determine the optimal approach, all of which favoured treatment. One method, which used statistical adjustment for volume, demonstrated conventional statistical significance (p value 0.049). The authors concluded that CT densitometry is more sensitive than physiological and health status indices and therefore is more likely to detect a treatment effect than traditional measures. The rate of decline in lung function was reproducible when compared to the results from Dirksen et al a decade earlier.(129)

The RAPID study was the most recent and largest RCT investigating the efficacy of AAT augmentation with 180 patients randomised to weekly infusions of 60mg/kg AAT or placebo.(53) The study was intentionally powered to detect a significant treatment effect on annual loss of lung density as measured by CT densitometry. Secondary outcomes included physiological indices (change in FEV_1 % predicted and change in D_{LCO}), health

status measures e.g., St Georges Respiratory Questionnaire (SGRQ), and exacerbation frequency. Initial follow up was 24 months with an optional open label extension study for a further 2 years.(142) CT images were obtained at total lung capacity (TLC) and functional residual capacity (FRC) with the primary end point of CT density decline intended to utilise a combination of the two. Images obtained at TLC and FRC were also examined in isolation as secondary end points. Results showed a trend towards reduced decline in lung density when measured with both TLC and FRC, though the difference was not statistically significant ($p=0.06$). When TLC was used alone there was a significant difference in decline in lung density in favour of treatment ($p=0.03$) with an annual difference of 0.74g/L per year. Using TLC alone is the more usual approach in emphysema quantification since TLC represents full inspiration which is easier to achieve and reproduce consistently in patients with lung disease compared to FRC. Use of TLC also limits the impact of air trapping on CT analysis since trapped air at FRC could be interpreted as emphysema and skew the results. There was no difference in physiological indices or health status outcomes. Those originally on placebo who switched to treatment for the open label extension trial exhibited reduced decline in lung density toward rates similar to the original treatment group. This was further supported by analysis of desmosine levels, which showed that this marker of elastin degradation altered in line with augmentation and related to CT density.(105)

There is consistent and reproducible demonstration of a reduction of loss of lung density across all three described RCTs of augmentation therapy vs placebo. A meta-analysis of the three studies confirmed this (Figure 1.6), and showed an overall reduction in rate of lung density decline by 0.79 g/L/year versus placebo (95%CI 0.29 to 1.29, p value 0.002).(143)

Similar analysis of mean change in FEV₁% predicted and DL_{CO} show no significant change.(143)

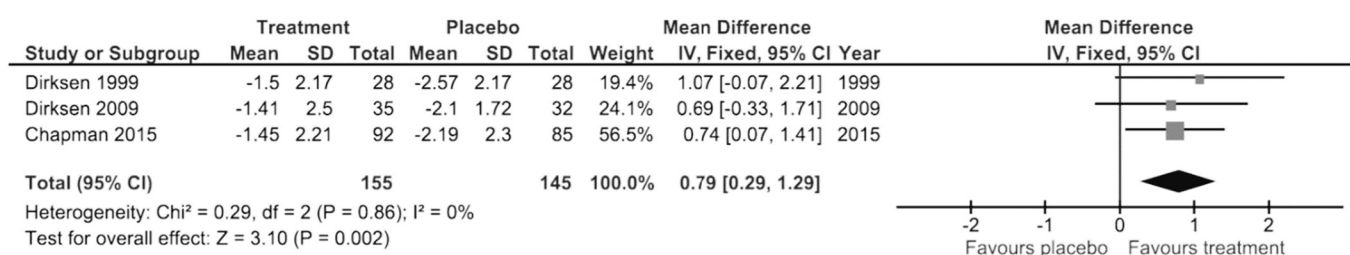


Figure 1.6: Meta-analysis of three randomised control trials of AAT augmentation therapy vs placebo. Dirksen 1999,(129) Dirksen 2009,(130) Chapman 2015.(53)

One of the main criticisms of using CT lung density as a surrogate marker for progression of emphysema is that it may not equate to conventional methods of measuring efficacy,(144) namely mortality, decline in FEV₁, change in QOL or health status and reduction of pulmonary exacerbations. Recent studies contradict this and suggest there is an association between lung density decline and both mortality(145) and QOL.(146) A recent ERS statement on the treatment of lung disease in AATD agrees that CT lung density is a relevant clinical measure.(126) In practice, implementing an adequately powered RCT with sufficient follow up time to detect a significant difference in conventional methods of measuring respiratory outcomes is not feasible(15) and it may be sensible to rely on CT density measures instead. Alternatively, well designed and large prospective studies could also be used to address whether use of AAT augmentation therapy to reduce the basal

elastin degradation rate results in improved mortality and other conventional outcomes. This forms part of one of the hypotheses of this thesis and which will be described later in Section 1.8 to 1.10.

The safety of AAT augmentation therapy has been demonstrated across numerous studies in a large number of patients. Similar adverse outcomes between treatment and control groups were seen in the RAPID study(53) though this outcome was not reported in the other RCTs. Patients typically have weekly infusions at 60mg/kg of body weight, either administered at home by a district nurse or at an infusion centre. Intravenous access varies but is usually via a temporary intravenous canula though occasionally indwelling venous lines are used. The standard 60mg/kg of body weight dose in use is based on studies demonstrated that trough AAT serum concentrations are above the protective threshold of 11 μ M/L. The threshold was derived from PiSZ heterozygotes having AAT levels above this threshold having no increased risk of developing pulmonary emphysema,(18) although this approach has been debated, with one study finding that once emphysema develops these patients have an equal rate of decline compared to PiZ patients.(147) In a post-hoc analysis of the recent RAPID study, rate of lung density decline was inversely proportional to the trough serum AAT level with no evidence of plateau throughout the measured range.(53) Patients with a higher body weight and higher pre-treatment AAT serum level tended to have higher trough serum AAT levels.(53) These findings suggest scope for individualising dosing regimes for AAT augmentation therapy. Further research on this is required before meaningful conclusions can be drawn. Most show serum levels tend to fall below therapeutic threshold before 14 days and therefore weekly administration is likely to be the most common regime in future practice. Pharmacokinetic studies have shown evidence for feasibility and safety of fortnightly(148, 149) administration.

Another factor when considering the use of AAT augmentation therapy is its cost. In contrast to usual COPD management, AAT augmentation therapy is an expensive treatment. One recent study in the US analysed the cost of health care for 9117 AATD patients over 53,872 patient years, 1142 of whom were augmentation users between 1993 and 2015.(150) The total annual cost for receiving patients AAT augmentation therapy was \$127,537; two thirds of this was attributed to the direct drug costs and administration. This is in comparison to \$15,874 for those not on treatment. In 2015 the mean annual cost of augmentation therapy alone was \$85,975. The price is somewhat justified by the complex process of manufacturing AAT augmentation therapy which requires collection of pooled human plasma followed by purification with the added cost of drug administration.

Direct administration of AAT via inhaled preparations of AAT have been attempted including two small early phase trials(151, 152) demonstrating potential feasibility. The results of a large phase II RCT completed in 2016 conducted by Kamada demonstrated a higher rate of annual exacerbations in the treatment group (3.12 in the AAT-treated group and 2.67 in the placebo group, $p=0.31$). Plans for a phase III trial are underway(153) though this may be delayed due to withdrawal of the Marketing Authorisation Application (MAA) with the European Medicines Agency (EMA).

Alternative approaches to exogenous AAT augmentation therapy from pooled human plasma have been explored. Use of recombinant or transgenic AAT was initially a promising solution to the costly process of collecting pooled human plasma and the associated infection risk from weekly transfusion of blood products. However, recombinant AAT had a shorter half-life than AAT from pooled human plasma owing to its rapid renal clearance and considerably reduced activity against serine proteases.(154) The presence of trace amounts

of animal AAT in transgenic AAT production caused systemic antibody responses in early studies.(155)

1.6 Surgical treatment of AATD related lung disease

Before considering the different surgical approaches to lung disease in AATD it is worth noting preoperative considerations to optimise the patient for surgery and give the best chance for a successful outcome.

General measures should be employed in the first instance such as smoking cessation, adequate bronchodilation, control of exacerbations, chest physiotherapy and pulmonary rehabilitation. Theoretically, since AAT is an acute phase protein, augmentation will ameliorate excessive inflammatory changes associated with invasive surgery.(156)

Special anaesthetic considerations for patients with emphysema should also be discussed prior to surgery such as intraoperative management of small airways collapse and increased anatomical dead space which is increased in both AATD related and smoking related emphysema.(157)

1.6.1 Lung volume reduction (LVR)

The characteristic development of panacinar emphysema and subsequent lung hyperinflation is a key factor causing breathlessness in AATD lung disease as hyperinflation adversely affects chest wall and lung mechanics thus compromising efficiency and effectiveness of breathing.(158) It has been demonstrated that targeted surgical resection or use of endoscopic devices to deflate these emphysematous areas in a carefully chosen COPD population can lead to improved morbidity and mortality outcomes. These approaches can be used either as an alternative to lung transplantation or as a bridging procedure to delay transplantation.(159) In usual COPD there are generally more

favourable outcomes for those with heterogenous emphysema and, in the case of endobronchial procedures, absence of collateral ventilation from adjacent lobes.(160, 161)

Before considering the evidence, it is worth discussing the disparity between emphysema in AATD and usual COPD. Emphysema associated with AATD is typically panacinar (encompasses the whole acinus) with a basal distribution. This is in contrast to the centrilobular (affecting only the proximal portion of the acinus) and apically distributed emphysema often seen in usual COPD. This observation is important as these differences may affect the generalisability of results of clinical trials of LVR in usual COPD to individuals who have AATD.

1.6.1.1 Lung volume reduction surgery (LVRS)

Lung volume reduction surgery (LVRS) aims to reduce airway hyperinflation by surgically resecting diseased portions of lung tissue and was one of the first techniques employed to achieve this. Hence, this is the area in which evidence of an effect in usual COPD is most abundant.

The outcomes from 16 patients with AATD within the National Emphysema Treatment Trial study (NETTs) were reported in a post-hoc analysis by Stoller et al (162) in 2007. Ten patients underwent bilateral LVRS. Though the numbers were relatively small, mortality at 2 years was significantly higher in the treatment group compared to medical treatment alone (20% vs 0% respectively). When compared with AAT replete individuals, those with AATD had a smaller increase in exercise capacity as assessed by 6-minute walk test and a diminished and shortened duration of FEV₁ improvement. Another study in 2006 retrospectively looked at unilateral LVRS outcomes in 17 AATD patients and 35 AAT replete controls.(163) Findings concluded that although there was an increase in FEV₁ and

dyspnoea scores at 3 months and 6 months after surgery for the AATD group, these increases were not sustained significantly after 12 months. The improvement was sustained in AAT replete patients.

Since these early trials were conducted there have been significant improvement in the techniques employed for LVRS. This includes more careful selection of patients for surgery, minimally invasive approaches such as video assisted thoracoscopy (VATS) and better intraoperative techniques. Overall, there have only been a small number of patients in a limited number of trials thus far and more research is required to adequately assess LVRS as a suitable treatment for AATD related hyperinflation.

1.6.1.2 Endobronchial Valves (EBV) and Endobronchial Coils (EBC)

EBV utilises the insertion of one-way valves into the airways of affected lungs with the aim to reduce the overall volume of that portion of lung. EBV are considered less invasive than traditional LVRS and in recent years there has been movement towards EBV as the preferred method to reduce hyperinflation.(164, 165) The most recent RCT for endobronchial valves randomised 68 patients with emphysema and radiological absence of collateral ventilation equally to either receive EBV or standard medical therapy.(160) They reported after 6 months a statistically significant increase in FEV₁ (mean 161 ml ,95% CI 80 to 242) and distance covered on 6MWT (mean 60m ,95 CI 35 to 85). However, this study only included 7 patients with AATD (EBV group n = 4, control group n = 3).(160) Another case series describes the insertion of endobronchial valves for 15 AATD patients with heterogenous emphysema.(166) Three patients had early complications that necessitated exclusion from the study (recurrent pneumonias, coughing up the valve and pneumothorax). The other 12 underwent up to 4 years follow up with sustained improvement in FEV₁ and QOL. Two patients were able to stop oxygen therapy. A further

smaller case series observed similar encouraging results.(167) These preliminary studies are promising but further research into this area is needed.

Another potential option is use of endobronchial coils (EBC) to achieve regional parenchymal volume reduction and reduction of lung compliance by restoring elastic recoil of lung tissue.(168) It has also been suggested that coils could be suitable for homogenous emphysema.(164) Older studies excluded AATD and hence research in this area is limited. One recent post-hoc analysis of the REVOLENS study(169) analysed outcomes in 6 patients with AATD who received EBC with comparison to 84 non-AATD patients and observed similar efficacy of treatment between two groups. Patient numbers were insufficient to analyse end points for AATD alone. The rarity of AATD and previous exclusion of AATD patients from larger RCTs has resulted in a relative paucity of data in the area of LVR for AATD associated emphysema. The encouraging results for EBV have meant that specialist LVRS centres have started referring AATD patients for these therapies, and it is hoped that this, and in Europe the new AATD registry (EARCO)(170) will lead to the gathering of more robust evidence in the future.

The overriding message from the literature to date for both LVRS and EBV is that careful selection of patients with significant hyperinflation is vital and should be reserved for those with significant burden of lung disease. This should be conducting via a multidisciplinary approach including respiratory physicians, thoracic surgeons, radiologist and interventional bronchoscopist with expertise in LVRS. Referral should be reserved for lifestyle limiting breathlessness who are medically optimised including a recent program of pulmonary rehabilitation.(126)

1.6.2 Lung transplant

Though the mainstay of AATD treatment is to delay development of pulmonary emphysema, lung transplantation may be necessary if lung disease has significantly progressed. In all cases, patients should be fully optimised medically before transplantation referral is made. This includes ensuring smoking abstinence, pulmonary rehabilitation and inhaled therapy adherence. Detailed assessment of other medical co-morbidities should also be made. This is especially important when considering liver disease which often coexists in patients with AATD.(171) Some transplant centres routinely perform liver biopsy in lung transplant candidates although the presence of liver cirrhosis does not preclude them from lung transplantation. There are instances of dual lung and liver transplant being performed with favourable outcomes.(126, 172, 173)

Since the early 1990's lung transplantation has been an established treatment for end stage lung disease in AATD(174) and is the fourth most common indication for lung transplantation behind cystic fibrosis (CF), interstitial pulmonary fibrosis (IPF) and COPD.(156) Lung transplantation in AATD accounted for 5.4% of all lung transplants worldwide between 1995 and 2014.(175, 176) As a proportion, more patients with AATD related lung disease are transplanted than usual COPD and is performed in approximately 7-13% (156, 174) of all patients with severe AATD. The difference in rates is likely due to the younger age and lower co-morbidity burden of AATD patients relative to usual COPD.(177) Though AATD is considered a subset of COPD there are distinct differences between patients who undergo lung transplantation with AATD or usual COPD. AATD patients are typically younger (up to 10 years) and have less cigarette smoke exposure compared with usual COPD.(178) Furthermore, AATD patients have fewer comorbidities such as hypertension or diabetes. This is logical when considering the natural progression

of AATD including imbalances of proteinases within lung tissue and the more aggressive nature of emphysema development. Despite their reduced age, AATD patients have similarly poor FEV₁ and a worse DLCO at time of transplant referral when compared to usual COPD.(156)

In the literature there are difficulties in accurately assessing the merit of lung transplantation in AATD, largely due to conflicting results of multiple retrospective cohort analyses. In addition, studies have been carried out at different international centres with varying health care infrastructures meaning generalisation of results are limited. The majority of studies have focused on comparisons of either transplanted AATD vs transplanted usual COPD patients or transplanted vs non-transplanted AATD patients.

The benefits of fewer co-morbidities may be offset by the underlying antiprotease deficiency and its relevance to intraoperative and post-operative complication rates. One US study retrospectively comparing post-surgical outcomes between bilateral lung transplants in AATD and usual COPD patients reported higher rates of airway dehiscence (3.5% vs 1.5% p value = 0.001), more treatment for rejection within 12 months of transplant (41% vs 33% p value = 0.002) and increased infection related mortality (10.5% vs 8.1% p value = 0.045(179) in the AATD group. It has been suggested that this observation is related to the underlying pathophysiology of AATD and its direct effect on wound healing and airway inflammation/infection respectively. Studies have also shown increased activity of elastases in patients with AATD post lung transplantation.(180, 181)

Another study found increased rates of acute naïve lung hyperinflation in unilateral lung transplant(182) which could be explained by the increased lung compliance associated with pan-lobular emphysema. This factor along with the younger age at time of transplantation

accounts for the movement towards bilateral lung transplantation in AATD patients in recent years. In the US, in 2013 over 80% of lung transplants were bilateral compared to around 34% in 1990.(179) Overall, recent evidence suggests that early complications of lung transplantation are more common in AATD recipients when compared with usual COPD. Whether augmentation would ameliorate these effects is unknown.

There is conflicting evidence in terms of overall survival when comparing outcomes for lung transplantation in AATD and usual COPD. The most recent analysis by Gulack et al(179) examined data from 3166 usual COPD patients and 715 AATD patients. Their findings were that for bilateral lung transplant, AATD was not associated with a survival difference up to 1 year; HR: 1.19, 95% CI: 0.89, 1.58, or beyond 1 year; HR: 0.94, 95% CI: 0.78,1.14, after lung transplant when adjusting for covariates. These results are similar to those reported by Burton et al(183) and Banga et al.(174) Other studies have reported higher early mortality from sepsis in AATD, reduced 10-year survival (184) and a smaller survival benefit compared to usual COPD.

Two studies comparing differences in mortality between transplanted and non-transplanted AATD patients are also contrasting in their results. Stone et al(178) reported no difference in post-surgical survival when adjusting for FEV₁, age, sex, SGRQ score and gas transfer. QOL increased post-transplant in this study, as would be expected. Conversely a Swedish study(185) showed that transplantation increased survival from 5 to 11 years compared to matched non-transplanted AATD.

The contradicting evidence presented above highlights the heterogeneity in study design and the inherent weaknesses of retrospective analysis of data, thus their results should be interpreted caution. Ideally, higher quality data such as RCTs or large prospective cohort

studies should be carried out to assess survival with more accuracy. However, conducting such studies is likely to be expensive, impractical, time consuming and possibly unethical. Though efficacy of lung transplant in AATD for a survival benefit remains uncertain, there are clear benefits relating to QOL(178) and therefore lung transplantation remains an effective palliative procedure for end stage lung disease in AATD.

1.7 Future therapeutic approaches

Any future therapy intended to treat or prevent the progression of AATD related emphysema should either a) increase serum levels of AAT, via exogenous administration or endogenous production of AAT or b) inhibit the destructive effects of NE in lung tissue.(15)

One alternative to the exogenous administration of AAT is to facilitate increased endogenous secretion via genetic therapy through replacement or insertion of genes capable of producing functional AAT. Genetic therapy is an attractive prospect since AATD is caused by a single, known genetic mutation of the SERPINA1 with a relatively short sequence which could theoretically be delivered by viral or liposomal vectors.

Several routes of administration have been explored, lungs being the obvious target for treatment of pulmonary disease. Delivery via a nebuliser is feasible though penetration of drug molecules are impaired in lung disease and may not achieve sufficient alveolar concentrations with conventional nebulisers.(152) Use of novel nebuliser devices such as the AKIRA² device may be a more efficient method of achieving this.(152) Direct intratracheal administration is another option and has been demonstrated in animal models.(186) This may not always be suitable as there are risks with bronchoscopy in patients with lung disease.

Other sites for administration, for example muscle tissue(187) or nasal mucosa,(188) would allow ectopic secretion of AAT and subsequent action in the lungs where it is required. This is possible as AAT is a secreted protein and functions predominantly in either plasma or the extracellular space.

The first description of in vivo AAT gene transfer in an animal model was published in 1991.(189) Since then the results of a small number of phase I clinical trials in humans investigating different methods of achieving novel gene therapies using viral and liposomal vectors have demonstrated potential safety and feasibility in a small number of patients. (187, 190) The only published phase II trial using rAAV1 vectors(191) demonstrated a 3 to 5% increase of target serum AAT concentration for those receiving therapy. Though modest, this is promising since these are early studies with scope for dose escalation or more efficient vectors.(192) Two further phase I/II clinical trials were completed recently with results yet to be published (clinicaltrials.gov reference NCT02168686, NCT00377416) Gene therapies have been comprehensively reviewed in a recent book on AATD.(193)

Briefly, an exciting prospect is gene editing via a knockout-knockin approach using CRISPR. This technique effectively aims to remove the mutant AAT gene and replace it with a normal functioning AAT gene. This would simultaneously treat lung and liver disease by increasing AAT levels in serum and removing the burden of polymerised AAT mutants within hepatocytes. Murine models have demonstrated its effectiveness in AATD.(194, 195)

An alternative approach to delaying progression of pulmonary emphysema in AATD is to target and block the destructive activity of NE in the lung. A phase II multicentre, double-blind, placebo controlled RCT (clinicaltrials.gov reference NCT03679598) is underway as a proof-of-concept study and to assess the safety and tolerability of an oral NE inhibitor in

patients with AATD. The same drug was tested in another phase II RCT of 625 patients with usual COPD.(196) There was no difference in the primary outcome of FEV₁ or any of the secondary outcomes including exacerbation frequency after 12 weeks. This was a relatively short follow up period for such outcomes; hence a conclusion of no effect may not have been justified. The hypothesis is that AATD patients have increased pulmonary NE activity and will benefit more than those with usual COPD. Even though this is a promising prospect, there are some concerns that NE inhibitors could decrease the antimicrobial defences of the lung.(197, 198)

1.8 Strategies to limit disease progression in AATD related lung disease

So far, I have discussed generally how AATD related lung disease presents and how AECOPD and emphysema decline contribute to the disease burden. I introduced the idea that precision medicine could lead to earlier detection of exacerbations and more targeted therapies that could ultimately slow down lung destruction that leads to advanced emphysema. I also discussed that the basal rate of emphysema progression can be slowed with augmentation therapy.

The overall aim of this thesis is to comprehensively assess the feasibility and efficacy of approaches to limiting disease progression in patients with AATD related lung disease. AATD related lung disease, in comparison to usual COPD, has a stronger emphysema phenotype due to protease-antiprotease imbalance. As such, therapies/interventions towards reducing emphysema decline are logical targets to reduce mortality and improve patient reported QOL.

I propose two determinants of emphysema decline: 1) accelerated elastin degradation secondary to pulmonary exacerbations and 2) overall basal elastin degradation rate.

Accelerated elastin degradation decline occurs during episodes of increased inflammation, such as during AECOPD. Basal elastin degradation rate is specific to each patient and may have multiple influential factors including smoking status(199), AAT phenotype(200), other lung comorbidities(201) and epigenetic factors that are not yet known.(202)

In the following sections I will present the rationale for exploring two interventions to reduce the rate of emphysema decline in patients with AATD related lung disease before outlining the main hypotheses for this thesis. I will do so by comparing two identical fictitious patients with AATD related lung disease (Figure 1.7). Patient A (red line) receives usual COPD care which is currently the standard treatment for patients with AATD in the UK.(203) Patient B (blue line), receives usual COPD care plus two additional interventions/strategies; 1) enhanced symptom/biomarker monitoring and 2) alpha-1 antitrypsin augmentation therapy.

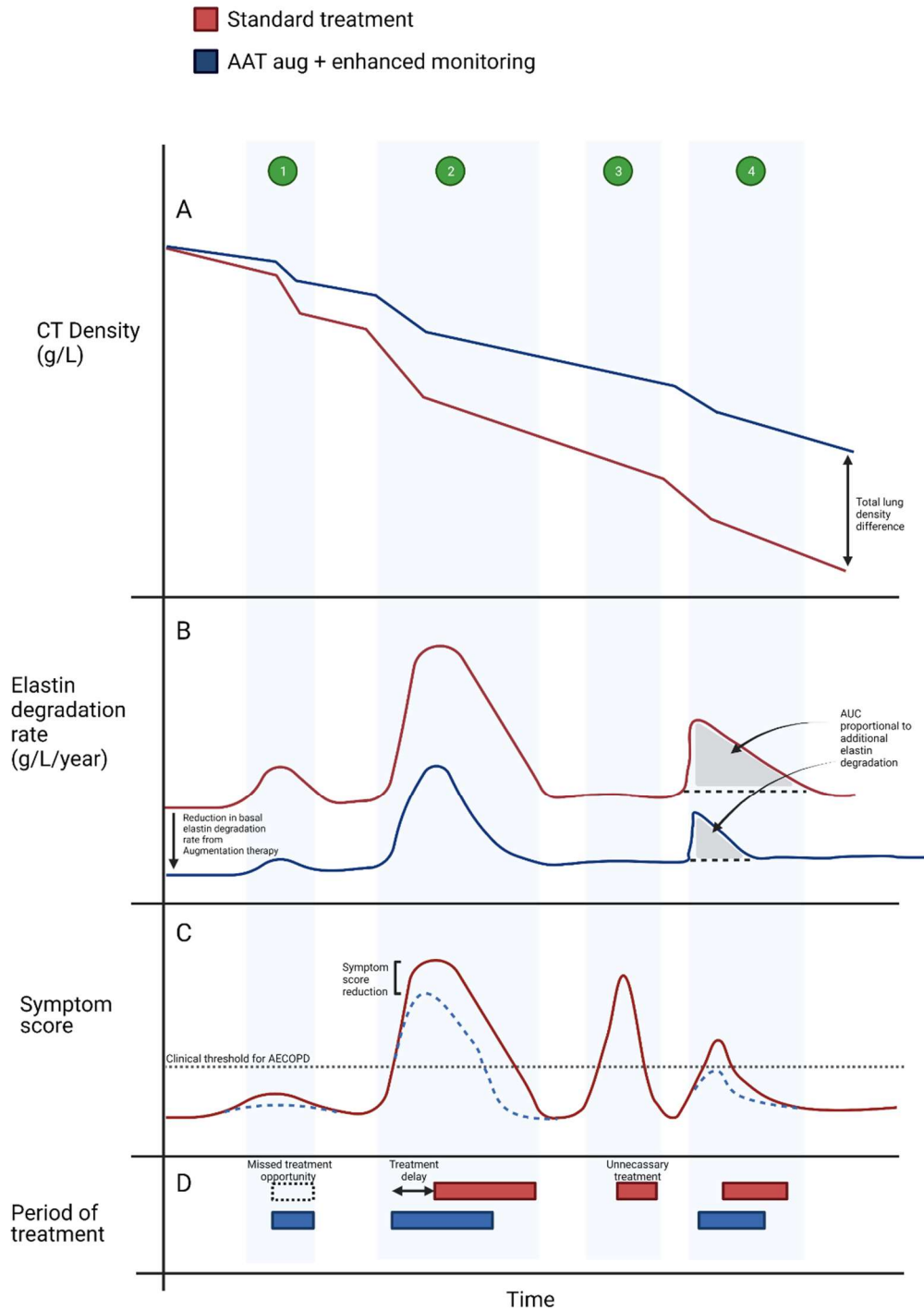


Figure 1.7: Comparison between Patient A (standard COPD treatment) and Patient B (AAT augmentation therapy + enhanced monitoring) and the theoretical outcomes for A) total CT density, B) elastin degradation rate, C) symptom score and D) periods of treatment.

1.8.1 Enhanced symptom/biomarker monitoring

This section will present two monitoring modalities that could be used to detect the early development of AECOPD in patients with AATD; symptom diaries and home biomarker monitoring.

1.8.1.1 Symptom diaries

As discussed earlier in this chapter, current standards for diagnosis of COPD/AATD exacerbations, particularly in the community, rely almost exclusively on changes in symptoms which are often misinterpreted or ignored.

Symptom diaries were developed as a way of accurately recording exacerbations in real time as opposed to a treatment-based definition or patient recall which inherently led to unreported exacerbations being missed. Symptom diaries formed two possible functions; 1) as a PRO tool in clinical trials of drugs that target exacerbations and 2) as a health monitoring tool to detect exacerbations early and facilitate prompt treatment.(204)

In recent years, daily recording of symptoms has moved from written diary cards to an electronic symptom diary (eDiary). The advantage of this is that exacerbations can potentially be reviewed in real time (rather than waiting for paper diaries to be sent) and the analysis process can be automated with computer algorithms. A large proportion of studies to date have used symptom diaries as part of randomised controlled trials to assess the performance of an intervention against placebo or standard therapy. There have been fewer studies looking at the feasibility and challenges associated with implementing an eDiary in a real-world setting.

Table 1.3: Comparison of studies that have used symptom diaries to assess COPD with a focus on exacerbations

Study	Cohort	Design	Assessment tool/device	Exacerbation definition	Adherence	Results
RCTs						
Chatwin, 2016 (205)	COPD (N = 38) + chronic respiratory failure due to other causes (N = 30). O2 sats <90% or on oxygen	Randomised crossover trial 6 months telemonitors vs standard care	Philips Motiva system – HR, O2 sats, weight, BP + symptom questionnaire (not specified). Required installation at home	Hospital admission	No reported	Increased hospital admissions and home visits No SD in QoL or time to next exacerbation
Ho, 2016 (175)	COPD N = 106 Mean age 80.2 years	RCT telemonitoring vs standard care 2 months post discharge for AECOPD	Symptom diary (breathlessness, sputum volume, sputum colour) + HR + BP + O2 sats + Temperature + weight. Access via internet with own device/computer	Hospital admission Clinical judgment via telephone	Not reported	Reduction in time to readmission for AECOPD
Tabak, 2014 (206)	COPD N = 29 (71% screened patients excluded due to no internet access at home).	Pilot RCT of telemedicine intervention vs standard care	Symptom diary + decision support was part of 4 modules (web-based exercise program, activity coach, consultation module). Access via web-based portal.	Hospital/eme rgency room visits Symptom based definition	For symptom module adherence: 82.8% (range 44.4 to 93.6%)	Health care providers play important role in patients' adherence to telehealth.

Post-hoc analyses of RCTs.						
Frent (2019) - (207)	COPD N = 453	FU 52 weeks Post-hoc analysis of subset of FLAME RCT	EXACT-PRO – electronic device not described	Anthonisen criteria and HCRU	Mean compliance 91.0 – 93.6%.	NS difference in capture of HCRU. 22.2% of HCRU captured by EXACT tool.
Ejiofor, 2017 (208)	AATD N = 46	Post-hoc analysis of subset of RCT (nebulised AAT vs placebo) FU > 52 weeks	Bronkotest – eDiary (Kindle Fire)	Anthonisen criteria and HCRU	No reported	Untreated episodes shorter than treated. Dyspnoea associated with treatment of exacerbation.
Kulich, 2015 (209)	COPD N = 209	Post-hoc analysis of SHINE study (dual bronchodilator vs single bronchodilator)	Symptom diary – administered twice a day	Did not assess AECOPD -	90.4% at baseline, 81.3% at week 26	Breathlessness associated with higher impact of AECOPD
Vijayasaratha and Stockley, 2012(210)	AATD N = 23	Post hoc analysis of EXACTLE trial (AAT augmentation vs placebo)(130) 24-month FU 11 patients on AAT for trial	Bronkotest – paper diary	Anthonisen criteria	Not reported. Of 164 episodes no missing data was available for 81 episodes	Frequency and duration of exacerbations related to baseline KCO
Vijayasaratha and Stockley, 2008(211)	AATD N = 19	Post hoc analysis of EXACTLE trial(130) 24-month FU	Bronkotest – paper diary	Anthonisen criteria and HCRU	Not reported	Higher total symptoms scores for treated vs untreated exacerbations. Early return of well-being to normal may influence decision to treat exacerbations

Observational studies						
Boer, 2018 (212)	N = 54, outpatient COPD and rehabilitation centres	Prospective study, up to 3 months FU Computer algorithm advised patients on management.	Adaptive Computerised COPD Exacerbation Self-Management Support (ASSESS) – symptoms + O ₂ sats + spirometry + temperature.	Symptom-based definition and HCRU	97.9% - one patient excluded for non-adherence. Data also collected on paper to avoid missed results	Sensitivity 97.4%, Specificity 65.6%. False positive rate 34.4% across 725 exacerbations and additional contact with healthcare professionals
Johnston, 2013 (213)	COPD Data collected 2007/2008 N = 49	Prospective cohort study 12 months follow up	Symptom diary not specified Issues with smart phone (Blackberry 8700) Reminder alert	Anthonisen criteria and HCRU	>99.9% Intensive nurse follow-up	Higher exacerbation frequency during Christmas period. Rhinovirus positive exacerbations were longer than negative ones
Sund, 2009 (204)	COPD N = 18	Prospective study to assess feasibility of symptom diary 6-month FU	Symptom diary (breathlessness, fatigue, sputum volume and sputum colour) + spirometry. Xda PDA device – SIM card used to send data	Variation of Anthonisen criteria	77% (range 70 – 92%) of total study days recorded. Adherence factors not explored	Reduction in hospitalisation – no comparator group 27% of treated AECOPD did not meet Anthonisen criteria
Pinnock et al 2013 (214) –	COPD N = 265	RCT telemonitoring vs standard care (1:1)	Symptom diary + O ₂ sats. Touch screen device issued.	Hospitalisation	Not reported	No difference in time to next hospital admission or QoL.

HCRU; health care resource use, N; number, RCT; randomised controlled trial, QoL; quality of life

Table 1.3 references recent studies that have attempted to capture AECOPD using symptom diaries, some alongside other monitoring devices to aid detection of exacerbations. The studies listed vary widely by design, length, exacerbation definition and intervention which makes direct comparison of results difficult.

Regardless of whether a diary performs well at predicting AECOPD, patient adherence will dictate the success of eDiary use in the real-world and therefore is important to consider. Adherence to a symptom diary between studies from Table 1.3 ranged from 77%(204) to >99.9%(213) with some failing to report adherence/compliance. The adherence seemed to depend on multiple factors, the most important of which was intensity of follow up. In Johnston et al,(213) who reported >99.9% adherence, the study was supported by research nurses who gave daily feedback to patients with worsening symptoms and called them on the telephone to discuss symptoms further if required. They also visited their homes if an exacerbation was suspected and visited them in hospital for severe exacerbations. The authors acknowledge that this level of follow up would be difficult to replicate in a multicentre trial and would be costly to continue this for patients in a real-world setting. This intensive follows up likely contributed to the high adherence levels.

By contrast, the lowest reported adherence rate was by Sund et al(204) with an average of 77% who tested the diary in an environment similar to a real-world situation with limited follow up. Other studies excluded or withdrew patients that did not adhere to using the symptom diary and therefore do not represent real-world data.

For the purpose of the following scenarios in Figure 1.7, a theoretical symptom score would accurately record an increase in symptom severity during exacerbations and return to a 'baseline score' during periods of stable disease.

1.8.1.2 Biomarkers

The predictive power of symptom diaries would be strengthened if paired with a biomarker that accurately represents the underlying pathophysiological process. In this context, a biomarker represents a measured analyte within either blood, sputum or urine that gives an indication to underlying disease activity. This could be an immunomodulatory cytokine directly involved with the inflammation cascade, a by-product of elastin degradation as a surrogate of lung damage or an extracellular matrix protein, amongst other pathways. Ideally, the biomarker will rise with an increase in activity and fall when activity reduces. It will also be specific to the underlying disease process and remain suppressed during periods of stable disease(215).

In addition to a highly performing biomarker, the measurement device used by patients must be accurate, quick and simple to use in order for it to be adopted by patients. Lateral flow immunoassays are an example of this.

In reality, biomarkers and quantitative tests are rarely 'perfect'. For the following demonstration we will assume that the biomarker performs as intended with an equally robust quantitative test.

The use of both a symptoms score and biomarker measurement will now be hypothetically compared to standard COPD care (Figure 1.7).

1.8.1.3 Scenario 1: missed/unreported exacerbations

Scenario 1 (green dot) of Figure 1.7 shows how the use of enhanced monitoring may reduce total elastin degradation. Both Patients A and B have a sub-clinical increase in symptoms that are coupled with an increase in elastin degradation rate. Patient A (red line – standard treatment) does not receive treatment as they use perceived symptoms alone to inform treatment decisions. Patient B, who has enhanced monitoring of their symptoms and

biomarkers of disease activity, is alerted to this rise in disease activity and small uplift in symptoms and takes precautionary treatment. This settles the increase in disease activity and ultimately reduces the overall loss of CT density (Figure 1.7A), represented by a less steep decrease in the overall CT density graph and an earlier return to basal elastin degradation rate.

1.8.1.4 Scenario 2: AECOPD with accelerated elastin degradation rate

Scenario 2 (Figure 1.7) represents an AECOPD where the rate of elastin degradation increases secondary to underlying lung damage. The aetiology of such an event could be infective or environmental and contributes to emphysema decline. Patient A, in response to worsening symptoms, takes their rescue pack and recovers back to the basal elastin degradation rate and baseline symptom score. Patient B, however, is alerted earlier to a rise in elastin degradation rate and worsening severity of symptoms and takes their rescue pack sooner. Their overall symptom peak is less (Figure 1.7C – blue dotted line) and they return to their baseline symptoms earlier. They also have a smaller degree of increase in elastin degradation rate. This results in a smaller impact on the overall CT density represented by a slower and shorter trajectory on the CT density graph (Figure 1.7A). This is also shown in scenario 4 where the area under the curve is smaller for Patient B (Figure 1.7).

1.8.1.5 Scenario 3: AECOPD without increase in elastin degradation rate

Certain exacerbations may not contribute to underlying lung damage and therefore treatment should be aimed at symptom control only. In scenario 3 (Figure 1.7), both patients experience a rapid worsening of their symptoms, possibly due to psychological or cardiogenic factors. Patient A uses a rescue pack and their symptoms quickly return to normal. Patient B does not take a rescue pack as their elastin degradation biomarkers did not increase. Both patients have the same burden of symptoms and recover at the same

time to their baseline symptom score. This is an example of when enhanced monitoring could be used to avoid unnecessary treatment of AECOPD.

Note that across all scenarios each patient receives the same length of treatment meaning the risk of side effects is theoretically the same. Optimal timing of treatment has maximised the reduction in elastin degradation and minimised risk of unnecessary side effects.

Enhanced monitoring over the lifetime of a patient could result in preserved CT density and ultimately slower decline in emphysema.

1.8.2 AAT augmentation therapy.

The second intervention is AAT augmentation therapy. In Figure 1.7, the overall basal elastin degradation rate is lower for Patient B compared to Patient A and therefore the trajectory of CT density decline is reduced. Theoretically, the changes in elastin degradation rate and symptom score burden could also be reduced during AECOPD for those with AAT augmentation therapy.

The effect of both treatment interventions is preserved CT lung density which has been shown to be associated with mortality and improved QOL.(145) Using this framework, in the next section I will present my rationale for the hypotheses tested in this thesis and briefly outline how I will aim to achieve this.

1.9 Thesis rationale and hypotheses

In the above discussion I have outlined a package of interventions to reduce the rate of emphysema decline in patients with AATD related lung disease.

Enhanced monitoring of symptoms and biomarkers that are specific to exacerbations and emphysema decline in patients with AATD are certainly exciting prospects that would redefine precision medicine for patients with AATD related lung disease. There are no such

studies that have explored the combination of symptom diary and biomarker analysis in AATD exacerbations.

To reduce the overall basal rate of emphysema progression, AAT augmentation therapy is already used in many countries including the US and parts of mainland Europe. Though randomised control trials have demonstrated evidence that CT density is preserved compared to placebo, whether this translates to tangible outcomes such as mortality, QOL or exacerbations is yet to be determined. This fact has prevented UK patients with AATD from accessing AAT augmentation therapy and is therefore an important area to explore.

From the above discussion I have developed three main hypotheses to be addressed within this thesis. The first two relate to enhanced monitoring of symptoms and biomarkers to reduce the impact of exacerbations on emphysema progression whilst the third refers to the overall basal elastin degradation rate:

- 1) Symptom scores can independently determine AECOPD in patients with AATD related lung disease including unreported exacerbations. It is also feasible to implement an electronic symptom diary (eDiary) for patients at home.
- 2) Molecules relating to elastin degradation, inflammation and extracellular matrix turnover will be elevated during AECOPD and return to baseline on recovery. Measurements of such biomarkers by patients at home is feasible.
- 3) AAT augmentation therapy slows the rate of QOL decline and reduces mortality compared with patients who do not receive AAT augmentation therapy.

I will now present how these hypotheses will be tested via the aims of this thesis.

1.10 Aims

1.10.1 Aim 1: describe a cohort of AATD with frequent exacerbations.

The first aim is to describe a cohort of frequent exacerbators with AATD related lung disease and to set out the general methods of how patients were recruited and how samples

were collected, presented in Chapter 2. This cohort will be used to collect data that addresses the first two hypotheses.

1.10.2 Aim 2: eDiary feasibility and effectiveness

To be able to test if symptom diaries are able to detect exacerbations to allow early recognition and treatment, I will assess the feasibility and effectiveness of the Bronkotest symptom score in electronic diary format. This will be performed in the cohort of frequent exacerbators described in Aim 1 with AATD related lung disease, the results of which will be presented in Chapter 3.

1.10.3 Aim 3: biomarkers of AECOPD in AATD

It remains unclear if biomarkers relating to elastin degradation, inflammation or extracellular matrix turnover are altered during AECOPD in AATD related lung disease, particularly in urine. I aim to assess the relationship of proposed urinary biomarkers of exacerbations in the stable state, during exacerbations and upon recovery. I also aim to assess the feasibility and validity of a novel urinary lateral flow testing kit that can be used by patients at home. To fulfil this aim, I will measure urinary biomarkers of the same cohort of frequent exacerbators with AATD related lung disease, paired with symptoms, in order to establish if they relate to exacerbations. The results of this will be presented in Chapter 4. The most successful biomarkers will be combined with eDiary data to test if a composite prediction algorithm can be used to determine early onset of an exacerbation.

1.10.4 Aim 4: outcomes for augmentation naïve vs augmented AATD related lung disease

The final aim looks at the role of AAT augmentation therapy in reducing the overall basal rate of emphysema progression and if this effects mortality or QOL outcomes. This will be explored in Chapter 5 using two separate prospective cohorts of AATD patients; one treated with AAT augmentation therapy and one receiving standard COPD care. This will determine

if reducing the basal rate of elastin degradation actually improves outcomes rather than CT density alone.

1.11 Conclusion

AATD is an autosomal co-dominant condition that predisposes the affected individual to early development of emphysema and liver disease. Pulmonary exacerbations are key aspect of the associated morbidity of the disease with similarities to exacerbations in usual COPD. As such, exacerbations are treated in same way. There is a lack of evidence specifically aimed at exacerbations in AATD. This is partly down to exclusion of AATD patients from randomised control trials of drugs to treat or prevent exacerbations of COPD, in addition to its low prevalence. There is currently no evidence that augmentation helps prevent exacerbations or is effective in treating exacerbations acutely. Precision medicine is emerging including use of biomarkers to help distinguish phenotypes of exacerbations and to help further understand the pathophysiology of exacerbations in usual COPD and AATD related lung disease.

One of the key factors influencing disease progression in AATD related lung disease is advancing emphysema. This is thought to be facilitated by increased protease activity within lungs. Increased inflammation from pulmonary exacerbations contributes to a more rapid decline in emphysema in addition to the accelerated basal rate of lung destruction compared with usual COPD. Tailoring treatments to reduce basal emphysema progression and limiting the destructive effects of exacerbations are two ways in which the rate of emphysema development can be slowed.

This thesis aims to assess a package of interventions designed to slow the rate of emphysema decline in patients with AATD related lung disease. The efficacy and feasibility

of home monitoring devices that can detect exacerbations earlier and distinguish whether conventionally defined exacerbations lead to lung tissue destruction or not will be explored. This will allow for a more personalised treatment approach. This thesis will also evaluate if use of augmentation therapy to slow emphysema decline reduces mortality and slows decline in QOL.

2 ENHANCED MONITORING IN EXACERBATIONS OF AATD

RELATED LUNG DISEASE - METHODS

2.1 Introduction

Pulmonary exacerbations of COPD in AATD contribute significantly to overall impact of the disease on symptoms and QOL.(216) As discussed in the introduction, the heterogenous nature of the presentation and aetiology of such episodes makes developing targeted therapies challenging. Decisions to treat exacerbations in primary care are guided by symptoms alone. Equally, clinical trials that assess effectiveness of drug therapies for AECOPD mainly use a treatment or healthcare use based definitions that do not address unreported exacerbations or exacerbations that were unnecessarily treated.

Specific to AATD, frequent pulmonary exacerbations accelerate emphysema decline.(217-219) By instigating earlier and targeted treatments for exacerbations in patients with AATD related lung disease the rate of emphysema decline could be slowed. One of the main hypotheses of this thesis is that enhanced monitoring of symptoms and biomarkers of pulmonary exacerbations, for example elastin degradation, could help achieve this.

The current chapter sets out the overall design and methods for the 'Elastin degradation in exacerbations of AATD lung disease' study which aims to investigate if daily symptom diary recording and regular monitoring of urinary biomarkers can predict exacerbations effectively. It will also assess if such technology can be feasibly rolled out in a cohort of frequent exacerbators with AATD related lung disease.

2.2 Study overview

The 'Elastin degradation in exacerbations of AATD related lung disease' study was a prospective observational study of frequency exacerbators with AATD related lung disease

with follow up over a period of 18 months. Each patient received standard COPD care as part of the study in line with GOLD guidelines.(1)

2.2.1 Protocol

The original protocol for this study was agreed in November 2016 with a further protocol amendment in March 2017.

2.2.2 Ethical and safety considerations

Formal ethical approval was sought from the Research Ethics Service, Health and Care Research Wales and Health Research Authority (REC reference number 16/WA/0352) prior to approval of the original study protocol. As this was an observational study the risks involved for patients were minimal and were mainly related to patient sampling such as venepuncture.

2.2.3 Funding/support

This study was funded by a research grant from the Alpha-1 Foundation (Florida, USA) in May 2016. Urine cube monitors were supplied by Mologic (Bedford, UK). Desmosine measurements by mass spectrometry were funded by a supplement to the grant, as a collaboration between pH Pharma and the Alpha-1 Foundation.

2.2.4 Inclusion criteria

Inclusion criteria for AATD patients in the study were as follows:

- Subjects capable of giving informed consent.
- Subjects with PiZ, Znull AATD or rare genotypes of similar severity.
- Subjects with FEV₁/FVC ratio <0.70 and less than the lower limit of normal (LLN).
- Subjects who had a history of ≥ 2 AECOPD in the previous year.

Inclusion criteria for healthy controls included no history of AATD or lung disease (excluding well controlled asthma).

2.2.5 Exclusion criteria

Exclusion criteria for the study were as follows:

- Inability to give informed consent.
- For AATD subjects: lung function and genotype that was outside the range specified in the inclusion criteria.
- For healthy control subjects: any sign of infection on the day of assessment.

2.2.6 Outcomes

In line with the hypothesis and aims of this thesis, Table 2.1 summarises primary and secondary outcomes for this study including the chapter in which each outcome is addressed. Outcomes relating to symptom scores were recorded using the Bronkotest eDiary. This is an electronic symptom diary adapted from Bronkotest diary cards(211) which have been used in previous studies of patients with AATD and COPD(208). Outcomes relating to QOL use the COPD assessment test (CAT) and the St Georges respiratory questionnaire (SGRQ). CAT is a simple and widely used 8 question test to assess a patient's symptoms of COPD including breathlessness cough, sputum and overall well-being.(220) The maximum score is 40 with a minimal clinically important different (MCID) of 2 points.(221) SGRQ is a disease-specific measure of health status for use in COPD.(222) It has 3 domains (impact, symptoms and activity) with an overall total score measured from 0 to 100 and a MCID of 4.(223)

Table 2.1: Summary of primary and secondary outcomes for 'Elastin degradation in exacerbations of AATD related lung disease' study.

	Outcome Measure	Thesis Chapter
Primary Outcomes		
Characterise elastin degradation at stable state and start of exacerbation in AATD patients who have COPD	Stable, 'Day 1' and 'Day 56' measurements of urinary desmosine, NE, TIMP1 and CRP and AAT.	Chapter 4
Assess feasibility urinary monitoring by AATD patients	Adherence to use of urine monitor Visual analogue scale for ease of use of urine monitor	Chapter 4
Secondary Outcomes		
Describe the time course of changes in elastin degradation from 'Day 1' to 'Day 56'	Stable, 'Day 1' and 'Day 56' measurements of urinary desmosine, NE, TIMP1 and CRP and AAT.	Chapter 4
Describe patterns in other specified urinary biomarkers	Measure urinary markers of extracellular matrix turnover, endothelial function and inflammation.	Chapter 4
Assess feasibility and utility of a smartphone app for Bronkotest	Adherence of Bronkotest eDiary Visual analogue scale for ease of use of eDiary	Chapter 3
Verify health status during exacerbations of AATD	CAT score and Bronkotest score during stable, exacerbation ('Day 1') and recovery phase ('Day 56') of exacerbation.	Chapter 3
Compare elastin degradation in AATD to age matched healthy controls	Measure desmosine, NE, TIMP1, CRP and AAT in healthy controls	Chapter 4

2.2.7 Sample size calculation

Unpublished preliminary data in usual COPD (included in the grant application for this work; see Appendix 7.4.5) suggested that 88 pairs of samples ('Day 1 and 'Day 56'), assuming that all exacerbations are Anthonisen types I or II, would be required to detect a difference in urinary desmosine of the same magnitude with 80% power and $\alpha=0.05$. This rises to 111 pairs if no such assumption regarding AECOPD type is made. If 60% of patients continued to have either 2 or 3 annual exacerbations over an 18-month period, with 10% allowance drop out, 55 patients should equate to approximately 88 to 120 exacerbations.

2.2.8 Participant identification and screening

In order to reduce rate of screen failures, an existing AATD research database was used to highlight potential patients for inclusion in the study. Patients were also approached during weekly NHS AATD clinics if they had previously been known to meet eligibility criteria for the study.

2.2.9 Recruitment

2.2.9.1 AATD patients

Figure 2.1 represents the recruitment timeline. All patients seen at the AATD NHS clinic at Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, were initially screened by checking FEV₁ and AAT phenotype in advance of clinic. If initial screening was passed, patients reporting 2 or more treated pulmonary exacerbations annually were approached for inclusion in the study and invited for a consent/baseline visit. Those patients already known to be frequent exacerbators were approached by telephone in advance of clinic so that consent and baseline visit could be arranged on the same day as clinic.

2.2.9.2 Healthy controls

Healthy controls were recruited to act as a comparison group for biomarker analysis.

Although age matching was not specifically used, control patients of a similar age to the AATD cohort were sought. No inclusion criteria were specified but patients were not permitted to take part if they had active lung disease, excluding well controlled asthma or a known history of AATD.

Recruitment of healthy controls was through relatives of AATD patients and advertisement to colleagues at the University of Birmingham and University Hospitals Birmingham, Queen Elizabeth hospital. Recruitment rate declined after the first 37 patients due to the limited pool of healthy volunteers willing and available to take part. As such, application was made

to extend recruitment to Heart of England NHS Foundation Trust, which has since become part of University Hospitals Birmingham. This led to increase in recruitment rate. This can be seen clearly in Figure 2.1. In total, 75 healthy controls were consented to take part and provide samples with 71 completing the recruitment process. My appointment as a research fellow is marked and all recruitment and follow up visits after this time were done by me.

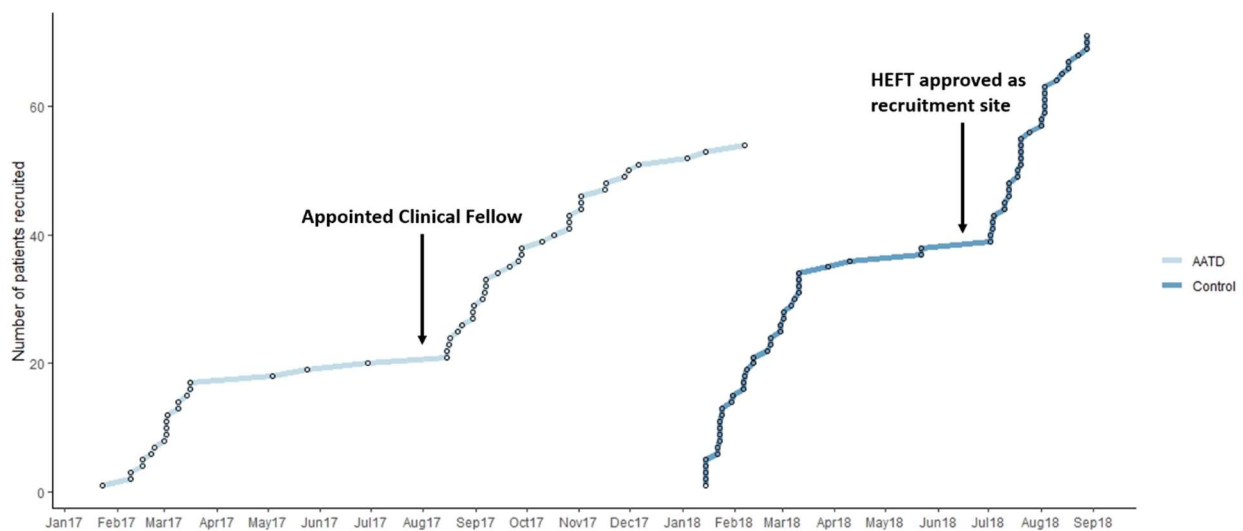


Figure 2.1: Recruitment timeline for AATD patients and healthy controls

2.2.10 Consent

All patients were guided through a patient information sheet and given adequate time to consider if they wanted to take part. Capacity was assessed in accordance with the accepted definition of mental capacity. Once fully informed, patients signed a consent form in person and retained a copy for their own records.

2.2.11 Trial procedures

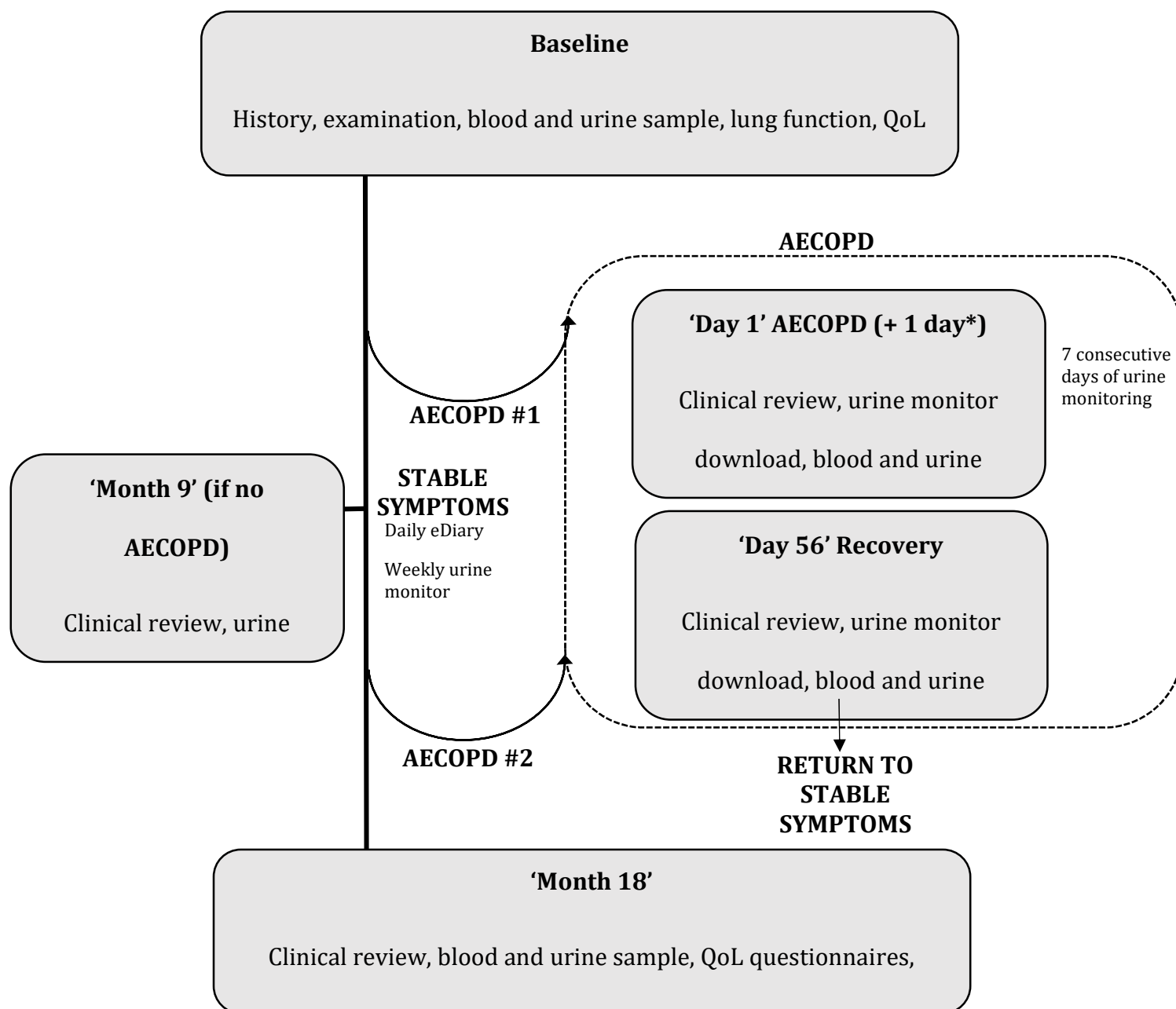


Figure 2.2: Overview of 'Elastin degradation in exacerbations of AATD related lung disease'. Study site visits are signified by grey boxes. Processes for AECOPD are represented by the dashed box. Urine monitoring occurs daily from Day 1 of an exacerbation for 7 days followed by weekly testing as per stable state.

*A protocol amendment was made to allow study participants to visit within 7 days of an exacerbation

2.2.11.1 Baseline visit

Patients were consented to the study if they had not already done so. A detailed consultation including past medical history, medications, social history and examination was performed. Lung function and CT images were reviewed to confirm obstructive lung disease and to check for any radiological evidence of bronchiectasis. Up to 20mls of whole blood was taken in addition to a urine sample. A fresh urine sample was used to train patients on how to use the urine cube. A further 10mls of urine was retained in plain specimen pots and taken to the laboratory for further processing and storage.

For QOL, CAT and SGRQ questionnaires were issued to patients on their arrival to the study site and were filled out prior to consultation.

Patients were issued with an eDiary and shown how to use it including logging in with username and password, charging, connecting to the internet and how to navigate their way through the questions.

A POC urine lateral flow device, referred to as a 'urine cube' colloquially by study participants, was also issued. Using urine collected during the same visit patients were shown how to use the urine cube and if necessary were asked to do it again themselves to check understanding. Participants were informed to keep the urine cube plugged in during the testing period. They were asked to complete a POC lateral flow urine test on a weekly basis on the same day of the week. If they felt they were having an exacerbation they were asked to perform a urine test daily for 7 days and then revert back to weekly measurements.

2.2.11.2 Exacerbation visit ('Day 1')

Patients were asked to make a telephone call to the study site as soon as they thought they were having an exacerbation which required treatment with antibiotics or steroids.

Symptoms of an exacerbation were checked via telephone and additional medical advice was given if required. Patients were then invited for a clinical assessment (called a 'Day 1' visit). Initially, patients were required to visit within one day of the onset of symptoms. However, due to the wide geographic spread of patients across England and Wales a protocol amendment was made to allow for patients to be seen within 7 days of onset of symptoms. This action was taken as (a) the home monitor and eDiary were able to pick up most outcomes remotely and (b) there weren't enough patients willing/able to travel during the first days of their illness. This coincided with the period of limited AATD recruitment in Figure 2.1, where all those willing to travel were recruited early, and recruitment improved again after my appointment and approval of the amendment.

During the 'Day 1' study visit patients had a medical consultation with a doctor and the onset of an exacerbation was confirmed. Vital signs were recorded and a focused medical examination was performed. If appropriate, clinical blood tests were sent including full blood count (FBC), C-reactive protein (CRP) and urea and electrolytes (UE). CXR was available if deemed clinically required. Up to a further 20mls of blood and 10mls of urine was taken to the laboratory for processing and storage (see Section 2.4.1.1). CAT scores were also taken as a measure of QOL during an exacerbation.

2.2.11.3 Recovery visit ('Day 56')

Patients were also invited to visit the research site two months following the onset of symptoms, called a 'Day 56' visit, to collect samples during the recovery from an exacerbation. Details of the previous exacerbation were checked and recovery confirmed. Examination and vital signs were performed and blood and urine tests taken and sent to the laboratory. CAT score tests were also recorded.

2.2.11.4 'Month 9'

If a patient had not exacerbated during the first 9 months from enrolment, they were invited back to the study site for review. Patients' daily symptoms and medications were checked and stable blood and urine samples were taken, plus QOL scores recorded.

2.2.11.5 End of study visit ('Month 18')

After 18 months patients were invited back for an end of study review. Patients provided blood and urine samples as for previous visits. Patients returned their urine cubes and eDiary devices for data download. Patients also filled out a questionnaire detailing their experience of using the eDiary and urine cube and completed a CAT and SGRQ questionnaire.

2.2.11.6 Healthy controls

Healthy controls underwent medical review including medical and social history and current and past medications. Spirometry was performed without bronchodilation (Micro Medical MicroLab 3500 spirometer) to establish presence or absence of obstructive lung disease. They also provided blood and urine samples as for previous AATD patient visits. AATD diagnosis was ruled out by either level or genotyping following patient consent. Those found to be carriers of a deficient AAT genotype were informed of the results and additional genetic counselling offered.

2.2.12 Withdrawal criteria

If a patient had chosen to withdraw from the study no specific action was taken. Patients were asked to return the study equipment and complete an exit questionnaire if appropriate to find out details of why they wished to withdraw.

2.2.13 Study visit data collection

Baseline medical data was collected using a pre-made case report form (CRF). This data was then recorded electronically on NHS computers which were in compliance with local

information governance policies. CRFs were stored in a study file in a locked office and patient identifiable information was removed.

2.2.14 Statistical analysis

All statistical analyses for patient characteristics and study visits were performed using R(224) in R Studio version 3.6.1(225) with the following packages: tidyverse,(226) ggplot2,(227) tableone,(228) ggsignif,(229) and geodist.(230)

Baseline characteristics for patients and healthy controls were assessed using means and standard deviations for normally distributed variables. For non-normally distributed variables medians with 25th and 75th quartiles for distribution were used. Distance between the study site and patient residence was calculated from the Haversine formula using longitude and latitudes.(230) Simple logistic regression was used to assess impact of distance from study site to likelihood of attendance for exacerbation visits. For comparative tests between QOL measures and biomarker concentrations; t-tests were used for normally distributed data and either the Wilcoxon signed-rank test or Kruskal-Wallis test, depending on the package used and the number of groups compared, for non-normally distributed data.

2.3 eDiary

The following section describes detailed methods relating to the eDiary which was issued to patients to track symptoms during the course of the study. Results from the data generated from patient use of the eDiary will be presented in Chapter 3.

2.3.1 eDiary device

All 55 patients that were recruited to the study were issued with a Kindle Fire HD 8" (sixth generation) during the baseline visit. This device was chosen as it was deemed easy to use and the most cost-effective available Android device. Designing a separate application that

could be used on personal smart phones was considered but the process of designing and an app and making it available to download presented a challenge that was easier solved by issuing individual devices. This also allowed inclusion of all patients, regardless of whether they owned a suitable device, and standardised the user experience. Each device was preloaded with the Take Part app. Patients were given a unique patient identification number and password to gain access to the app. The format of this was as follows; username: patXX, password: exacXX (XX denoting the patient unique identifier). A sticker on the back of the device had a unique serial number, the last 2 numbers of which corresponded to their unique patient identifier number.

Patients were trained during the baseline visit on how to use the device and were encouraged to demonstrate its use to the study investigator by filling in the eDiary as an example of their current symptoms to check understanding. They were also shown how to connect the device to Wi-Fi and how to charge the device.

2.3.2 Take Part application

The Take Part application was specifically created for this project using the paper Bronkotest diary, which had previously been validated and used in COPD and AATD patients.(208, 211) In order to convert the paper-based diary to an electronic version an iterative process of conversion of each question was followed by the eDiary company with the chief investigator of the grant that funded the project (Professor Alice Turner), aiming for a direct visualisation of the paper diary with few changes, in order to ensure that a validated system was being used, and not a newly developed scoring method. A system of cloud-based storage compliant with appropriate security standards (ISO 27001) was set up; specifically, it employed built-in enterprise security measures. At-rest data was 256-bit Advanced Encryption Standard (AES) encrypted on both client devices (web browser and

Kindle app) and server infrastructure. Data was transferred between client and server via Transport Layer Security (TLS)/Secure Sockets Layer (SSL) secure transport. Access to the solution required verified user name and password authentication, in addition to password and PIN protection on the user's device. Role Based Access Control (RBAC) restricted which functions a user (patient or researcher) may perform. Personal identifiers (such as name or date of birth) were segregated from the PRO tool such that data viewed or extracted for research and analysis purposes was pseudo-anonymised. Audit logs captured details of who/what/when/how/why key administrative features are performed.

After development it underwent beta testing with approximately 15 members of the research team in order to identify any bugs prior to going live with patients; this involved research team members completing the diary daily as if they were a patient and feeding back to the eDiary company on usability of the application and any faults in either design or functionality that were discovered with daily use. This occurred for approximately 2 weeks per user. Further troubleshooting of the system occurred in the first 1-2 months of use with patients based on data received. The layout of the application is illustrated in Figure 2.3.

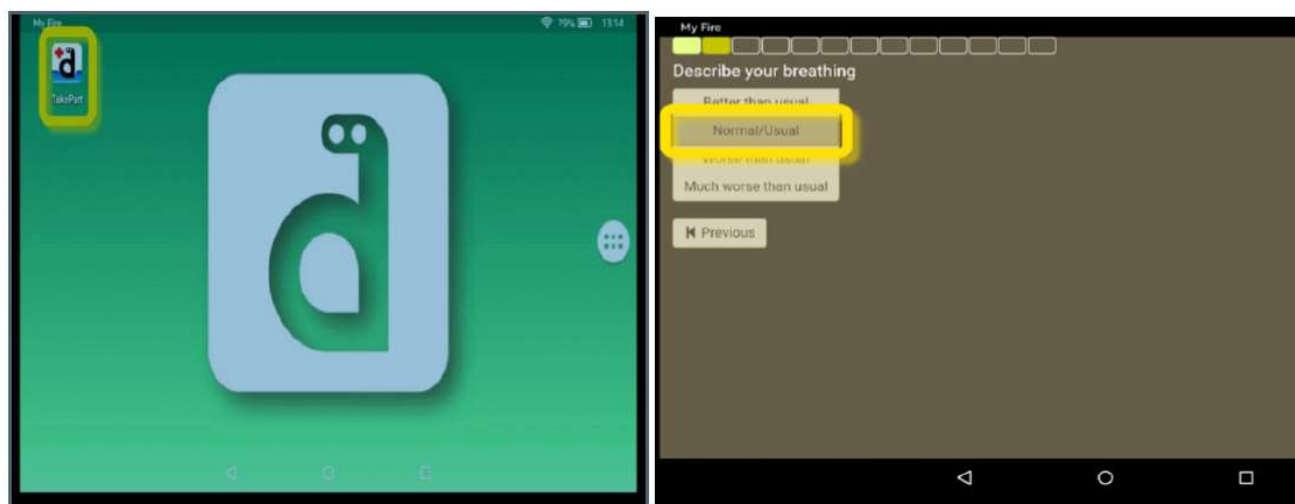


Figure 2.3: TakePart app A) home screen with application pre-loaded on device and B) example of how questions are displayed within the app.

2.3.3 Bronkotest eDiary

The Bronkotest eDiary is presented in Table 2.2 with each of the question domains and responses. This is the paper diary used previously(211) but in a digital format. The Bronkotest symptom score, like other PRO scores for exacerbations, incorporated domains of the usual exacerbation symptoms in addition to a well-being score into a quantifiable symptom score. When designed, questions were intentionally worded to be able to detect the same magnitude of difference in subjective symptoms such as breathlessness by comparing their current breathlessness levels to their usual normal baseline. For example, the first question asks: 'Describe your breathing' with four possible responses: better than usual, normal/usual, worse than usual and much worse than usual. Regardless of baseline dyspnoea or COPD severity, the daily score would only change if there was a deviation from baseline, such as with a pulmonary exacerbation. This is also the case for general well-being/feeling. During the original study using Bronkotest,(211) it was noted that breathlessness and feeling/well-being were strongly associated with treated exacerbations. The relevance of this is illustrated with the following example. During the onset of an

exacerbation, if a patient went from having no sputum (0 points for sputum amount, 0 points for sputum colour and 0 points for sputum type), to having 'some' sputum (2 points) which was 'colour 4' (4 points) and with 'sticky liquid' type consistency (2 points), then there would be an additional gain of 8 points. In contrast, a change in dyspnoea from 'normal/usual breathing' (2 points) to 'breathing worse than usual' (3 points), would only mean a change in 1 point. This imbalance led to the decision to use a weighting factor of 5 for the breathing to account for the significance in these symptom in a patient's decision to treat an exacerbation. The remaining domains each have a score of 1 attributed to them. For cold and flu symptoms a maximum of 1 point is added for any symptom combination. The maximum achievable score is 63 with a minimum score of 11.

In addition to symptom domains that contribute to the overall symptom score, there were a series of free text options that give more detail on treatment including whether antibiotics were used, whether other treatments were used e.g., steroids, additional bronchodilators, and if a GP or hospital doctor was seen with further option to include their advice. There was a final comments response which allowed the patient to report anything else they thought was relevant. These were designed to give extra detail to the eDiary and to aid decisions about defining exacerbations during analysis

Table 2.2: Bronkotest domains and responses with corresponding weighting and symptom score

Question/statement	Responses	Weighting	Score
Describe your breathing	Better than usual	5	1
	Normal/usual	5	2
	Worse than usual	5	3
	Much worse than usual	5	4
What colour is your sputum? (colour chart displayed on eDiary)	No sputum	1	0
	Colour 1 (Clear)	1	1
	Colour 2 (Mucoid)	1	2
	Colour 3 (Purulent)	1	3
	Colour 4 (Purulent)	1	4
	Colour 5 (Purulent)	1	5
The amount of sputum you produced?	None	1	0
	A little	1	1
	Some	1	2
	Moderate	1	3
	A lot	1	4
Type of sputum	Watery	1	1
	Sticky liquid	1	2
	Semi solid	1	3
	Solid	1	4
How do you feel?	Better than usual	5	1
	Normal/usual	5	2
	Worse than usual	5	3
	Much worse than usual	5	4
How often do you cough?	Rarely	1	1
	Occasionally	1	2
	Frequently	1	3
	Persistently	1	4
Do you have chest pain?	No	1	0
	Yes	1	1
Do you have cold or flu symptoms?	No	1	0
	Yes	1	1
How did you sleep?	Slept well	1	1
	Woke once	1	2
	Woke twice	1	3
	Woke more than twice	1	4

2.3.4 Data collection and monitoring

Data was uploaded via an internet connection to a cloud-based server hosted by Microsoft Azure. This central database was accessible by study organisers only. The concept was to allow remote monitoring of patient symptoms though in reality it was mainly used to check initial patient adherence to the study. The study design was set up to assess feasibility and any feedback given to patients on symptoms and whether to treat exacerbations or not would have biased the results of the study. Instead, patients were left to complete the symptom diaries without direct input based on these scores but were able to contact the study group via telephone or email for clinical advice, as would be the case with a normal COPD/AATD service.

During the study, there were technical difficulties with remote monitoring of data and subsequently data could no longer be monitored live. On completion of the study, eDiary devices were returned and the data manually downloaded as a *.txt* file instead. Data was then exported into a Microsoft Excel spreadsheet and merged with other patient data before analysis (Figure 2.4).

2.3.5 Data cleaning

Prior to all data being extracted from the eDiary system, data cleaning was piloted with data from the first patients to complete the study to plan the optimum way of dealing with such large quantities of data. This was first done in Microsoft Excel. It became apparent that the volume of data was not appropriate for use in Excel since repetitive operations using point-and-click via a graphical user interface (GUI) can lead to errors. It also means that if later in the analysis if there was a mistake in the data cleaning process then the whole operation would have to be repeated, costing considerable time and effort.

R was used as alternative way to deal with the large quantities of data. R is a free, open-source programming language that uses written scripts of code to perform data cleaning tasks, statistical analysis and data visualisation. It also had more sophisticated statistical analysis potential and allows for fully customised graphs, figures and tables. The other advantage is that once the code is written, only minor changes are needed to the original code to change the analysis and to explore the results. There is also a written document of what changes were made to the data with the source data preserved in its original format. This ensures that data cleaning is transparent and errors can be identified later on if necessary. This would not be possible with Microsoft Excel as there is no trail of operations.

Figure 2.4 illustrates the data handling methods employed. In R Studio(225) I used the following packages for data tidying and imputation: tidyverse,(226) janitor,(231)

Amelia.(232)

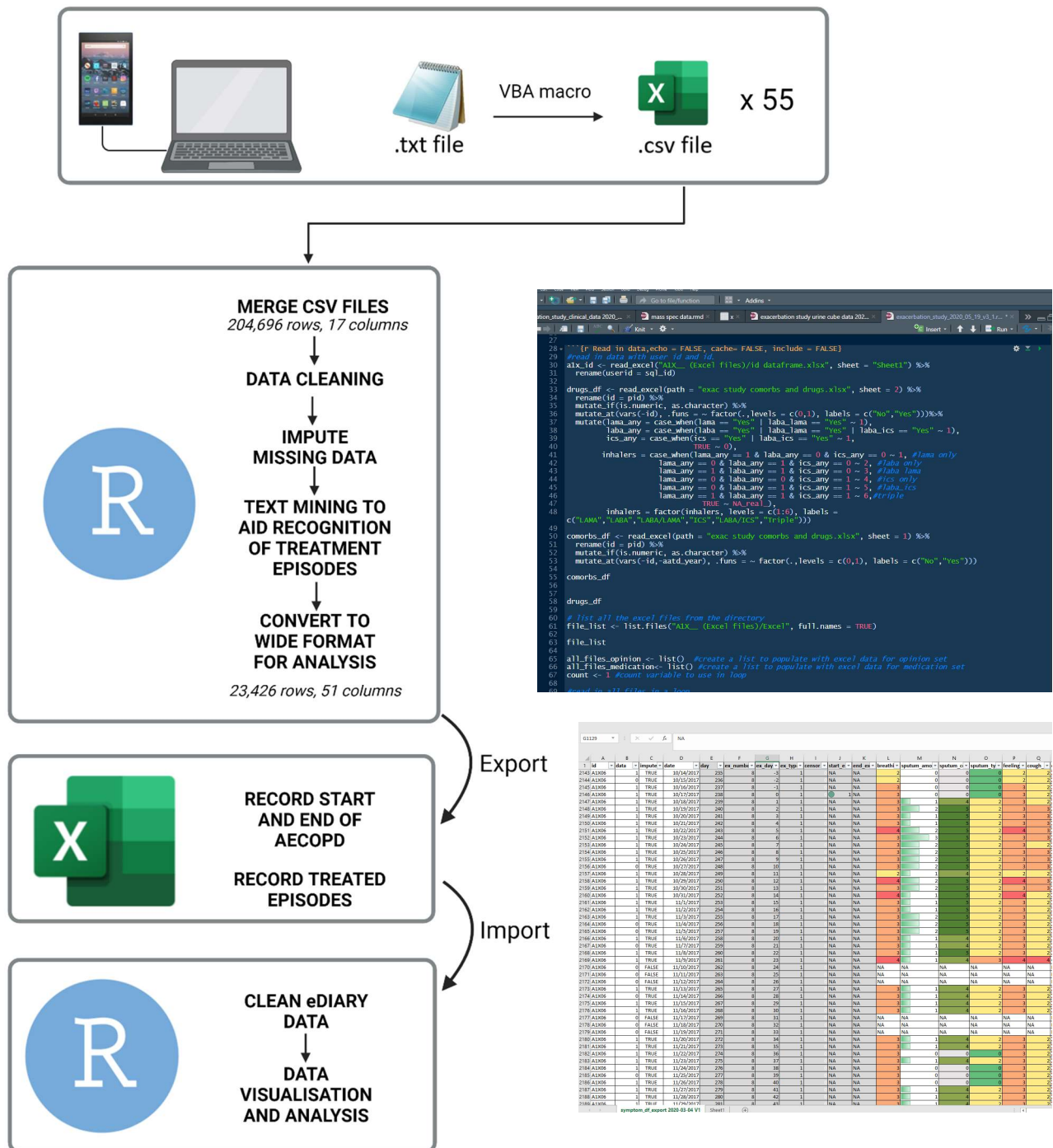


Figure 2.4: Process for data download and cleaning including examples from R Studio and Microsoft Excel.

2.3.6 Imputation of missing data

Since this study involved daily data collection by patients for 18 months with a relatively light touch from researchers in terms of reminders about the data (in order to replicate real life) it was inevitable that some missing data would occur, and a solution to this for analysis purposes was required. Many large datasets, such as genome-wide association studies (GWAS), use imputation as a method of minimising the losses to analysis that would otherwise accrue from missing data. It has been used for symptom data to detect AECOPD previously(233). I decided this would be appropriate in my study. Any missing dates were recorded as 'NA' (used to denote missing values in R). The first day of data was defined as the earliest entry by date and the last day of data was defined by the latest entry by date. To maximise the value of the data, imputation was used according to the following rules; 1) if ≤ 2 days of data was missing the most recently available data was used for imputation; 2) any data that formed part of 3 or more consecutive days was left as missing data. This was reset once there were 5 consecutive days of stable symptoms to ensure an exacerbation was not missed or included in stable data fields.

To aid recognition of treatment characteristics text mining and regular expressions were used. This identified string variables from free text responses that represented medications used for treatment of an exacerbation namely steroids and antibiotics. The following search terms were used and returned as 'TRUE' if the criteria were met. The criteria for antibiotics were as follows:

'antibiotic/anti/amox/clari/clary/dox/cipro/coamox/clav/azith/azyth/levo/flox'. For steroids it was as follows: *'pred / steroid / solone'*. Data was also manually reviewed to ensure data was not missed.

2.3.7 eDiary: data analysis

Symptom and treatment data were analysed for each patient to determine the number of exacerbations that occurred and any corresponding treatment. Treated episodes that did not fall within a defined exacerbation were recorded as 'unclassified' exacerbations. Any use of antibiotics or steroids that was not directly for the treatment of pulmonary exacerbations were not included as treated episodes.

This process is summarised in Figure 2.4. A long format dataframe was imported in Microsoft Excel with conditional formatting applied to columns to highlight times when a symptom domain changed from baseline and aid interpretation. Figure 2.4 includes a screen shot of one patient's data denoting an exacerbation using Microsoft Excel. This shows the change in colour for breathing, sputum amount and sputum colour. This data was then analysed by a clinician to determine the exacerbation start day, end day and Anthonisen type. Resolution of an exacerbation was achieved if symptoms were deemed to have resolved after 5 days. Any exacerbations that had incomplete data were censored at the point where data was missing until symptoms were stable again for 5 consecutive days. The analysis then carried on from this point.

The use of a computer algorithm was explored at length to see if it was feasible to assess the symptom diary data and determine the number of treated and untreated events with Anthonisen criteria defined exacerbations. After several iterations it was found to be insufficiently accurate so this was not explored any further.

2.3.8 eDiary: statistical approach

Baseline characteristics for all patients are presented as means and standard deviation for normally distributed continuous variables. Medians and interquartile ranges are presented for non-normally distributed variables.

2.3.8.1 Missingness

All data was downloaded directly from the eDiary. Dates of data entries were collected and ordered from the earliest to the latest day available and any missing days in between filled in. Three devices were not returned due to the loss to follow up with consequent loss of data. Other patients had almost no data at all (approx. 6 patients). This was checked and was not due to software or data processing errors and was down to lack of patient engagement.

Multivariable regression was used to assess influence of baseline characteristics on missingness of data. Variables for the model were selected if deemed relevant to the analysis or if $p < 0.2$ on univariate analysis. Assumptions for regression were assessed and deemed to have been met.

2.3.8.2 Exacerbation characteristics

Exacerbation characteristics were compared using the following statistical tests as part of the 'tableone' package in R Studio.(228) For continuous variables, t-tests were used for normally distributed data and the Kruskal-Wallis test for non-normally distributed data. Chi-squared tests were used to compare categorical variables. Normality of continuous variables was determined first by comparison of mean, median and their respective variances. Histograms were also constructed to visualise the distribution. For borderline cases of normality, Q-Q plots were examined.

Average symptom score data by day was compared visually using mean symptom score for Anthonisen type, seasons and treated vs untreated exacerbations.

2.3.8.3 Symptom score analyses

Receiver operator characteristic (ROC) curves were constructed to explore the diagnostic ability of the symptom score across a range of definitions for change in symptom score

(daily change, 3-day rolling mean and 7-day rolling mean). Sensitivity and specificity for the range of symptom scores was explored to determine the optimum approach. Symptom scores were calculated using weighted scores as discussed above (Table 2.2).

2.4 Biomarkers of exacerbations

Two primary methods were used to investigate urinary biomarkers of pulmonary exacerbations in AATD related lung disease; ELISA and lateral flow cytometry. A small cohort of serum biomarkers were also measured using mass spectrometry. First, I will describe the process for collecting and analysing urine samples using ELISA. The second part will describe POC urine testing kits that were issued to study participants including how the samples were analysed using lateral flow cytometry.

2.4.1 Enzyme linked immunosorbent assay (ELISA)

This section refers to all patient samples taken during study sight visits for baseline, exacerbation recovery and stable state assessments as illustrated earlier in Figure 2.2.

2.4.1.1 Sample processing and storage

Figure 2.5 illustrates the process for sample storage during the study. All samples were taken to a University of Birmingham laboratory located on the main hospital site at the Queen Elizabeth Hospital, Birmingham.

Whole blood was centrifuged at 4°C (3000rpm for 15 minutes, Hettich Centrifuge Universal 320R) within 1 hour of collection and separated into 3 x 500µl aliquots. Urine was separated in up to 10 x 1ml aliquots. All samples were recorded in a data log and then stored in a swipe access freezer room at -80°C.

All stored samples were appropriately labelled in accordance with the procedures to comply with the 1998 Data Protection Act; specifically, they were only identifiable by a number with the link back to identifiable data being held separately. Biological samples collected from participants were transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities met the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

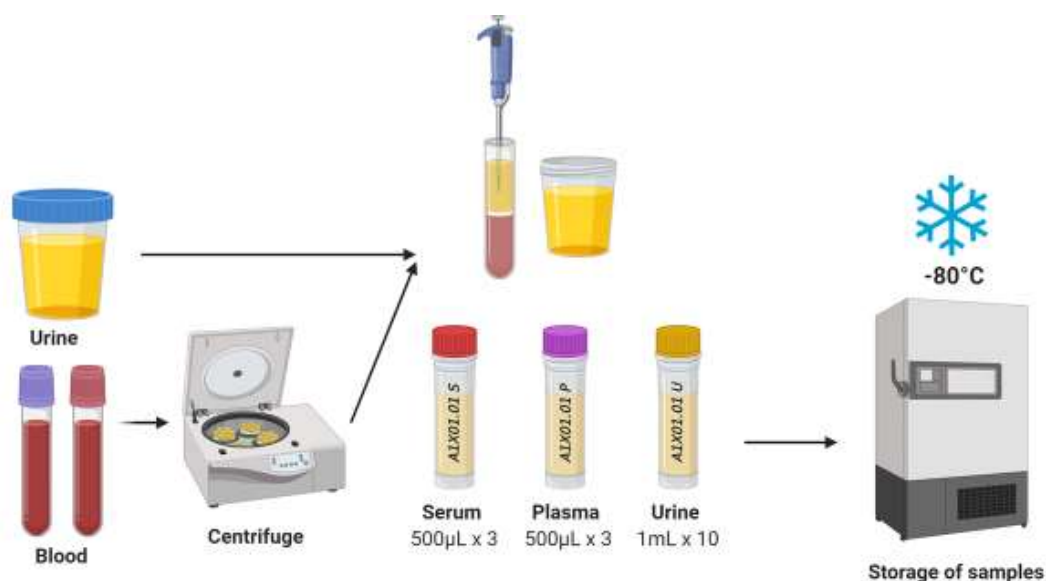


Figure 2.5: Sample procession procedure for the 'Elastin degradation in exacerbations of AATD related lung disease' study.

2.4.1.2 Sample transportation

Urine samples were transported in two batches via courier on ice to Mologic (Bedford, UK) where ELISA testing would occur. All samples were checked and stored at -80°C within 10 minutes of arrival.

2.4.1.3 Sample Analysis

Patient sample sets with at least one of each of baseline, 'Day 1' and 'Day 56' samples available were taken forward for further analysis by ELISA. Table 2.3 and 2.4 summarise

the biomarkers assessed and the respective ELISA kit/procedure used which are further detailed below. Biomarkers were selected based on previous work performed in patients with usual COPD.(234-236)

Table 2.3: Reagents and dilutions used for Mologic ELISA protocol

Assay	Coating antibody	Detecting antibody	Top standard (ng/mL)	Standard dilution	Sample dilution	volume needed (µl)
AAT	AAT Fab 1521 at 2µg/ml	AAT Fab 1951- Alkaline phosphatase conjugate at 1/30,000 dilution	80	1 in 2	20	15
B2 microglobulin	Sheep anti β-2-microglobulin diluted to 1µg/ml	HRP Conjugated sheep anti-human beta-2-Microglobulin (NS16/1/2014) 1/20,000	50	1 in 4	100	3
Fibrinogen	CF1765 affinity purified, 901.5µg/ml	CF1766-alk phos, 1/4000	40	1 in 2	2	150
NE	NE 1871 at 1.5µg/ml	NE 1241- Alkaline	25	1 in 2	10	30

		phosphatase conjugate 1/25,000				
RNASE3	Affinity purified sheep polyclonal (SA466)	Conjugated sheep anti- human RNASE3, 1/20,000 (SA466)	10	1 in 2	4	100
Desmosine	Ovalbumin/desmos ine conjugate, 1µg/ml	CF1316 alkaline phosphatase 50µl	200	1 in 2	5	0
fMLP	ovalbumin-fMLP conjugate at 2µg/ml	Sheep anti fMLP CF1573 alkaline phosphatase solution 50µl	50	1 in 2	5	0

Dilution buffer: 1%BSA/PBST, block: 1%BSA/PBS

Table 2.4: Details of commercial ELISA assays

Assay	Company	Cat No	Top standard	units	Standard dilution	Sample dilution	Volume needed (µl)
Creatinine	R&D	KGE005	20	mg/dl	1 in 2	20	15
Cathepsin B	Duaset	DY2176	4	ng/mL	1 in 2	10	0
CC16	Duaset	DY4218	2	ng/mL	1 in 2	10	0
MMP8	Duaset	DY908	4	ng/mL	1 in 2	10	0
MMP9	Duaset	DY911	2	ng/mL	1 in 2	10	100
MPO	Duaset	DY3174	4000	pg/mL	1 in 2	10	0
NGAL	Duaset	DY1757	5	ng/mL	1 in 2	100	3
RBP4	Duaset	DY3378	1500	pg/mL	1 in 2	2000	1
TIMP1	Duaset	DY970	2	ng/mL	1 in 2	10	0
TIMP2	Duaset	DY971	2	ng/mL	1 in 2	10	0
CRP	Duaset	DY1707	1000	pg/mL	1 in 2	10	0
Cystatin C	Duaset	DY1196	2	ng/mL	1 in 2	500	1

IL-6	Duoset	DY206	600	pg/m L	1 in 2	2	50
B2 microglobulin	Abcam	108885	50	ng/m L	1 in 4	100	3
Fibrinogen	Abcam	108841	80	ng/m L	1 in 2	2	50
Chitinase-3- like- protein	Duoset	DY2599	2	ng/m L	1 in 2	10	0
MMP Substrate	Enzo	BML-P276- 001	2000	ng/m L	1 in 3	neat	10

The process for all Mologic assays is detailed below with the various reagents used summarised in Table 2.3. The remaining biomarkers were assessed using commercial ELISA kits with processes adherent to the manufacturer's instructions (Table 2.4). All samples were tested on the same day in all assays.

A Maxisorb™ plate (Thermo Fisher Scientific, Waltham, MA, USA) was sensitised with the coating antibody (Table 2.3) in PBS overnight at ambient temperature using a 100µl/well. Plates were then washed with TBST in a plate washer. The plate was then blocked with 1% BSA in PBS for 1hr at ambient temperature with 120µl/well. This was again washed with TBST in a plate washer. Urine samples were diluted at 1/20 in 1% BSA in PBST.

The standard solution was diluted using dilution values displayed in Table 2.3 (standard dilution). For AAT for example, AATD stock was diluted at 1mg/ml at 1/100 (10µl + 990µl 1% BSA in PBST) to get 10µg/ml solution. Then, 10µg/ml solution was diluted at 1/125 (4µl + 496µl 1% BSA in PBST) to get 80ng/ml top standard. This 80ng/ml standard was then serially diluted at 1 in 2.5 (200µl + 300µl 1% BSA in PBST) to prepare remaining standards. The same procedure was repeated for other assays using the ratios from Table 2.3.

Wells were then incubated using the standard solution prepared at different dilutions as described above, 100µl/well. Urine samples were diluted at various dilutions depending on the assay being used (Table 2.3, sample dilution) in 1% BSA in PBST, 100µl/well.

The standards and samples were incubated for 1.5hr at ambient temperature with shaking, 100µl/well.

Plates were washed with TBST in a plate washer. Then, there was incubation with the detecting antibody in 1%BSA in PBST for 1hr at ambient with shaking, 100µl/well. Plates were washed again with TBST in plate washer. Next, pNPP substrate was added to the appropriate wells. Readings were taken at 405nm in the BMG Omega plate reader, 100µl/well. BMG Mars software used for sample calculations.

2.4.2 Isotope-dilution liquid chromatography-tandem mass spectrometry

To further characterise elastin degradation during stable, exacerbation and recovery states, serum desmosine was analysed using isotope-dilution liquid chromatography-tandem mass spectrometry. This was performed by an independent laboratory (Medical Research Institute, School of Medicine, University of Dundee, UK) commercially using previously described methods.(237) Patients with at least one baseline and one 'Day 1' study site visit were selected with 'Day 56' (recovery) samples sent if available.

2.4.3 Lateral flow cytometry

Lateral flow immunoassays are an ideal system in POC testing as they use simple equipment and results are available within minutes.(238) They can be used for both qualitative and quantitative monitoring in non-laboratory or resource limited settings e.g., in a patient's home or in GP practices.(239) Commercial kits are widely available, an example being home pregnancy testing kits.

Lateral flow immunochromatography works in the following way and is illustrated in Figure 2.6. For this particular urine test kit, sandwich ELISA has been used since type of assay is better at detecting larger molecules with multiple binding sites.(240)

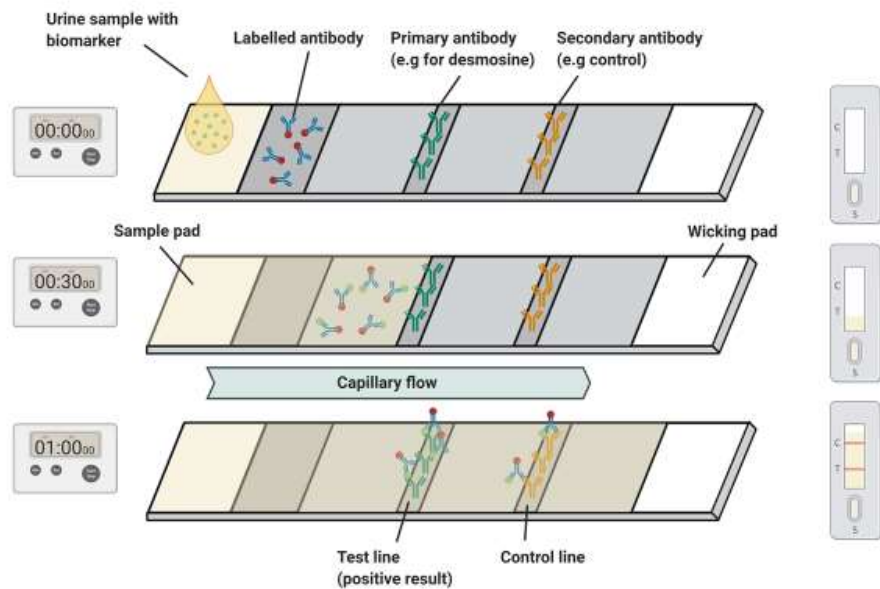


Figure 2.6: Mechanism for how lateral flow cytometry works

First, a mid-stream urine sample is applied to an absorbent pad. The urine then migrates along the assay where it encounters a labelled antibody specific to the analyte of interest. The analyte and labelled antibody become bound together where they migrate together along the assay. The test line contains an area of fixed antibody specific to the target analyte. The antibody/analyte complex migrates to the test line and binds to the fixed antibody. The labelled component provides a visual indication that there is analyte present within the test sample. Importantly, the higher the concentration of the target analyte, the more densely stained the test line becomes. This concept can be used to quantify the concentration of analyte within the test sample by using an optical density reader. This optical density reading can then be compared to a standard curve where known concentrations of the test analyte are plotted against the optical density.

2.4.3.1 Lateral flow device (urine cube)

The following section describes the urine device used for lateral flow testing at home. The urine cassettes (assay devices) were specially made by Mologic to test five different

molecules; A1AT, CRP, NE, TIMP1 and desmosine. The optical reader (Figure 2.7) known as the 'urine cube' to test subjects, was used to calculate the optical density of the test line for each of the five analytes. Cassettes, known as 'urine sticks' to study participants, were issued in batches of 15 via post upon request or when visiting the study site for an exacerbation. This was to allow at least 8 weekly urine samples during the stable state and a further 7 test sticks to be used in case of an acute exacerbation that required treatment. The box number was recorded and patients were instructed to use the urine sticks from the initial box first before moving on to the next one to ensure the batch number for the urine stick could be tracked. As the urine cassettes were made in batches, concentration curves were generated for each batch to ensure optical density readings would be accurate (Appendix, Section 7.4.2, Figure 7.2).

The optical density device included within the Head Start® home testing kits (Figure 2.7), issued at the baseline visit, was operated via a single button to switch on and then initiate the cube to start reading the cassette device. Urine cubes sat in a plastic baseplate which allowed consistent reading of the urine cassette. There was an internal watch battery which gave power though the recommendation was to plug the device in whilst in use to ensure data was not lost. The process of reading took 10 minutes (Figure 2.8). Data was then stored on the device accessed via linking the device to a computer with compatible software located at the study site. Patients were asked to bring their urine cube with them during every visit to the study site so that data could be downloaded and checked if data was being recorded correctly.



Figure 2.7: Lateral flow assay cube reader (red), base plate (grey), connect wire (black) and plug (white) with spare batteries

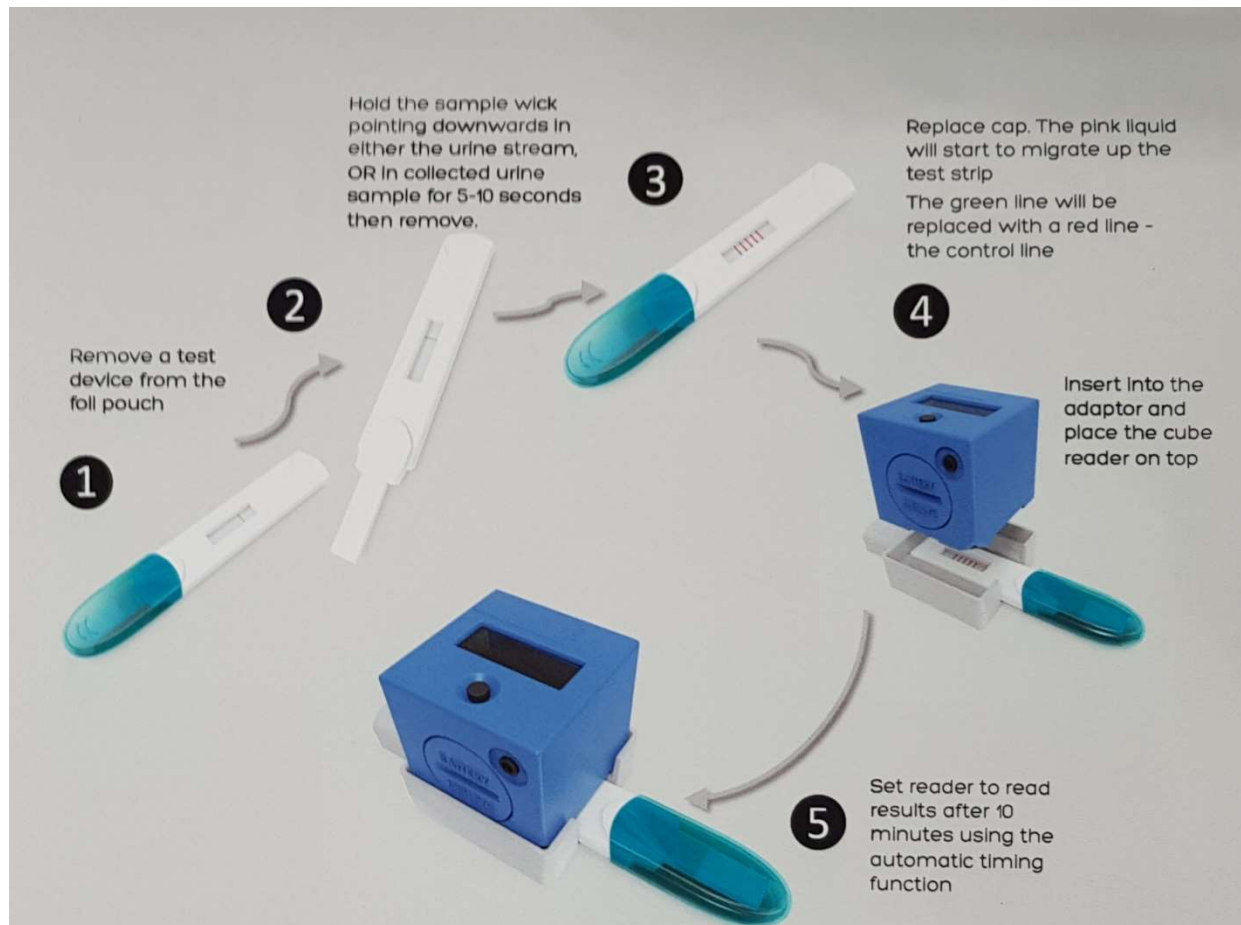


Figure 2.8: Process for using urine cassette and lateral flow assay optical reader.

There were issues with some patients operating the device. Some got a 'FAIL' message when completing the analysis procedure and were unsure if data was being recorded. This led to repeat measurements on certain days for some patients. Other patients, despite reportedly following the operation procedure, failed to record any data on their urine cube. In some cases, the date was reset to an arbitrary date (usually 23rd March 2016), likely due to the device losing power due to mains connection not being used. This particular issue led to some dates being difficult to assign to the correct exacerbation day, although the chronology of samples could be tracked with a unique test identifier. These issues were addressed by retraining participants on their devices if needed or replacing any potentially faulty devices. Longer power cables were requested and issued to ensure all patients were able to use the device with the mains plug engaged to ensure data was not lost or reset due to lack of power.

All optical density data was collected during patient visits or at the end of the study if the patient was not able to visit the test site during the course of the study. This was then sent to Mologic where optical data could be transformed back to analyte concentrations using the standard curves created for each urine cassette batch. At this point, data quality was checked including adjustment of incorrectly recorded dates. The cleaned data was then returned for analysis.

2.4.4 End of study questionnaire

An important outcome to determine was whether use of a POC urinary biomarker at home was feasible. To assess feasibility, once all participants had returned their urine cube at the end of the study, they were issued with an end of study questionnaire (see Appendix, Section 7.4.1, Figure 7.1). Section 2 of the questionnaire related directly to how easy the urine cube was to use with two questions measured on a visual analogue scale (VAS).

Question one asked 'How easy were the urine sticks/monitor to use?' measured from 1 (very easy) to 10 (very difficult). Question 2 asked 'How often did you forget to measure your urine?' measured from 1 (almost weekly) to 10 (never). Sub questions to question 2 were 'Why do you think this was?' and 'Were there any other barriers to testing urine at home?' with a free text box to fill out. All answers were transcribed and analysed once all responses were collected.

2.4.5 Biomarker: data analysis

For ELISA data three distinct groups were assigned; stable, exacerbation and recovery.

Stable state samples included baseline, 'Month 9' and 'Month 18' results. In line with other studies in the literature, samples were compared using raw values and creatinine corrected values.(80)

For lateral flow immunoassay results, sample measurements around the time of an exacerbation were compared in groups as follows; -14 to -7 days, -7 to 0 days, 0 to 3 days, 4 to 7 days and >7 days. This was to maximise the number data points for each period of an exacerbation. Treated exacerbations were identified via eDiary data where day 0 represented the first day of a symptom defined exacerbation rather than the first day of treatment.

Stable state samples were identified by selecting patient samples that occurred outside the window of an exacerbation coupled with a breathing score of either 'Normal/usual' or 'Better than usual' since breathlessness has been shown to be the highest indicator of exacerbation treatment in a previous AATD cohort.(208)

For end of study questionnaire data, visual analogue scores (VAS) data were displayed on histograms and visually analysed. Free text responses were concatenated together and

frequency of common words calculated. Free text replies were also examined with their themes identified.

2.4.6 Biomarker: statistical analysis

For ELISA data, comparisons were made between stable and exacerbation results and exacerbation and recovery results using Kruskal-Wallis test since the distribution for all biomarkers was deemed to be non-normal by histogram and Q-Q plot inspection. This was repeated for creatinine corrected values.

Lateral flow cytometry data was compared using Kruskal-Wallis test for the following pairs; -14 to 7 days and 0 to 3 days, -7 days to 0 days and 0 days to 3 day, 0 to 3 days to 3 to 4 days and 0 to 3 days to ≥ 7 days. Daily data throughout the course of an exacerbation was explored with visual comparisons of median and median absolute deviation from -14 days through 14 days after the onset of symptoms. For lateral flow data median plots, only data with 12 or more values available on each day were chosen as some days, particularly the weeks prior to an exacerbation, had too few measurements for meaningful analysis.

Stability of stable state lateral flow cytometry data was analysed visually by comparing the mean and standard deviation values across each biomarker for each individual

Quantitative data from the end of study questionnaires was explored via simple descriptive statistics and histogram analysis.

For qualitative analysis, all free text responses from the end of study questionnaire were amalgamated and specific themes identified. Each theme was split into two groups; those with a VAS score for remembering to perform the urine test ≥ 6 and those < 6 to explore any differences between patients who perceived themselves to adhere well to the protocol and those who did not. A score of 6 was chosen since this was the median score. A word cloud

was constructed from counts of specific terms to further enrich specific themes from the data.

2.5 Results

In this chapter I will outline the main patient characteristics and clinical presentations during the study. Results collected from eDiary data and biomarker analyses are presented in Chapters 3 and 4 respectively.

2.5.1 Patient characteristics

The baseline characteristics of all patients in the study are summarised in Table 2.5. Fifty-five patients were originally included in the study with one patient withdrawing within the first few days without data collection as they no longer wanted to participate. Patients were diagnosed with AATD between 1990 and 2017. Other patients who withdrew or those lost to follow up were still able to submit some data and therefore are included. The majority of patients were male (61.1%) with a mean age of 55.1 years (range 38 to 73) which is typical for an AATD cohort.(210) BMI was normally distributed (mean 24.6 kg.cm⁻²). Most patients were ex-smokers with two current smokers and six patients having never smoked. Current or ex-smokers had a median smoking pack year history of 19.0 years. The cohort studied had relatively severe disease; mean FEV₁ % predicted was 41.4% with the majority of patients having an mMRC of either 3 or 4. This was reflected in the PRO scores; median CAT score was 27 and median SGRQ total was 65.4 at baseline. Median annual exacerbation rate was 3.0 (IQR 2 to 3.25) and around a third of patients had CT evidence of bronchiectasis. The mean eosinophil count was 0.18 x 10³ cells/μL (±0.09). The majority of patients had confirmed emphysema (92.6%) with 20.4% having bronchiectasis. One patient had previously undergone lung volume reduction surgery for advanced emphysema.

Table 2.5: Patient demographics for 'Elastin degradation in exacerbations AATD related lung disease' study.

n	55
Female (%)	21 (38.9)
Age (mean (SD))	55.14 (9.13)
BMI (median [IQR])	24.64 [22.48, 30.42]
Smoking status (%)	
Non smoker	6 (11.1)
Ex-smoker	46 (85.2)
Current smoker (%)	2 (3.7)
Pack year history (median [IQR])	19.00 [8.00, 25.00]
FEV ₁ /L (median [IQR])	1.24 [1.00, 1.68]
FEV ₁ % predicated (mean (SD))	41.40 (16.01)
FVC/L (mean (SD))	3.73 (1.13)
FVC % predicted (mean (SD))	89.74 (22.90)
FEV ₁ /FVC ratio (mean (SD))	0.37 (0.11)
KCO % predicted (mean (SD))	57.02 (16.62)
mMRC score ¹ (%)	
0	1 (2.0)
1	6 (12.0)
2	13 (26.0)
3	25 (50.0)
4	5 (10.0)
CAT score (median [IQR])	27.00 [20.00, 30.00]
SGRQ total (median [IQR])	65.39 [53.06, 75.03]
Annual exacerbation rate (median [IQR])	3.00 [2.00, 3.25]
CT evidence of bronchiectasis (%)	16 (35.6)
Oxygen therapy (%)	5 (9.3) ¹

¹ 5 missing data points

2.5.2 Healthy controls

Initially healthy control patients were tested for AATD using phenotyping or genetic sequencing. Of the 71 control subjects, 53 had AAT phenotype determined with the following results: 45 PiM, 5 PiMZ, 5 PiMS, 1 PiS and 1 PiFM. The remaining characteristics are summarised and compared to the AATD cohort in Table 2.6.

Table 2.6: Baseline characteristics for healthy controls compared to AATD participants.

	AATD	Control	P
n	55	71	
Female (%)	21 (38.9)	32 (45.1)	0.61
Age (mean (SD))	55.14 (9.13)	54.83 (8.63)	0.845
Smoking status (%)			<0.001
Non smoker	6 (11.1)	38 (53.5)	
Ex-smoker	46 (85.2)	29 (40.8)	
Current smoker	2 (3.7)	4 (5.6)	
Pack year history (median [IQR])	20.00 [10.00, 25.50]	8.00 [2.25, 21.50]	0.015
BMI (median [IQR])	24.64 [22.48, 30.42]	26.22 [23.63, 29.63]	0.367
FEV ₁ , L (mean (SD))	1.35 (0.51)	3.06 (0.80)	<0.001
FEV ₁ % predicted (mean (SD))	41.40 (16.01)	99.99 (16.73)	<0.001
FVC, L (mean (SD))	3.73 (1.13)	4.07 (1.09)	0.098
FVC % predicted (mean (SD))	89.74 (22.90)	109.29 (18.40)	<0.001
FEV ₁ /FVC ratio (mean (SD))	0.37 (0.11)	0.76 (0.09)	<0.001

2.5.4 Geographical distribution of patients

The home location for study participants is displayed in Figure 2.9. There is a wide geographical spread of patients throughout England and Wales. Red points indicate any patient that visited the study site for review during an exacerbation, so called 'Day 1' visits. Those that did not are denoted by blue points. The study site at the Queen Elizabeth hospital is displayed as an orange triangle. There was no relationship between distance the patient lived from the study site and the likelihood that they visited the site for a 'Day 1' consultation as assessed by simple logistic regression ($p = 0.452$).

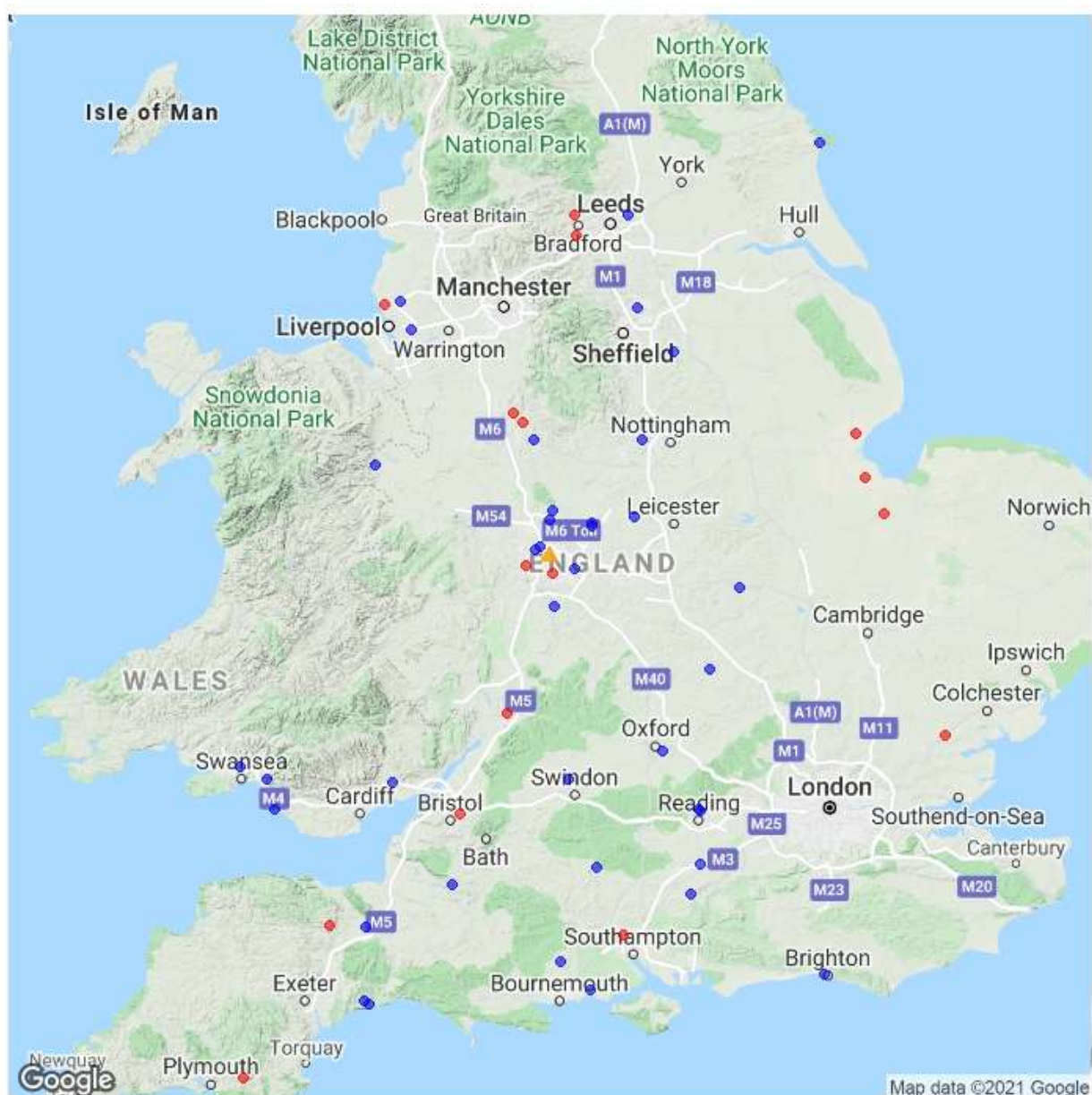


Figure 2.9: Geographical distribution of patients in elastin degradation of AATD related lung disease study.

2.5.5 Exacerbation visits

In total, excluding 'Month 9' and 'Month 18' visits, there were 122 individual visits to the study site. There were 24 exacerbation visits ('Day 1') across 16 unique patients. This value was lower than anticipated but was offset by a satisfactory number of exacerbations captured with the urine cube device by patients at home. Urinary levels of desmosine

correlate with plasma levels(80) so the primary outcome to characterise elastin degradation during stable state and exacerbations can be addressed. Of the 24 exacerbation visits, 18 were followed up with recovery visits ('Day 56'). Clinical measures during site visits and CAT scores are compared in Table 2.7 between baseline, exacerbation and recovery days. Oxygen saturations were not recorded.

Table 2.7: Clinical and QOL measures for study visits of AATD patients (includes only those with 'Day 1' visit).

	Baseline	Exacerbation ('Day 1')	Recovery ('Day 56')	<i>P</i>
n	16	24	18	
Systolic BP (mmHg) (median [IQR])	133.00 [121.00, 143.00]	135.00 [125.00, 141.00]	131.00 [128.00, 141.00]	0.895
Diastolic BP (mmHg) (median [IQR])	84.00 [74.50, 93.50]	86.00 [78.00, 95.00]	86.00 [83.00, 92.50]	0.705
Heart Rate (beats/min) (mean (SD))	76.08 (15.09)	84.79 (13.39)	78.78 (19.27)	0.346
Temperature (C) (mean (SD))	36.44 (0.45)	36.39 (0.45)	36.35 (0.24)	0.89
Respiratory Rate (breaths/min) (median [IQR])	17.00 [16.00, 18.00]	18.00 [18.00, 21.50]	18.00 [18.00, 20.00]	0.097
CAT score (mean (SD))	25.00 (8.12)	25.95 (5.55)	23.24 (8.56)	0.52

During exacerbations the majority of patients received antibiotics (87.5%) with amoxicillin being used most often (50%). The median duration of antibiotic use was 7 days.

Prednisolone was taken for less than half of episodes (45.8%), also for a median of 7 days.

Two episodes were classified as severe with hospitalisation.

2.5.6 COPD Assessment Tool (CAT)

The CAT questionnaire was filled out by all participants visiting the study site including baseline, 'Day 1' and 'Day 56' visits. Figure 2.10 shows comparisons between CAT scores at baseline, exacerbation and recovery. In Figure 2.10A, there appears to be an average increase in CAT score between baseline and exacerbation which is logical. This difference is small and statistically not significant. The plot also shows that patients with higher baseline CAT scores tended to have similar or sometimes lower CAT scores during exacerbations. Figure 2.10B shows comparison between exacerbation and recovery. This shows an average decrease following exacerbation which is expected but is skewed by outliers with typically high baseline CAT scores. A sensitivity analysis was performed to assess those with a baseline CAT score ≤ 25 which is shown in Figure 2.11. When the patients with more severe baseline symptoms were removed the trend is much clearer between baseline and exacerbation (Figure 2.11A), and exacerbation and recovery (Figure 2.11B).

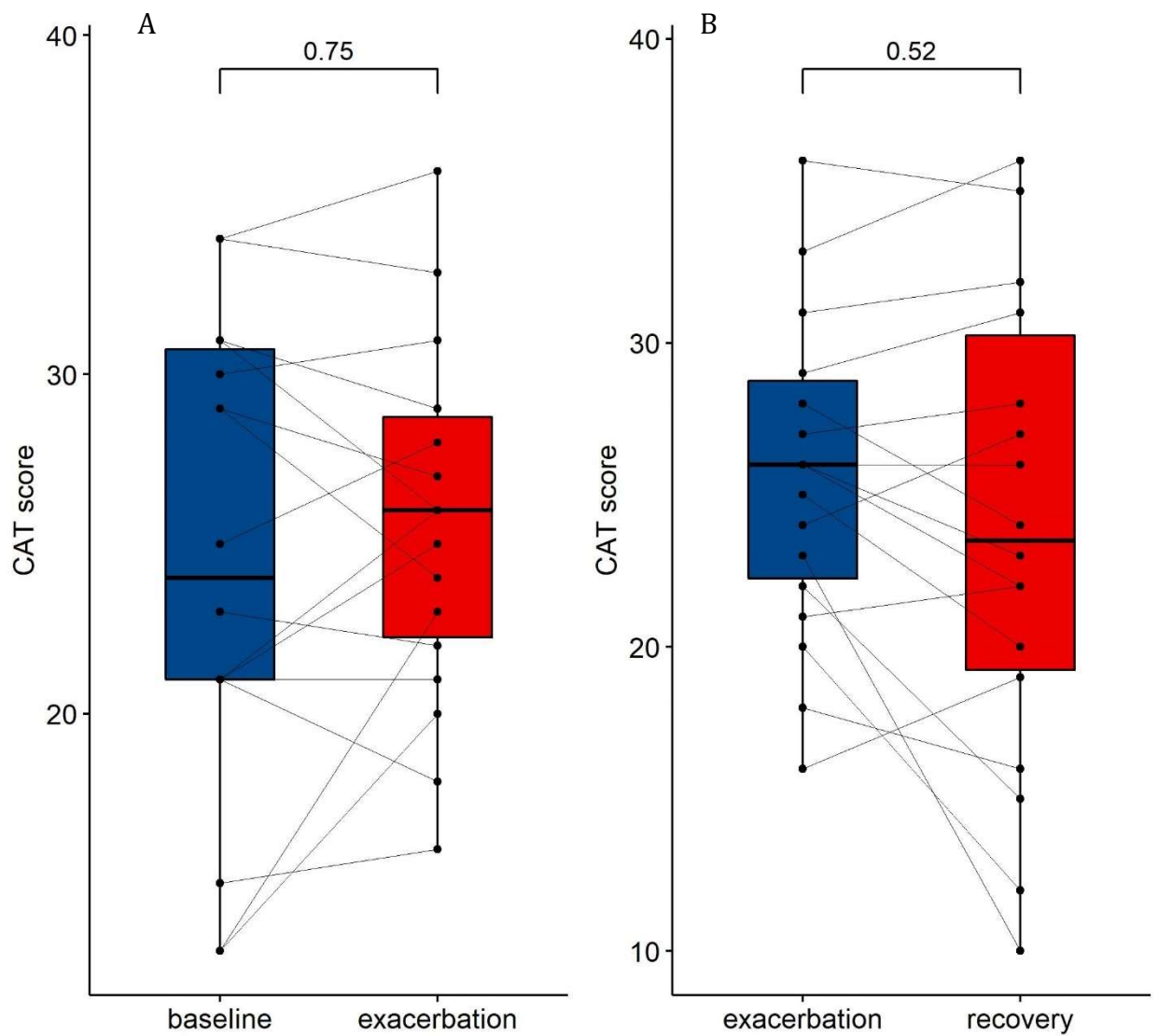


Figure 2.10: Pairs plot comparing A) baseline vs exacerbation and B) exacerbation vs recovery. For A, this includes all patients that 'Day 1' and 'Day 56' visit for each exacerbation. $N = 15$ with 9 unique patients.

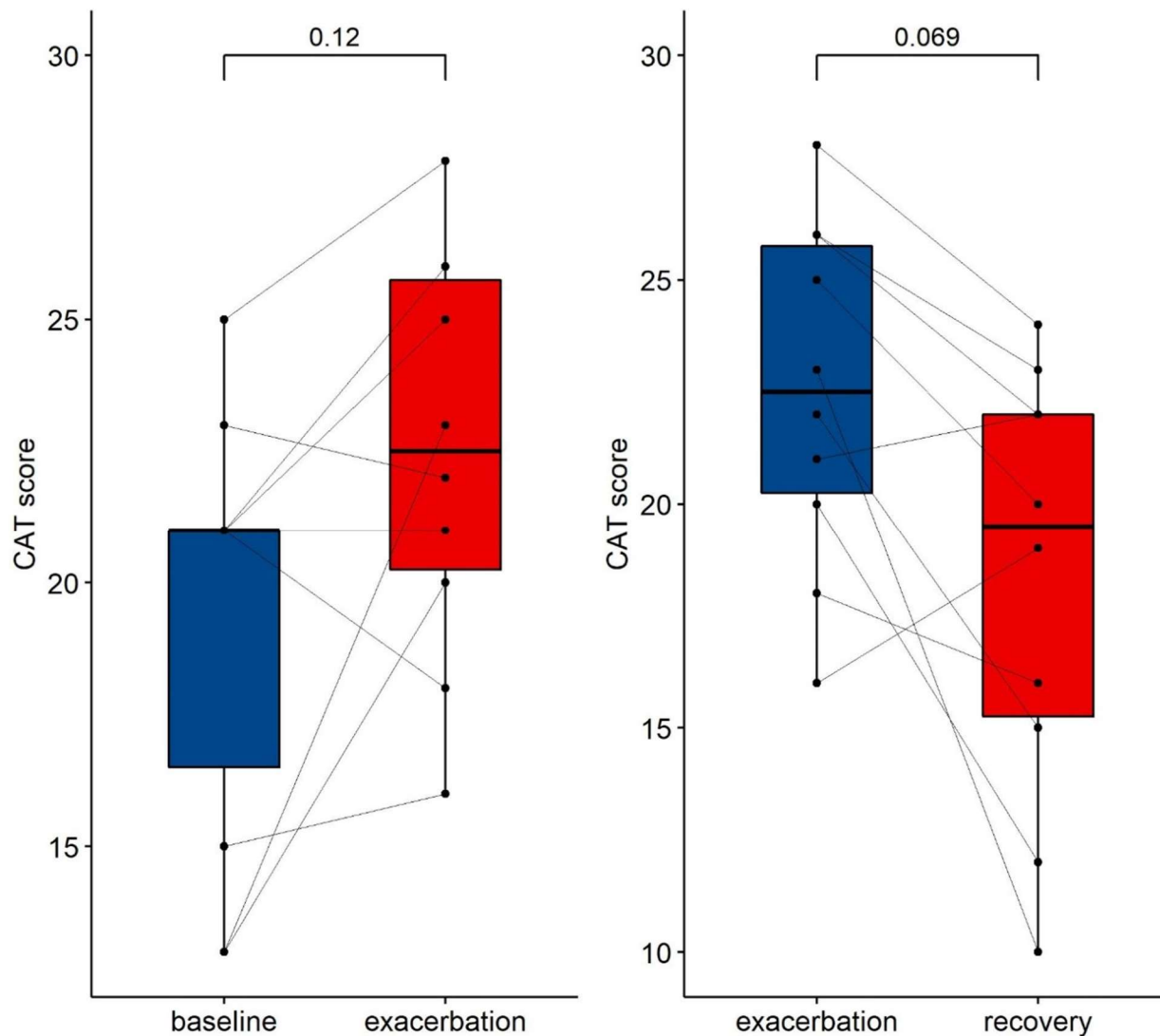


Figure 2.11: Pairs plot comparing of all patients with baseline CAT score ≤ 25 for A) baseline vs exacerbation and B) exacerbation vs recovery. For A, this includes all patients that had a 'Day 1' visit. B includes only those that had both a 'Day 1' and 'Day 56' visit. B includes those that had both a 'Day 1' and 'Day 56' visit for each exacerbation.

2.5.7 St Georges Respiratory Questionnaire (SGRQ)

SGRQ scores were collected at baseline and at 'Month 18' signifying the end of the study.

Figure 2.12 shows pairs plots for the three separate domains and the total score at baseline and 'Month 18' and demonstrates how QOL scores progress over 18 month in AATD patients with frequent exacerbations. There appear to be small average increases in activity

and impact domains suggesting these deteriorate over time. No measures reached statistical significance.

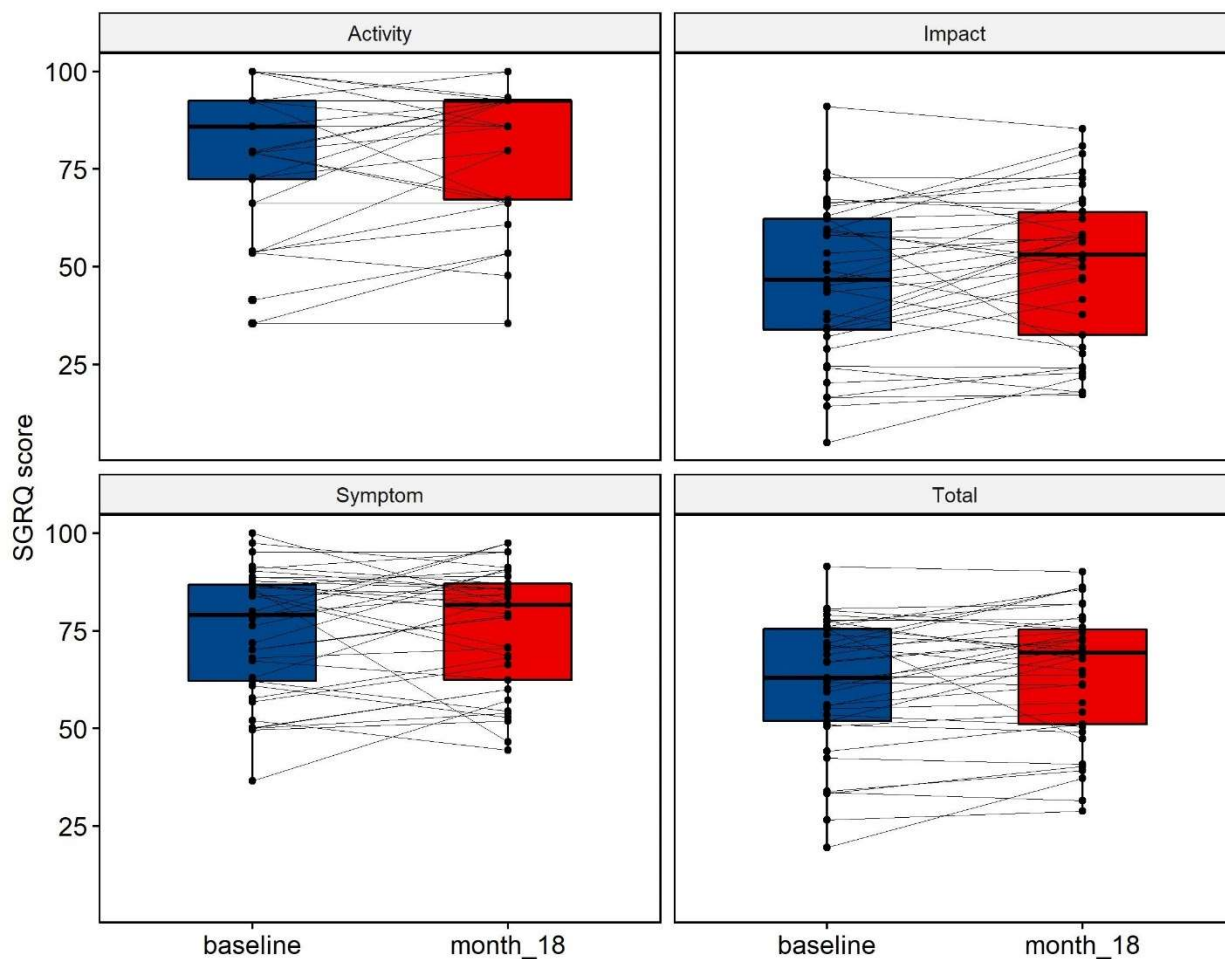


Figure 2.12: Pairs plots for each domain of the St. Georges Respiratory Questionnaire from baseline assessment to 'Month 18' at the end of the study. Each domain comparison was non-significant $p > 0.05$.

2.6 Discussion

This chapter has outlined the methods for the 'Elastin degradation in exacerbations of AATD related lung disease' study which aims to address the hypothesis that enhanced monitoring with an eDiary and urinary biomarker measurements can predict exacerbations and can be feasibly achieved in a population of patients with AATD related lung disease. I

have also presented some preliminary results from clinical data collected during site visits. In the following paragraphs I will discuss the successes and challenges associated with the oversight of the study.

2.6.1 Recruitment

This study had adequate and timely recruitment of AATD patients as demonstrated by Figure 2.1. The main success for recruitment was the existing AATD data base which allowed screening of existing research participants in advance. I was also able to use a weekly NHS AATD clinic to recruit patients. For new patients this was more difficult as they were often recruited to the existing AATD observational study at the same time which was time consuming. There were a few patients who were recruited on their first visit to the study site that subsequently withdrew or were lost to follow up thereafter. Long standing patients seemed more likely to attend the study site reliably. This is a form of selection bias as patients who are more likely to attend then get recruited to other studies. This is a well-known issue in COPD studies and may lead to results that may not be generalisable overall to the study population.(241) In this study the primary outcome was to assess feasibility of an eDiary and home urine monitor and therefore targeting all eligible participants was appropriate to ensure a representative study sample.

Healthy participants were also recruited, mainly hospital and university staff and the family of patients were targeted. There were 11 patients heterozygous for a known deficient AAT allele and one S homozygote found from the healthy control cohort which led to the need for appropriate genetic counselling. The PiS who was a non-smoker declined further follow up. Another PiMZ individual was concerned of any future inheritability to their children and sought further testing of their partner. These alleles are relatively common in the general Caucasian population which confers little to no risk of emphysema unless they are

smokers.(242) To a lay person who does not understand this fully may be alarmed when learning about a 'deficient' allele which should be appropriately explained prior to any test being carried out. This is something that I would consider more thoroughly for future studies.

2.6.2 Cohort characteristics

This cohort of patients with frequency exacerbations is similar in age and severity compared to previous studies of exacerbations in AATD related lung disease.(208) Overall severity was higher than what would be expected compared to age matched usual COPD which is typical for the AATD population.(208) All patients were frequent exacerbators, who are seen at a tertiary outpatient respiratory clinic, were prescribed a combination of LAMA/LABA/ICS in line with GOLD guidelines.(1) One patient did not tolerate LAMAs and therefore took a LABA/ICS only. This means any variability in biomarkers or symptom scores are unlikely to be due to differences in inhaled therapy. Medication adherence was also enquired about to ensure this was the case.

The majority of patients continued to have frequent exacerbations which were captured using the eDiary with parallel measurement of urinary biomarkers by lateral flow cytometry. Some patients did not have any exacerbations during the 18-month follow up period. We know from studies in COPD that annual exacerbation frequency is not stable over time(243) and therefore it was expected for some patients to not have any exacerbations. A good predictor of future exacerbations is having had exacerbations in the previous year(93), therefore targeting frequent exacerbators was the best strategy for maximising captured events.

There were low levels of diagnosed cardiovascular disease within the groups (hypertension 14.8%, hypercholesterolaemia 9.3%, ischaemic heart disease, hypertrophic cardiomyopathy and atrial fibrillation all 1.9%). This potentially reduces the overall contribution of extrapulmonary sources of elastin degradation products, such as vascular tissue, and means that results are more reflective of underlying lung tissue turnover.

2.6.3 Site visits

The number of study site visits for 'Day 1' exacerbations was lower than anticipated from the original calculation prior to the study reaching only 27.3% over the lowest required estimate. This is likely for a number of reasons.

Firstly, the geographic spread of patients was large. Figure 2.9 illustrates that patients were from all over the UK, including Plymouth, which by car is over 3 hours away. Less than a third (29.6%) of patients were from the West Midlands. This was a substantial physical barrier to clinical review and collection of samples. This could be improved in the future by either a) only recruiting locally or b) setting up additional satellite study sites to improve reach of the study. Local recruitment may limit the number of patients recruited to the study and setting up satellite study sites would incur additional cost or be impractical. Cost was the limiting factor in this case.

Secondly, having AECOPD makes a patient feel short of breath and unwell. Having to travel to the study site for assessment during the early stages of an exacerbation may be challenging. Given that the AATD cohort in the study mainly have moderate to severe COPD, this likely explains why there were lower numbers of 'Day 1' exacerbation visits. Patients may also have prior plans, such as work or childcare commitments, that meant they could not accommodate an unscheduled visit at short notice. This is one of the main reasons for

the protocol amendment to allow the patient to visit within 7 days of an exacerbation. The disadvantage of this amendment was that samples taken for biomarker analysis could be at different time points during an exacerbation. It was for this reason that not all patients had clinical bloods taken during 'Day 1' visits since by the time they were seen most symptoms had improved and there was not a clinical justification. Clearly the delays in sampling may impact upon the results for samples collected during study visits which will be discussed in the context of the biomarker results in Chapter 4.

Any financial implications for patients were minimised by patients being fully reimbursed for any travel or parking costs. The study budget unfortunately could not allow overnight stays which could have helped with patients who lived further distances to attend during an exacerbation. Though the numbers visiting the study site for exacerbations was disappointing, this did not detract from the primary outcomes to assess feasibility of using a urine monitor and eDiary to measure biomarkers during exacerbations, during recovery and in the stable state. The original purpose of exacerbation visits was to check that symptoms were consistent with true pulmonary exacerbations rather than related to other cause such as cardiac failure or psychological factors. There was adequate data collected from the eDiary to allow accurate diagnosis of exacerbation phenotypes based on the Anthonisen criteria⁽⁶¹⁾ alone which is in line with other studies assessing exacerbation characteristics in COPD.^(233, 244, 245) Sampling of urine during these visits was an additional method to help further validate lateral flow cytometry of urine against ELISA – validations had already been completed in the usual COPD population, but not in AATD.

2.6.4 Quality of life measures

This study used two different QOL measures; CAT and SGRQ.

Interestingly, CAT score was more useful when patients had lower baseline scores, presumably because at the higher limits of CAT there is less potential for patients to deteriorate. The currently agreed MCID for CAT is 2 points. Only 36.8% of exacerbations met this threshold with a mean difference of 0.84 points (± 4.02) compared to baseline scores. For those with a baseline CAT score ≤ 25 the MCID threshold was met in 60% of exacerbations. During recovery visits the average decrease in CAT score was -1.95 (± 4.55) with 47.4% of patients having a ≥ 2 -point improvement in their CAT score compared to at the start of the exacerbation. This increased to 81.8% for those with baseline CAT scores of ≤ 25 . These results suggest CAT is more useful as a marker of exacerbations for less severe patients, though the timing of scoring should be taken into consideration.

SGRQ was not designed to detect differences during exacerbations but is more sensitive as a measure of overall health-status and can be used to track overall symptom burden. Despite small numbers there was an overall increase in the average total SGRQ score. This was mainly driven by impact and activity scores. Symptom scores were less predictable with some patients improving over time and others worsening. This is in keeping with the heterogenous symptoms displayed with usual COPD and possibly 18-months of follow up is too short to detect large changes in overall SGRQ scores.

2.7 Conclusion

This chapter has set out the study design and methods for the 'Elastin degradation in exacerbations of AATD related lung disease' study, the main aim of which is to establish if enhanced symptom and biomarker monitoring can be used to reduce the impact of exacerbations and ultimately reduce the rate of progression of emphysema. It also aims to determine if currently available technology is feasible in a cohort of patients with AATD related lung disease with frequent exacerbations.

There were challenges in implementing a widely geographically distributed study over a long time period in a rare disease, with adaptations that resulted in adequate recruitment for the primary aims. Patients generally exhibited baseline phenotypic characteristics typical of an AATD population, and patterns of symptom worsening that would be expected in AECOPD.

The following chapter presents results collected from the eDiary portion of this study and explores if use of such a diary is able to predict exacerbations to help aid early treatment and reduce the impact of such episodes. It also aims to characterise how exacerbations present in the described cohort whilst simultaneously assessing feasibility.

3 ENHANCED MONITORING IN EXACERBATIONS OF AATD

RELATED LUNG DISEASE - RESULTS (i): eDIARY

3.1 Introduction

Until recently, exacerbations were defined by either crude symptom-based definitions or more commonly by health care use, especially in clinical trials. Event-based definitions are indirect measures of exacerbations and do not take into account unreported or untreated exacerbations which can account for up to two thirds of exacerbations.(213, 246) This led to a lack of measurable outcomes in trials of drugs targeting reductions in frequency or severity of exacerbations. The majority of PRO scores for exacerbations have been tested in the context of randomised controlled trials where follow up is intensive and adherence is constantly monitored to ensure accurate recording of data. These trials have also focused on usual COPD as opposed to AATD related lung disease and few studies have aimed to validate a PRO symptom score in this population.(208) With advances in the field of precision treatment of exacerbations and exacerbation risk in COPD(247) it may be useful to combine more robust symptom data with clinical biomarkers. The end goal would be to develop advanced diagnostic tools that recognise and facilitate the early treatment of exacerbations leading to reduced harm to patients. In the context of AATD related lung disease, this could mean a slower rate of emphysema decline. However, real-world data around electronic symptom diaries and symptom scores is lacking, especially in combination with biomarkers and specifically in AATD.

This chapter presents the results of eDiary data collected from the 'Elastin degradation in exacerbations of AATD related lung disease' study as set out in Chapter 2. During this

chapter I will address the following research questions that align with hypothesis 1 (Section 1.9) and aim 2 (Section 1.10.2):

- 1) Can symptom scores independently determine AECOPD in patients with AATD related lung disease?
- 2) Is it feasible to implement an eDiary for patients at home?

3.2 Results

The baseline characteristics of all patients in the study are summarised in Chapter 2, Table 2.5. Of the total 55 devices issued, three devices were not returned due to the loss to follow up with consequent loss of data. Six patients had less than two weeks of data. This was checked and was not due to software or data processing errors and was down to lack of patient engagement.

3.2.1 Feasibility of an eDiary

3.2.1.1 General feasibility

A questionnaire was filled out by participants at the study site relating to ease of use of the eDiary. A visual analogue scale of 1 to 10, 1 defining 'very easy' and 10 'very hard', was used to quantify ease of use (Figure 3.1) with the majority of patients giving a score of 1 suggesting it was very easy to use. The highest score given was 8.

An open question with a free text response for ease of use of the eDiary was included to explore barriers or ways to improve the design. The major themes that emerged included forgetfulness, being too busy to fill out the eDiary, depression/anxiety and life events e.g., bereavements, weddings. Other comorbidities were reported as being a factor. Three patients with generally stable symptoms commented that they 'felt like they were filling in the same values each day' and 'found it repetitive'. Another said it was 'another device to carry with [them] on work trips'. Some participants commented on the readability of the device; one stating that the colour chart was difficult to see and recommended that a paper

chart would be better as the colours would be more representative of their sputum colour. Finally, some patients remarked that they felt too well and that filling in the eDiary seemed trivial or pointless.

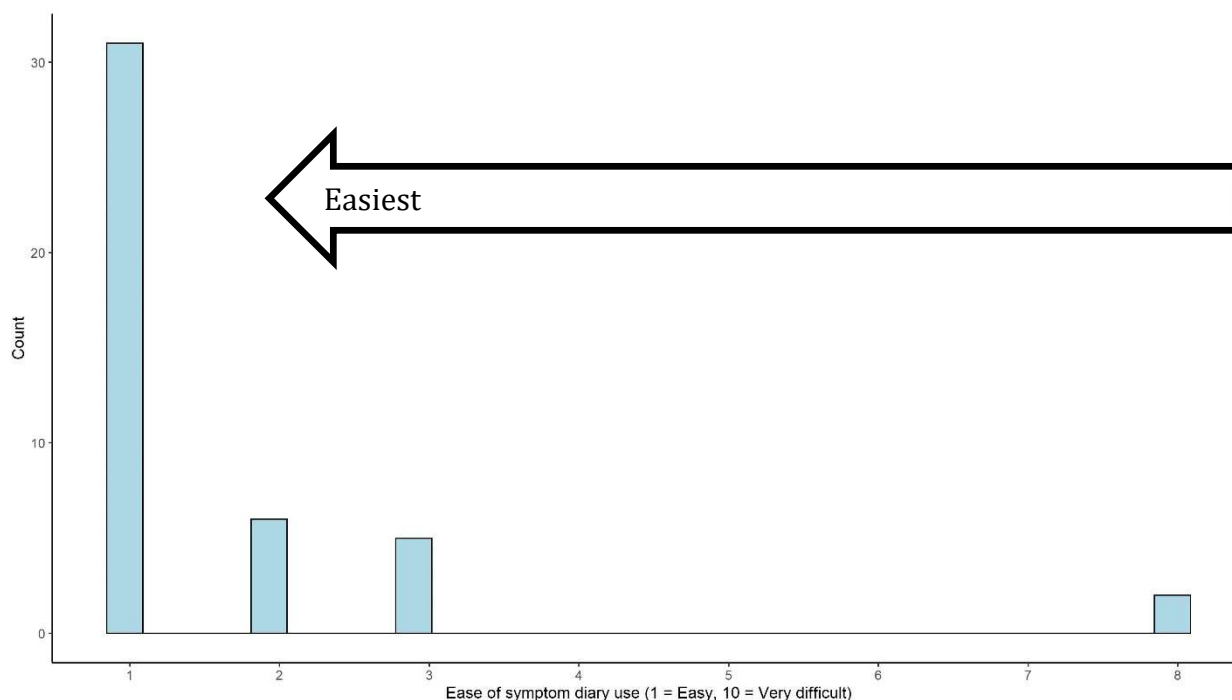


Figure 3.1: Visual analogue scores for the ease of use of the eDiary

3.2.1.2 Timing of device use

The times of day that patients completed the eDiary were also assessed to check recording behaviour. Patients were not instructed to fill out the eDiary at a particular time of day. A histogram of the time all responses were recorded is presented in Figure 3.2. There are two distinct peaks; the largest in the evening around 23:00 with another smaller peak during mid-morning. The majority of entries were made between 18:00 and midnight. There were a small number of entries occurring after midnight which would be considered the following day. No entries were made between 03:00 and 04:00.

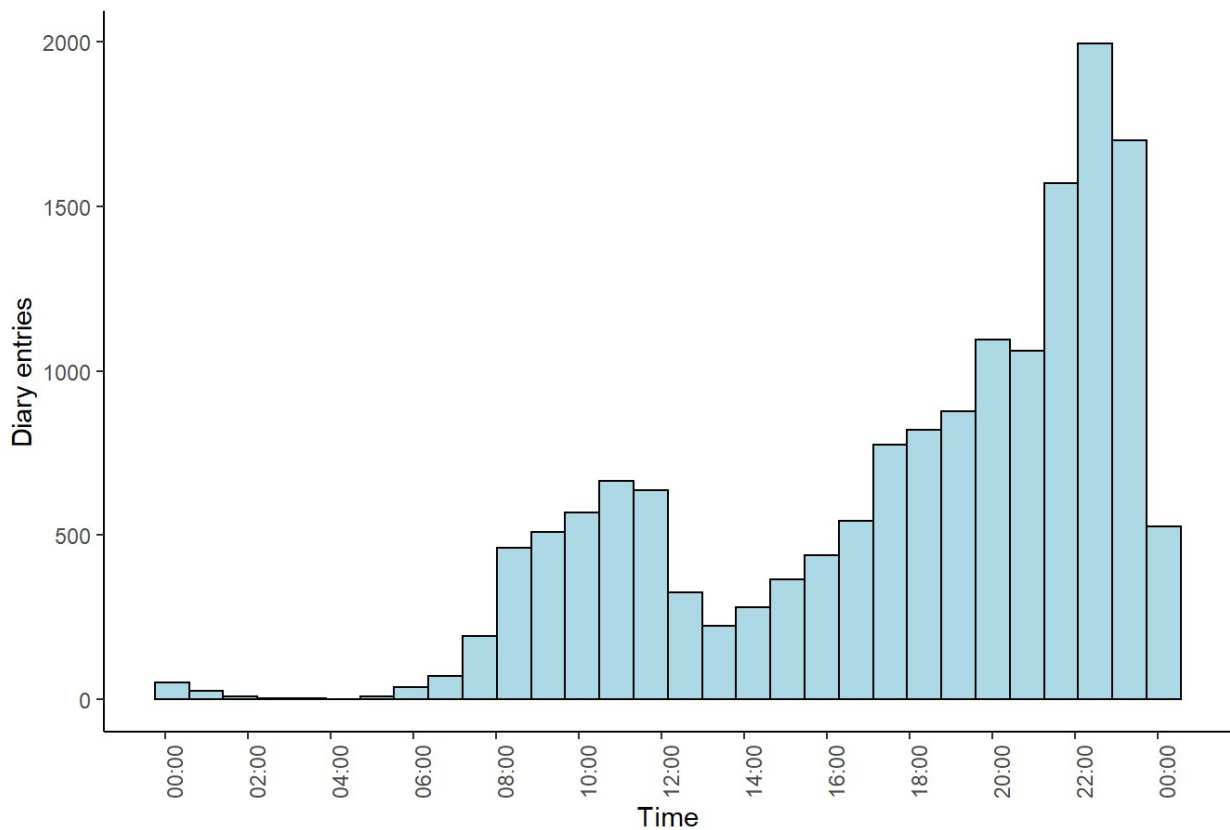


Figure 3.2: Histogram of the time of day the eDiary was filled out

3.2.1.3 Data missingness and imputation.

Percentage of missing days is presented for the raw data and data following imputation

(Figure 3.3). Missingness was reduced by 11% using the described imputation method.

Figure 3.4 shows the reduction in missing values by each patient. For participants with high levels of missing data the imputation algorithm was not as successful. This is likely because these patients did not engage for long periods and as such most of their missing data formed part of a consecutive chain of unfilled eDiary days. For patients that were well engaged the imputation algorithm was more successful with much larger reductions in missing values. This would include patients who were well engaged but may have forgotten the odd day.

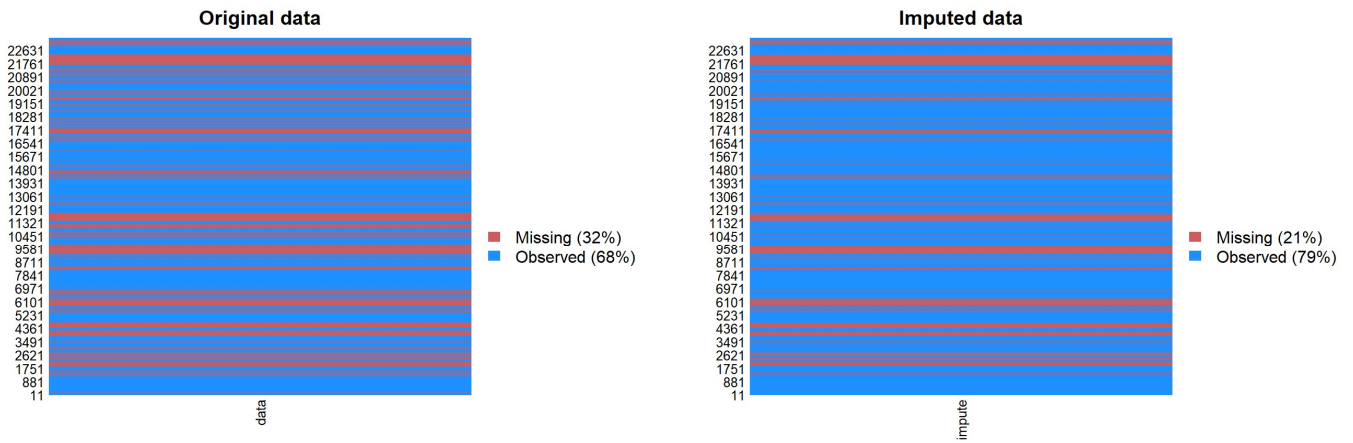


Figure 3.3: Missingness of data in original and imputed dataset

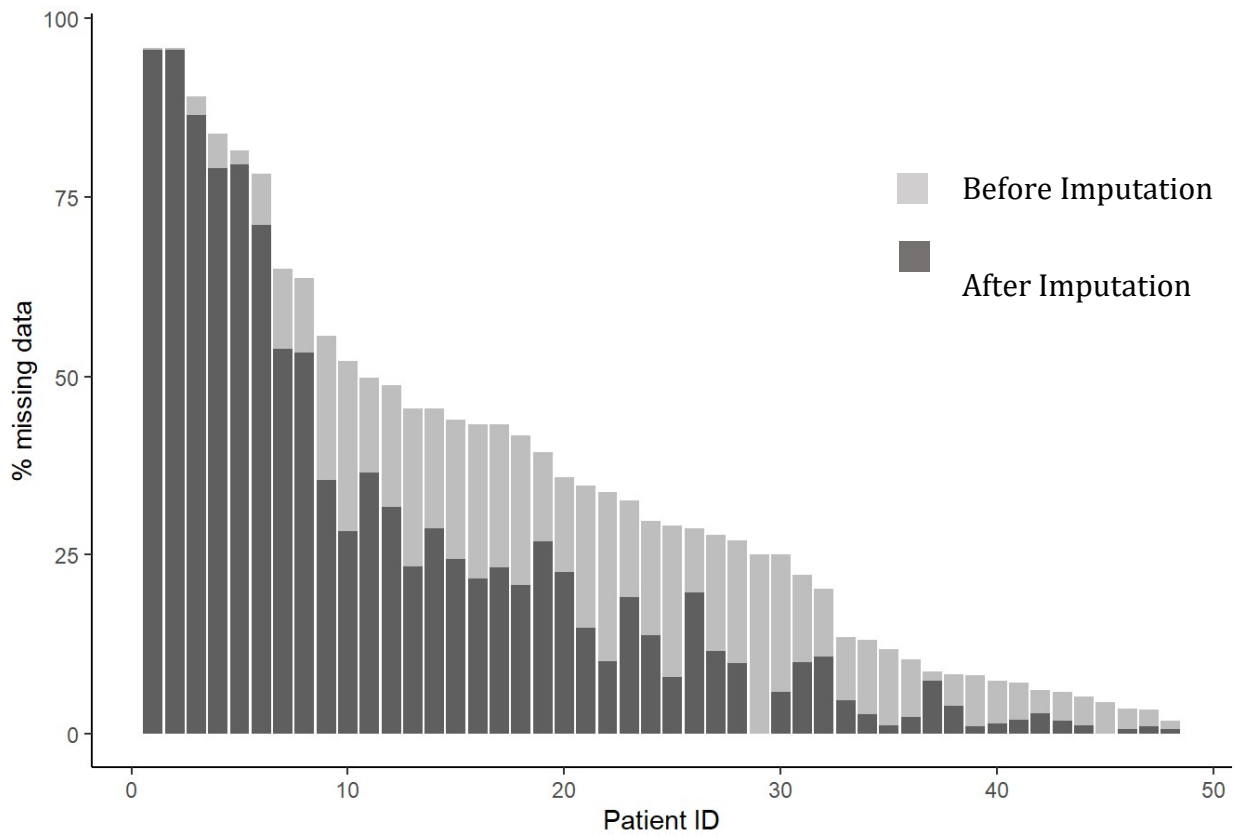


Figure 3.4: Reduction in % missing data after imputation

Percentage of missing values was used as a surrogate marker of patient adherence, which is part of feasibility, in the sense that it describes how feasible it was to enter daily data over a long period. Table 3.1 shows baseline characteristic of patients split into terciles of percentage of missing data. Mean age was higher in tercile 1 (least missing data) compared with tercile 2 and 3. BMI was higher in tercile 3 compared to the other terciles. FEV₁ percentage predicted was on average 8.7% and 14.3% higher for tercile 2 and 3 respectively. Employment status was also different between terciles; 18.8% of patients in tercile 1 were employed compared to 76.5% and 33.3% in terciles 2 and 3.

Table 3.1: Patients' baseline characteristics split by missingness tercile

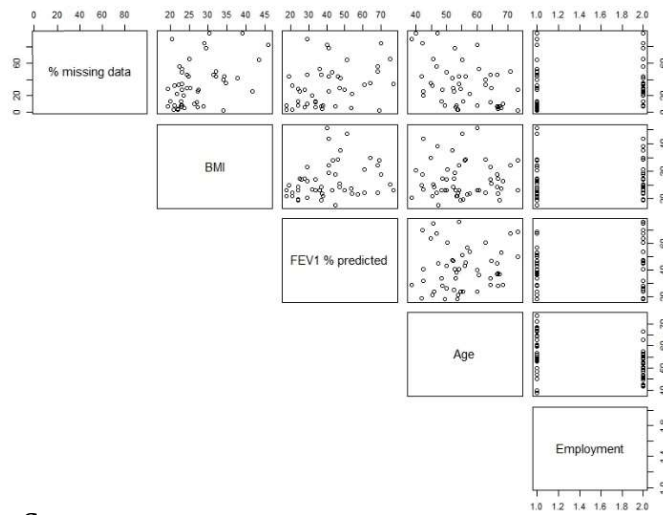
	1st Tercile (least missing)	2nd Tercile	3rd Tercile (most missing)	<i>P</i>
n	16	17	15	
Missing entries % (mean (SD))	7.39 (3.50)	31.68 (7.20)	66.33 (19.30)	<0.001
Sex (%)	4 (25.0)	9 (52.9)	7 (46.7)	0.238
Age (mean (SD))	60.54 (8.01)	53.41 (8.93)	53.00 (9.98)	0.037
BMI (mean (SD))	24.03 (3.82)	27.42 (6.60)	30.08 (7.71)	0.033
FEV ₁ % predicted (mean (SD))	33.99 (13.41)	42.67 (16.75)	48.33 (16.32)	0.052
COPD severity (%)				0.405
Moderate	2 (12.5)	5 (31.2)	5 (38.5)	
Severe	7 (43.8)	6 (37.5)	6 (46.2)	
Very Severe	7 (43.8)	5 (31.2)	2 (15.4)	
MRC score ² (%)				0.669
0	0 (0.0)	0 (0.0)	1 (7.1)	
1	2 (12.5)	3 (21.4)	1 (7.1)	
2	5 (31.2)	4 (28.6)	2 (14.3)	
3	7 (43.8)	5 (35.7)	9 (64.3)	
4	2 (12.5)	2 (14.3)	1 (7.1)	
CAT (mean (SD))	25.80 (8.63)	25.71 (7.21)	24.71 (6.91)	0.914
SGRQ total (mean (SD))	63.54 (15.07)	61.94 (17.26)	64.62 (14.65)	0.896

² Missing MRC score data points hence values do not add up to N

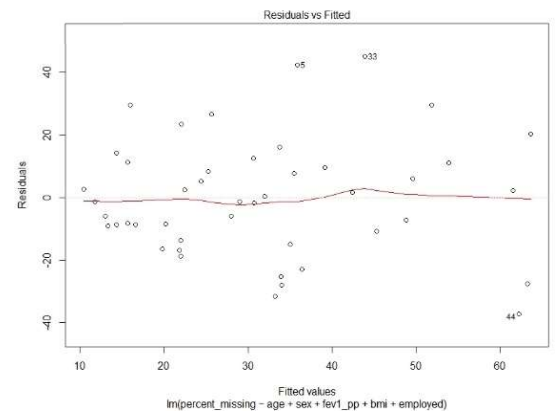
Smoking status (%)				0.34
Non smoker	0 (0.0)	2 (11.8)	3 (20.0)	
Ex-smoker	15 (93.8)	15 (88.2)	11 (73.3)	
Current smoker	1 (6.2)	0 (0.0)	1 (6.7)	
Employment status (%)				0.011
Employed	3 (18.8)	13 (76.5)	5 (33.3)	
Retired	9 (56.2)	4 (23.5)	7 (46.7)	
Unemployed	4 (25.0)	0 (0.0)	3 (20.0)	

To further explore the relationship between missingness and baseline characteristics and to adjust for confounders a multivariable regression was performed (Table 3.2). Assumptions for regression were assessed and deemed appropriate (Figure 3.5). Missingness was on average 1.6% lower per year of age increase. FEV₁ percentage predicted was also shown to be related to missingness with an average 0.5% higher missingness for each 1.0% increases in FEV₁. The differences for BMI and employment status were not sustained in the multivariable regression.

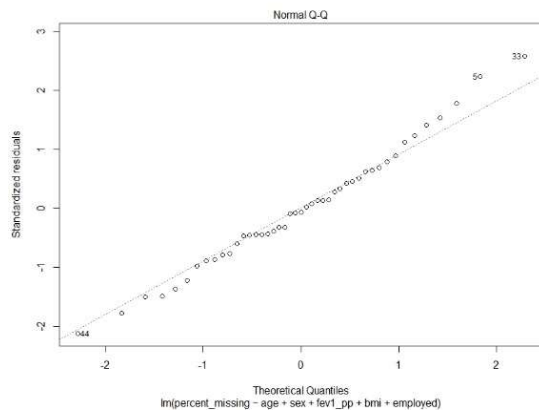
A



B



C



D

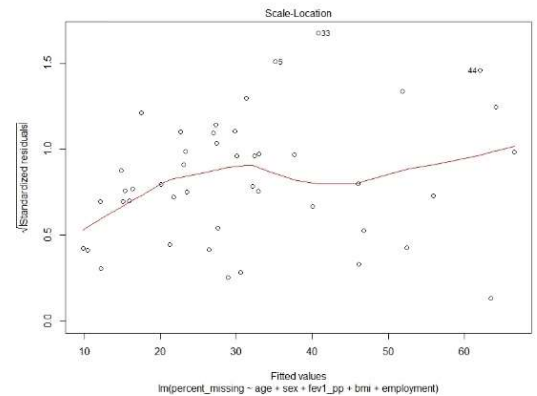


Figure 3.5: Testing assumptions of multivariable regression. A) pairs plot, B) linearity of data, C) normality of residuals, D) homogeneity of variance pair plots (A) confirmed no clear relationship between variables.

Assumptions of linearity of data were checked using a plot of residuals vs fitted values (B) with no pattern seen. Normality of residuals was confirmed using a Q-Q plot (C) with most data points hitting or being adequately close to the fitted line. Homogeneity of variance was checked using a scale location plot (D) and although there appeared to be a slight positive trend, given the relatively small number of patients this was deemed acceptable. Log transformation of the outcome variable did not improve the assumptions and therefore transformation was not used.

Table 3.2: Multivariable regression for missing data (%)

Outcome measure: Missing data %			
Predictors	Estimates	CI	P
(Intercept)	68.14	13.11 – 123.18	0.020
Age	-1.59	-2.49 – -0.68	0.001
Female	4.24	-8.65 – 17.14	0.523
FEV ₁ % predicted	0.52	0.11 – 0.93	0.018
BMI	0.86	-0.15 – 1.87	0.105
Employed	Ref		
Retired	14.96	-2.76 – 32.68	0.106
Unemployed	1.59	-17.54 – 20.71	0.872
Observations	45		
R ² / R ² adjusted	0.412 / 0.319		

A sensitivity analysis was performed that excluded patients that had made less than 90 entries (representing at least 3 months of data) to remove non-engagers or those that used the eDiary for a short period of time (Table 3.3). In this group 49% of data was missing suggesting that compliance decreased the longer the patient used the eDiary. The relationship between age and missingness is retained whilst results for unemployment show a strong correlation. There were only three unemployed individuals included within the sensitivity analysis who all had high levels of missing values (48.8, 89.1 and 95.9% each).

Table 3.3: Sensitivity analysis showing multivariable regression for missing data (<90 entries)

Outcome measure: Missing data %			
Predictors	Estimates	CI	P
(Intercept)	44.75	-8.51 – 98.01	0.116
Age	-1.12	-2.01 – -0.22	0.025
Female	8.85	-4.89 – 22.60	0.222
FEV ₁ % predicted	0.35	-0.04 – 0.74	0.097
BMI	1.25	0.27 – 2.24	0.022
Employed	Ref		
Retired	14.02	-3.85 – 31.89	0.141
Unemployed	33.86	10.11 – 57.62	0.012
Observations	26		
R ² / R ² adjusted	0.575 / 0.441		

3.2.2 Characteristics of exacerbations in AATD

During the course of the study there were 271 Anthonisen defined exacerbations captured using the eDiary. There were a further 13 episodes that were treated despite Anthonisen criteria not being met; these have been labelled 'unclassified' exacerbations. Table 3.4 groups and compares exacerbations by Anthonisen criteria.

Table 3.4: Comparison of exacerbations by Anthonisen type

	Type I	Type II	Type III	P
n	140	94	37	
Season (%)				0.128
Autumn	41 (29.5)	28 (29.8)	10 (27.0)	
Spring	31 (22.3)	18 (19.1)	14 (37.8)	
Summer	23 (16.5)	25 (26.6)	7 (18.9)	
Winter	44 (31.7)	23 (24.5)	6 (16.2)	
Duration (median [IQR])	10.00 [5.00, 22.50]	5.00 [2.25, 10.00]	2.00 [0.00, 5.00]	<0.001
Severity (%)				<0.001
Mild	62 (44.6)	65 (69.1)	28 (75.7)	
Moderate	72 (51.8)	26 (27.7)	8 (21.6)	
Severe	5 (3.6)	3 (3.2)	1 (2.7)	
Treatment delay/days [IQR])	1.00 [0.00, 4.00]	0.00 [0.00, 2.00]	0.00 [0.00, 1.00]	0.201
Peak symptom score (mean (SD))	44.65 (5.43)	40.15 (5.88)	37.92 (5.19)	<0.001

The majority of exacerbations were type I (51.7%). There was no difference in hospitalised episodes between the three groups although there were significantly more moderate exacerbations in type I episodes i.e., treated exacerbations. There were no differences in treatment delays between groups. The peak symptom score during an exacerbation was on average 4.5 points higher than type II and 6.5 points higher than type III exacerbations (Figure 3.6).

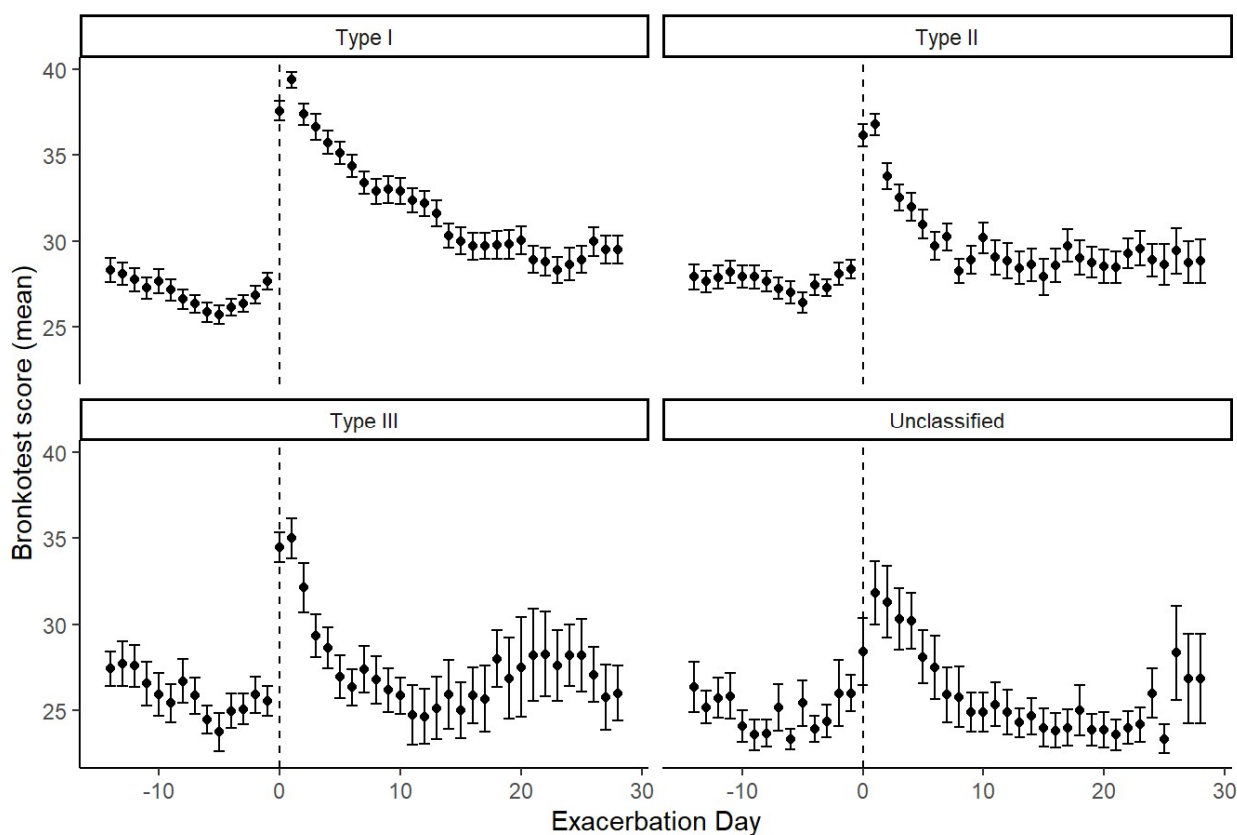


Figure 3.6: Bronkotest symptom score (mean) at different timepoints of an exacerbation compared by Anthonisen type.

Type I events were treated more frequently and lasted longer than type II and type III episodes; 39.6% of untreated episodes were type I compared to 61.6% in treated episodes ($p < 0.001$). Treated events also had a higher symptom score peak compared to untreated episodes (44.0% and 40.1% respectively, $p < 0.001$). Figure 3.7 shows the average symptom score at different time points before and after a treated and untreated exacerbation. There is little difference in symptom score in the 14 days before an exacerbation. On day -1 of an exacerbations (the day before) there is a significantly higher symptom score in treated episodes vs untreated episodes. For both treated and untreated episodes the peak tends to be on the second day of an exacerbation and was significantly higher in the treated group. The symptom score returns to baseline earlier for untreated episodes compared to treated episodes.

Treated episodes were significantly longer than untreated episodes; treated 8.0 days [IQR 4.0 to 17.0], untreated 5.0 days [IQR 2.0 to 11.0] $p < 0.001$ (Figure 3.7).

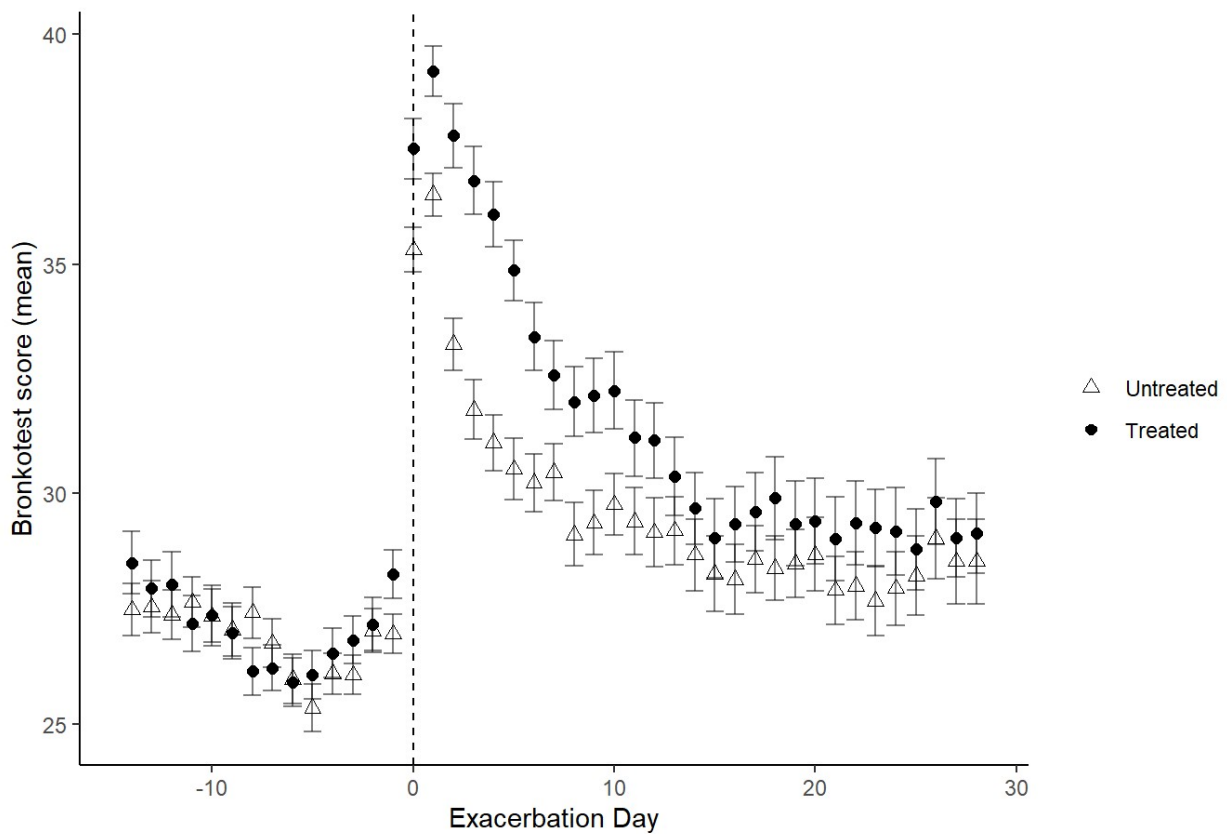


Figure 3.7: Bronkotest symptom score (mean) at different time points of an exacerbation for treated and untreated episodes

Of 125 treated episodes, 106 (84.8%) were associated with both feeling worse and worse breathing on the first day of treatment (antibiotics, steroids or both). Four (3.2%) were associated with feeling worse alone and 4 (3.2%) with worse breathing alone. Eleven (8.8%) treated exacerbations had neither worse breathing or felt worse on the first day of treatment.

Seasonality of exacerbations was also explored. Table 3.5 describes exacerbation characteristics based on either spring/summer or autumn/winter. There was no difference in treated vs untreated episodes or Anthonisen type exacerbations. Exacerbations in autumn/winter were longer than those in spring/summer (median difference of 1.0 day, $p = 0.047$). Figure 3.8 shows mean symptom score over time by season.

Table 3.5: Comparison of exacerbations between Spring/Summer and Autumn/Winter

	Spring/Summer	Autumn/Winter	<i>P</i>
n	123	159	
Anthonisen Type (%)			0.254
Type I	54 (43.9)	85 (53.5)	
Type II	43 (35.0)	51 (32.1)	
Type III	21 (17.1)	16 (10.1)	
Unclassified	5 (4.1)	7 (4.4)	
Treatment (%)			0.372
Antibiotics	37 (30.1)	41 (25.8)	
Prednisolone	2 (1.6)	3 (1.9)	
Both	14 (11.4)	30 (18.9)	
None	70 (56.9)	85 (53.5)	
Duration (median [IQR])	6.00 [2.00, 11.00]	7.00 [3.00, 15.00]	0.047
Severity (%)			0.818
Mild	70 (56.9)	85 (53.5)	
Moderate	48 (39.0)	68 (42.8)	
Severe	5 (4.1)	6 (3.8)	
Treatment delay (median [IQR])	1.00 [0.00, 3.00]	1.00 [0.00, 4.00]	0.772

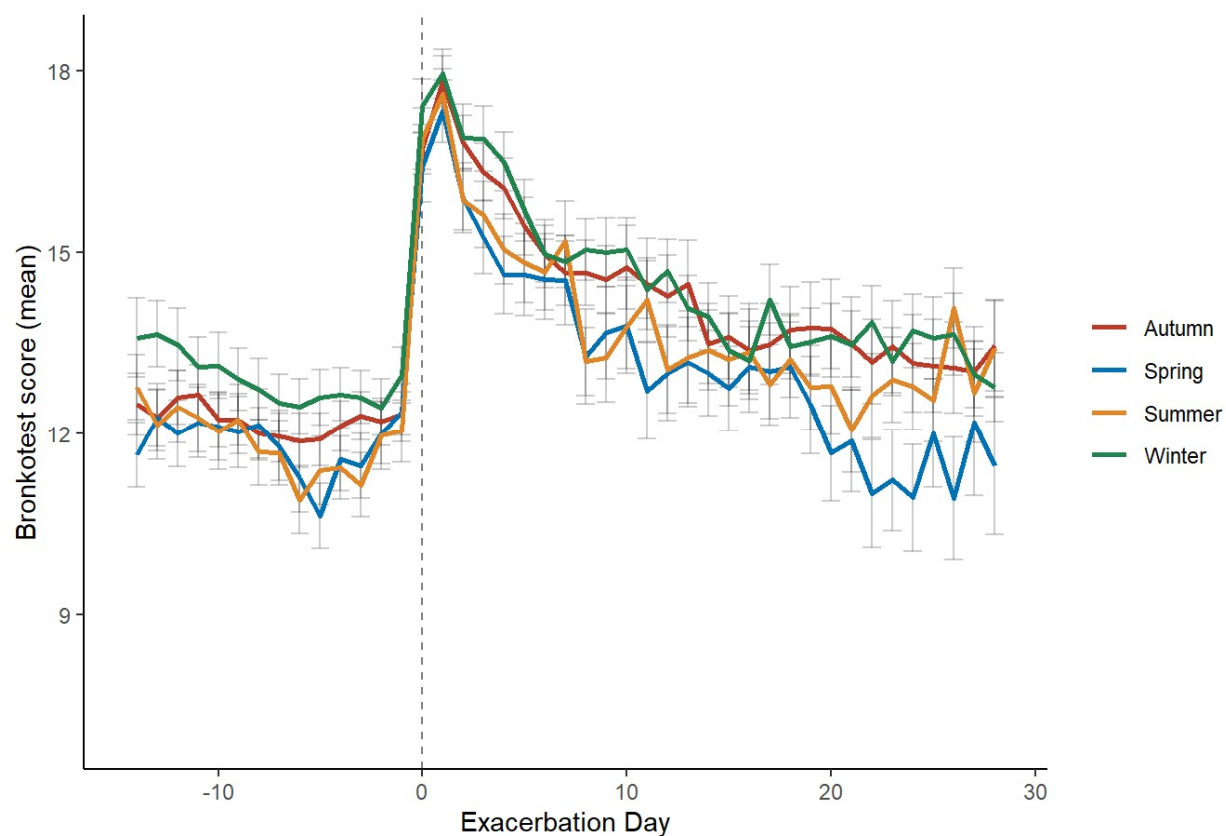


Figure 3.8: Bronkotest symptom score by season over time

3.2.3 Choosing a method to assess difference in symptom score

I considered two methods of detecting an exacerbation based on symptom score. The first and main way of doing so was to compare the score to a clinician determined ‘gold standard’ of whether an exacerbation had occurred based on symptoms. This enabled classification into treated and untreated events, as many other studies in COPD have done. The second way was to use individualised data to determine a patient’s ‘normal baseline’ and then see what level of deviation would be sufficiently far from their normal to represent a possible event. The second approach is in some ways agnostic to a clinician’s view, in that it depends solely on the number of standard deviations from the individual mean to say whether an event might be occurring. However, if this did not concur with a clinician view, then effectively it would be raising false positives. For this reason, it was challenging to implement such an approach in addition to difficulties in establishing an

effective period of stable symptoms for each patient. Consequently, the former analysis approach was used.

Prior to testing the accuracy of either method at detecting Anthonisen defined exacerbations, different ways of deriving a change in symptom score were explored.

The simplest measure of using daily difference was compared to 3-day and 7-day rolling means respectively. Figure 3.9 shows a short sample of real data from a patient enrolled in the study with their symptom score plotted against time. Daily difference (black), three day rolling mean (red) and 7-day rolling mean (blue) are presented. The daily difference (black) shows large fluctuations and spikes in symptom scores. There is a smoothing of the symptom score over time with increasing days used in a rolling mean.

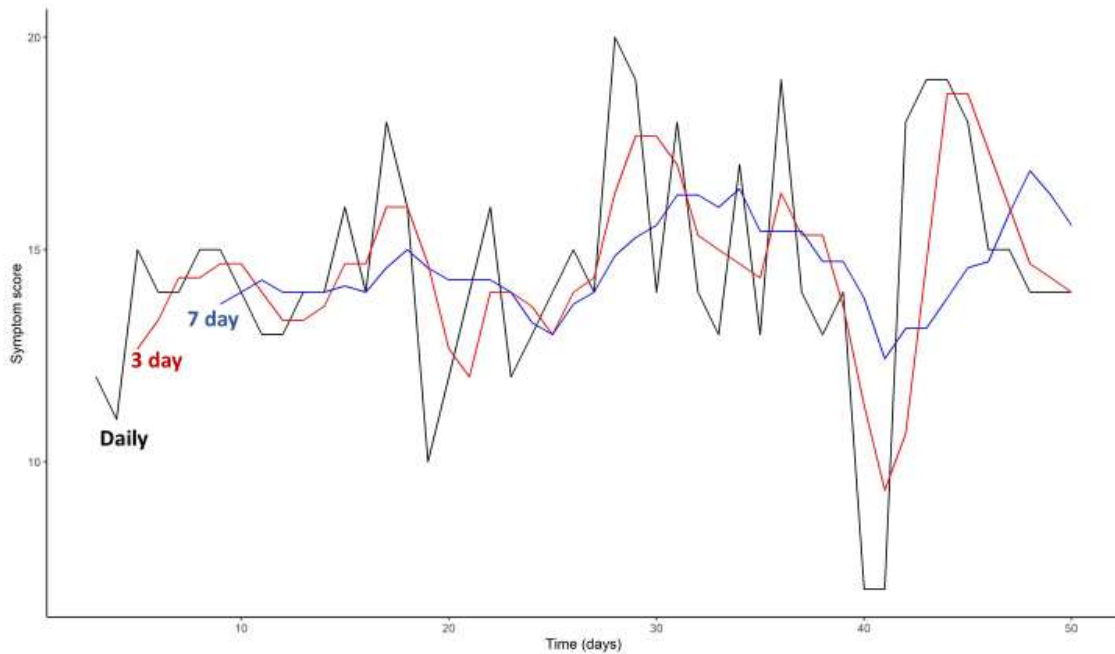


Figure 3.9: Comparison daily (black), 3 day mean (red) and 7 day mean (blue) measurements for calculating change in Bronkotest symptom score.

To assess how the measurement method affects overall performance a comparison between methods was made using ROC curves (Figure 3.10). This was used to compare the three methods (daily, 3-day rolling mean and 7-day rolling mean) for all exacerbations and type I exacerbations alone. As expected, it appears that the symptom score is better at recognising type I exacerbations compared with all exacerbations. Since type I exacerbations are clinically the ones that treatment is targeted at, they were selected to take forward for

further analysis. Next, separate methods of measuring difference in symptom score were assessed. Figure 3.11 shows a more focused look at identification of type I exacerbations in sensitivity and specificity ranges that are likely to be clinically useful (note the scale for the x and y-axis). Daily measurements had a drop of specificity for lower values of sensitivity compared to the other methods. Both 3-day and 7-day rolling means appeared to perform similarly with overlap of curves across the range of sensitivity and specificity values. All methods had a similar sensitivity. A 3-day rolling mean was selected to take forward to the

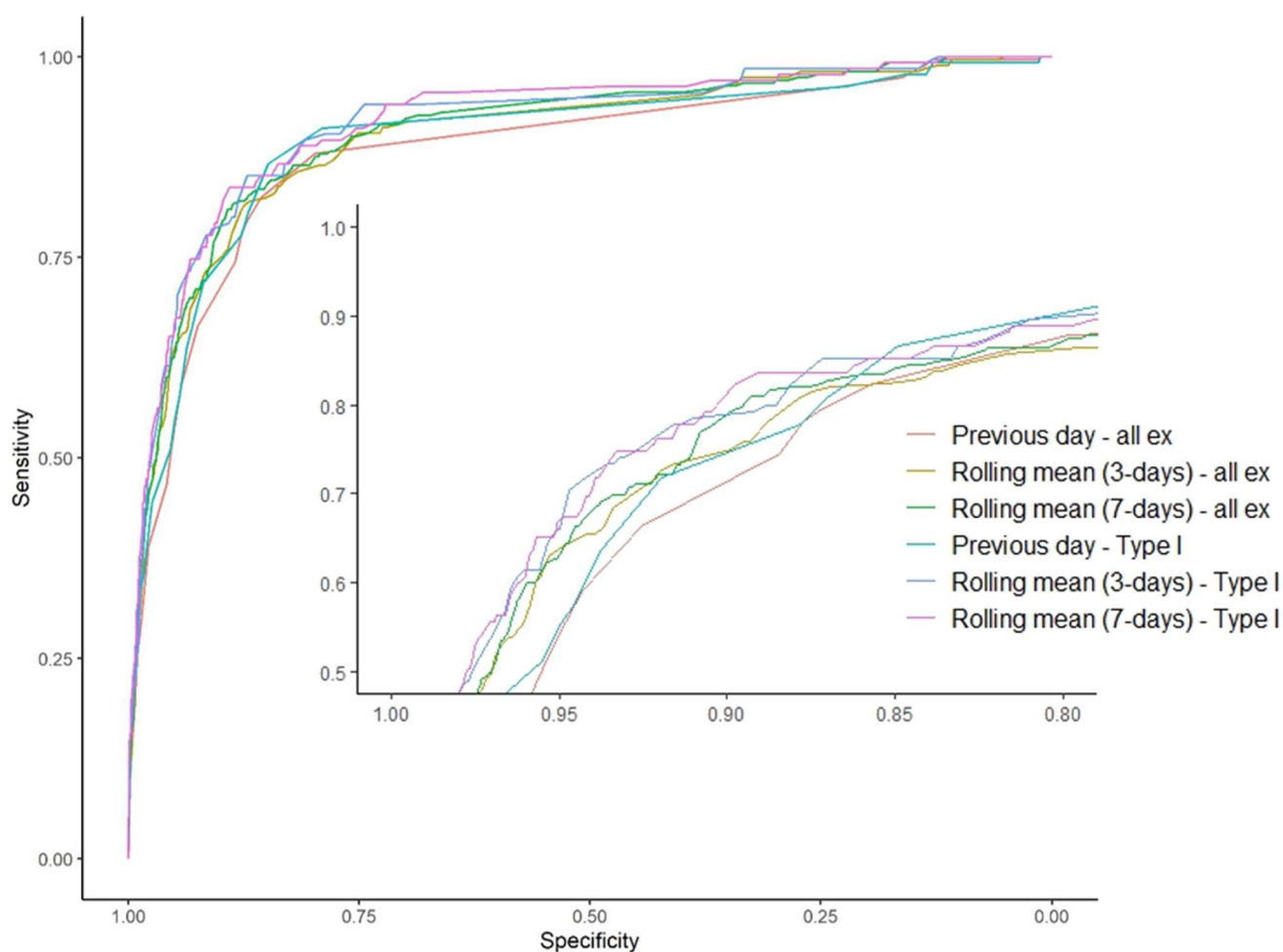


Figure 3.10: ROC curves comparing all exacerbations (ex) and Anthonisen Type I exacerbations for daily, 3 day mean and 7 day mean.

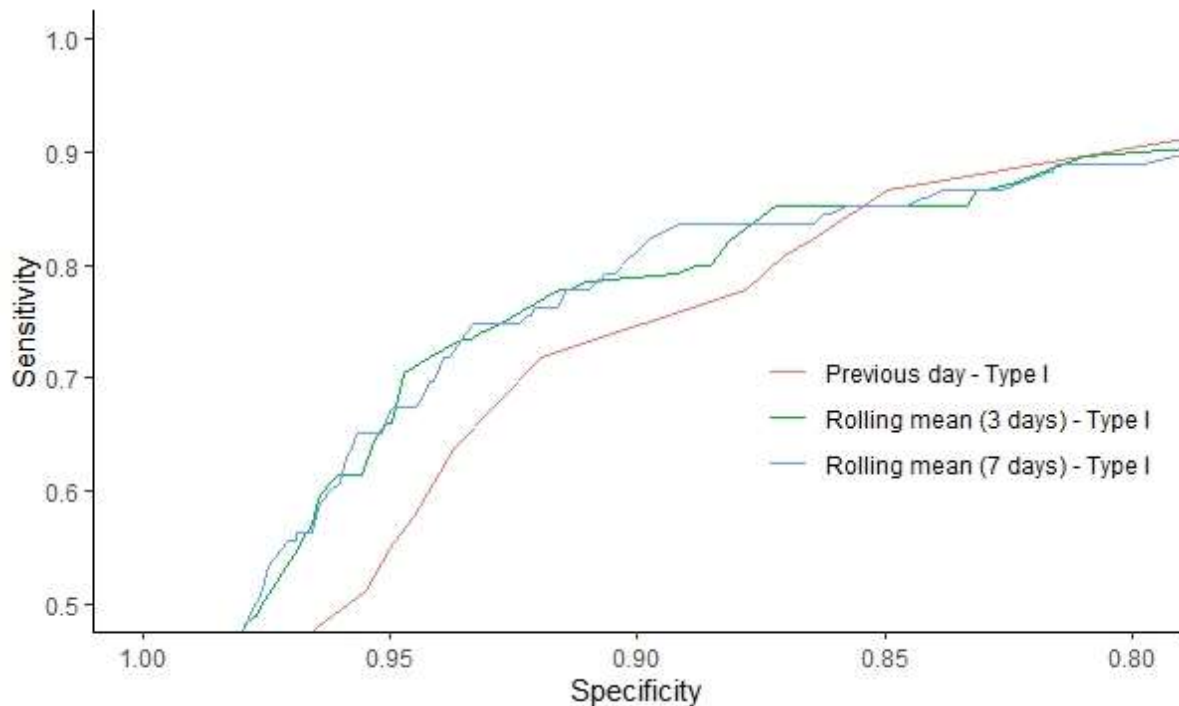


Figure 3.11: ROC curve of type 1 exacerbations for daily, 3 day mean and 7 day mean.

next part of the analysis as it was felt to be the most balanced of all the measures and suffered the least from missing values or influence of recurrent exacerbation episodes.

3.2.4 Choosing a symptom score threshold

The next step was to explore the influence of a symptom score threshold on the ability to detect or rule out an exacerbation. Figure 3.12 plots sensitivity and specificity (y-axis) against a range of symptom score thresholds. The Youden index is also displayed. This is calculated as sensitivity + specificity – 1. It is used to determine the threshold at which there is the maximum overall score for combined sensitivity and specificity. For this analysis the Youden Index is at a symptom threshold of 3.83 but does not vary much until a threshold score of 7 where it begins to decrease.

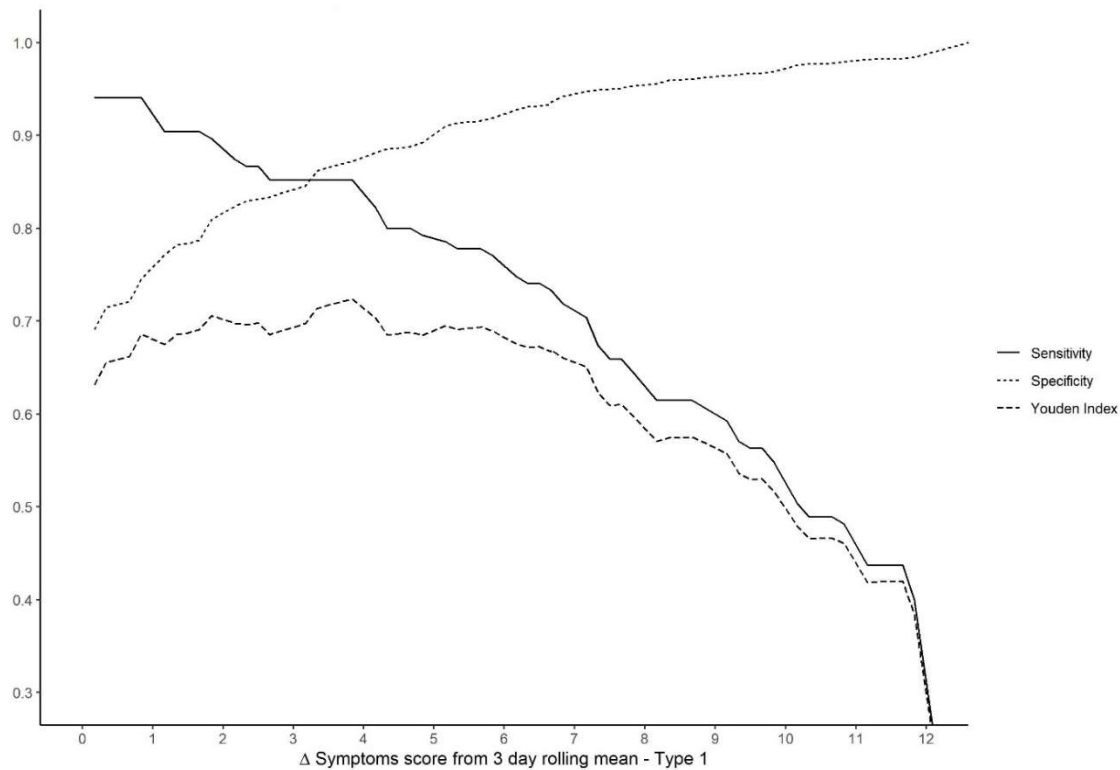


Figure 3.12: Effect of symptom score threshold on sensitivity, specificity and Youden index.

In this dataset there are 12,541 days of patient data that are either a stable symptom day or the first day of an exacerbation as defined by Anthonisen criteria. Days that were either part of an exacerbation (other than the first day) or days where data was not completed are not included within this total. First day exacerbations represent 135 entries (1.01%). Figure 3.13 shows the rate of false positives or false negatives as the symptom score threshold is increased and is accompanied by Table 3.6 which shows values of sensitivity and specificity for integer values of threshold symptom scores. In this case a false positive represents patients who are not exacerbating being defined as having an exacerbation. A false negative result represents patients who are having an exacerbation with a symptom score change below the symptom score threshold for exacerbation and therefore are not identified as having an exacerbation. The former would result in overtreatment of patients and the latter a delayed or missed opportunity to treat an exacerbation.

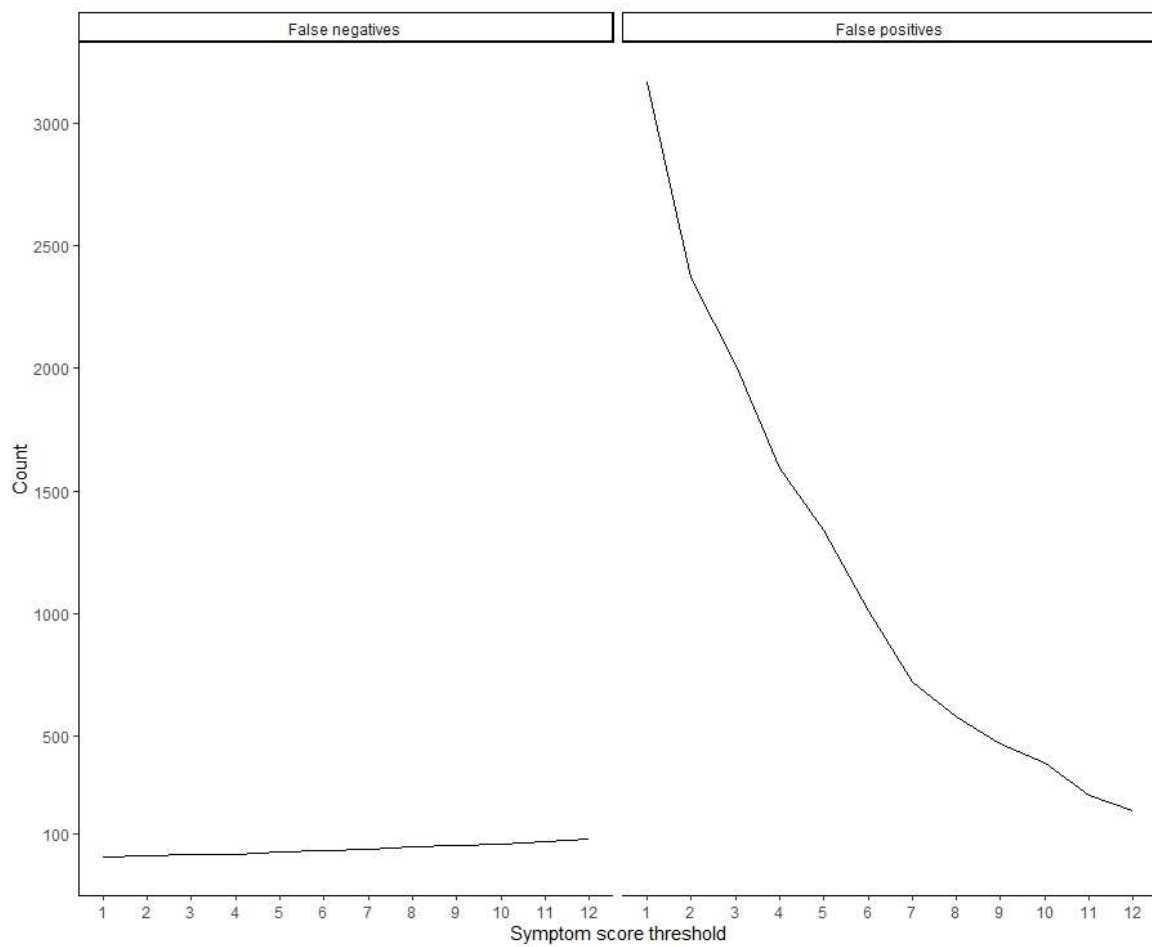


Figure 3.13: Plot demonstrating influence of change in symptom score threshold on false negative and false positive rates

Table 3.6: Sensitivity and specificities across a range of integer symptom threshold scores.

Threshold	Sensitivity	True positives	False negatives	Specificity	True negatives	False positives	PPV	NPV
1	0.94	127	8	0.75	9,264	3,170	3.85	99.91
2	0.90	121	14	0.81	10,064	2,370	4.86	99.86
3	0.85	115	20	0.84	10,420	2,014	5.40	99.81
4	0.85	115	20	0.87	10,841	1,593	6.73	99.82
5	0.79	107	28	0.89	11,093	1,341	7.39	99.75
6	0.77	104	31	0.92	11,424	1,010	9.34	99.73
7	0.72	97	38	0.94	11,711	723	11.83	99.68
8	0.64	87	48	0.95	11,853	581	13.02	99.60
9	0.61	82	53	0.96	11,965	469	14.88	99.56
10	0.55	74	61	0.97	12,045	389	15.98	99.50
11	0.48	65	70	0.98	12,176	258	20.12	99.43
12	0.40	54	81	0.98	12,237	197	21.51	99.34

3.2.5 Predicting future exacerbations

Frequency of treated exacerbations and the total number of treated exacerbations days were compared with patient baseline characteristics including FEV₁ % predicted, KCO, CAT score and SGRQ. For frequency of treated exacerbations there was no correlation between any of the four baseline characteristics.

For total number of days during a treated exacerbation, FEV₁% predicted (Figure 3.14A, estimate -1.74, 95% CI -2.47 to -0.99, $p < 0.001$) and KCO % predicted (Figure 3.14B, estimate -1.65, 95% CI -2.28 to -1.02, $p < 0.001$) were negatively correlated.

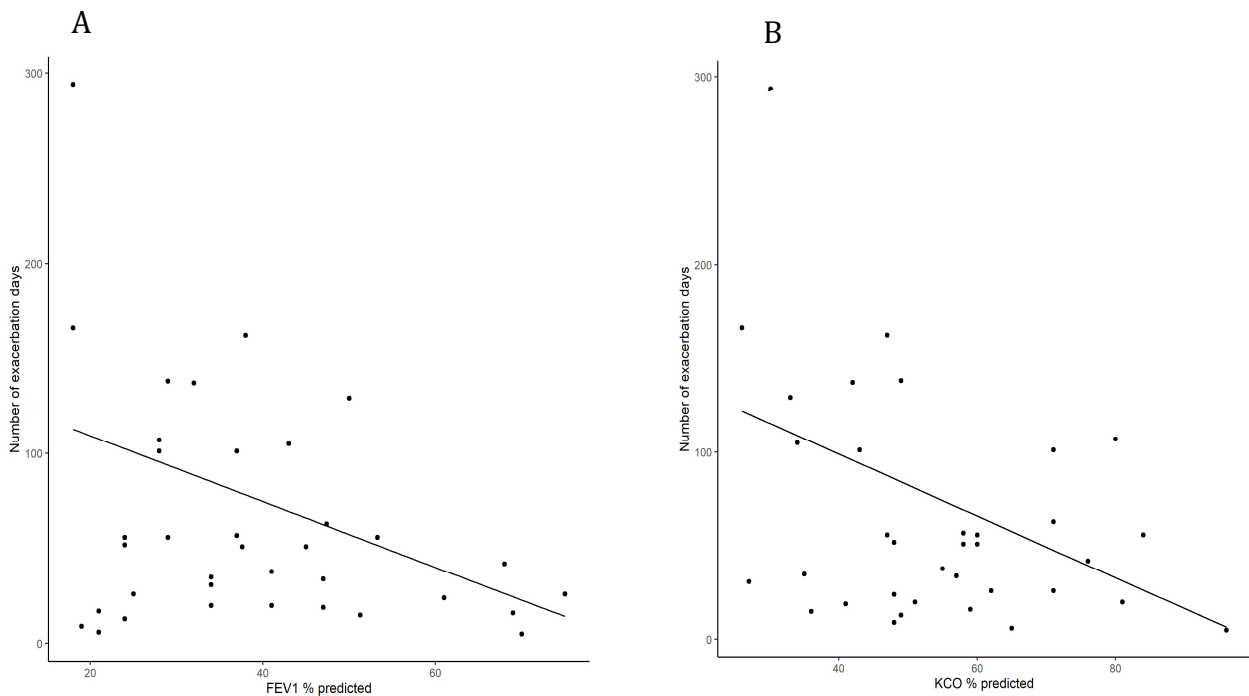


Figure 3.14: Comparison of total number of days for treated exacerbations and A) FEV1 % predicted and B) KCO % predicted.

3.3 Discussion

This study builds on work by Vijayasaritha and Stockley(210) who used Bronkotest diary cards in patients with AATD related lung disease. They found that treated exacerbations are likely to be more severe, findings which mirror those from this study. They also found that episodes that are associated with breathlessness and poorer well-being were more likely to be treated. In this thesis chapter I am not only able to give a more detailed analysis on the characteristics of exacerbations in a larger number of AATD patients but also provide useful insights on the feasibility of implementing an eDiary in this cohort over an 18-month period. I have also attempted to give a range of useful symptom scores for Bronkotest that could be used in clinical practice for enhanced monitoring to detect exacerbations either alone or in combination with biomarkers of exacerbations.

3.3.1 Feasibility of using an eDiary

Most patients reported the eDiary as feasible to use when asked directly, however variability of time of completion and adherence suggested that this was not the case in daily life where reporting was inconsistent.

3.3.1.1 Patient factors

Patient factors are an important barrier to introducing a new healthcare technology.(248) Even if a new health care technology is found to be efficacious its overall success depends on patient acceptance and engagement. In this section we will discuss factors that may influence engagement and ways in which the design of such products/interventions could be tailored to minimise the effect.

Comparing terciles of missingness shows that data were more consistently collected in older patients and in those who were either unemployed or retired. This could be explained by retired patients having more time to fill out the eDiary and incorporate it into their

schedule. Confounding may be present, especially with older patients being more likely to be retired. Being older generally means that any children are older or grown up, so there may be fewer non-work-related distractions. Patients who are younger with dependants may not have the time or be distracted by childcare responsibilities. Older patients may also have more severe disease and find more benefit from reviewing their symptoms daily. Fitter or younger patients with stable symptoms could lose interest with repeated days with the same score. One comment from a patient during the end of study feedback stated that they 'were filling out the same [answers] every day.' An eDiary could be prioritised for use in patients after severe exacerbations, or during periods of higher exacerbation frequency, rather than used throughout life. It would also be important to ensure that patients have the time to complete the eDiary, and this is explored fully with patients in a consultation. Whilst this was done for our research study the variation in completion suggests more time could have been spent on this. Repeated consultations may be required to gain full engagement with a regular eDiary. Another option at the beginning of the symptom diary could ask 'are your symptoms the same as yesterday' although this may lead the patient into a false sense of security and not consider small changes in symptoms which may signify the beginning of an exacerbation.

Somewhat surprisingly, patients with worse disease were found to be more consistent with entering eDiary data. Again, this may be confounded by older patients having worse disease. Patients with severe disease are more likely to be medically retired, regardless of age. In reality, interplay between age, employment status and disease severity could be present and such variables should be considered in patients for clinical trials that involve an eDiary and for use in clinical practice. Table 3.3 shows a multivariable regression of factors that were shown to have association at an individual level. After adjustment it

showed that older age and lower FEV₁ % had a lower percentage of missing data. This analysis adds weight to the influence of age and disease severity to study engagement and adherence to an eDiary. With regard to age this result may be somewhat unexpected given the assumption that younger patients would have a better grasp of technology and therefore be more adherent/able to use it. However, smart phones, tablets and other technologies have been around for a sufficient amount of time for such technologies to be acceptable to people of all ages. Furthermore, though employment did not show a close correlation in the multivariable regression, it may be because the study was too small to detect a difference in a three-category nominal variable. Future work could be adjusted to use hours per week worked to further explore the influence of employment status on adherence though this data was not collected. This study has given new insights into factors that influence adherence to eDiary use in a COPD population that have not been explored elsewhere.

I was able to study the time-of-day participants filled out the eDiary. Most patients entered data towards the end of the day which is in line with other studies.(249) There was an additional peak during mid-morning. Patients may be responding based on symptoms for the morning alone and therefore afternoon or evening symptoms could be missed. In contrast, patients with poor morning symptoms that improve throughout the day could under represent their symptoms if the eDiary is filled out in the evening time when sufficient time has passed. For future studies it may be advantageous to instruct patients to complete the eDiary at night or to fill the questions out based on a reflection of their symptoms for the prior 24 hours. This would avoid missing periods of symptoms that happened at certain times of day. Alternatively, more than one eDiary entry could be recorded. This was done in one study which explored symptoms throughout the day during

stable disease and reported reasonable adherence rates over 2 weeks.(209) This approach increases the burden of filling out an eDiary and may put patients off using it as a symptom monitoring tool.

3.3.1.2 Device and application factors

The format in which the symptoms are accessed and data collected is fundamental to the successful implementation of telemedicine.(248) In the published literature there is often little detail on the methods in which electronic symptom diaries are delivered. This makes direct comparisons between studies and establishing the best approach challenging. My study aimed to explore device factors to help inform the future use of electronic symptom diaries in research trials and in clinical practice.

There were some issues with remote monitoring of patient data in this study. The first was the volume of data being sent; patient symptoms were recorded and pushed daily. This volume of uploaded data led to duplication in some instances. This was due to the data synchronisation coding instructing data to be pushed at the end of questionnaire completion and not enough time being allowed for safe data transfer before the eDiary was either powered down or put in standby by the patient. The resulting back log of data eventually overwhelmed the server. Subsequently it was not practical to access data remotely early on in the study. Another problem was when all patients temporarily lost access to the TakePart application on 1st January 2019 and were unable to record their symptoms. This was caused by prior programming of the application to complete data collection in 2018. This was changed and patient access to the application was reinstated thereafter with only a few days of data capture lost. Toward the middle of the study, once initial patient adherence was checked, the remote monitoring of patients was abandoned and telephone checks were carried out thereafter. Although remote monitoring of patients

was not successful, this was advantageous in assessing feasibility of implementing symptom monitoring. It meant that patients had to be self-motivated to complete the eDiary, as they would if the eDiary was used long term in a real world scenario. In clinical practice it would require a dedicated health care professional to continually check and monitor patient symptoms, which may be an overly paternalistic model of healthcare, rather than a partnered model that tends to be preferred for guided self-management.(250)

Another consideration is the type of device used to collect data. In this study a single device preloaded with the TakePart application was issued to patients. This ensured that each patient had the same experience of using the app and did not disadvantage those without a smart phone. The application was not available to Android or Apple devices via their respective online stores and patients would not be able to use their smart phones. Since this study was a pilot of the Bronkotest questionnaire in application form, it was reasonable to use the application on a preloaded device. In case of issues with remotely uploading data, as with this study, the data could still be recovered once the devices were returned. This would have been more difficult if patients had used their own smartphones with potential loss of data. It would be important to pilot a universally available application before rolling it out into a larger study to ensure data integrity.

There are some disadvantages to using a single device issued to patients. If the device is lost, misplaced or damaged then study participants are unable to continue completing their eDiary. From the feedback at the end of the study one patient moved house and lost the device in the process and was unable to complete the questions. Patients often own a smart phone or another device and an additional device could be inconvenient. Kindle Fires require separate charging, are generally much larger and therefore less portable than a

smart phone and are not carried about one's person as often as smart phones. One patient did acknowledge this point in their feedback by saying that 'it was another device to take with [them] on work trips.' Another lost the charging cable and didn't inform us until an exacerbation review visit. Use of a smart phone app that is universally available would allow access from any smart phone device and likely be more convenient to the patient and result in better uptake. It could be a cheaper option as the initial cost of devices would be avoided. The upkeep of servers for running of the app would still need to be built in to any costing. The app could also be updated in real time if bugs or errors occurred and user experience could be upgraded with help of feedback.

It is clear that user behaviour during a study and the internal conditions used to test the device in the pilot stages prior to use in a study are not the same. Therefore, the use of the eDiary for this study has been valuable in its future development and to find bugs on a smaller project before scaling it up to a larger study. This pilot study has led to creation of an Android and IOS version based on EXACT-PRO and the Bronkotest sputum colour chart which will be used by patients on their own smart phones and is due to be launched in the Colour COPD study.(251) This randomised control trial aims to assess the use of sputum colour charts to guide treatment of COPD exacerbations against usual care. A large number of decisions around the application design were informed by lessons learned from this study. The initial background coding is the same with an over haul of the user interface. The patients will have their own email log in details and are able to give feedback to the study in real time. The data synchronisation issues have also been resolved by ensuring pushing of data occurs during the entire time the app is running, not just at the end of when the questionnaire is filled out. There is also an on-screen prompt that lets the patient know when the data has successfully been uploaded. These measures have been put in place to

avoid the same issues that occurred during this feasibility study. Data is still backed up on the device being used in case of issues with the cloud database. The app can be updated in real time to fix any bugs and to respond to any feedback from patients. The daily interval can also be changed to avoid the reported issues with days of data being missed due to a midnight cut off.

One final consideration is the use of an alarm or feedback for patients. This could not only improve adherence but also increase satisfaction and reinforce positive behaviour

3.3.2 Comparison to other studies using symptom diaries

One of the main strengths of this study was the study of a real-world AATD cohort over a long time period. Previous studies attempting to detect AECOPD using symptom diaries (Chapter 1, Table 1.3) were considerably shorter (18 months in my study compared to an average of 7 months), and were used as part of clinical trials, meaning direct comparison is difficult.

Frent et al(252) found that EXACT-PRO recognised 22.2% of HCRU defined AECOPD. Boer et al(212) went into more detail and quoted high sensitivity (97.4%) but low specificities (65.6%) for their Adaptive Computerised COPD Exacerbations Self-management System (ACCESS,) a computer algorithm. ACCESS provided feedback to patients and gave recommendations on treatment and whether to consult with a health care professional or not. Using the quoted sensitivity and specificity levels there is a false positive rate of 34.4% meaning 725 additional cases where a patient is encouraged to contact a health care professional. The authors comment that the algorithm is overcautious but do not discuss the impact that this may have on resource allocation. One point of an exacerbation recognition system is to help lessen the burden of exacerbations on healthcare systems. This approach does the opposite and highlights the importance of balancing sensitivity and

specificity in coming up with an algorithm. Over 700 false positives in a 3-month period for 54 patients would likely overwhelm most COPD services if it necessitated review from a health care professional, whether by phone or other means.

Comparison between studies quoted in the introduction (Table 1.3) suggests that symptom diaries are more acceptable for shorter periods, a finding which is supported in the results of my study. Overall adherence dropped to 49% in study participants who engaged for the fully duration of the study compared to 68% overall.

The use of an eDiary may be better directed towards the post exacerbation period, for example, where a patient's symptoms may be unstable and symptom monitoring being a useful way to detect treatment failure and to avoid hospital admission. A recently published feasibility randomised control trial of hospitalised COPD patients with exacerbations has shown promising results with the use of a smart phone application to support patients in the post-exacerbation period(253) with lower rates of re-exacerbation and quicker recovery of CAT score.

One of the limitations of this study was that data was imputed to increase the yield of recorded exacerbations. The nature in which the symptom data was analysed meant that a sensitivity analysis using the raw, unimputed data could not be performed easily to assess its impact on the results. Simple imputation algorithms have been used previously in studies of COPD(233, 254). I predict that the impact of imputation on the results would not change the outcomes since it was replicating data only within a 48-hour period. Anything longer was recorded as missing data.

One point that deserves discussion is that telehealth use can increased health care use, especially telephone consultations.(205) This is a relatively unexpected outcome and certainly needs consideration when implementing this into real-world situations. It also

highlights the importance of selecting a suitable symptom score threshold (or algorithm if using other markers such as FEV₁ or oxygen saturations). It may also mean that current symptom diaries alone are not able to detect exacerbations and separate them from natural variations in COPD symptoms.

An alternative way to analyse symptom diary data is statistical process control (SPC), a process often used in engineering to identify fluctuations in normal process's and identify significant changes in signals. One study attempted the use of SPC charts to monitor FEV₁ amongst other parameters to identify exacerbations in 17 COPD patients.(255) This particular study suffered from poor adherence due to a complicated monitoring regime. It did show that physiological parameters like FEV₁ and oxygen saturations could be monitored easily. This technique is similar to using a symptom score threshold, but instead it uses a baseline patient data to set a threshold limit for an exacerbation. In reality, patient symptom baselines may fluctuate depending on factors such as weather or pollution. Using a rigid symptom score baseline may lead to substantial false positive or false negatives and is the main reason for adopting a more dynamic approach in my study.

3.3.3 Characterising COPD exacerbations in AATD patients

This is the largest study to date of pulmonary exacerbations in AATD using an eDiary to assess treated and untreated exacerbations. It has shown that Anthonisen type I exacerbations are longer in duration, are treated more often and typically have a higher symptom burden. These results are in keeping with a previous study that looked at exacerbations in a sub cohort of patients enrolled in a randomised controlled trial of AAT augmentation therapy.(208) Results also confirm that two of the main driving factors of treatment are increased breathlessness and worse feeling of wellbeing with 9 in 10 treated exacerbations having at least one of these symptoms present on the first day of treatment.

My study emphasises the burden of untreated exacerbations within this cohort; 45% of all Anthonisen type I exacerbations were untreated. This may be of particular concern in AATD patients who are known to have higher levels of inflammation compared with usual COPD and higher level of lung damage.(256)

One aspect which has not been presented before in AATD related exacerbations is seasonality. The results of this study demonstrate a difference in exacerbation duration occurring in autumn and winter compared with the warmer seasons albeit a small difference. The frequency of exacerbations was also higher in autumn/winter. This could provide evidence for the rationalisation of drugs used to reduce exacerbations during the summer months in AATD, such as long-term macrolide therapy. It also implicates viruses as a factor for exacerbations in AATD related lung disease since virus' are more prevalent during the autumn and winter periods.(257) This could be explored in future work by viral swabs or serology during events, though the geographical spread of patients and inability to attend our centre during exacerbations limited this endeavour during my study.

One strength of this study is the way it was able to present average symptom data across the whole course of an exacerbation including the period leading up to an event and the recovery phase (Figure 3.6, Figure 3.7). This has been performed in studies of COPD before but not in AATD.(103, 252) Symptoms generally peak by the second day of an exacerbation and then gradually recovered. Figure 3.6 shows type I exacerbations take longer to recover than type II or type III exacerbations. An interesting observation is the 13 unclassified exacerbations that represent episodes that patients acknowledged as exacerbations but did not meet Anthonisen criteria. Figure 3.7 shows a small peak in symptom scores of unclassified exacerbations that seems to persist for 4 to 5 days before returning to the

baseline value. This means there is another symptom outside of the typical Anthonisen criteria that is driving the use of oral medication for exacerbations. Reasons for this found during this study were a feeling of poorer general wellbeing and upper respiratory tract symptoms. Treatment of these symptoms could be driven by patient education or past learned experiences that a particular symptom then led to a significant exacerbation. There may also be other factors that were not captured with the symptom diary such as feelings of anxiety which are known to contribute to exacerbations(258) and would affect well-being. Patients could have perceived their symptoms to not warrant worse scores when filling in the symptom diary but decided to take treatment anyway. Unclassified exacerbations represented only a small proportion of overall treated exacerbations in this study but deserve consideration.

Figure 3.7 shows the difference in symptom progression and recovery for treated and untreated exacerbations. Similar to other studies, treated symptoms lasted longer and have a higher symptom peak. An interesting observation is the day preceding treatment for the treated exacerbations has a significantly higher symptom score. This suggests that worsening symptoms in the day before an exacerbation influences the treatment decision.

There are only two other studies that have described pulmonary exacerbations in AATD patients specifically. The most recent study by Ejiofor and Stockley(208) used symptom diary data derived from a randomised controlled trial of inhaled AAT augmentation therapy.(54) They also used the same Bronkotest symptom score and studied a similar number of patients ($n = 46$). There was no comment on missingness of data and how data was collected meaning further comparison to my study was limited. Data was sent via a transmission device, though the format in which the diary was completed and detail on how

it was sent was not discussed. All data analysis was manual, which would not be practical in real-life, especially if being used to warn patients about an impending exacerbation event. The study found treated exacerbations were longer and were likely to be associated with breathlessness. They also noted significant heterogeneity in the onset and recovery of symptoms. The original RCT included 168 patients whilst the sub-study of exacerbations only examined 46 patients, the reason of which is not clear. It may be that only the placebo group were used though this does not account for some patients. They also limited the study to patients filling out more than 1 year of entries. Study participants were reminded daily to complete the diary via an electronic signal, something which was not done with this cohort. The proportion of treated to untreated episodes was similar (233 episodes, 103 untreated, 130 treated in the Ejiofor and Stockley study compared with 284 episodes, 159 untreated and 125 treated in this study). By comparison, the age, sex distribution and FEV₁% predicted were remarkably similar in both groups with similar inclusion and exclusion criteria too.

3.3.4 Comparing the performance of Bronkotest to EXACT-PRO

The most well-known PRO score for exacerbations is the EXAcerbations of Chronic Pulmonary Disease Tool (EXACT). Following the first study in 2010,(259) EXACT has been developed as an outcome measure for exacerbations in several randomised controlled trials of drugs that aim to improve exacerbation frequency or severity.(249) The overall aim of its development was to produce an overarching PRO that could be used to compare outcomes of studies of exacerbations and to improve understanding of the disease process. Concerns over inconsistencies between healthcare use definitions and EXACT score still remain with one study reporting the tool missing treatment defined exacerbations(103), though it remains useful as a measure of exacerbation severity. In more recent studies investigators

moved from using symptom diary cards to an electronic format although exact details on how it was delivered were not reported.

Bronkotest was initially developed and used as part of the EXACTLE trial (130) which, like this study, included patients with AATD related lung disease. Bronkotest differs to EXACT-PRO as it uses a scale that allows for differences in patients baseline severity or symptoms by comparing to their 'normal' state. In contrast, EXACT-PRO, which has 14-domains, uses a 5-point Likert scale ('Not at all' to 'Extremely') with objective measures of breathlessness e.g., short of breath with personal care, short of breath with indoor activity. This approach may not record exacerbations equally among patients with varying baseline symptom severity. For example, a patient with mild disease who does is not typically breathless who then experiences breathlessness may have a larger increase in their score compared to a severe COPD patient who usually has poor baseline exercise capacity. A small increase in breathlessness score may be more significant for a severe COPD patient too, especially if their respiratory reserve is low. Bronkotest negates this by using breathlessness scales that are relative to a patient's baseline.

EXACT-PRO collects very detailed responses relating to exacerbations in addition to other symptoms that may not directly relate. In addition to domains for typical symptoms such as breathing, sputum and chest discomfort, the EXACT-PRO asks questions relating to psychological state, tiredness/weakness and limitation of activities. Both Bronkotest and EXACT-PRO collect data on sleep disturbance. Although more detailed responses are useful for characterising exacerbations, it may be advantageous to use a simpler symptom score that is user friendly, especially for daily use outside the detailed follow up of a randomised control trial where lack of intense follow up could lead to reduced adherence. However, use

of a very detailed questionnaire may give rise to novel findings that were not previously considered. This would make EXACT-PRO more useful in the context clinical trials.

Though the wording of the question domains for Bronkotest allows for recognition of change in symptoms relative to baseline, it means the responses are less specific and therefore not comparable in cross-section between patients. This is true for describing sputum amount and for cough frequency which uses arbitrary scales of volume and frequency. As long as patients were consistent with their responses then this would not affect the validity of results on an individual basis. In the first study using Bronkotest(211) sputum amount was given a volume indicator such as 'a tablespoon full' or 'eggcup full'. For this study, examples of what 'a little' or 'moderate' were given to patients during initial eDiary training but further prompts to aid quantification of sputum volume in the future could improve consistency of results.

One criticism of Bronkotest is how the scoring system was derived. The original study 'noted from [their] initial analysis that episodes in which breathlessness and well-being deteriorated were more likely to result in the patient changing their therapy' which were subsequently weighted by a score of 5 with all other symptoms being scored 1.(211) This is somewhat arbitrary and has not been formally validated. This should be taken into consideration when interpreting the results of my study which used Bronkotest.

3.3.5 Developing a symptom score threshold for Bronkotest

One of the key potential uses of a daily symptom diary outside the setting of a clinic trial is to inform patients and clinicians to worsening of a patient's symptoms so that assessment and treatment can be organised promptly. In the context of AATD related lung disease, this would minimise any damaging effects of exacerbations on the lungs and limit emphysema

progression. This aligns with the main aim to be addressed in this chapter. Symptom scores could also be used to monitor recovery and assess overall intensity of an exacerbation by calculating the total symptom score across the total time course of an episode.(75)

The development of a symptom score threshold would therefore be useful in both clinical trials and real-world patient management. A symptom score threshold is the number of points a symptom score must increase to represent an exacerbation. In a clinical trial this would enable recognition of unreported exacerbations. In a real-world setting, meeting a symptom score threshold could trigger a patient prompt or a clinician to further assess a patients need for additional treatment or facilitate early self-treatment.

In my study, the symptom score threshold for Bronkotest was explored in detail. A 3-day rolling mean was selected as this balanced out the large fluctuations that can occur day to day (such as with a daily difference) with the ability to recognise exacerbations that arise quickly, something that may be missed with a 7-day rolling mean. It was also deemed appropriate to explore the symptom score threshold for type I exacerbations alone as they are typically treated with oral steroids and/or antibiotics and deemed the clinically most important. Overall specificity and sensitivity for all exacerbations was worse than that of type I exacerbations alone. This can be expected as type II and type III exacerbations have lower peak symptom scores (Figure 3.6)

In this analysis we compared change in symptoms score to the previous day. This is different to EXACT-PRO which uses 2 or 3 days of consecutive increase in symptoms with a difference of 12 or 9 points respectively. Use of a symptom score triggered over consecutive days is something that could be explored in the future in this cohort and may be an

optimum approach, though it may miss the early parts of an exacerbation and delay treatment.

The sensitivity and specificity for a range of symptom score thresholds were then explored for type I exacerbations using a 3-day rolling mean compared to a physician assessment. A daily difference was not as good as ruling out exacerbations (worse specificity) since large fluctuations in symptoms may occur as part of the normal COPD disease. Use of a rolling mean smoothed out these fluctuations. As discussed in the results (Section 3.4.5, Figure 3.9), a balance between sensitivity and specificity needs to be struck for a real-life tool in order to avoid overtreatment, as well as undertreatment. Having a highly sensitive eDiary would mean there are less false positive results.

Though a sensible threshold for Bronkotest may be between 6 and 8, in reality it is unlikely that symptom scoring alone will be both sensitive and specific enough to be able to distinguish between regular fluctuations in symptoms and AECOPD. Some studies have used additional parameters, such as home oxygen saturations or hand-held spirometry(204, 212) with variable success.

Pairing the eDiary with a carefully chosen biomarker that acutely represents an exacerbation would enhance the predictive power of such enhanced monitoring and is yet to be explored in AATD related lung disease.

With two modalities (an eDiary and a biomarker) to measure the emergence of an exacerbation, it is important to determine how they would work together. Would they both be measured at the same time and the results combined into a composite score or would a positive result in one test modality then lead to the use of the second for confirmation?

Though a combination score is an attractive concept, daily biomarker measurement may not be acceptable to all patients. The results from the feasibility and acceptability portion of this work have shown that patients would not be willing to do this test on a daily basis (only a quarter of participants) and may prefer to do it once or twice a week (results are presented in Chapter 4, Section 4.1.1). This is understandable since the process of running the urinary biomarker analysis at home took up to 15 minutes. The eDiary used in this study took less than a minute for users with stable symptoms. Therefore, it seems logical to use an eDiary gated model with biomarker testing only occurring in response to the symptom score threshold being achieved. This is a more cost-effective model since the use of consumables for a biomarker analysis are more expensive than simply using an application on a smart phone.

With the combination of an eDiary with a well performing biomarker, the threshold symptom score could be set lower (more sensitive, less specific) to maximise the capture of true exacerbations. The biomarker threshold could then be finely tuned to eliminate non-exacerbation events by being more specific. This biomarker threshold could even be personalised depending on a patient's particular exacerbation phenotype.

The use of biomarkers to help identify exacerbations is the subject of the next chapter which will address the performance and feasibility of urinary biomarkers. It will also explore if the predictive ability of the eDiary can be enhanced by pairing it with urinary biomarkers and therefore limit the damage caused by exacerbations.

3.4 Conclusion

Symptom based definitions of exacerbations alone are not adequate to detect all episodes due to high prevalence of unreported exacerbations that have an impact on health status.

The use of an eDiary to capture symptoms accurately in real time is feasible and informative for characterisation of frequency and severity of exacerbations. Careful patient selection is important to ensure adherence and accuracy of results.

4 ENHANCED MONITORING IN EXACERBATIONS OF AATD

RELATED LUNG DISEASE - RESULTS (ii): BIOMARKERS OF

AECOPD

4.1 Introduction

Exacerbation prevention and treatment remain central to COPD guidelines due to the economic impact and the effect of such episodes on patients' QOL.(260) During this millennium, there have been few major advances in the acute treatment of AECOPD despite the recognition of the overall burden of exacerbations on the lived experience of COPD patients. Similarly, with AATD which shares many similarities with usual COPD, no advances have been made. Even the use of AAT augmentation therapy has shown no benefit in the reduction or treatment of exacerbations.(53)

A recent report has established ten research priorities that reflect the views of experts in the field of COPD exacerbations and COPD patients.(260) Two of the highest ranked priorities were 'What is the best way to tell the start of an exacerbation from day-to-day variation in symptoms?' and 'What is the best way to tell the difference between an exacerbation and a different cause of changing symptoms in a person with COPD?'. These questions naturally give rise to research designs that study biomarkers of AECOPD. If suitable biomarker(s) could be identified, in combination with an eDiary as discussed in the previous chapter, precision medicine treatment of exacerbations could emerge. For those patients with AATD related lung disease, where emphysema decline is more rapid than with usual COPD, such technology could improve mortality and QOL by reducing rate of emphysema decline.

The use of serum and sputum biomarkers have been extensively reviewed in AECOPD via the ECLIPSE study(261) with very few clinically useful biomarkers identified. Most of the samples from biomarker sub analysis of the ECLIPSE study excluded active or recent exacerbations and were related back to annual exacerbation frequency which by its self may not be a stable indicator of exacerbation phenotypes.(243) This illustrates the remaining uncertainty about the use of serum biomarkers to establish pathophysiological pathways in AECOPD and new treatment targets. This large cohort study had relatively few AATD patients within them.

An ideal biomarker for AECOPD should satisfy the following criteria as discussed by Stockley(215): 1) be central to the pathophysiological process underlying AECOPD, 2) be stable and only vary during exacerbations, 3) exhibit larger changes in biomarker concentration in more severe exacerbations, 4) return to baseline levels during recovery from an exacerbation and 5) be sensitive to treatments of exacerbations. Given the heterogeneity of AECOPD these criteria are unlikely to all ever be satisfied by a single biomarker.

The first step in investigating possible biomarkers is identifying what to sample from patients.

Urinary biomarkers have yet to be explored in depth for AECOPD. Urine analysis is a simple way of measuring biomarkers that does not require the use of lancets/needles and is not dependent on sputum production. An unpublished systematic review carried out in 2016 found that only 6 urinary biomarkers had been tested in AECOPD. Three groups investigating desmosine/isodesmosine (total AECOPD events, n=124) and found lower levels during stable COPD than AECOPD,(80, 106, 110) of which one (n=9 AECOPD) used a

longitudinal design. Two longitudinal studies of endothelin-1 excretion reported significantly lower levels during stable COPD than AECOPD(122, 123) which implicates endothelial dysfunction during AECOPD. There were also studies looking at urinary IPF_{2a}-III,(262) albumin (124) and *S. pneumoniae* antigen.(263, 264)

Continuing from the previous chapter, the following sections present results from the biomarker analyses for the 'Elastin degradation in exacerbations of AATD related lung disease' study. It will address whether home urine testing with POC urine lateral flow cytometry devices is feasible and whether urinary biomarkers are able to distinguish symptom-based definitions for exacerbations from stable disease.

4.2 Results

4.2.1 Feasibility

During the final study visit ('Month 18' visit), each study participant filled out a questionnaire assessing the ease of use of the urine cube and recorded any difficulties encountered during the study. Figure 4.1 shows results of patients perceived ease of use of the urine cube, measured on a VAS from 1 (easy) to 10 (very hard). The median score was 2 (IQR 1,6) with 42.9% patients scoring 1 and 7.1% of patients scoring 2, representing relatively easy use of the device. Almost a quarter (23.2%) of patients that had some difficulties with the device (scoring between 3 and 6) and 5.4% had significant difficulties (scoring ≥ 7). Over 90% of participants said that they would be able to use a smart phone in the future to transmit data electronically and 80.5% of patients would like to see an output from the cube as an indicator of the test result.

Patients were asked if they would be prepared to carry out testing long term and, if so, how often they would prefer to perform testing if this technology was commercially available to aid with detection of exacerbations. Responses for intervals between testing included

'daily', 'alternate days', 'twice weekly' and 'weekly'. Over 90% of respondents would be willing to use this technology long term with weekly testing being the most popular option (56.8%). A quarter of patients would be willing to perform the test daily and 18.9% twice a week. No patients reported wanting to do the test on alternate days.

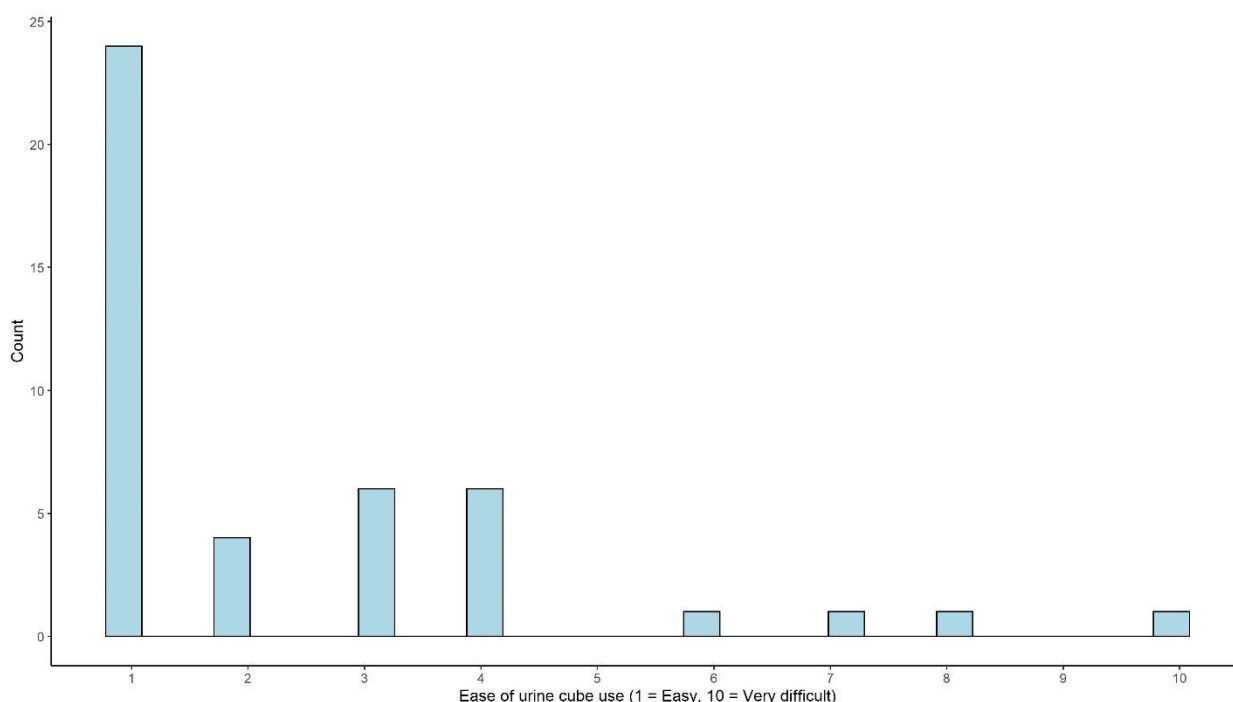


Figure 4.1: Ease of use of urine cube rated at the end of the study.

VAS was used to rate how often a patient thought they had forgotten to analyse their urine ranging from 1 (almost daily), to 10 (never). In between there were scores of 3 (once a week), 6 (once a month) and 9 (rarely). Figure 4.2 shows the distribution of responses. The median response score was 5.5 [2.25, 7.75]. Those scoring 8 or above accounted for 42.9% of responses with the majority forgetting to perform the urine test at least once a month.

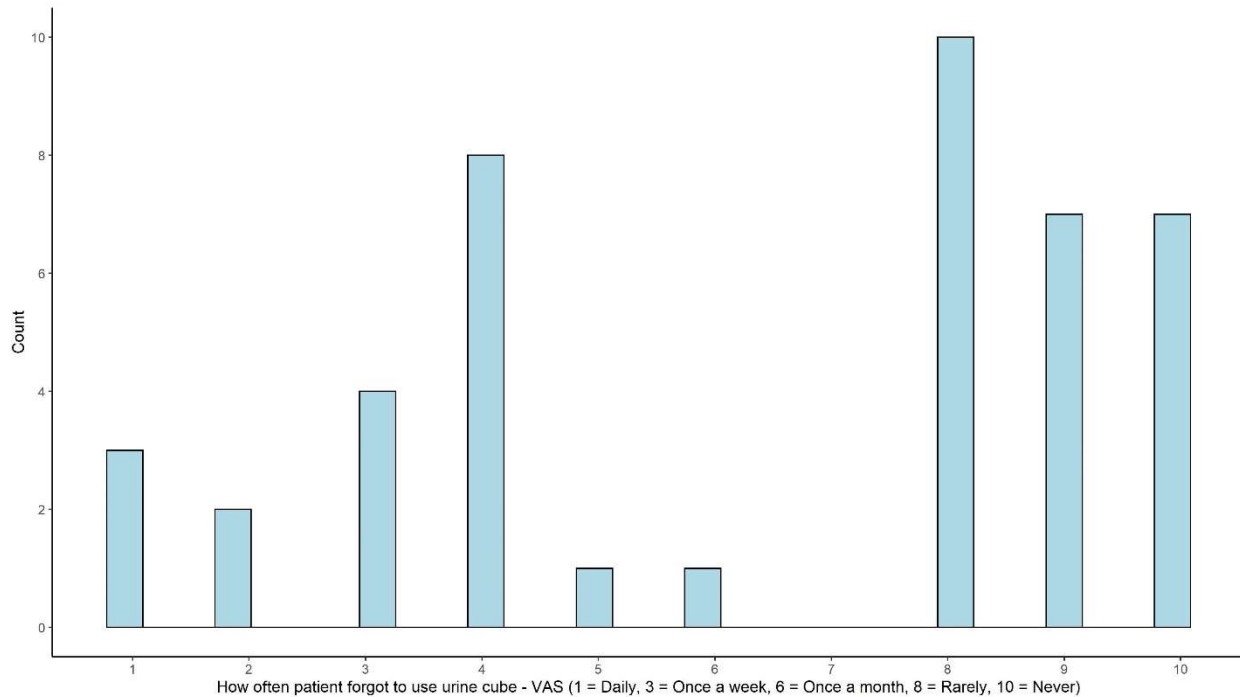


Figure 4.2: Visual analogue score (VAS) of how often patients reported forgetting to use the urine cube.

Patients were also asked to give written feedback on barriers to using the urine cube or reasons why they found it difficult to remember or use. Table 4.1 includes some key phrases from the feedback separated into themes and split by VAS for frequency of forgetting urine (higher scores represent better compliance). Figure 4.3 presents a word cloud of the frequency of words used in the free text feedback responses.

Table 4.1: Themes identified in patient feedback of urine device use split by visual analogue score (VAS) for frequency of urine device use.

	VAS ≥6 (better compliance)	VAS <6 (worse compliance)
Forgetting	'Work' 'Routine difficult when working' 'Easier to remember when part of a routine' 'Stuck to routine' 'Did it on a Saturday mostly' 'Busy life' 'Forgot the rules [of when to test]'	'Fibro [Fibromyalgia] fog' 'Busy lifestyle' 'Difficult to keep up habit' 'Bad memory' 'Could use text reminders to use urine test' 'Used phone reminder'
Urine cube issues	'Battery life very poor'	'Battery an issue [on urine cube]' 'Gave up on [the urine cube] as it kept saying 'error'' 'Date kept changing' 'The testing kit didn't work reliably and I didn't have time [to repeat the test]' 'Sometimes urine did not soak up', 'Sometimes [urine cube] didn't work properly' 'The odd error was time consuming' '10 minutes [for it to read]'
Comorbidities/illness	'Difficult to do when I had a chest infection'	'Breathlessness; had to use bottle by the side of my bed'
Other	'My wife reminds me to do the test'	'Family problems' 'Bereavement' 'Anxiety' 'Tiredness' 'Inconvenience'

4.2.2 ELISA: stable AATD vs healthy participants

Twenty control participants matched for age and sex were selected and samples put forward for Enzyme Linked Immunosorbent Assay (ELISA) measurement of urinary biomarkers for comparison to all AATD patients at baseline. The participant characteristics are compared in Table 4.2. Table 4.3 compares baseline urinary biomarkers between stable AATD and control participants. All biomarkers were grouped according to common pathways.

For elastin degradation pathways, median urinary desmosine concentration was 4.54 ng/mL higher ($p = 0.059$) in stable AATD patients (Figure 4.4A). There was no overall difference in NE levels though there were some notably high outliers in the stable AATD group (Figure 4.4B). AAT was significantly lower in the AATD group as expected (Figure 4.4C).

Inflammatory markers were also measured. Of note, CRP and RBP4, a molecule associated with increased inflammation in cardiometabolic disease(265, 266)B, showed a higher level in the urine of AATD patients compared to controls.

Extracellular matrix protein markers including MMP substrate, MMP8 and MMP9 showed no difference. Cathepsin B was significantly higher in AATD patients.

Markers of endothelial dysfunction were generally higher in AATD patients. The largest difference was with B2 microglobulin ($p = 0.029$).

Table 4.2: Comparison of all patients who had baseline urinary ELISA measurements.

	Control	AATD	P
n	20	54	
Female (%)	6 (30.0)	21 (38.9)	0.665
Age (mean (SD))	54.77 (10.66)	55.14 (9.13)	0.883
Smoking status (%)			0.001
Non smoker	10 (50.0)	6 (11.1)	
Ex-smoker	9 (45.0)	46 (85.2)	
Current smoker	1 (5.0)	2 (3.7)	
Pack year history* (median [IQR])	23.00 [10.00, 30.00]	20.00 [10.00, 25.50]	0.792
BMI (median [IQR])	26.90 [23.14, 30.05]	24.64 [22.48, 30.42]	0.652
FEV ₁ (mean (SD))	3.12 (0.71)	1.35 (0.51)	<0.001
FEV ₁ % predicted (mean (SD))	101.05 (15.08)	41.40 (16.01)	<0.001
FVC (mean (SD))	4.12 (0.94)	3.73 (1.13)	0.175
FVC % predicted (mean (SD))	108.05 (13.46)	89.74 (22.90)	0.001
FEV ₁ /FVC (mean (SD))	0.76 (0.05)	0.37 (0.11)	<0.001

*For those who smoked only

Table 4.3: Urinary biomarkers measured with ELISA compared between control patients and stable AATD.

	Control	Stable AATD	P
n	20	40	
Elastin degradation			
Alpha-1 antitrypsin (ng/mL)	26.48 [0.17, 96.95]	0.17 [0.17, 0.17]	<0.001
Desmosine (ng/mL)	3.04 [0.02, 7.14]	7.58 [0.02, 22.90]	0.059
Neutrophil elastase (ng/mL)	2.97 [0.02, 23.64]	0.02 [0.02, 18.67]	0.8
Extracellular matrix			
Chitinase-3-like protein 1 (ng/mL)	0.24 [0.00, 0.62]	0.00 [0.00, 0.84]	0.926
Cathepsin B (ng/mL)	4.36 [3.28, 5.90]	6.60 [3.80, 13.42]	0.048
MMP substrate (ng/mL)	0.02 [0.02, 1.70]	0.02 [0.02, 0.02]	0.24
MMP8 (ng/mL)	0.00 [0.00, 0.15]	0.00 [0.00, 0.44]	0.724
MMP9 (ng/mL)	0.77 [0.00, 1.51]	0.00 [0.00, 0.99]	0.172
TIMP1 (ng/mL)	1.32 [0.39, 2.82]	1.27 [0.35, 3.22]	0.95
TIMP2 (ng/mL)	2.97 [1.14, 4.37]	3.96 [2.08, 7.84]	0.082
Inflammation			
CRP (ng/mL)	42.19 [0.80, 182.3]	131.10 [0.80, 565.39]	0.081
Fibrinogen (ng/mL)	24.47 [19.37, 36.18]	28.83 [19.26, 40.77]	0.471
fMLP (ng/mL)	2.13 [0.04, 6.30]	5.56 [3.15, 8.41]	0.084
IL-6 (ng/mL)	0.10 [0.10, 0.10]	0.10 [0.10, 0.10]	0.577
MPO (ng/mL)	8312 [2269, 15,525]	2996 [799, 19,721]	0.515
RNASE3 (ng/mL)	0.00 [0.00, 0.00]	0.00 [0.00, 1.26]	0.01
CC16 (ng/mL)	20.00 [13.44, 20.00]	20.00 [4.53, 20.00]	0.391
Endothelial dysfunction			
B2 microglobulin (ng/mL)	81.65 [33.02, 133.43]	130.63 [73.50, 192.35]	0.029
Creatinine (mg/dL)	72.91 [40.20, 141.37]	90.69 [61.70, 129.23]	0.323
Cystatin C (ng/mL)	57.12 [36.01, 78.26]	77.55 [47.23, 138.46]	0.076
NGAL (ng/mL)	13.11 [10.86, 24.79]	19.33 [8.57, 30.43]	0.838
RBP4 (ng/mL)	0.24 [0.24, 9.37]	80.17 [0.24, 146.65]	<0.001

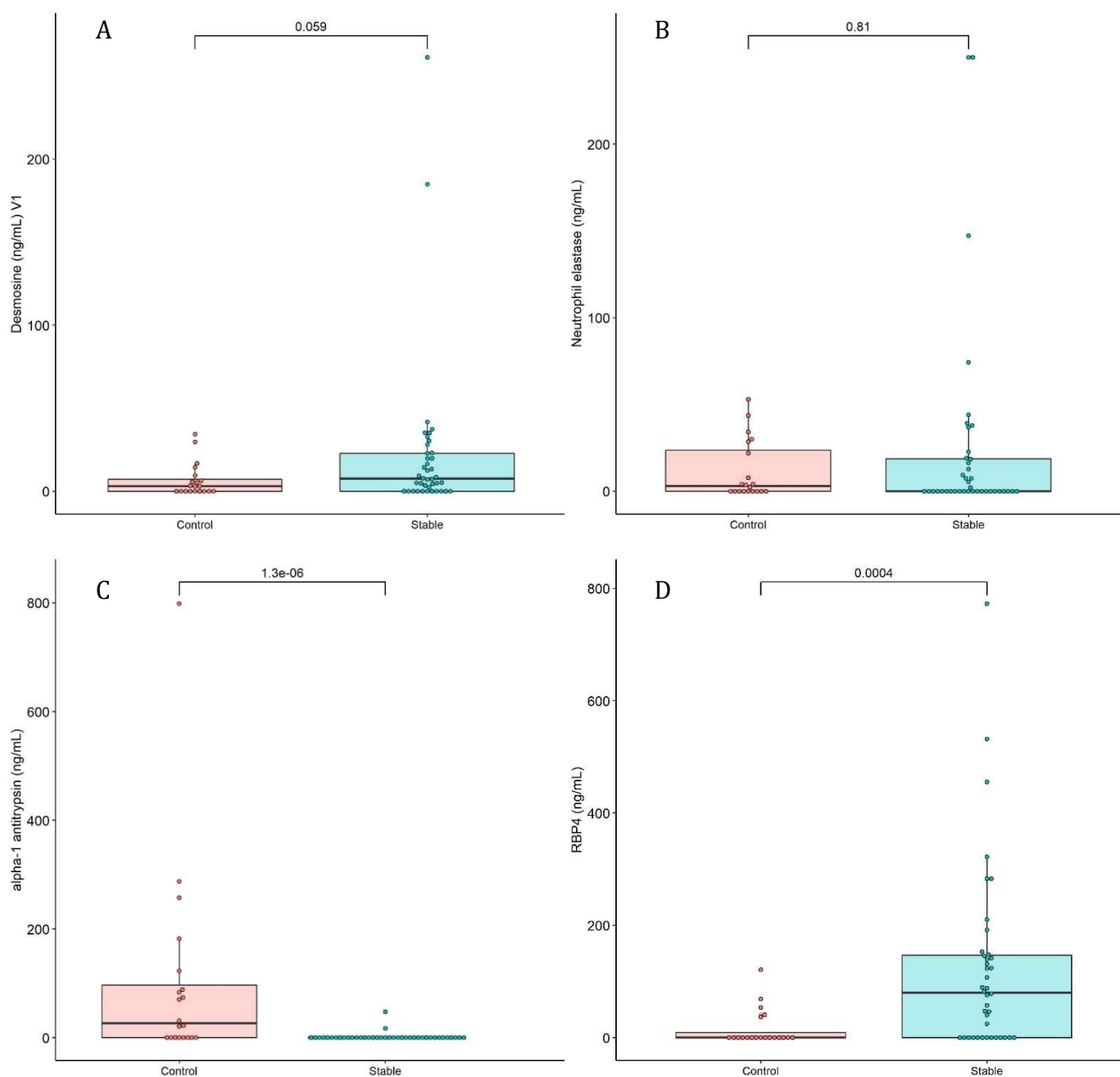


Figure 4.4: Urinary measures of A) Desmosine, B) neutrophil elastase, C) alpha-1 antitrypsin and D) RBP4 by ELISA for control and stable AATD patients.

4.2.2.1 Urinary desmosine levels and increasing age

Figure 4.5 demonstrates urinary desmosine levels during stable and exacerbating AATD patients and controls. Overall, there is no association between age and desmosine levels between all groups and individually as assessed by simple linear regression.

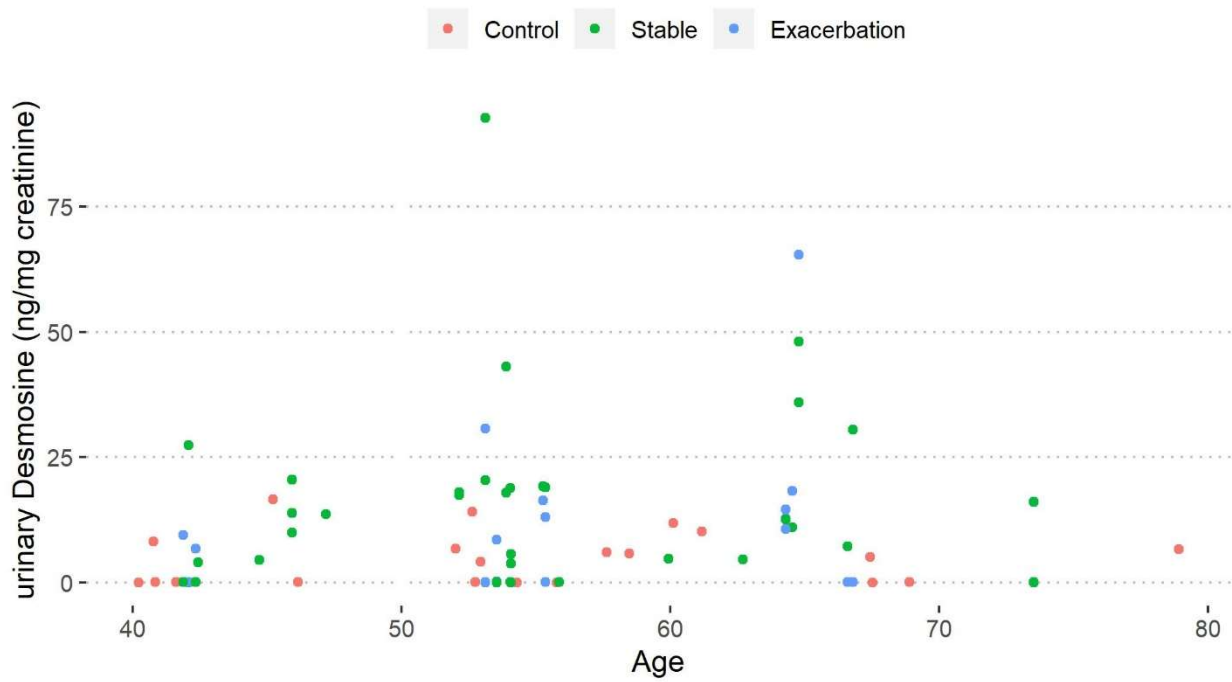


Figure 4.5: Urinary desmosine measurement compared to age for control, stable and exacerbations (linear regression $P = 0.257$).

4.2.2.2 Urinary desmosine and KCO

Figure 4.6 demonstrates urinary desmosine levels at differing values of KCO. There is no relationship between KCO and urinary desmosine levels in stable AATD patients.

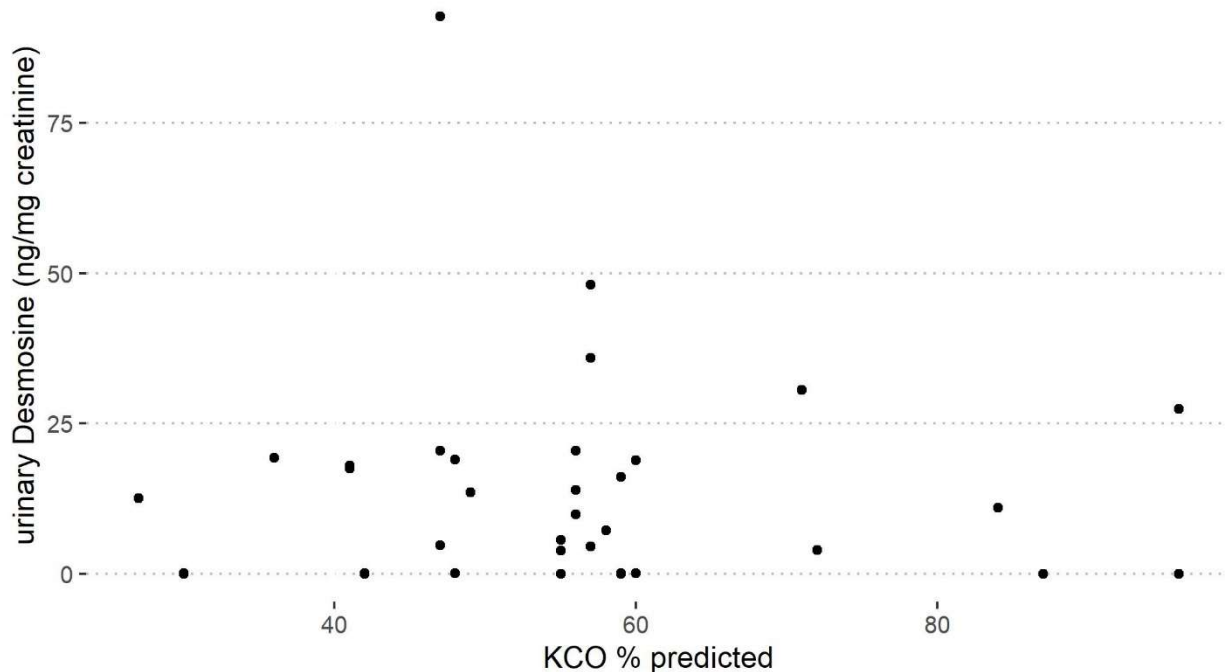


Figure 4.6: Urinary desmosine measurement compared to KCO % predicted for control, stable and exacerbations (linear regression $p = 0.860$)

4.2.3 ELISA: AATD patients during stable state, exacerbations and recovery

This section of the results refers to ELISA of samples taken during patient study visits. Table 4.4 presents stable (baseline, 'Month 9' and 'Month 18'), exacerbation ('Day 1') and recovery ('Day 56') samples. Table 4.5 presents the same results adjusted for urinary creatinine. In total there were 21 'Day 1' visits with 40 stable and 20 recovery visits.

Table 4.4: Urinary ELISA for stable, exacerbation and recovery.

	Stable ¹	Exacerbation ²	Recovery ³	P	
n	40	21	30	1 - 2	2 - 3
Elastin degradation					
AAT (ng/mL)	0.17 [0.17, 0.17]	0.17 [0.17, 0.17]	0.17 [0.17, 0.17]	0.968	0.232
Desmosine (ng/mL)	7.58 [0.02, 22.90]	4.53 [0.02, 9.75]	4.37 [0.02, 12.42]	0.186	0.874
Neutrophil elastase (ng/mL)	0.02 [0.02, 18.67]	0.02 [0.02, 3.83]	0.02 [0.02, 1.88]	0.268	0.537
Inflammation					
CRP (ng/mL)	131.1 [0.80, 565]	148.05 [0.80, 297.8]	153.48 [81.0, 355.3]	0.617	0.931
Fibrinogen(ng/mL)	28.83 [19.26, 40.77]	22.44 [14.51, 33.19]	20.2 [17.0, 32.7]	0.354	0.893
fMLP (ng/mL)	5.56 [3.15, 8.41]	5.13 [0.04, 6.38]	4.43 [0.04, 7.07]	0.34	0.706
IL-6 (ng/mL)	0.10 [0.10, 0.10]	0.10 [0.10, 0.10]	0.10 [0.10, 0.10]	0.099	0.82
MPO (pg/mL)	2995 [799, 19,720]	1215 [413, 1870]	815 [84.0, 2,992]	0.033	0.862
RBP4 (ng/mL)	80.17 [0.24, 146.65]	53.06 [0.24, 118.90]	57.57 [0.24, 99.56]	0.644	0.683
RNASE3 (ng/mL)	0.00 [0.00, 1.26]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.016	0.354
CC16 (ng/mL)	20.00 [4.53, 20.00]	7.47 [3.66, 20.00]	9.97 [2.79, 20.00]	0.19	0.907
Extracellular matrix					
Chitinase-3-like protein 1 (ng/mL)	0.00 [0.00, 0.84]	0.53 [0.00, 1.18]	0.21 [0.00, 0.76]	0.172	0.189
Cathepsin B (ng/mL)	6.60 [3.80, 13.42]	4.17 [2.25, 8.17]	4.68 [2.33, 8.77]	0.06	0.528
MMP substrate (ng/mL)	0.02 [0.02, 0.02]	0.02 [0.02, 0.02]	0.02 [0.02, 0.02]	0.83	0.151
MMP8 (ng/mL)	0.00 [0.00, 0.44]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.165	0.956
MMP9 (ng/mL)	0.00 [0.00, 0.99]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.052	0.821
TIMP1 (ng/mL))	1.27 [0.35, 3.22]	1.20 [0.00, 2.32]	1.28 [0.37, 3.49]	0.819	0.664
TIMP2 (ng/mL)	3.96 [2.08, 7.84]	2.27 [1.32, 2.94]	2.60 [1.20, 6.30]	0.029	0.688
Endothelial dysfunction					
B2 microglobulin (ng/mL)	130.63 [73.50, 192.35]	87.38 [45.72, 165.34]	86.74 [39.43, 158.59]	0.122	0.702
Creatinine (mg/mL)	90.69 [61.70, 129.23]	61.08 [30.47, 89.55]	56.69 [31.21, 110.42]	0.015	0.54
Cystatin C (ng/mL)	77.55 [47.23, 138.46]	67.58 [27.68, 104.57]	67.32 [23.34, 107.27]	0.273	0.939
NGAL (ng/mL)	19.33 [8.57, 30.43]	8.04 [5.33, 16.34]	6.52 [4.95, 16.13]	0.014	0.673

Table 4.5: Urinary ELISA for stable, exacerbation and recovery (creatinine corrected)

	Stable ¹	Exacerbation ²	Recovery ³	P	
n	40	21	30	1 - 2	2 - 3
Elastin degradation					
AAT (ng/mg creatinine)	0.20 [0.13, 0.32]	0.34 [0.21, 0.60]	0.30 [0.15, 0.55]	0.025	0.348
Desmosine (ng/mg creatinine)	8.56 [0.13, 18.93]	8.51 [0.08, 14.57]	5.38 [0.09, 11.79]	0.439	0.969
NE (ng/mg creatinine)	0.08 [0.02, 21.08]	0.07 [0.03, 11.60]	0.07 [0.02, 2.10]	0.952	0.579
Inflammation					
CRP (ng/mg creatinine)	197 [3.59, 672]	269.6 [3.63, 614.5]	211.1 [88.63, 521]	0.773	0.848
Fibrinogen (ng/mg creatinine)	34.79 [17.26, 46.94]	50.54 [28.18, 84.36]	38.72 [28.75, 59.73]	0.045	0.284
fMLP (ng/mg creatinine)	5.35 [2.32, 6.65]	6.22 [0.18, 9.08]	4.39 [0.14, 6.71]	0.412	0.157
IL-6 (ng/mg creatinine)	0.15 [0.09, 0.41]	0.16 [0.11, 0.33]	0.18 [0.09, 0.32]	0.585	0.528
MPO (pg/mg creatinine)	3,064 [816, 19,775]	2,909 [536, 4307]	1780 [117, 3723]	0.236	0.379
RBP4 (ng/mg creatinine)	84.84 [0.78, 132.5]	104.62 [2.36, 188.0]	74.80 [0.92, 141.1]	0.242	0.433
RNASE3 (ng/mg creatinine)	0.02 [0.00, 1.14]	0.01 [0.00, 0.02]	0.01 [0.00, 0.01]	0.23	0.267
CC16 (ng/mg creatinine)	14.96 [4.37, 23.52]	13.67 [4.52, 32.74]	15.14 [1.99, 39.08]	0.738	0.833
Extracellular matrix					
Chitinase-3-like protein 1 (ng/mg creatinine)	0.01 [0.00, 0.72]	0.78 [0.01, 2.17]	0.21 [0.01, 0.83]	0.008	0.088
Cathepsin B (ng/mg creatinine)	8.20 [5.13, 11.71]	10.05 [6.12, 11.02]	8.05 [4.15, 12.27]	0.952	0.954
MMP substrate (ng/mg creatinine)	0.02 [0.01, 0.05]	0.04 [0.02, 0.11]	0.03 [0.01, 0.05]	0.054	0.221
MMP8 (ng/mg creatinine)	0.00 [0.00, 0.42]	0.01 [0.00, 0.01]	0.01 [0.00, 0.01]	0.387	0.66
MMP9 (ng/mg creatinine)	0.01 [0.00, 1.14]	0.01 [0.00, 0.01]	0.01 [0.00, 0.02]	0.649	0.969
TIMP1 (ng/mg creatinine)	1.22 [0.35, 2.76]	2.21 [0.01, 5.89]	3.06 [0.26, 4.81]	0.379	0.863
TIMP2 (ng/mg creatinine)	4.29 [3.18, 5.84]	4.56 [3.20, 5.17]	4.68 [3.23, 5.38]	0.785	0.893
Endothelial dysfunction					
B2 microglobulin (ng/mg creatinine)	137.74 [89.18, 205.7]	180.4 [121.7 265.3]	125.99 [86.58, 195.8]	0.167	0.058
Cystatin C (ng/mg creatinine)	84.15 [44.50, 147.8]	93.32 [36.61, 212.9]	93.07 [29.21, 163.1]	0.616	0.774
NGAL (ng/mg creatinine)	17.82 [9.47, 37.45]	15.83 [6.01, 40.82]	12.96 [4.16, 23.75]	0.693	0.301

4.2.3.1 Elastin degradation

There was an overall increase in creatinine adjusted AAT level during exacerbation compared to the stable state (median increase 0.14 ng/mg creatinine, $p = 0.025$), though AAT levels were low overall. There was no relationship between desmosine or NE found.

4.2.3.2 Inflammation

None of the biomarkers that represent general inflammation showed a difference between groups. There was an overall increase in CRP during exacerbation with subsequent reduction during recovery though the results were not statistically significant.

4.2.3.3 Extracellular matrix

Similarly, most of the biomarkers that represent extracellular matrix turnover showed no direct relationship. There was a small increase in Chitinase-3-like-protein when adjusted for creatinine during exacerbations compared to the stable state. The lower limit of the 95% confidence interval was similar suggesting the majority of samples did not reach the lower limit of the assay sensitivity.

4.2.3.4 Endothelial dysfunction

Urinary creatinine was significantly lower during exacerbations suggesting glomerular filtration may be reduced compared to stable state or recovery individuals. There was no difference in levels between B2 microglobulin, Cystatin C or NGAL.

4.2.4 Mass spectrometry of plasma desmosine

Mass spectrometry was performed on a subset of plasma samples to explore the relationship between paired urinary and plasma samples. In total, 98 samples were included for measurement (stable; 66, exacerbation; 16, recovery; 15). Median values were as follows: stable 0.31 ng/mL (IQR 0.25 to 0.4), exacerbation 0.34 ng/mL (IQR 0.24 to 0.49), recovery 0.33 ng/mL (IQR 0.29 to 0.43).

Plasma desmosine did not show any difference between the stable state, during an exacerbation or at recovery as measured by mass spectroscopy (Figure 4.7).

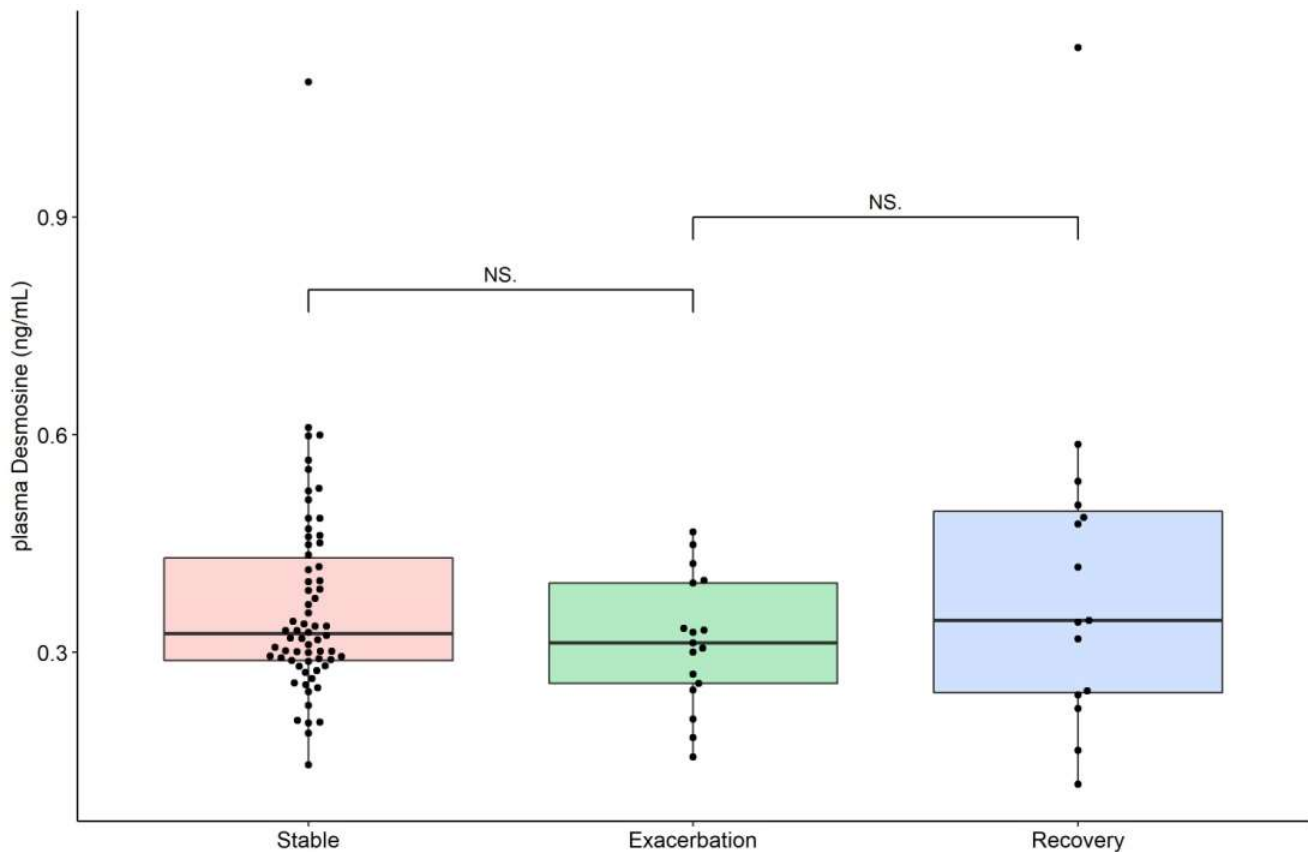


Figure 4.7: Plasma desmosine measured with mass spectrometry during stable, exacerbation and recovery states

4.2.5 Lateral flow immunoassay

The following section presents results collected from the POC urine cube devices which used lateral flow cytometry to determine biomarker levels. The biomarkers assessed were AAT, NE, TIMP1, CRP and desmosine. Data from the home lateral flow assay device (urine cube) were available from 30 individuals with 2052 results per biomarker tested. Data losses were down to failure for patients to return urine cube at the end of the study (n = 10) and insufficient data being recorded (n = 14). The median number of urine samples analysed per participant was 71 (range 12 to 107).

Table 4.6: Lateral flow results for all treated exacerbations at different time periods

	-14 to -7 days ¹	-7 to 0 days ²	0 to 3 days ³	4 to 7 days ⁴	>7 days ⁵	<i>P</i>			
n	74	77	86	124	226	1 - 3	2 - 3	3 - 4	3 - 5
AAT (ng/ml)	49.4 [24.8, 86.6]	58.3 [27.7, 103.1]	72.2 [34.0, 136.6]	62.04 [33.8, 107.7]	57.0 [30.0, 97.4]	0.034	0.201	0.4	0.075
NE (ng/ml)	2.90 [0.00, 4.89]	2.90 [0.00, 6.01]	2.46 [0.00, 4.52]	1.57 [0.00, 3.95]	1.05 [0.00, 4.08]	0.714	0.552	0.407	0.208
TIMP1 (ng/ml)	6.20 [1.62, 15.7]	8.18 [3.59, 20.5]	4.35 [1.78, 13.2]	5.56 [1.67, 14.5]	7.76 [2.95, 16.4]	0.44	0.017	0.537	0.008
CRP (ng/ml)	0.32 [0.19, 2.00]	0.53 [0.24, 3.54]	0.99 [0.27, 7.26]	0.73 [0.19, 3.09]	0.35 [0.18, 1.55]	0.006	0.246	0.183	0.001
DES (ng/ml)	100.0 [64.8, 100.0]	100.0 [55.2, 100.0]	100.00 [76.83, 100.00]	100.00 [50.6, 100.00]	100.0 [71.5, 100.0]	0.655	0.445	0.282	0.923

4.2.5.1 AAT

For the entire dataset, 8 (0.39%) AAT samples were below the lower limit of detection for the assay range (0 ng/ml). A further 99 (4.82%) were above the threshold of 250 ng/ml for selected assays.

Figure 4.8 presents urinary lateral flow assay results for AAT. Figure 4.8A demonstrates an overall increase in AAT in the days leading up to an exacerbation between -14 to -7 days and 0 to 3 days (median difference 22.8 ng/ml, $p = 0.034$). Figure 4.8B provides a breakdown of daily values.

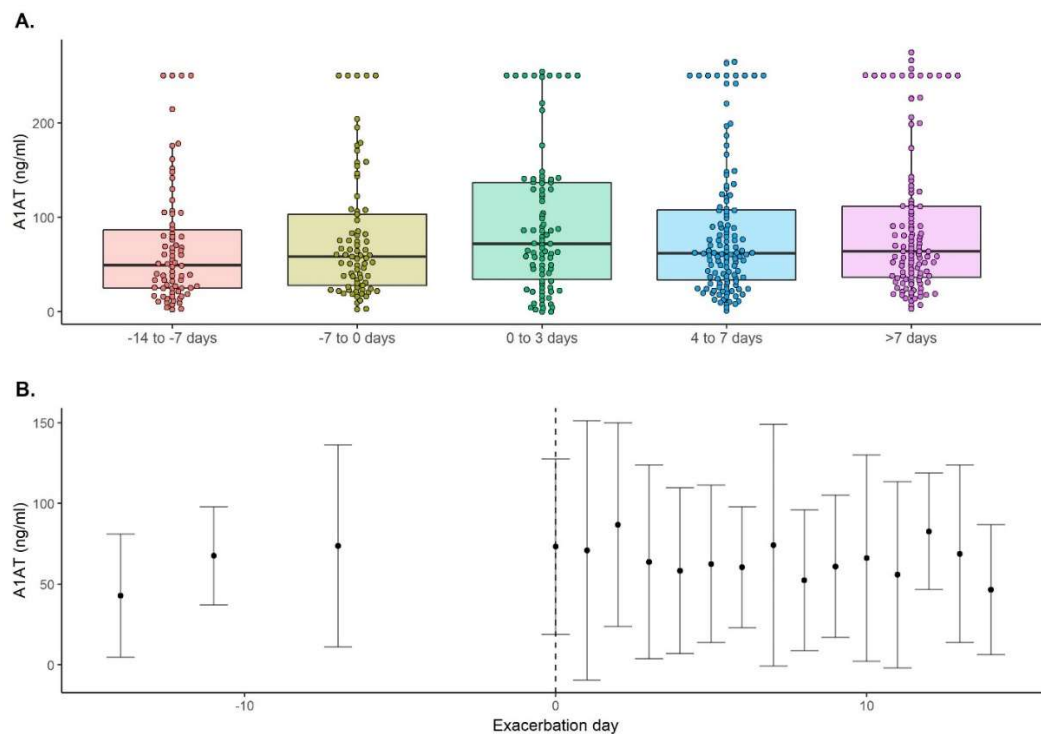


Figure 4.8: Lateral flow measurements of AAT for treated pulmonary exacerbations A) Bee swarm plot showing actual values across time, B) median AAT measurement across time. Error bars represent the upper and lower limit of the median absolute deviation (MAD).

4.2.5.2 NE

For the entire dataset of NE samples, 806 (39.3%) samples were recorded as below the lowest limit of the assay range (0 ng/ml).

Figure 4.9 presents urinary lateral flow assay results for AAT. There is no difference between urinary NE levels at different time points during an exacerbation.

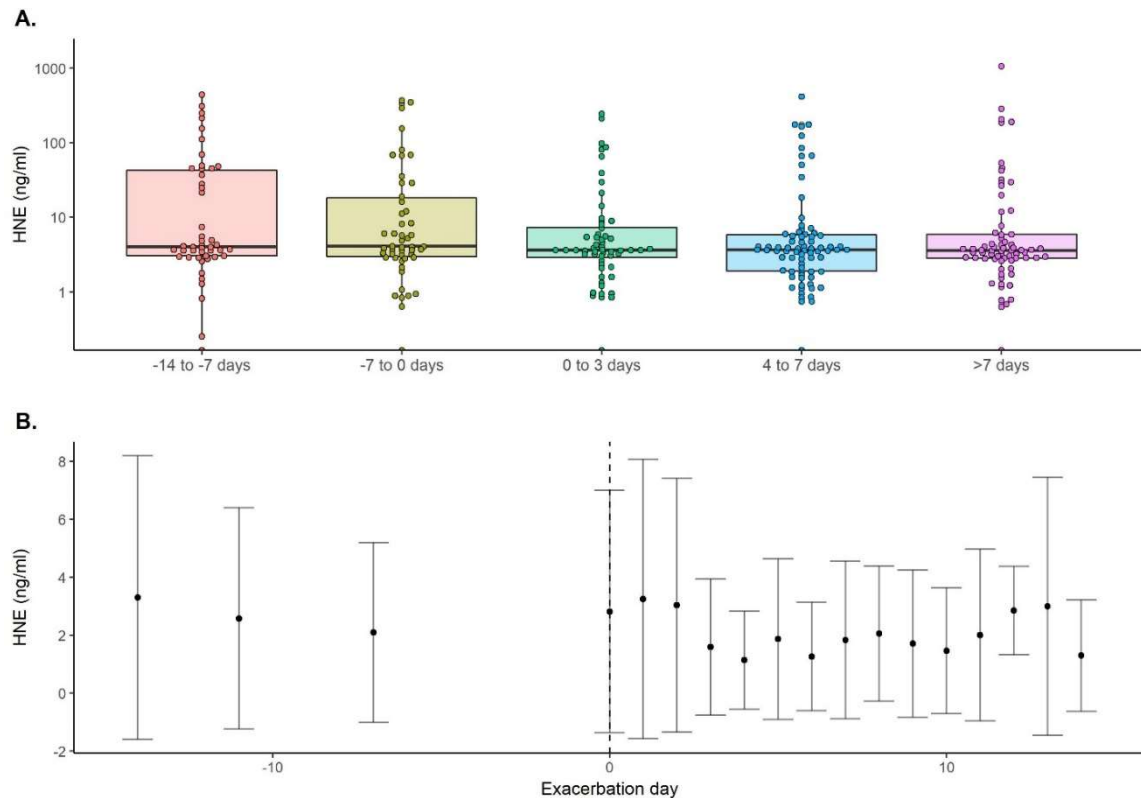


Figure 4.9: Figure 4.9 : Lateral flow measurements of NE for treated pulmonary exacerbations A) Bee swarm plot showing actual values across time, B) median NE measurement across time. Error bars represent the upper and lower limit the median absolute deviation (MAD).

4.2.5.3 *TIMP1*

For the entire dataset for TIMP1, there were 22 (1.1%) samples that were undetectable within the range of the assay for TIMP1. The lowest median value for urinary TIMP1 was during 0 to 3 days. There were significantly higher levels during -7 to 0 days and ≥ 7 days when compared to 0 to 3 days ($p = 0.017$ and 0.008 respectively). This is shown in Figure 4.10.

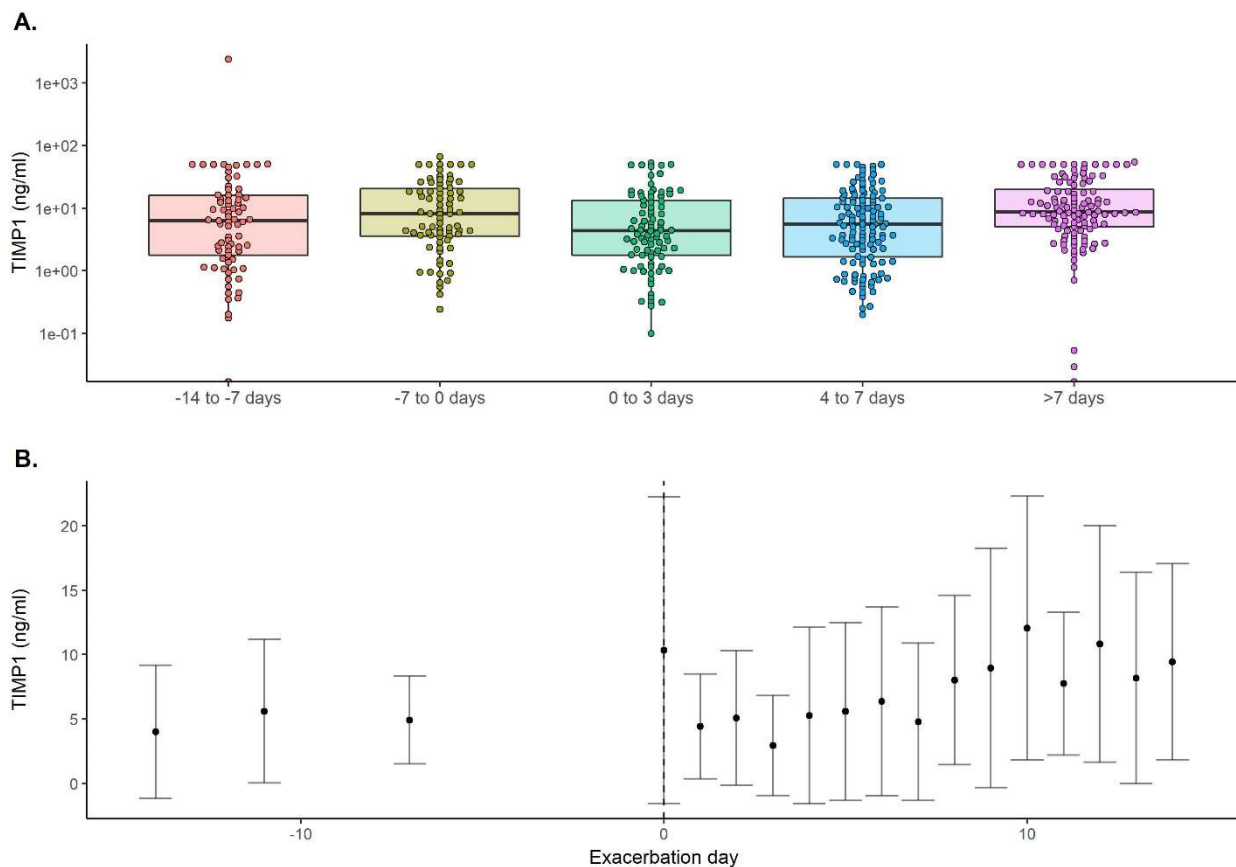


Figure 4.10: Lateral flow measurements of TIMP1 for treated pulmonary exacerbations A) Bee swarm plot showing actual values across time, B) median TIMP1 measurement across time. Error bars represent the upper and lower limit the median absolute deviation (MAD).

4.2.5.4 CRP

For CRP, 115 (5.6%) samples were deemed undetectable by the lower limit of the assay range. There was a trend for CRP to increase gradually until 0 to 3 days and then reduce during recovery with a median difference of 0.67 ng/mL compared to -14 to -7 days ($p = 0.006$). This is shown in Figure 4.11.

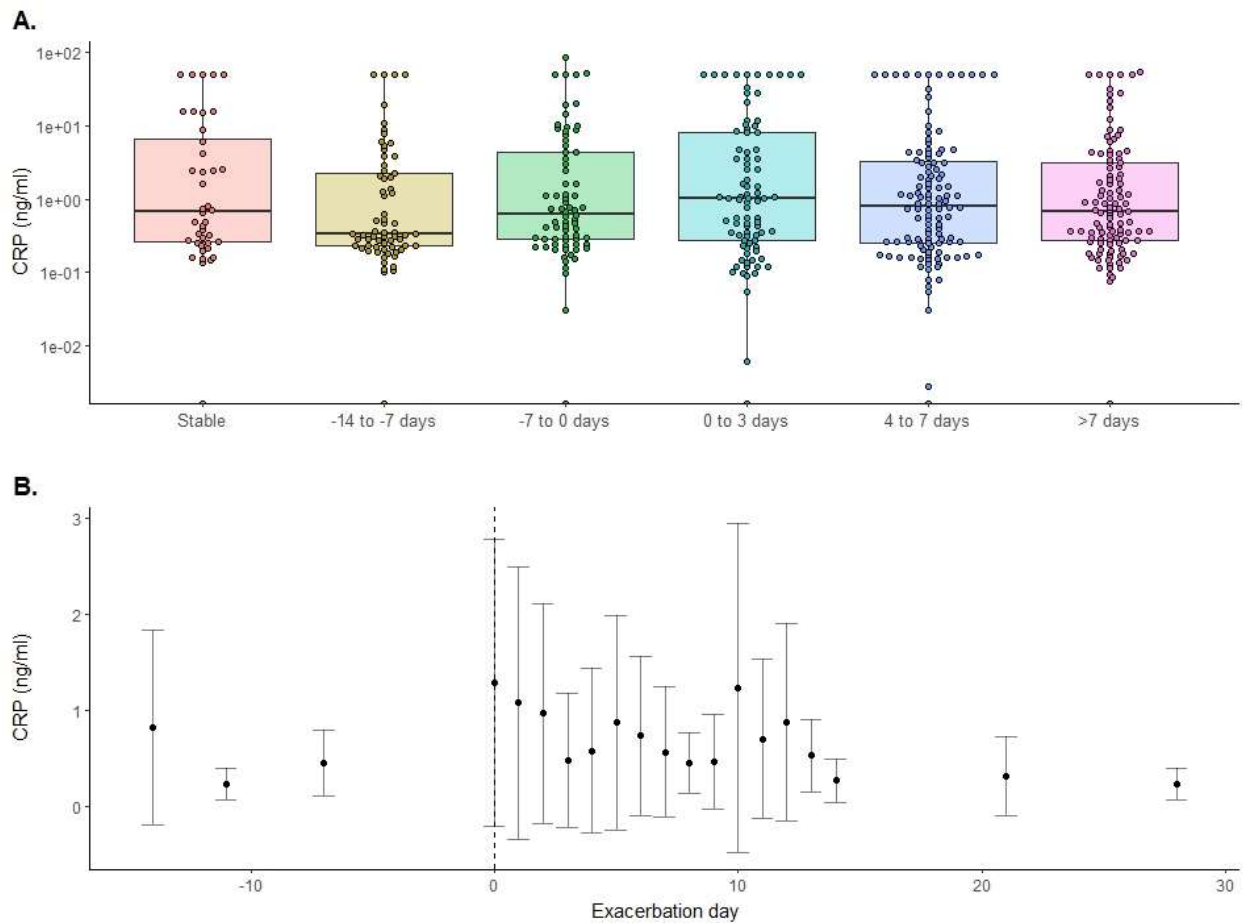


Figure 4.11: Lateral flow measurements of CRP for treated pulmonary exacerbations A) Bee swarm plot showing actual values across exacerbation day, B) median CRP measurement across time. Error bars represent the upper and lower limit the median absolute deviation (MAD).

4.2.5.5 *Desmosine*

For desmosine measured by lateral flow there were 1027 (50.0%) samples that met the upper limit of the concentration range for the desmosine assay and were therefore capped at this level (100 ng/ml). This is reflected by all median values for each group being 100 ng/ml. There was no relationship seen between groups (Figure 4.12).

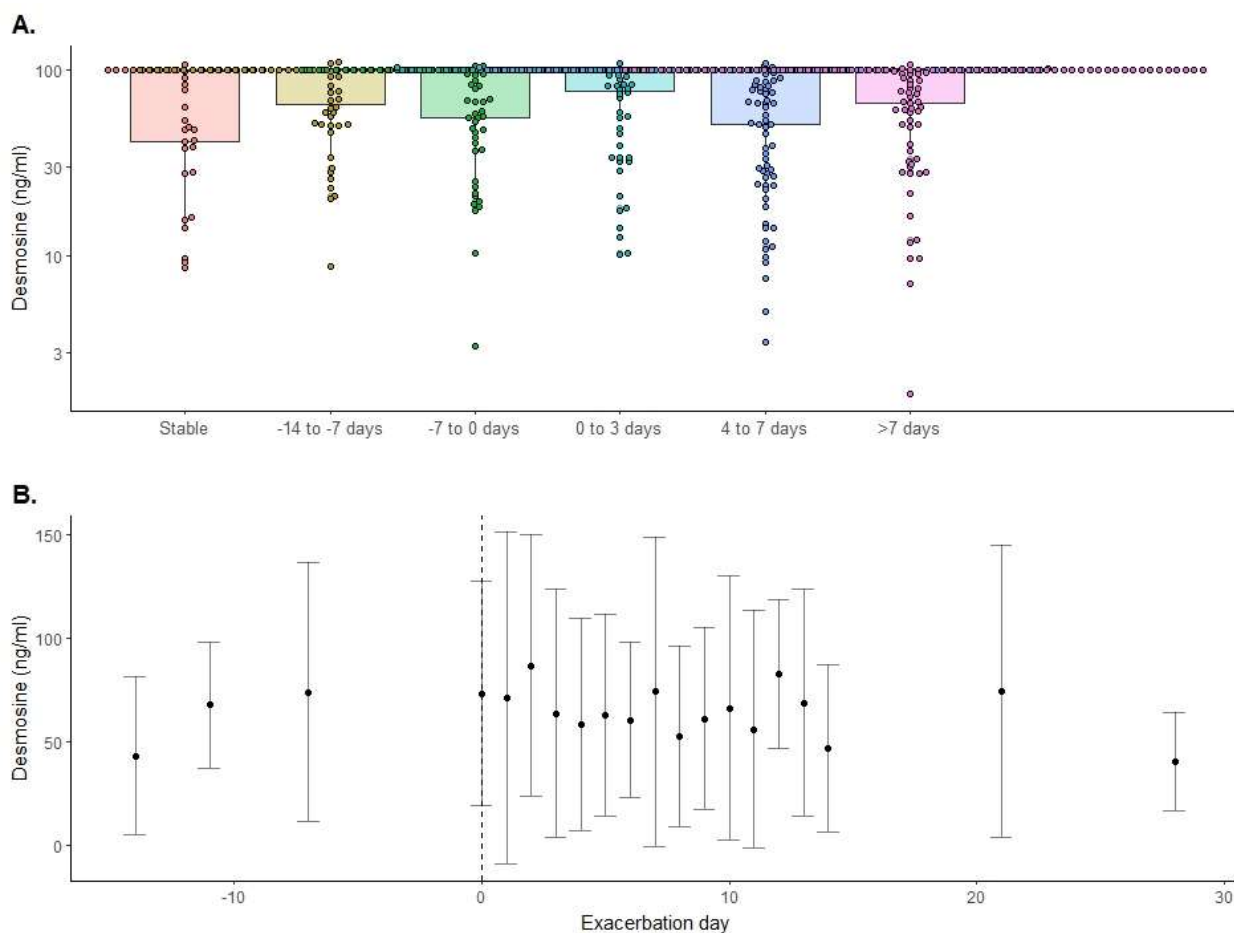


Figure 4.12: Figure 4.12: Lateral flow measurements of Desmosine for treated pulmonary exacerbations A) Bee swarm plot showing actual values across time, B) median desmosine measurement across time. Error bars represent the upper and lower limit the median absolute deviation (MAD).

4.2.5.6 Stability of biomarkers for individual patients

For all lateral flow biomarkers there was a variable range of means and standard deviation during the stable state. Figure 4.13 shows a representative plot of 10 patients for CRP; the best performing biomarker. For some patients, there was a clear distinction between the stable state and during an exacerbation (e.g., A1X15, A1X29). For others, there was no difference seen. A detailed breakdown of all biomarkers is available in the appendix (Section 7.4.3, Figure 7.3).

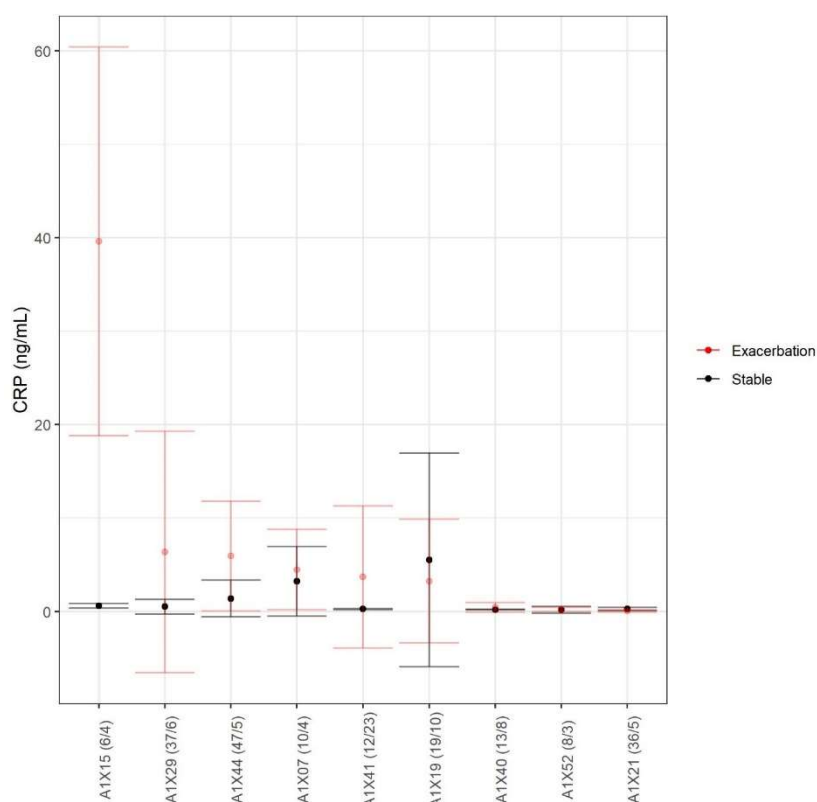


Figure 4.13: Sample of 10 patients with mean (SD) during stable state (black) and exacerbations (red). X axis - brackets represent (N samples stable/N samples exacerbation).

4.2.5.7 Predictive ability of biomarkers in combination with an eDiary

From the lateral flow assay results, the two best performing biomarkers, CRP and TIMP1, were taken forward for ROC curve analysis in combination with symptom scores generated from the eDiary to determine if the prediction algorithm was improved with their addition. Figure 4.14 presents three ROC curves on the same graph; eDiary alone, eDiary plus CRP and eDiary plus TIMP1. For eDiary plus CRP and eDiary plus TIMP1 an eDiary gated approach was used with a symptom score threshold cut off of 4. This cut off prioritised specificity to ensure as many true exacerbation events were captured as per discussion in Chapter 3, Section 3.3.5. There was no added benefit of using CPR or TIMP1 in conjunction with the eDiary across the entire range of biomarker cut off values.

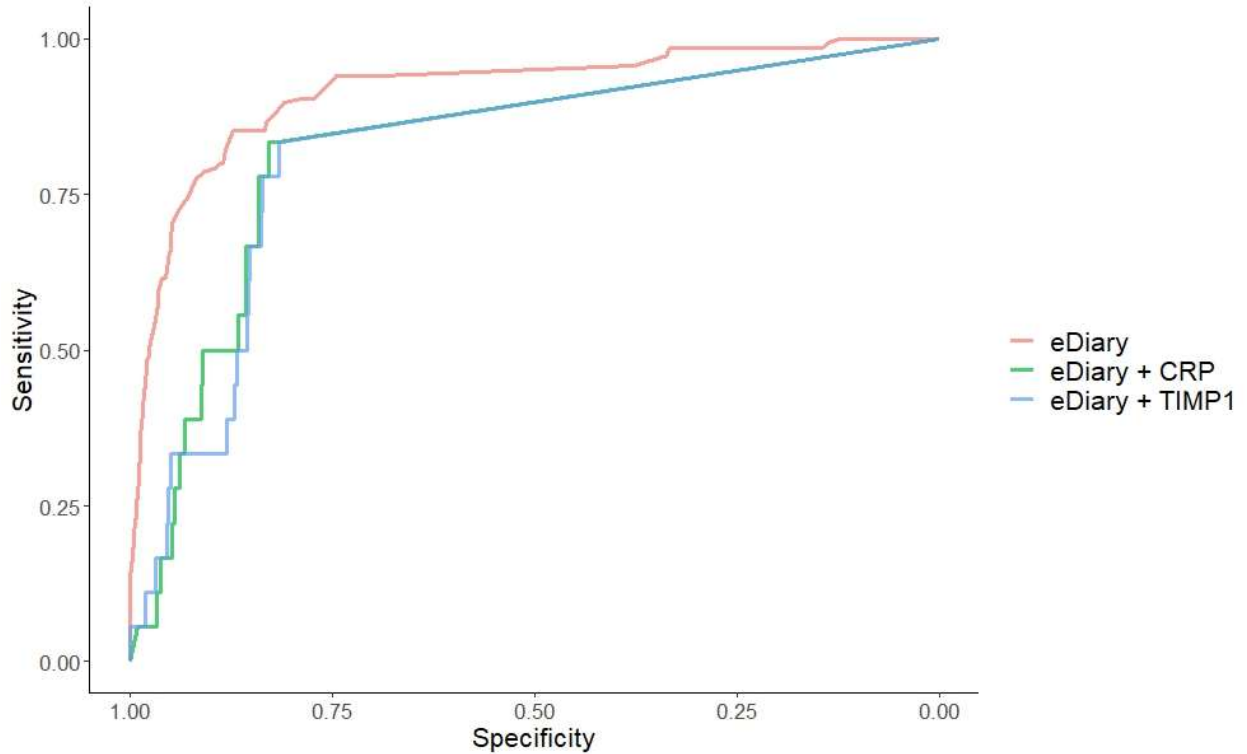


Figure 4.14: Comparison of ROC curves between eDiary alone or in combination with CRP or TIMP1. The eDiary gate threshold was set at 4.

4.3 Discussion

My study is the first to attempt to assess the feasibility and efficacy of a POC urine device to characterise multiple urinary biomarkers during the time course of an exacerbation in patients with AATD related lung disease. The lateral flow test data alone captured 2052 days of biomarker data paired with well characterised symptom data via an eDiary. This dataset is one of the largest recorded for exacerbations in AATD and has provided valuable insights into a poorly studied area. I will first discuss results of the ELISA and mass spectrometry analyses from samples collected during the study site visits followed by the feasibility and quantitative results of the POC urinary lateral flow assays.

4.3.1 ELISA

4.3.1.1 Stable AATD vs control

The results from this study support previous findings in usual COPD that desmosine levels in AATD patients are higher during the stable state compared to controls(80). This suggests that elastin turnover is higher which is in keeping with the underlying pathophysiology of AATD. AATD patients could be expected to have higher levels of desmosine compared to usual COPD too given the reduction in AAT as a serine protease inhibitor. However, the magnitude of difference in urinary desmosine was less than that between controls and stable COPD in a study by Huang et al(80). In contrast to their study, I was able to age match patients which may in part explain this. If using creatinine correction, the difference in urinary desmosine increased. Mean desmosine levels for controls were 4.64ng/mg (IQR 0.06, 7.11) compared to 8.56 ng/ml (IQR 0.13, 18.93) for AATD ($p = 0.041$). Using data from a previous unpublished study urinary desmosine in COPD patients was on average 3.85 ng/mg creatinine (0.48-6.79) compared with our stable AATD cohort (8.56 ng/mg creatinine (0.13, 18.93)). CRP and TIMP2 were also shown to differ between AATD in the stable state and healthy controls giving support to the hypothesis that inflammation and extracellular matrix turnover are relevant in the pathophysiology of AATD.

None of the other measured biomarkers showed a difference between stable AATD and controls which is surprising given the shared pathophysiological pathways. This result highlights the heterogenous nature of urinary biomarkers, particularly in the stable state.

4.3.1.2 Stable AATD vs exacerbation vs recovery

The results comparing urinary biomarkers in stable AATD to exacerbation and recovery periods was largely negative which was disappointing. Before outlining the main limitations

of the ELISA portion of this chapter, I will briefly discuss selected biomarkers from these analyses.

There were three biomarkers measured relating to elastin degradation included AAT, NE and desmosine. Results for AAT show that urinary levels tend to increase leading up to the start of an exacerbation and then reduce. This is physiologically feasible given that AAT is an acute phase protein.

Measurements of NE was affected by the limitations of the assay and low levels of detected analyte. Previous studies have shown that accurate measurement of NE is difficult due to rapid inhibition and its detection in both the free and inhibitor-bound form.(217) This bears no relevance to NE activity and its destructive potential which is thought to be higher in patients with AATD and during neutrophil derived inflammation in AECOPD. Surrogate markers that are specific to NE activity include the fibrinogen cleavage protein A α -Val³⁶⁰ which may better represent activity of NE during AECOPD and the stable state.(217)

Desmosine, a protein specific to the breakdown of elastin(237) is thought to be central to the pathophysiology of emphysema and it has been touted as a potential biomarker of lung damage during exacerbations.(80) Disappointingly, large numbers of samples fell above the threshold for desmosine measurement. This may reflect a narrow assay concentration that was not optimally targeted to the range of outcomes for patients with AATD.(267)

There were 8 separate urinary biomarkers representing various parts of the inflammatory cascade. Myeloperoxidase (MPO), showed an overall decrease between stable and exacerbation states (median difference -1780 ng/mL, $p = 0.033$). This effect was not seen in creatinine adjusted values. Previous studies have shown that sputum MPO increases during the initial phases of AECOPD that represent increased influx of neutrophils.(112) It is

possible that urinary MPO levels are reduced during an exacerbation as there is increased secretion into the airways with reduced urinary clearance. Urinary MPO has not been studied in the context of AECOPD before.

Conversely, though there was no detectable change in fibrinogen levels during an exacerbation compared to stable patients in raw values, there was an overall increase in fibrinogen for creatinine adjusted values (median difference 15.8 ng/mg creatinine, $p = 0.045$). When using Bonferroni adjusted p -values this result is not significant. Fibrinogen has been shown to relate to future risk of exacerbation from the ECLIPSE study(268) but is a non-specific biomarker of inflammation(269). It is therefore difficult to discern inflammation of an AECOPD from other sources. For these reasons fibrinogen is less likely to be a good candidate biomarker for enhanced monitoring of exacerbations in AATD related lung disease.

The creatinine adjusted median for CRP increased during an exacerbation and reduced during recovery though the deviation of values meant that the result was not statistically significant. Other biomarkers of inflammation studied showed no relationship.

Extracellular matrix proteins are associated with homeostasis outside of the cell and in health maintain airway infrastructure. My hypothesis was that during AECOPD there would be an increase in proinflammatory proteins and a reduction in their inhibitors. From the ELISA results alone, only Chitinase-3-like-protein showed an increase during AECOPD and subsequent reduction with recovery. None of the other measured proteins showed a difference. MMP8, MMP9 and MMP substrate showed very low levels which may be related the timing of sampling and reductions in analyte concentration. This is discussed in more detail in the next section.

4.3.1.3 *Limitations of ELISA analyses*

There were 21 urinary biomarkers tested across four broad pathways; elastin degradation, non-specific inflammation, extracellular matrix turnover and endothelial dysfunction.

Testing large numbers of biomarkers simultaneously can lead to increased risk of type 1 errors (false positives). Statistical methods, such as use of Bonferroni correction of significance testing parameters, is one way to achieve this. Since most of the ELISA sample results were largely negative, this is unlikely to have impacted upon our main conclusions. Using the Bonferroni correction method, the accepted p-value would reduce to 0.0024 (0.05/21) for all ELISA analyses and would render any results 'non-significant'. There is increasingly less emphasis placed on p-values in high impact journals in the reporting of results. The magnitude of effect in the ELISA analyses presented, even if conventionally significant, had very small effect sizes.

The lack of observed differences between biomarkers is impacted by the timing of urine sampling in the context of exacerbations, one of the most important limitations of this portion of the study. Collecting samples in a timely manner is challenging in a widely dispersed community-based study population. The design of this study aimed to capture patients that would ordinarily have self-treated exacerbations in the community based on symptoms alone. Though patients were asked to attend the study site during the first few days of their exacerbation there were often delays. By the time patients had attended the study site they may have already had a few days of treatment where the pathophysiological changes of an exacerbation had diminished. The reason for such delays were largely due to the overall design of the study. Since AATD is relatively rare, inherently the geographic spread of patients would be wide in order to achieve the number of patients for an adequately powered study. Travelling long distances during times of worsening symptoms

is also not ideal. As such the overall validity of the results comes into question since most 'exacerbation' samples were taken when peak of the symptoms had passed and treatment had already been started.

In trials of hospitalised patients optimum timing of samples is more easily achievable as patients undergo routine tests include collection of blood and urine on the first day of their admission. The day of admission is usually when the patients' symptoms have peaked or when initial therapy with oral steroids or antibiotics has failed. This ensures that samples taken during this time truly reflect the patient in the exacerbation state. This trial design selects out a more severe population of exacerbators. This group of patients, although important, only represents a small proportion of total exacerbations.

Some of the assays also suffered from low sample concentrations (or high in the case of desmosine as it was a competitive ELISA). One of the potential causes of this was multiple freeze/thaw cycles which is known to diminish the accuracy of testing(270). The samples from my study were initially stored in a -20°C freezer until a 10x10 sample box was filled, at which point it was moved to a -80°C freezer. Samples were also transferred on ice to minimise any risk of the samples thawing. In addition, multiple aliquots of urine were stored to ensure that all analyses could be performed on urine that had only had one thawing. It may be that analyte sample levels were truly low. One study has shown lower levels of urinary peptides in PiZ individuals compared to PiM though none of the proteins from my study were included.(271)

If I were to plan this study again, I would arrange for patients to collect samples at home and either send them directly to the study site or to freeze them at home and then bring it with them during study site visits. This may incur issues with freezing/thawing and

subsequent changes to the biomarker concentrations but would mean samples were taken during the first few days of an exacerbation when biomarker changes are likely to occur.

Another improvement would be the use of satellite sites dotted around the country which would significantly reduce the amount of time that patients would need to travel to be reviewed and have samples taken. It could also increase the likelihood that a patient would attend and therefore increase the overall numbers of patient episodes captured. This study design would be more expensive than the use of a single site.

4.3.2 Mass Spectrometry

As a sensitivity analysis for ELISA, plasma serum desmosine was measured to see if changes existed within the blood in comparison to urine. Huang et al have previously shown raised plasma desmosine levels in COPD exacerbations compared to healthy volunteers.(80) My results corroborate urinary findings that there was no difference between the stable state, during an exacerbation or recovery. Given sample numbers were relatively low and were generally not taken during the peak time of an exacerbation, these results may not fully represent the pathophysiology during the first days of an exacerbation. Huang and colleagues also compared their measurements to healthy controls rather than stable AATD patients. AATD patients are likely to have higher basal desmosine levels due to enhanced proteinase activity which would exaggerate any differences observed.

4.3.3 Feasibility of urine cube device

The use of POC devices to assess biomarkers in COPD is the next step in pioneering precision medicine to improve the treatment of AECOPD, particularly those usually treated in the community.(272) Regardless of whether a potential biomarker is indicative of AECOPD, the device in which it is measured must be acceptable and easy to use. During the COVID-19 pandemic, lateral flow assays kits have been the subject of intense media

attention and scrutiny(273) following the international roll out of home testing kits for SARS-CoV-2 infection. The aim of such tests was to rule out current infection and test for prior infection and immunological response.(274) There was controversy about some antibody tests carrying near 100% sensitivity which was met with scepticism.(274) The urine cube in my study differed slightly in the sense that it aimed to measure a concentration of the analyte as opposed to giving a binary outcome.

My study was able to robustly test the opinion of patients on the ease of use of a POC urine device to measure biomarkers of AECOPD over an 18-month period. The urine cube was well liked by the patients who used it (Figure 4.1). The use of a urine monitor has advantages over blood testing as patients may be less familiar or indeed scared of using needles/lancets to acquire blood for a test. From qualitative analysis of the exit questionnaire data it is clear that there are factors that contribute to a patient's overall acceptance and adherence to a new technology. In my study it was found that those who were able to incorporate the use of the urine cube into their usual daily routine were the best at adhering to the protocol.

One issue with using a POC device that is operated by patients is how to access data in a timely manner. This is crucial if this type of device is to be used on a larger scale in clinical trials assessing technology to aid management decisions during exacerbations. In this study, the data was stored on the urine cube and data could only be accessed during visits to the study site. From a biomarker analysis point of view this did not matter as exacerbations were not designed to be assessed in real time. The main aim was to assess patient acceptance and feasibility of the urine device. However, there were occasions when study participants were unaware that they were using the urine cube incorrectly or there

was a fault with the urine cube. This meant that data was being lost. The only way to identify such problems was during patient study site visits when urine cube data could be accessed. In some instances, data was not being recorded. This was realised during the 'Month 9' check-up visit or at the end of the study. Though frustrating, this observation was vital in assessing the feasibility of this particular version of the urine cube and has led to the development of upgrades to the device which will hopefully address these issues for future studies.

Another smaller study has used the same technology previously in 10 hospitalised COPD patients from presentation to one month afterwards.(236) Adherence and success levels were much higher overall in comparison to my study, though during the same initial time period of 1 month adherence rates were similar.

Following feedback from my study there have been some adaptations made to the urine cube hardware. Firstly, a small, external battery pack has been added. This ensures that even if the device is unplugged from mains electricity the urine cube still has power. This avoids data loss. Early testing of this feature has been promising and seems to address the issue with battery longevity.

The other adaptation was installation of Bluetooth® connectivity to allow real time transfer of data to the study site. This feature allows 1) remote monitoring of patient data, 2) the ability to check patient adherence and 3) troubleshooting in real time and to confirm data capture. Remote monitoring of patient data is an obvious advantage. For example, if a patient's urine result for one of the specified biomarkers were to cross a predefined threshold in combination with an elevated eDiary score, action could be taken; either consultation with their GP or to start their rescue pack. For future trials, it may be possible

to use the urine cube to aid treatment decisions for pulmonary exacerbations compared to usual care alone.

Another advantage of Bluetooth® connectivity would be checking patient adherence to urine cube use. Manual checking of a database to determine which patients are non-adherent may be achievable in a small trial but would be labour intensive for clinical trial staff in larger studies or in a real world setting. A computer algorithm could be designed to alert study staff to patients that do not adhere to the study protocol and prompt a telephone call to the patient. Equally, the computer algorithm could be designed to automatically send text or email messages to study participants. This would reduce the burden of follow up for study staff.

Finally, issues with data recording could be addressed via telephone or video calling to see why data was not being stored. In the current study, this could not be assessed until the cube was physically plugged into the computer at the study site. With the new Bluetooth® adaptation, the correct recording of data could be checked and then rechecked following advice or retraining of patients on the urine cube process.

Other studies have explored novel ways to incorporate the use of smart phones for POC biomarker analysis(275) including using a smart phone camera as an optical reader for a lab-on-a-chip ELISA device which could be used.

4.3.4 Lateral flow immunoassays

This study was the first to quantify multiple urinary biomarkers of AECOPD in patients with AATD over a long follow up period. These data were also paired with detailed symptom score measurements to enable accurate timing of exacerbations and the recovery period.

CRP appeared to be the most promising urinary biomarker found which is in keeping with previous studies looking at serum measurements during AECOPD.(66, 276) CRP is well known to be a stable analyte and is implicated in inflammatory, infective and neoplastic aetiologies.(277) Hurst and colleagues(276) examined serum for 36 biomarkers in 90 patients with treated and symptom confirmed AECOPD from the London COPD cohort, of which CRP was the most selective. This showed an overall increase in serum CRP during AECOPD compared to the stable state. Though unable to predict exacerbation alone, it was predictive in combination with classical symptoms of an exacerbation (AUC 0.88, 95% CI 0.82 to 0.93, $p < 0.0001$). They also found that CRP concentration did not link with AECOPD severity. My study was not able to determine if there was a linear relationship between AECOPD severity and CRP concentration mainly due to the majority of patients having moderate exacerbations. Sub-analysis of CRP concentration and symptom score or change in symptom score from baseline showed no relationship either.

The most surprising outcome was related to TIMP1. It was shown to reduce during AECOPD and rebound back to pre AECOPD levels by day 7 of a treated AECOPD. This is somewhat in contrast to unpublished data from urinary measures in usual COPD though other studies have shown that TIMP1 is lower in patient with emphysematous COPD compared to those COPD patients without emphysema.(278) It has been suggested that an increased MMP/TIMP ratio could be responsible for the increased turnover that results in emphysema progression.(279) It is possible that TIMP1 is used up during times of increased inflammation hence the increase in MMP/TIMP ratio and the findings in my study that TIMP goes down.(280) This may also be pronounced in AATD related lung disease, where emphysema predominates. This finding will need further validation in a larger cohort of patients.

AAT also showed a small increase during exacerbations. As a well-known acute phase protein, it increases even in AATD individual and has led to the common practice of measuring CRP at the same time as AAT(281) to ensure that AAT levels aren't interpreted incorrectly as being within normal limits resulting in a missed AATD diagnosis.(282) This finding is reassuring in my study as a well-known phenomenon has been shown with this data and may go a small way in validating its efficacy.

The other biomarkers studied, namely NE and desmosine, did not show any difference which is likely to have related to the assays themselves. The assay designed for this study may not have included a suitable range of NE concentrations. This could also be related to the small batch production of urine cassettes and subsequent differences in concentration curves for each batch. In future, larger batches could be made to reduce the risk of inter-measurement bias or to use a single set of urine cassettes per patient.

Some lateral flow devices are unable to cover the entire range of measurement for a particular molecule/analyte.(283) Oh and colleagues(283) were able to negate this issue by measuring serum CRP across three different concentration ranges on the same cassette.

This may be something that could be used to increase the range of analyte measured.

Adding creatinine to the assay could also improve accuracy and help account for fluctuations in sample concentration due to non-AECOPD factors like dehydration or diuretic therapy.

Sandwich ELISA in particular, the basis for the POC lateral flow immune assay in this study, can be prone to inaccurately low concentration read outs in the presence of very high analyte concentrations. This phenomenon is known as the 'hook effect' (284, 285) due to the characteristic hook like appearance to the end of a concentration curve where the

detected signal strength drops abruptly as concentration increases. This is due to high levels of antigen decreasing the ability for antibodies to form immune complexes.

One of the limitations of this study with regard to biomarker analysis is that all patients were on an inhaled corticosteroid which could flatten any inflammatory signal. Given this is a cohort of frequent exacerbators it is expected that all patients will be on GOLD guideline adherent treatment and will be the case for any prospective cohort study. Other non-COPD causes such as cardiovascular disease and venous thromboembolism could not be excluded as the cause of a patient's symptom deterioration, but were not clinically suspected.

POC measurements, as opposed to tests carried out within a primary or secondary care setting, have the advantage of being performed at the time when a patient has symptoms and in some cases before treatment has even begun. The results of the lateral flow assay portion of this chapter are therefore likely to better represent periods of true exacerbation in contrast to the ELISA results for reasons discussed above. POC devices, although convenient for patients, may have less accuracy compared to testing in laboratories. The inevitable compromise between accuracy and convenience will need to be carefully balanced to ensure an optimal approach to future implementation of such devices.

The main objective of routine urinary lateral flow measurement in this study was to establish a suitable biomarker(s) to use in combination with an eDiary to diagnose exacerbations and to guide treatment in an effort to stem emphysema decline.

Though there was a small average increase/decrease in levels of CRP and TIMP1 respectively, on an individual patient basis this association was not reproducible. As such the addition of these biomarkers to the data from the eDiary described in Chapter 3 to the prediction model did not improve the overall performance (Figure 4.14).

Clearly there were certain patients who had reproducible changes in their biomarkers during exacerbations (Figure 4.13) though this study was not powered enough to be able to perform sub-analyses of such patients.

A much larger number of patients with a wide range of exacerbation aetiologies would be required to be able to discern an exacerbation which leads to lung damage. This would balance the signal to noise ratio generated from incorrect measurements, treatment of non-significant exacerbations and the heterogenous nature of the severity of exacerbations.

The most promising biomarker prior to the study was desmosine as discussed in the introduction to this thesis. The uncertainty around the accuracy and validity of the lateral flow assay for desmosine specifically have meant that the question still remains if desmosine can be used as a surrogate marker of lung damage in AECOPD of AATD related lung disease. This study has, however, provided valuable data to the team at Mologic who will continue to develop this assay further.

Repeating a similar study to more accurately determine if biomarkers can be used to improve prediction of exacerbations in a hope to slow the rate of emphysema decline will be challenging. This is unlikely to be feasible in patients with AATD unless replicated on a global scale with dozens of study sites recruiting patients. This concept may be better approached via usual COPD patients and then validated in an AATD cohort thereafter.

Discovery of other novel pathophysiological pathways and subsequent biomarkers may be another route to understanding the complex nature of exacerbations, including cardiopulmonary biomarkers such as pro-BNP and troponin T.(286, 287)

4.4 Conclusion

This chapter has shown that a POC urine monitor is feasible to implement and generally well accepted by patients with AATD related lung disease and frequent exacerbations. CRP and TIMP1 were particularly promising biomarkers, but overall changes were small. Addition of these biomarkers to results from an eDiary did not improve the overall diagnostic performance of a prediction algorithm.

The final results chapter of this thesis changes focus to the second treatment target presented in the main introduction and asks the following question: can reduction in the basal rate of emphysema decline via augmentation therapy improve overall mortality and reduce QOL decline in patients with AATD related lung disease?

5 COMPARISON OF OUTCOMES IN AUGMENTATION NAÏVE AND AUGMENTED PATIENTS WITH ALPHA-1 ANTITRYPSIN DEFICIENCY

5.1 Introduction

The main focus of this thesis so far has been on methods to limit emphysema progression from AECOPD by monitoring symptoms and urinary biomarkers. Not all patients with AATD suffer exacerbations frequently, if at all. Equally, those who are frequent exacerbators may only suffer their effects for limited times during the year. As such, an additional therapy to enhanced monitoring that reduces of the basal elastin degradation, presented in the main introduction, is central to limiting overall emphysema decline. This chapter aims to address this final hypothesis.

Correction of the protease-antiprotease imbalance is the basic concept behind AAT augmentation therapy. This involves intravenous administration of AAT purified from human plasma. AAT augmentation therapy was approved in the US and several other countries in the late 1980s based on its ability to increase human blood and lung levels of AAT. It is currently the only specific disease modifying treatment for patients with AATD and has been shown to slow emphysema progression as measured by CT lung density.(53, 129, 130) Following results of the RAPID trial,(53) the European Medicines Agency approved one form of AAT augmentation therapy in Europe. However, the efficacy of AAT augmentation therapy has been questioned as randomised control trials (RCTs) have so far failed to show clinically or statistically significant results in widely accepted clinical endpoints such as lung physiology, mortality and QOL.(144) Designing an adequately powered RCT that is large enough and long enough to detect disease modifying effects is

not feasible,(288) especially since AATD is a rare disease and emphysema, even in AATD, progresses relatively slowly.

Since a large and prolonged RCT approach has not been possible in this condition, it seems logical to explore other experimental designs to evaluate clinically meaningful endpoints. For example, prospective cohort studies have the potential of studying a greater number of patients with longer follow up.(289-291)

In the US, augmentation therapy has been administered to patients since 1989 whereas the UK is yet to approve funding. Both cohorts adopt a westernised healthcare infrastructure with similar ways of managing other therapies pertinent to AATD lung disease e.g., inhaled therapies(42) and transplantation(178, 292). By comparing both highly characterised patient cohorts I aim to address whether AAT augmentation therapy improves survival and health status in patients with AATD related lung disease which may indicate that emphysema decline is effectively slowed.

5.2 Methods

Analysis was performed studying overall mortality, time to lung transplant and QOL outcomes in patients with AATD related lung disease in two large prospectively followed cohorts which aimed to characterise the progression of AATD related lung disease. The first cohort includes patients in the United States who received augmentation therapy. The comparison cohort included UK patients who received standard COPD care.

5.2.1 Ethics, protocol approval

The study protocol including an analysis plan was finalised in July 2018 and subsequently approved by the Western Institutional Review Board (WIRB) along with ethical approval. Both cohorts had prior ethical approval for the collection and analysis of data and the additional protocol and ethical approval was sought in order for data to be shared.

5.2.2 Information governance and data transfer

Careful attention was made to information governance and data transfer, particularly when sending data from the US for data merging and analysis. In the US, data transfer rules are governed by the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Data fields that were deemed patient identifiers were adjusted to be HIPAA compliant. This included censoring of exact dates e.g., birth, death, recruitment, transplant, and as such only year of dates were transferred. Data fields of control/UK patients were treated in a similar manner to maintain consistency. Data was transferred in password protected documents with no patient identifiable information available. Patients were only identifiable by a unique number.

Data from the US was transferred in stages, initially baseline demographic data in August 2018 followed by transplant and QOL data. Mortality data was continually updated following repeated data cleaning from the US group and finalised in May 2019.

Data for the UK cohort was extracted from a central database and variables aligned with those from the US cohort. Mortality data was cross checked and updated where necessary.

5.2.3 Inclusion and exclusion criteria

Adult patients (≥ 18 years old) with lung disease and a severe AAT genotype (defined as PiZ, PiZNull, PiNullNull, and other rare variants) were deemed eligible for inclusion in analyses. PiSZ, PiS and PiM heterozygotes were excluded from the analysis. The rationale for this was based on these phenotypes having a less severe presentation of progression of emphysema and that patients with severe deficiencies would benefit most from AAT therapy. Individuals who had a lung transplant prior to their start year were not eligible for inclusion.

5.2.4 Primary and secondary outcomes

Overall length of survival (all-cause mortality) was selected as the primary outcome since this was deemed the most important outcome that could not be addressed by a randomised controlled trial. A prespecified censor date of 31st December 2016 was used to allow adequate time for databases of mortality to be updated to ensure all patients were coded correctly. Time to lung transplant and patient reported QOL were secondary outcomes. Time to lung transplant was selected since it represents patients who have experienced significant deterioration in lung related health in addition to the health care cost implications for transplanted patients. Patient reported QOL using the St Georges Respiratory Questionnaire (SGRQ)(222) was selected to assess impact of augmentation therapy on QOL. SGRQ was used as it is widely used in clinical trials of COPD and has an accepted minimal clinically important difference; an increase of 4 points.(223) SGRQ was also routinely captured in both cohorts from around 2007 onwards allowing direct comparison. SGRQ measures impact on overall health, daily life, and perceived well-being in patients with respiratory disease. The total score is calculated from three domains: symptoms, activity and impact. Each domain is scored from 0 to 100, with higher scores representing worse QOL.

5.2.5 Patient selection/recruitment

Figure 5.1 summarises patient selection. Study size was determined by a fixed available sample of eligible patients that were prospectively followed as part of a separate ongoing cohort study in both the UK and US. All patients had given prior informed written consent before the analysis was carried out.

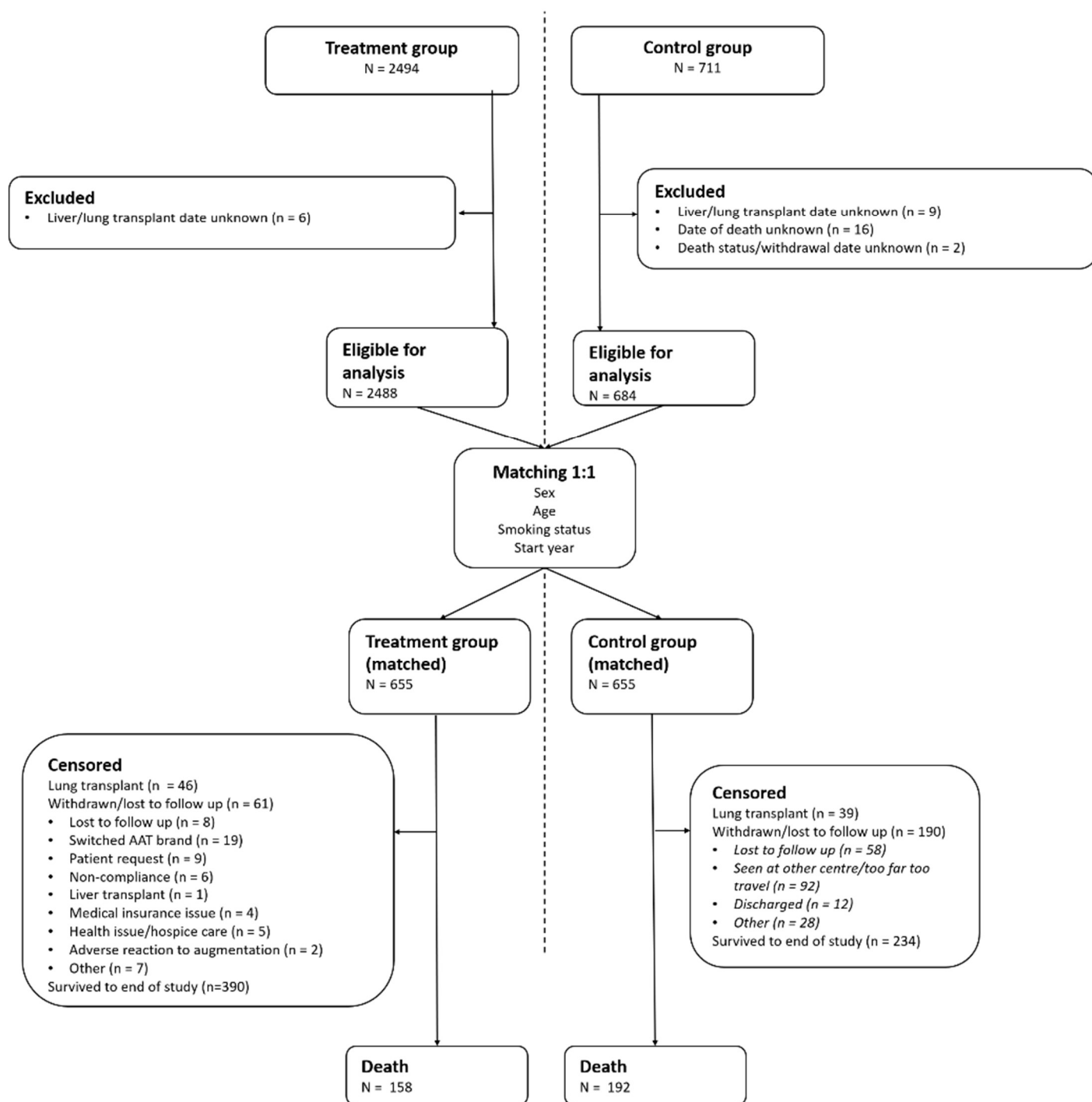


Figure 5.1: Study flow diagram

5.2.6 Treatment cohort

The treatment group consisted of individuals in the US who had been prospectively followed by AlphaNet, a not-for-profit organisation that provides a telephone-based disease management program designed for individuals with AATD-associated lung disease who are prescribed augmentation therapy.(293) Referral to AlphaNet occurs after an individual has been prescribed augmentation therapy by a physician. At present, AlphaNet follows the majority of individuals in the US who are prescribed augmentation therapy for lung disease due to AATD.

AlphaNet began providing basic disease management to patients with AATD in 1999, which consisted of monthly phone calls from trained AlphaNet coordinators. These unstructured phone calls were designed to support patients but did not involve data collection. Since 2008 AlphaNet has utilised structured telephone interviews to collect extensive information from individuals enrolled in AlphaNet. The current analyses include individuals who completed a baseline assessment with AlphaNet between 2008 (when the baseline assessment was implemented) and the end of 2015. The baseline assessment uses patient self-reports to collect demographic information, AAT genotype, year in which the patient began using augmentation therapy, current smoking status and smoking history, and information relating to the prescription and delivery of AAT augmentation therapy. All individuals in the treatment cohort self-reported at baseline that they have lung disease and currently use augmentation therapy.

Self-reported outcomes are consistent with the treatment guidelines and insurance requirement for receiving augmentation therapy in the US. QOL was measured consistently from 2007 onwards using the St. Georges Respiratory Questionnaire (SGRQ), Individuals who are enrolled in AlphaNet complete the SGRQ approximately once per year.

The initial source of data for mortality was direct notification from the patient's family. All individuals who were reported deceased or who were lost to follow up as of December 31st, 2016 were cross-checked against data provided by the US National Death Index to determine whether the patient was deceased and to verify or capture date of death.(294) Records of patients with missing data were rechecked and excluded if not available.

5.2.7 Control cohort

The control cohort consisted of patients with AATD who had never received AAT augmentation therapy, from an observational study of patients in the UK. Processes for study visits have been described previously.(295) Participants were referred from primary and secondary care from 1996 to the present day. Their follow up included annual medical history and physical examination, post-bronchodilator pulmonary function tests, SGRQ and AAT genotyping. CT thorax was either performed by the referring physician or as part of the cohort study to confirm presence of emphysema. Sources of data for mortality included notification from the patient's family or general practitioner or directly from a central NHS data base. Patients in this control cohort who had received short periods of augmentation therapy as part of a clinical trial or for other indications, e.g., panniculitis, were excluded. Records of patients with core missing data were rechecked and excluded if this was not available.

5.2.8 Cohort matching

Nearest neighbour propensity matching from the UK data base with eligible US patients was performed using R 3.6.0(225) and the *MatchIt* package(296) in a 1:1 fashion without replacement with a caliper of 0.2. Calipers are used to improve the closeness of match between cohorts; the lower the number the closer the matching. The following variables for matching were taken at the time of initiation of augmentation therapy (augmentation

group) or time of baseline assessment (control group): sex, age and patient reported smoking status (never-smoker, former smoker or current smoker). Former smokers included patients with self-reported cessation of smoking prior to initiation of augmentation therapy or baseline assessment.(140) In the control group, never smokers were defined as subjects having smoked less than 20 packs in their lifetime, as used in other studies of AATD.(199) In the augmentation cohort, non-smokers were defined as subjects having smoked less than 1 pack in their lifetime. To reflect the improvements in care of AATD related lung disease over time in both cohorts(297) a fourth continuous matching variable was used, 'baseline year', which was defined as start of augmentation for the treatment group and time of initial review at an AATD centre for the control group. Balance of covariates was checked and deemed satisfactory prior to statistical analysis. Covariate balance was assessed by comparing absolute mean differences between unmatched and matched cohorts (Figure 5.2) and means using t-tests and chi squared tests for normally and non-normally distributed variables. To check the performance of matching, an

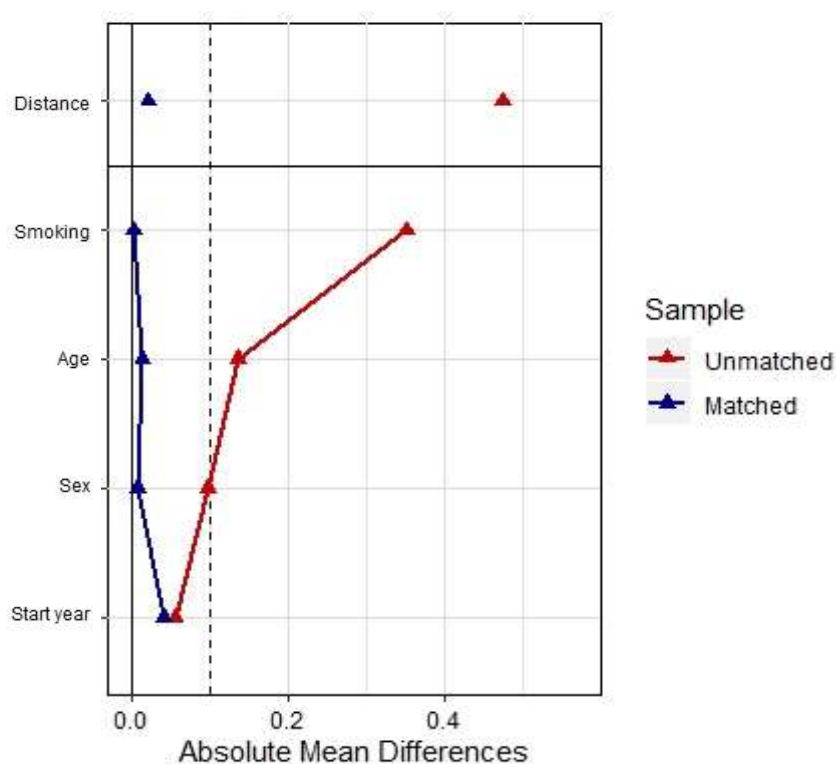


Figure 5.2: Love plot demonstrating mean difference between matching variables before and after matching

exploratory analysis using cox proportional hazards was also performed using the same matching variables as covariates.

Love plots (Figure 5.2) are used to demonstrate covariate balance when matching. The x-axis shows a standardised absolute mean difference before matching (red) and after matching (blue). This is plotted for each of the matching variables (y-axis). The plot clearly shows that the matching process reduced the overall mean difference for each matching variable.

5.2.9 Statistical analysis

All statistical analyses were performed using R version 3.6.0.(225) The *survival* package(298) was used for all time to event analyses using the Kaplan-Meier method for both survival and time to lung transplant.(299) Survival was calculated from the time of augmentation therapy initiation (augmentation group) or baseline assessment (control

group) until year of death. A censor date was set prior to analysis, such that patients alive after December 31st, 2016 were censored. In addition, lung transplant recipients were censored in the year of transplantation. Those who were lost to follow up were censored at the time of withdrawal or the last recorded follow up visit. All survival times for the augmentation group were reported as integer values (years) in order to comply with the deidentification standards of HIPAA; control group survival times were transformed to mirror this and thus reduce potential bias. Time to lung transplant was calculated from start of augmentation therapy for the augmentation group or baseline for the control group to year of surgery.

The way in which the US cohort data was collected from 2008 onwards meant that patients starting augmentation therapy prior to 2008 had survival times back calculated from the year augmentation therapy was initiated. As such, an exploratory analysis using patients seen from 2008 onwards was performed to check the effect of this on the results. Lung function data was deemed to be more reliable in the augmentation group i.e., was within the year of baseline assessment rather than an older record, and as such FEV₁ percentage predicted was used as an additional matching variable in the post 2008 sub-analysis.

QOL data was used for patients seen from 2007 through December 31, 2016, since reliable data was available for both cohorts during this time period. Scores recorded after lung volume reduction surgery or lung transplant were excluded from the analysis. The dependent variable was defined as change in SGRQ total score calculated as annual change/year for all patients with 3 or more recorded SGRQ scores. Covariates for the model identified by prior univariate analysis were included if the coefficient p value was ≤ 0.1 . The selected covariates were sex, age, first recorded SGRQ total score and treatment group

(augmentation or control). Multivariable linear regression assumptions were tested and deemed to be achieved including normal distribution of variables, linear relationship between independent and dependent variables and homoscedasticity.(300) These assumptions were assessed using visual inspection of histograms and Q-Q plots, pairs plots and plots of standardised residual values vs fitted values respectively. A separate exploratory analysis was performed looking at time to achieve MCID for SGRQ using Kaplan-Meier method in a similar way to the mortality and lung transplant analysis.

5.3 Results

Patient characteristics for whole and matched cohorts are summarised in Table 5.1. Control patients were more likely to be male, were marginally older, and more likely to be current smokers; matching eliminated these differences. In the augmentation group, the most common reason for withdrawal was patients switching to a different brand of augmentation therapy (32.2%). Other reasons included patients opting out of the treatment programme (13.5%), patients being transferred to hospice care (5.1%), non-compliance (10.2%), liver transplant (1.69%) and issues with medical insurance (6.78%). In the control group the most common reason for withdrawal/loss to follow up was either discharge from NHS follow up or living too far from the research site. Exact reasons were not recorded for withdrawal.

Table 5.1: Baseline characteristics for whole and matched participants

	Whole cohort			Matched cohort		
	Control	Augmentation	<i>P</i>	Control	Augmentation	<i>P</i>
N	684	2488		655	655	
Female (%)	269 (39.3)	1217 (48.9)	<0.001	263 (40.2)	272 (41.5)	0.65
Age (mean±SD)	52.2 (10.1)	50.9 (10.7)	0.005	52.2 ± 10.1	52.3 ±11.0	0.82
Smoking (%)			<0.001			0.36
Never-Smoker	114 (16.7)	638 (28.4)		114 (17.4)	88 (14.4)	
Ex-smoker	470 (68.6)	1469 (65.2)		468 (71.5)	451 (74.1)	
Current Smoker	98 (14.4)	144 (6.4)		73 (11.1)	70 (11.5)	
AAT phenotype (%)			0.24			0.28
ZZ	655 (95.8)	2404 (96.6)		627 (95.7)	634 (96.8)	
Z Null	11 (1.6)	32 (1.3)		11 (1.7)	13 (2.0)	
Null Null	1 (0.1)	12 (0.5)		1 (0.2)	0 (0.0)	
Other	17 (2.5)	40 (1.6)		16 (2.4)	8 (1.2)	
Lung transplant (%)	41 (6.0)	143 (5.7)	0.88	39 (6.0)	46 (5.5)	0.81
Mortality (%)	202 (29.5)	551 (22.1)	<0.001	192 (29.3)	158 (24.1)	0.039
Median follow up (years) [IQR]	7.00 [4.00, 11.00]	9.00 [4.00, 16.00]	<0.001	7.00 [4.00, 11.00]	9.00 [5.00, 15.00]	<0.001

N; patient number, AAT; alpha-1 antitrypsin, IQR; interquartile range

FEV₁ is not included as this data was not available until 2008 in the treated cohort.

5.3.1 Mortality

Kaplan-Meier survival analysis was conducted to determine if AAT augmentation therapy prolonged time to death from all-causes (Figure 5.3). For the whole cohort (Figure 5.3a), the augmentation group survived longer with 10-year survival being 15.8% higher compared to controls (augmentation: 84.8%, 95% CI 83.2 to 86.5%, control 69.0%, 95% CI 64.6 to 73.7%, p value <0.001). Similar results were seen after matching, albeit with a smaller magnitude of effect (Figure 3B; mean difference of 12.4%, augmentation: 81.5%, 95% CI 78.1 to 85.2%, control: 69.1%, 95% CI 64.7 to 73.9%, $p <0.001$, respectively). A sensitivity analysis examined differences in mortality since standardised assessments were introduced in the augmentation group in 2008 and found no clinically or statistically significant difference in mortality up to 8 years (Figure 5.4); this is consistent with the point at which the 95% confidence intervals for mortality begin to separate in the matched cohort analysis (Figure 5.3).

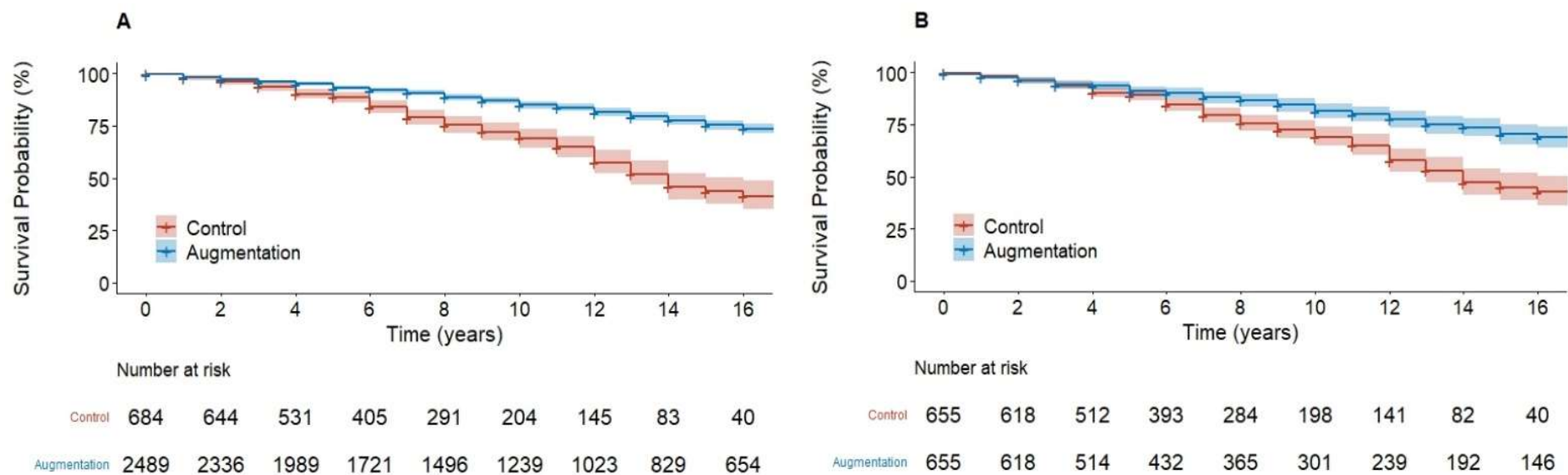


Figure 5.3: Kaplan-Meier survival plots for patients on augmentation therapy vs controls for A) whole cohort and B) matched cohort

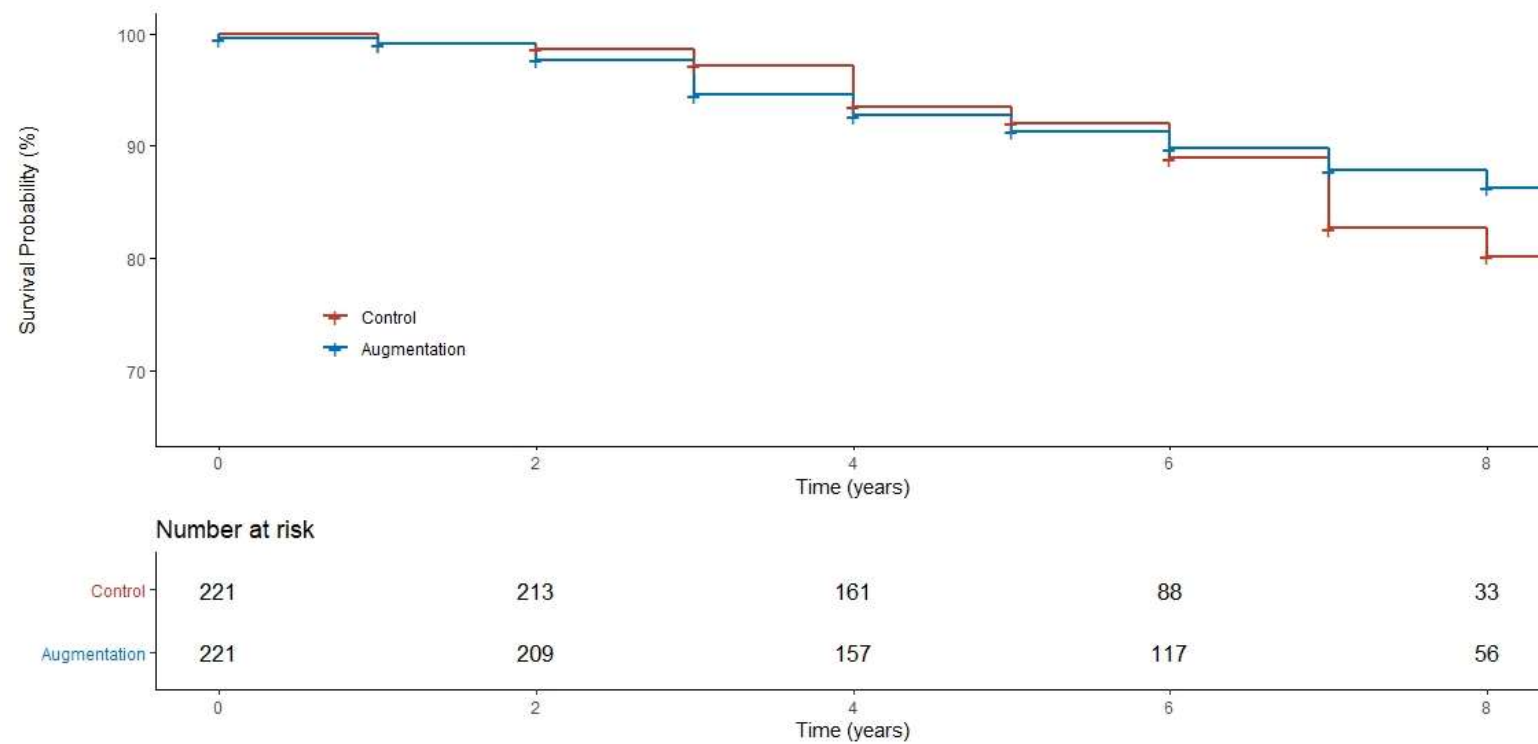


Figure 5.4: Kaplan-Meier survival plot in patients seen from 2008 onwards

An exploratory analysis using cox proportional hazards was performed. Covariates were the same as those used for matching. Figure 5.5 represents the whole cohort. Augmentation therapy appears to improve survival (HR 0.54, 95%CI 0.46 to 0.65, $p < 0.001$). Age has a hazard ratio of 1.06 (95% CI 1.05 – 1.07) which can be interpreted as for each one-year gain in age the risk of death across the time of the study is 6% higher. Similarly, ex-smokers have a HR of 3.77 (95%CI 2.81 – 5.05) compared to non-smokers. Baseline year was also shown to have a large impact on survival though the direction of effect seems counterintuitive. The results show that patients seen from 2007 onwards have a HR of 3.64 (95%CI 2.69 – 4.94). To explore whether this effect is seen in both groups, a split analysis was performed by looking at control (Figure 5.6) and augmentation therapy groups (Figure 5.7) in isolation. This shows the effect of baseline year has opposite effects in each group. For the control group patients tend to survive longer when their baseline year is later (2007 onwards HR 0.32, 95%CI 0.18 – 0.54) compared to those seen before 1997. In contrast, patients from the augmentation group had a significantly higher hazard of dying if seen from 2007 onwards with reference to before 1997 (HR 9.93, 95%CI 6.78 – 14.5, $p < 0.001$). Further exploration of reasons led to looking at deaths per group per year (Figure 5.8). This shows that no patients were recorded as dying prior to 2008 in the augmentation cohort and is likely the source of bias relating to the above results.

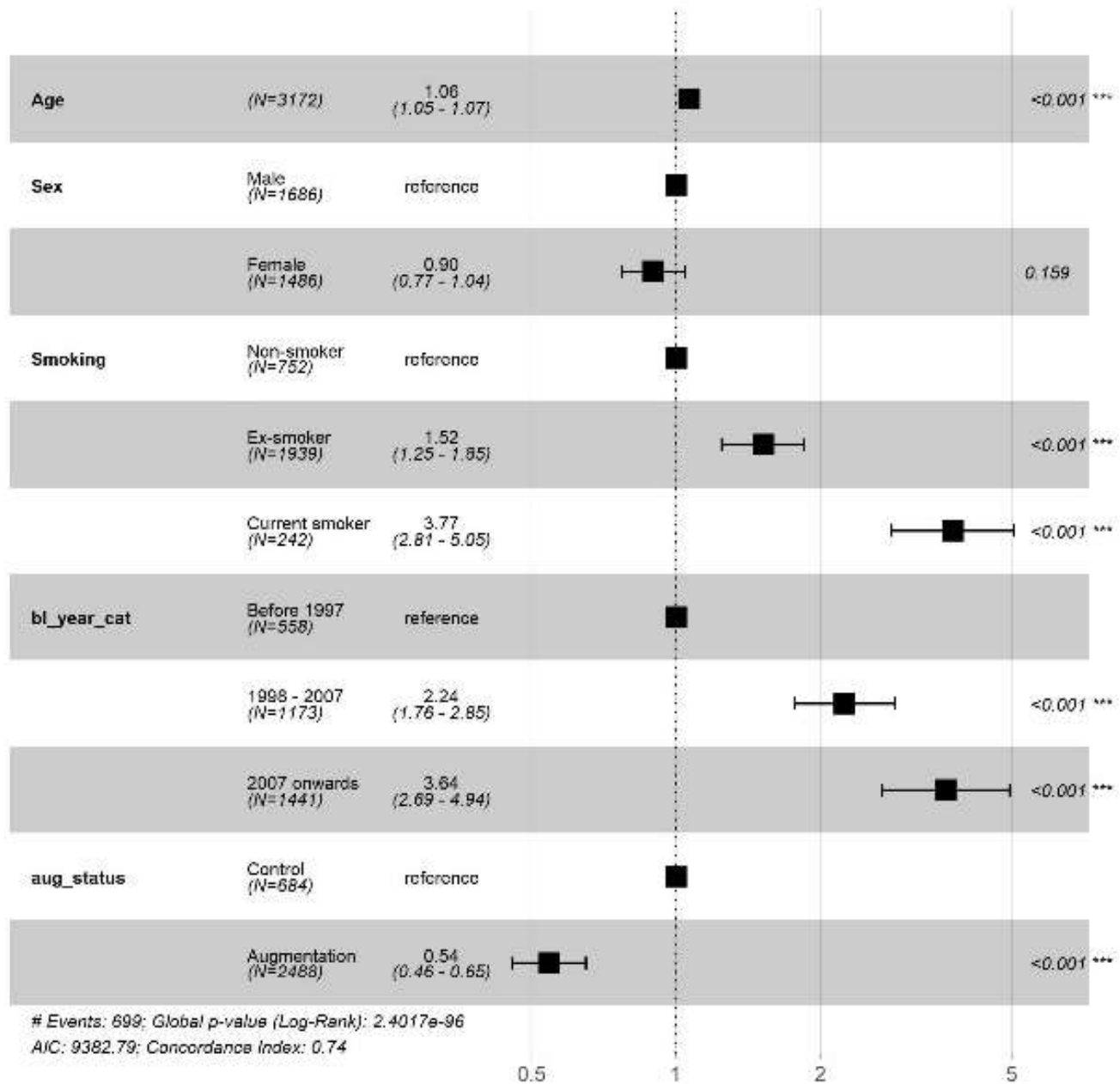


Figure 5.5: Forest plots of cox proportional hazards for whole cohort.

bl_year_cat; baseline year, aug_status; group variable

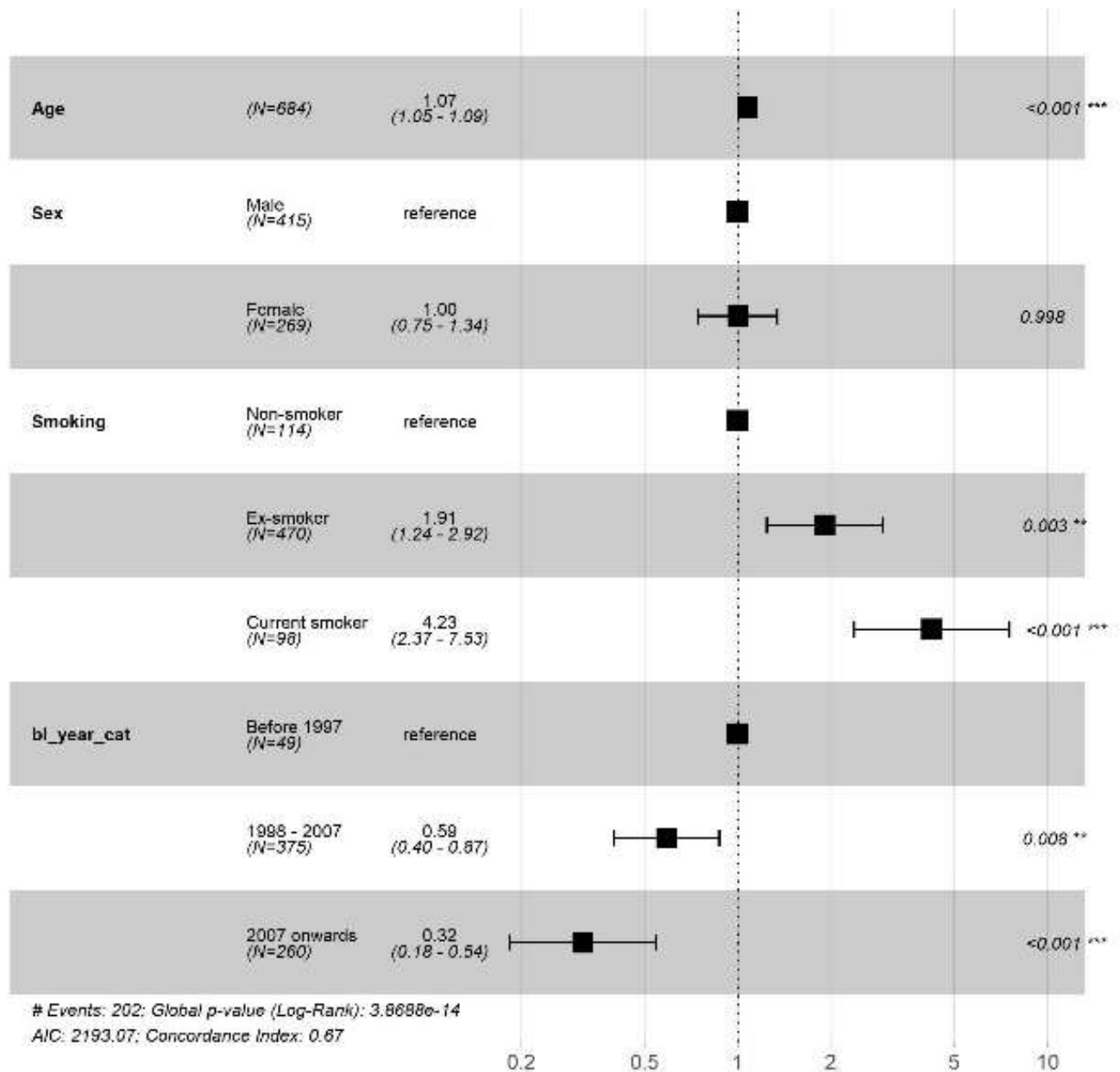


Figure 5.6: Forest plots of cox proportional hazards for control group.

bl_year_cat; baseline year, aug_status; group variable

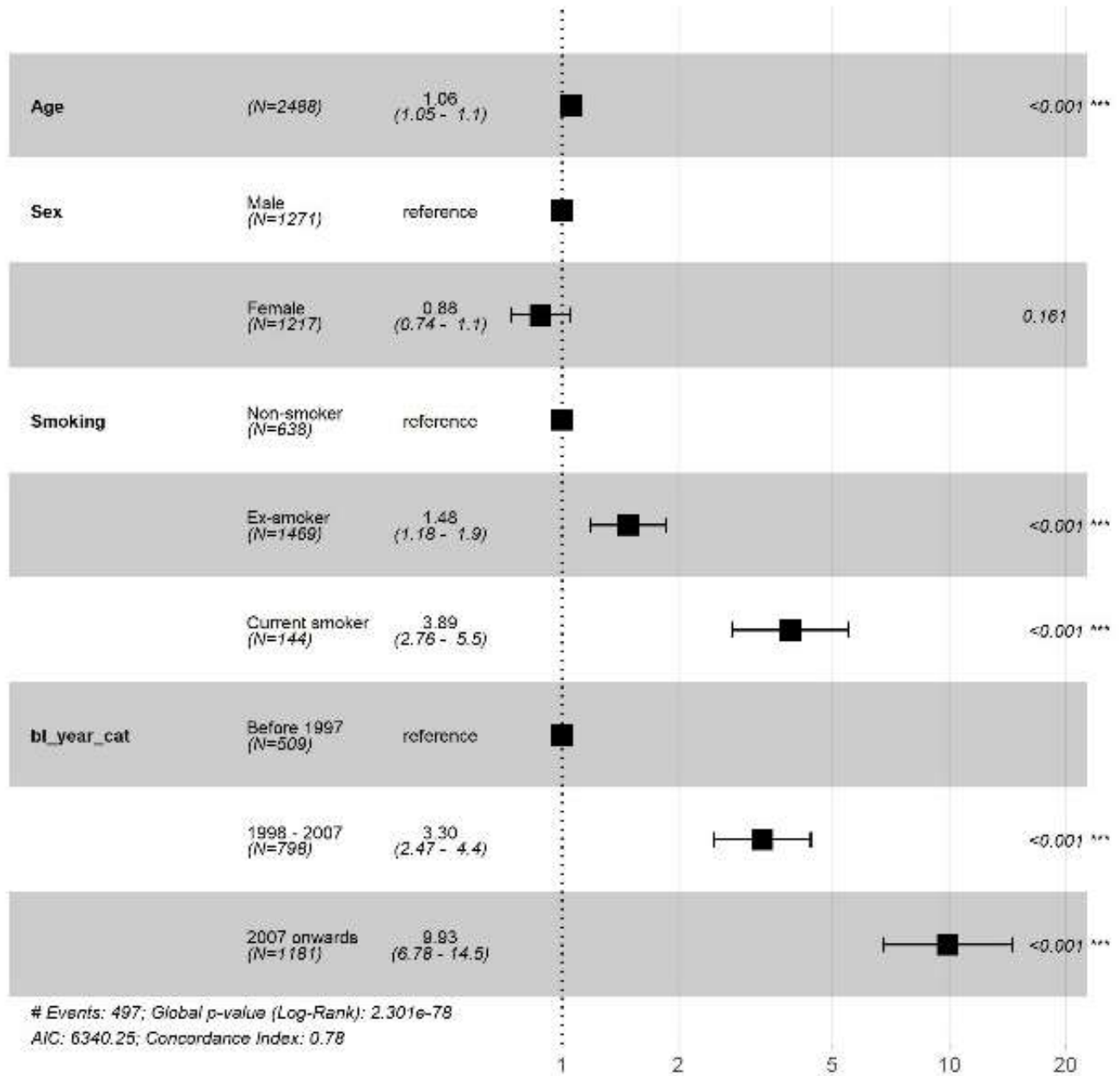


Figure 5.7: Forest plots of cox proportional hazards for augmentation group

bl_year_cat; baseline year, aug_status; group variable

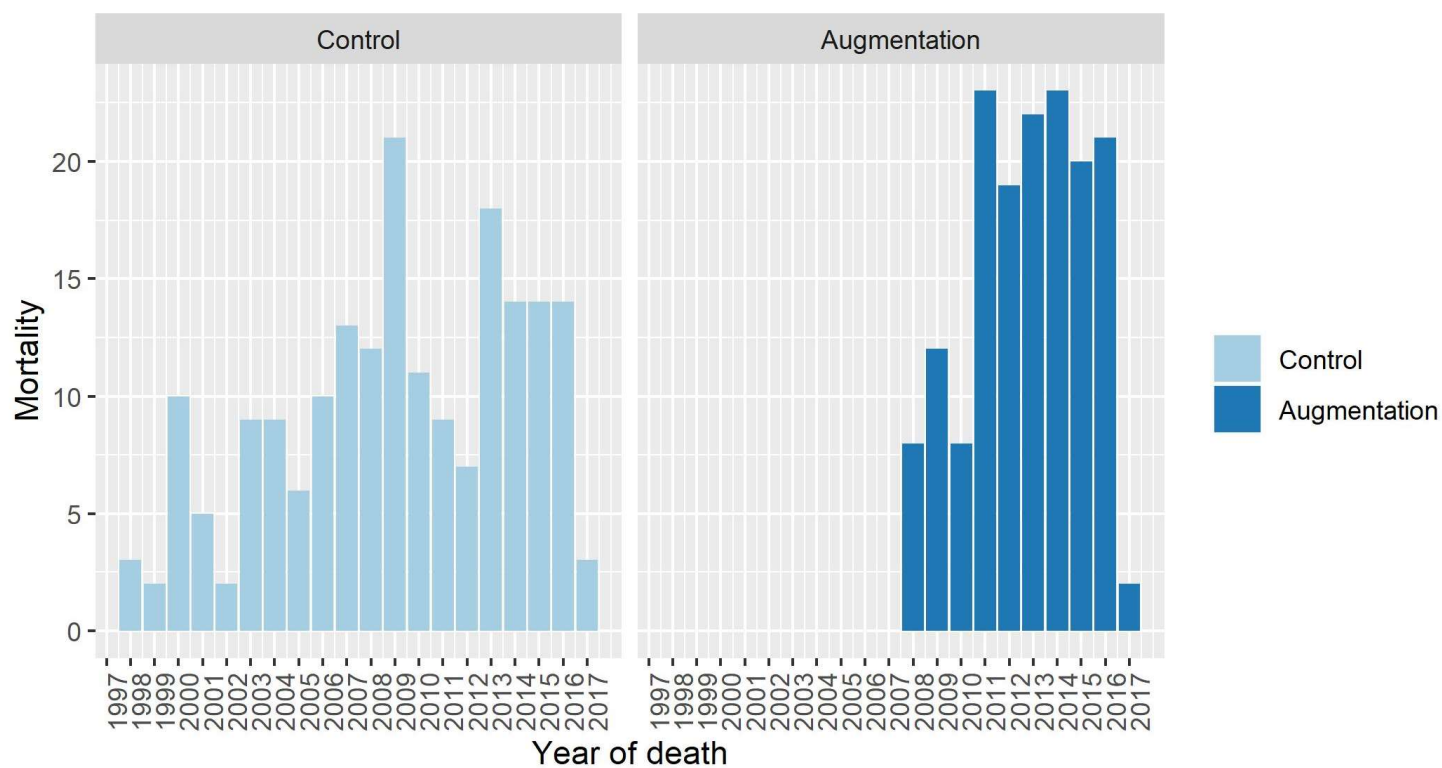


Figure 5.8: Number of deaths per year for control and augmentation groups

5.3.2 Lung transplant

A secondary analysis examined overall time to lung transplant between groups. The demographics of patients receiving lung transplantation during follow up are summarised in Table 5.2; due to lower numbers of patients and since there were no statistically significant differences no matching was conducted. The results show a delay to intervention with lung transplant in the augmentation group with median time difference of 8.0 years (augmentation: 13 years, 95% CI 13 to 15 years, control: 5 years, 95% CI 4 to 8 years, $p < 0.001$). This is illustrated in Figure 5.9. There were insufficient number of patients to conduct a sub-analysis of post 2008 patients.

Table 5.2: Baseline characteristics for lung transplant patients

	Control	Augmentation	<i>P</i>
N	41	143	
Female (%)	13 (31.7)	65 (45.5)	0.16
Age (mean $\pm$ SD)	47.3 $\pm$ 6.4	44.7 $\pm$ 7.8	0.059
Smoking (%)			0.21
Never-Smoker	2 (4.9)	19 (14.1)	
Ex-smoker	36 (87.8)	102 (75.6)	
Current Smoker	3 (7.3)	14 (10.4)	
AAT phenotype (%)			0.16
ZZ	40 (97.6)	137 (95.8)	
Z Null	0 (0.0)	5 (3.5)	
Null Null	0 (0.0)	1 (0.7)	
Other	1 (2.4)	0 (0.0)	
Median time to lung transplant (years) [IQR]	5.0 [3.0, 9.0]	13.0 [8.5, 17.5]	<0.001

N; patient number, AAT; alpha-1 antitrypsin, IQR; interquartile range. *As baseline characteristics were similar, patients were not matched

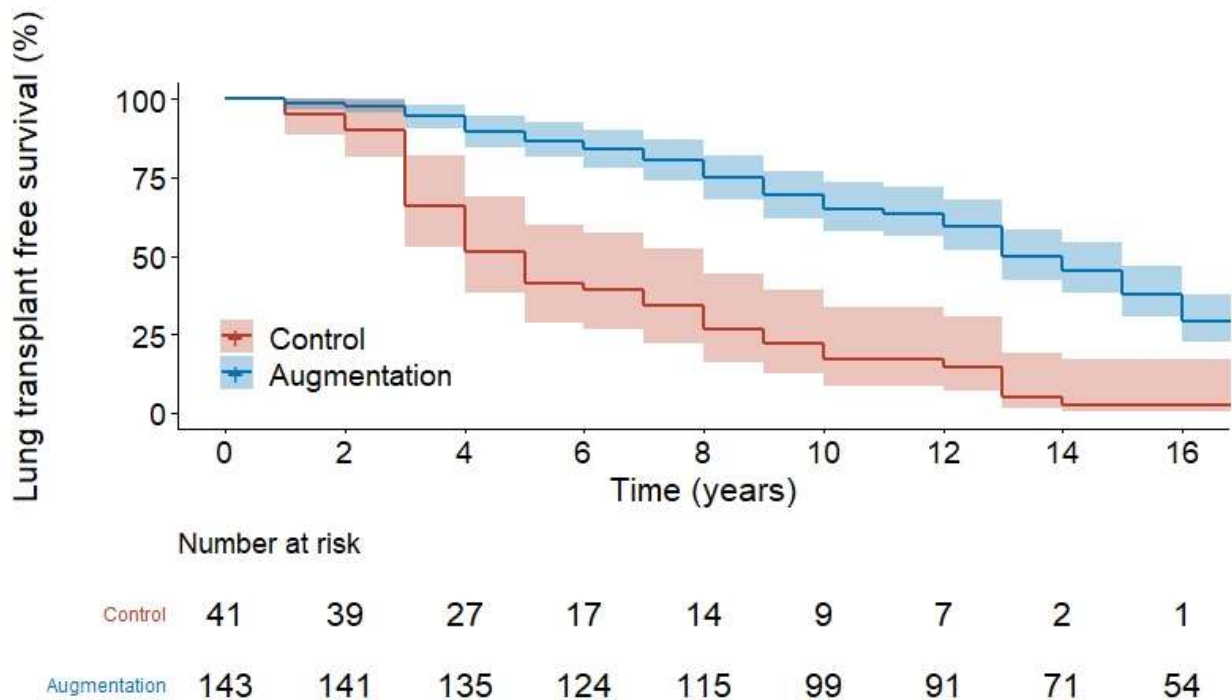


Figure 5.9: Kaplan-Meier plot of time to lung transplant for patients on AAT augmentation therapy vs controls

5.3.3 Quality of life

Patient characteristics for the QOL analysis are summarised in Table 5.3. Consistent with the features of the whole group, there was a higher proportion of active smokers in the control group; small differences in age and sex were not significant. SGRQ symptom scores were lower in the augmentation group although SGRQ total scores were similar. The mean slope of SGRQ total score was 1.19 points/year higher in the control group.

Results of multiple regression for annual SGRQ total score decline are reported in Table 5.4; annual progression of SGRQ total for the control group was on average 1.3 points higher/year in control group patients compared to those receiving augmentation therapy (95% CI 0.41 to 2.19, $p=0.004$) after adjustment for sex, age and baseline SGRQ total score. Whilst slope of SGRQ was influenced by the starting score the effect was marginal, being only -0.07 points per additional starting SGRQ point (95% CI -0.09 to -0.05, $p<0.001$).

Table 5.3: Baseline characteristics for all patients with ≥ 3 SGRQ total measurements.

	Control	Augmentation	P
N	115	426	
Female (%)	47(40.9)	215 (50.5)	0.085
Age (mean $\pm$ SD)	55.38 $\pm$ 10.06	55.5 $\pm$ 10.46	0.91
Smoking (%)			0.039
Never-Smoker	33 (28.7)	132 (32.7)	
Ex-smoker	73 (63.5)	261 (64.6)	
Current Smoker	9 (7.8)	11 (2.7)	
AAT phenotype (%)			0.58
ZZ	108 (93.9)	406 (95.3)	
Z Null	3 (2.6)	12 (2.8)	
Other	4 (3.5)	8 (1.9)	
Baseline FEV ₁ % predicted	55.86 $\pm$ 23.63	45.91 $\pm$ 20.68	<0.001
Baseline SGRQ score			
Total	44.90 $\pm$ 18.58	43.90 $\pm$ 19.14	0.62
Symptom	54.23 $\pm$ 22.90	46.59 $\pm$ 22.04	0.001
Activity	60.39 $\pm$ 22.88	62.35 $\pm$ 24.63	0.44
Impact	32.99 $\pm$ 19.36	32.46 $\pm$ 19.48	0.80
SGRQ follow up (years)	3.77 $\pm$ 1.57	4.72 $\pm$ 1.96	<0.001
SGRQ total annual slope	1.21 $\pm$ 4.11	0.02 $\pm$ 4.62	0.013

N; patient number, AAT; alpha-1 antitrypsin SGRQ; St. Georges Respiratory Questionnaire, CI; confidence interval, FEV₁; Forced Expiratory Volume in 1 second.

Table 5.4: Multivariable regression for annual decline in SGRQ.

	Estimate	95% CI	P
Group			
Augmentation	Ref	-	-
Control	1.30	0.41 – 2.19	0.004
Sex			
Male	Ref	-	-
Female	0.45	-0.29 – 1.19	0.23
Age (years)	-0.01	-0.05 – 0.03	0.65
Baseline SGRQ total	-0.07	-0.09 – -0.05	<0.001

*R*²/R² 0.1/ 0.09. †St. Georges Respiratory Questionnaire (SGRQ) total score is determined by the SGRQ-COPD questionnaire; scores range from 0 to 100 points, with higher scores indicating worse health status (minimum clinically important difference, 4-points. SGRQ total score is calculated from three separate health status domains; symptom, impact and activity scores. Ref; reference variable, SGRQ; St. Georges Respiratory Questionnaire, CI; confidence interval

Additional analyses examining symptoms alone, which appeared to differ between groups, are presented in the appendix (Section 7.5.1, Table 7.3).

Time to minimum clinically important difference in SGRQ using Kaplan-Meier curves is shown for all patients with ≥ 3 SGRQ data points (Figure 5.10A) and for patients matched on baseline characteristics (Figure 5.10B). For matched patients the median time to MCID for patients on augmentation therapy was 3.2 years (95% CI 2.9 to 3.5) compared to 2.0 years in the control group (95% CI 2.0 to 2.9), $p = 0.002$.

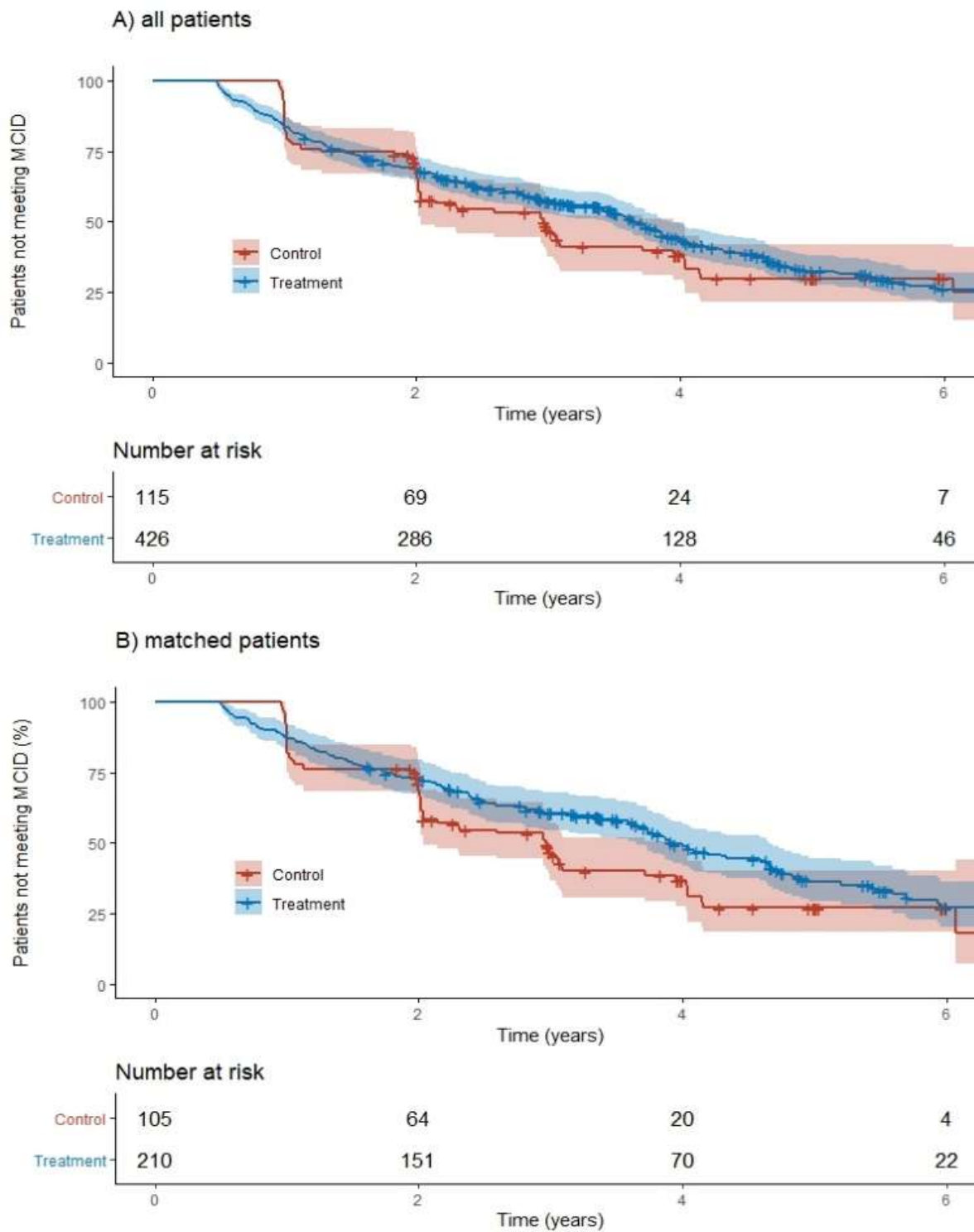


Figure 5.10: Kaplan-Meier plots of time to MCID for SGRQ total score for A) all patients and B) matched patients

5.4 Discussion

So far in this thesis I have discussed the challenges in implementing an enhanced monitoring system to limit the impact of exacerbations on emphysema decline due to the heterogeneous nature of exacerbations and the limited scope of specific biomarkers for exacerbations. This approach is fairly novel and it has not been established if such monitoring would actually result in reduced emphysema decline. In contrast, the use of augmentation therapy has compelling evidence for reducing emphysema decline as assessed by CT density. The results of this study suggest there is a reduction in QOL decline as defined by SGRQ though the impact of augmentation therapy on mortality remains uncertain. In the following sections I will discuss the results of Chapter 5 in light of results from the preceding chapters.

5.4.1 Mortality

For any disease modifying drug, mortality is regarded as an important outcome and is feasible to assess in rapidly progressive pathologies as demonstrated in clinical trials for lung cancer.(301-303) Emphysema is slowly progressive and takes decades to deteriorate sufficiently to impact mortality. Whilst CT lung densitometry is the most direct measure of emphysema progression(143) its relationship to clinically relevant outcomes remains controversial.(144) CT density change has been the gold standard measure for trials targeting emphysema, and was later supported by a systematic review (n=112 studies) demonstrating relation of density to more widely accepted measures, such as FEV₁, QOL, exacerbations and mortality in studies of both usual and AATD related COPD.(304) The UK registry has also shown that CT lung density decline correlates with FEV₁ decline(305) and mortality in PiZ AATD, with the strength of the signal being greatest in the upper zone, where progression occurs later in the disease.(145) Since augmentation has consistently

been shown to reduce emphysema progression demonstrated by CT densitometry, it would be predicted to influence long term mortality too. This was the basis for exploring mortality to assess emphysema decline in patients with AATD related lung disease.

The initial mortality results in Figure 5.3 showed a survival benefit in favour of augmentation therapy for both the whole cohort and for patients matched on baseline characteristics. However, following exploratory analysis with cox proportional hazards (Figure 5.5, Figure 5.6, Figure 5.7), there was a large influence of a patient's baseline year for those in the augmentation group. With better quality COPD care and earlier recognition of AATD worldwide it would be expected that a patient's hazard of death would decrease the later they were seen at baseline. This is true for the control cohort (Figure 5.6), but shows the opposite for the augmentation cohort (Figure 5.7), which biologically did not seem feasible. Further exploration of the data led to the discovery of an immortal time bias in the augmentation group (Figure 5.8), occurring as a direct result of how the data was collected in the augmentation group. A new patient questionnaire was implemented in 2008 and therefore any patient dying prior to 2008 would not have been able to complete the questionnaire and would be missed from the database. Subsequently, any data prior to 2008 in this dataset cannot be used to assess mortality benefit unless the patients who died during that period are also included.

The discovery of this issue highlights the importance of data quality in a database centred research study, especially when data is collected from multiple sources that are not in direct control of the person performing the analysis. It has also underlined the principle of checking data and sensitivity analyses in order to test hypotheses robustly. Of course, over testing can also lead to issues with results. If data is analysed and looked at in a multiple

number of ways it increases the odds of finding a statistically significant result by chance.(306) The results can then be presented selectively which is a form of publication bias. In this study, this was avoided by pre specifying an analysis protocol and presenting results of all analyses together.

Looking at the post 2008 data, which includes only patients who started augmentation therapy within 12 months of their baseline questionnaire and therefore do not have risk of immortal time bias, the results show no difference in survival (Figure 5.4). There appears to be some separation at around 8 years in favour of augmentation, however. The mortality rate is small with only 10% of participants dying within the follow up period so the likelihood of a statistically significant result in a cohort of this size would need a large difference in mortality rate or a much longer follow up period. Since the start date was from 2008 and the censor date was from the end of 2016, only a maximum of 9 years data could be studied. The number of observed deaths at 8 years was also low (33 and 56 deaths for control and augmentation groups respectively).

There are notable differences between the cohort's baseline characteristics for those seen post 2008. Augmentation patients were significantly older and had substantially worse lung function than the control group (Table 5.1). Though matching may account for some of these variables there are important unmeasured variables such as healthcare infrastructure. I attempted to acquire data for socio-economic status which has a strong influence on all-cause mortality but this was challenging. Firstly, HIPAA limited the type of data that could be shared for the augmentation group. I was unable to get data on place of residence or direct measures of social class but were able to get income data for the US patients, albeit only for approximately a third of participants. Equally, I did not have income

data for UK patients and using other surrogates of socio-economic status, such as education or occupation, may not have been directly comparable. For these reasons I did not use socio-economic status as a matching variable. It is possible that a treatment effect does exist but was not detectable in the methodology used. Sensitivity analysis to determine the effect of unobserved confounding is one option to address this.(307)

The use of respiratory related mortality may have negated some of the issues discussed above and given a better representation of events related to severe emphysema and subsequent respiratory failure. Unfortunately, this data was not available from the US cohort and this could not be assessed. The lack of respiratory related mortality data undermines that main aim of this chapter and whether delay in emphysema decline leads to reduced mortality. It is unlikely that augmentation therapy reduces other cause mortality (e.g., cardiovascular disease, cancers) and for future studies data on respiratory mortality should be collected. Rate of lung transplantation and time to lung transplant may be alternative ways to explore this outcome.

5.4.2 Lung transplant

AATD is the fourth most common indication for lung transplant world-wide with approximately 5% of AATD patients receiving a lung transplant.(308) Two cohort studies(178, 309) estimated median survival after lung transplant at approximately 11 years with differing survival benefit despite improvement in QOL.(178)

The use of time to lung transplant as a surrogate marker of emphysema progression is reasonable since chronic respiratory failure and reduced lung function, both features of advanced emphysema, are two of the main reasons for lung transplant referral in AATD. The BODE score(310) has previously been used to risk stratify lung transplant patients; a

score >7 is typically considered acceptable for lung transplant referral in COPD and represents those at a high risk of death in the following 2 years.(311) This has received some recent criticism due to the original BODE cohort not being typical of a transplantable population.(311, 312)

Similar to the mortality results, an immortal time bias exists for augmentation patients starting augmentation therapy prior to 2008 and therefore any results relating to lung transplant from these data should be interpreted with caution. There were only 24 lung transplants performed in total from 2008 onwards and a meaningful sensitivity analysis of this cohort could not be achieved. Direct comparison of lung transplant rates between groups was not statistically different in matched patients either (1.8% and 2.7% for augmentation and control respectively, $p = 0.75$). As such, no clear consensus can be made about whether time to lung transplant is prolonged with augmentation therapy by delaying emphysema decline.

5.4.3 Quality of Life

There are significant challenges in detecting patient centred outcomes in trials of disease modifying therapies(288) as demonstrated by the two most recent AATD RCTs which included SGRQ as a secondary outcome. They found no difference between treatment groups.(53, 130) SGRQ, although widely used, is influenced by both disease development/progression and aging and requires large numbers of patients or long term follow up in chronic diseases such as AATD to detect a difference.(288) The current study found an average annual difference in total SGRQ decline of 1.3/year (representing a slower decline in QOL) in favour of augmentation therapy (Table 5.4). The SGRQ symptom domain also showed a slower decline in patients on augmentation therapy. With an MCID accepted as ≥ 4 points in total score,(223) our data suggest it would take 3 to 4 years on

augmentation before such a difference would become apparent. Demonstrating this has the added problem that patient participation in clinical trials alone improves both compliance and health care status and detecting such a clinical impact above a putative placebo effect is challenging.(288) This study represents real-world data independent of the influences of intensive follow up which characterise RCTs and is supportive of the role of augmentation to achieve better patient centred outcomes. The QOL portion of this study does not suffer the same immortal time bias since this analysis includes patients from around the same time that the new baseline questionnaire was introduced in the augmentation cohort, and is limited only to the period of active follow up.

Though the overall median time to MCID for matched patients (Figure 5.10) is shown to be longer in the augmentation cohort, there are additional considerations needed to interpret their significance and the morphology of the Kaplan-Meier curves deserve close attention. There is a difference in the smoothness of the curves for each group such that the augmentation group has a more linear trajectory compared to a stepwise descent of the control group. The steps of the control groups relate to the annual collection of data in comparison to the varied collection time points for the augmentation group. This means that the control group effectively only get the 'opportunity' to reach MCID once a year. For example, looking at year 1 of Figure 5.10 you can see that the control curve initially is above the augmentation curve but then suddenly drops below. This crossing happens twice in the first 2 years. Usually crossing of the two curves in survival analysis should be approached with caution as it may indicate bias or lack of difference between groups.(219) Looking at the data at annual time points only, there is separation of the curves after 2 years in the matched group (Figure 5.10b). This suggests augmentation therapy prolongs time to

meeting MCID if used for 2 years or longer whereas short term differences are unlikely to exist.

Relating QOL back to the main aim of this chapter is more difficult. Though severity of emphysema does relate to QOL, there are also many other factors that influence this and therefore it cannot directly answer our original hypothesis. The exploration of QOL in this chapter served more as an important measure for the approval of AAT augmentation therapy.

5.4.4 Strengths and limitations

The size and nature of the two parent cohort studies has allowed the study of up to 9 years follow up of a rare condition that is not possible within an RCT and more likely reflects real-world outcomes for unselected populations. Wide acceptance of augmentation therapy effectiveness among patients with AATD and their physicians in countries where it is available has also made enrolment into placebo-controlled RCTs increasingly difficult to explore the issues addressed here.

Despite the strength of the large number of patients and long term follow up, there are limitations. Some have already been discussed earlier in the discussion. In addition, fundamental differences between groups which would be minimised in an RCT were unavoidable given the cohort study design. As discussed above regarding the mortality analyses (5.4.1), the use of individual patient matching on parameters outlined here attempts to adjust for such variability in baseline characteristics though not all variables could be accounted for including healthcare infrastructure and socioeconomic background. Data collection methods also differed although the primary outcome of mortality is less likely to have been affected as it is a finite outcome confirmed in registry data. Previous

QOL studies have indicated that telephone or face-to-face administration of SGRQ are equally valid.(313) Control patients with radiologically or physiologically confirmed lung disease were included to match those in the treatment group. Data from unmatched patients seen from 2008 onwards suggest less severe lung disease in the control group, underestimating the mortality effect. In addition, the close professional follow-up of control patients, as with all chronic disease, will have a beneficial effect on disease management and outcomes which may have also influenced the comparisons. This effect would have minimised differences between the groups.

Ideally CT lung density measurements overtime would have strengthened any results relating to emphysema decline which were unfortunately not available in large enough quantity to make reasonable analysis in either group. The demonstration of preserved lung density in combination with a mortality signal would have confirmed my hypothesis that limitations of elastin degradation via augmentation therapy improves survival which unfortunately could not be answered adequately with this dataset. As such, this hypothesis could not be fully addressed.

Similarly, the differences in how exacerbation data was collected in both cohorts meant that adjustment for exacerbations could not be made, or indeed comparisons between exacerbation rates between cohorts. This comparison would have been extremely relevant to the overall hypotheses of this thesis and should be included into any future prospective studies with similar designs.

5.4.5 Cost implications for augmentation therapy

The results of this study were submitted academic in confidence to decision making bodies (NICE) to aid interpretation of cost-effectiveness models submitted by a manufacturer of

augmentation therapy who had applied for their product to be used in the NHS. Before considering the cost of augmentation specifically, it is worth discussing the concept of cost effectiveness in relation to orphan disease and the policies the UK has adopted.

Orphan drugs; those that are used in rare conditions, are on average five times more expensive than non-orphan drugs.(314) This is in part due to high research and development costs with possibility of lower return on investment for drug manufacturers. This provides a less attractive commercial target for pharmaceutical companies, which deepens the issue.

Beyond the practicalities of cost, there are ethical considerations and whether it is fair to not fund a drug just because it is for a rare disease with higher research development cost. The 'rule of rescue' represents societies general urge to help individuals in need of immediate help regardless of cost.(315) This is in contrast to the balancing health gain for the whole population where allocation of resources to a rare disease will result in less health gain for the entire population. The tension between the greater good and fairness is something policy makers must consider.(314)

In 2013 the National Institute for Clinical Excellence (NICE) established the Highly Specialised Technologies (HST) program to review health care interventions for low prevalence conditions (less than two cases per 100,000). Criteria for approval include patient/carer/family impact and benefits other than health/social care. In 2016 a cost-effectiveness threshold of £100,000/quality adjusted life year (QALY) gained was introduced. This is substantially more than the £20,000 to £30,000 threshold used for non-HST technologies. Following public consultation, this approach was further amended to include a scaled weighting system. If interventions exceeded 30 QALYs it would be weighted by 3 with an incremental scale for interventions adding between 10 and 30 QALYs

ranging from 1 to 3. For example, if an intervention cost £250,000/QALY that added 35 QALYs in total the weighting system would reduce this number by a factor 3 to £83,300/QALY which would qualify it for approval. This amendment effectively prioritises therapies that have particular large positive effect. All therapies would be subject to the overall impact of a therapy on the NHS budget, hence the budget impact level for any therapy was set at £20 million. Any costs above this budget impact would need to be helped by management methods from the manufacturing company.

Though the UK has a defined threshold for approving health technologies in rare disease, this is not the same for some other countries. This poses an interesting ethical dilemma where there is worldwide difference in the delivery of drugs that are aimed at rare disease. This is clearly seen with the difference in prescribing practices of AAT augmentation therapy across Europe.(316)

Currently, the estimated annual cost of Respreeza (CSL Behring) is £57,200 based on 52 administrations per year for a person with average weight of 67 – 83kg at a dose of 60mg/kg. With added administration costs this would total £59,526 annually. By comparison the cost of standard COPD care in the UK is between £3,361 (FEV₁ >50% predicted) and £4,134 (FEV₁ < 30% predicted). Lung transplant is estimated to cost £76,698 in the first year and £9,260 per year thereafter.(317)

The QALY for augmentation therapy is difficult to define but was estimated at 1.51 QALYs during the Highly Specialised Technology Evaluation in 2018.(317) Based on a patient being on therapy for 7 years (giving a total lifetime cost of £422,198) the incremental cost-effectiveness ratio (ICER) was £237,822. AATD patients are likely to live longer than 7 years

according to the results of my study and as a result would lead to an even greater cost to the NHS.

The estimated overall budget impact in year 5 of drug implementation was £20,940,966 based on 90% population uptake in a population of 700 patients. This was likely an underestimation of the treatable population which AATD experts estimated at approximately 1,500 patients in the UK with PiZ/Znull AATD. There were concerns that availability of a disease modifying drug may encourage screening and therefore increase the population size eligible for treatment. This would have substantially increased the budget impact in excess of the £20 million limit.

Based on the above discussion augmentation therapy was rejected for approval for use in the UK. This was largely based on uncertainties with population sizes, eligibility criteria and impact of augmentation therapy on factors such as mortality and QOL.

This study has demonstrated that there may be a quality-of-life benefit which would be in favour of augmentation therapy; it also shows a mortality benefit is unlikely to exist below 8 years. It has also shown that AATD patients have a substantially longer life expectancy in recent years which would further push the ICER beyond an affordable option for use within the NHS. For augmentation therapy to be a reality in the UK it would have to be targeted at a small subset of severe patients with rapid progression of lung disease or be substantially cheaper. The definition of a rapid decliner remains uncertain. FEV₁ may be unreliable as it is patient effort dependant and is known to vary from day-to-day. DLCO may be a better surrogate compared to FEV₁.(143) Development of an MCID for CT lung density decline would be a step towards quantifying progression of emphysema in addition to better designed prospective studies that can assess more widely accepted outcomes.

5.5 Conclusion

AAT augmentation therapy improves QOL as assessed with SGRQ and may delay progression to MCID. There was no difference in mortality after 8 years; longer time points could not be assessed adequately due to data collection differences between cohorts. As such, the main hypothesis of this chapter could not be fully addressed.

The high cost of AAT augmentation therapy coupled with longer life expectancy than expected for AATD means that NHS funding is likely to be delayed for the only disease modifying drug currently available for patients with AATD related lung disease designed to reduce elastin degradation and rate of emphysema decline.

6 OVERALL DISCUSSION, CONCLUSIONS AND FUTURE WORKS

6.1 Overall discussion and conclusions

This thesis has set out to explore interventions that limit emphysema decline in patients with AATD related lung disease with the intention to improve overall QOL, reduce mortality and reduce the impact of exacerbations.

The first intervention involved close monitoring of symptoms and a panel of urinary biomarkers and formed the largest part of this thesis via the 'Elastin degradation in exacerbations of AATD related lung disease' study. Since this approach was fairly novel, the initial aim was to assess the feasibility of implementing remote patient monitoring alongside careful assessment of eDiary responses and a range of biomarkers to predict exacerbations.

The use of site visits during this study served two purposes: 1) to independently review and confirm exacerbation status with physician assessment and 2) to collect urine samples during different phases of an exacerbation and its recovery to validate the lateral flow immunoassays with ELISA. There were significant challenges encountered with this part of the study, mainly related to the number and timing of visits. As such, the ELISA analyses were underpowered and didn't capture the desired pathophysiological snapshots to validate the lateral flow immunoassays. This is reflected in the largely negative ELISA results reported in Chapter 4. It was able to confirm previously reported findings that urinary desmosine is higher in stable AATD patients compared to control subjects as stable samples were not impacted by timing. If this study were redesigned for the site visits it would require either delivery of samples via courier/post or the use of multiple study sites

to ensure samples were taken during the time intervals required. This would incur additional cost.

One of the studies main successes was data collected from the eDiary presented in Chapter 3. It confirmed the large burden of unreported exacerbations in patients with AATD related lung disease in addition to detailed characterisation of the episodes over the entire course of each episode. It also captured the influence of seasons which had not been done before in AATD related lung disease. Exploration of patient factors that influence adherence to eDiary technology was another strength, a topic lacking in the general literature. This information has already been used in the delivery of a larger cohort of patients with usual COPD which is currently in its recruitment phase.

Revisiting the first hypothesis set out in the introduction, the use of an eDiary alone for the early detection of exacerbations was neither sensitive or specific enough following in depth ROC curve analysis, though it was feasible to implement such technology. This aligns with observations following results from other studies assessing the performance of symptom diaries in patients with COPD(249, 318). Chapter 4 presented results from the lateral flow immunoassays which were performed alongside daily eDiary measurements in an effort to add another layer to the prediction algorithm. Two biomarkers, CRP and TIMP1, showed average changes over the time course of an exacerbation, including reverting back towards a baseline value upon recovery. This, however, was not reciprocated in individualised data, a vital feature needed for biomarkers to be successful in this setting. Addition of these results to the eDiary in a predictive model did not improve the overall performance. It is likely that over simplification of the pathophysiological process underlying exacerbations to a panel of biomarkers measured in urine with current technology is not feasible.

If the results from this study had shown positive results the next logical step would be to take it forward into a head-to-head randomised control trial of enhanced monitoring vs standard care. Since CT lung density has been established as a reproducible surrogate for emphysema decline this could be selected as one of the primary outcome variables.

Outcome measures of exacerbation frequency and severity could also be used. In reality, the issues with identifying a suitable panel of biomarkers that can accurately predict exacerbations alongside symptom scoring postpones future plans for now. If a more promising biomarker emerges then this could be repeated in a more carefully designed study. A better performing desmosine assay may be a suitable candidate.

Reflecting on the second hypothesis set out in the introduction, the question remains if the endless hunt for biomarkers in such a heterogenous disease entity will ever find the ideal candidate, or if the pursuit of such a biomarker is indeed futile. Biologically it seems reasonable to suggest that biomarkers that relate to a specific pathophysiological process will predictably change during episodes of increased disease activity. The work from this thesis highlights that proving this is extremely challenging. There was some signal seen when results were averaged between the cohort for CRP and TIMP1 in the lateral flow assay results.

Lessons could be learned from biomarker research in the area of cardiovascular disease. The Framington risk score, for example, was successful in predicting future risk of cardiovascular disease at the population level with many biomarkers having strong associations with cardiovascular events or death(319). The same biomarkers have so far failed to change the AUC to a meaningful extent in prediction models on an individual basis, leading to pessimism about biomarker research(320). This mirrors the search for

biomarkers of COPD exacerbations and elastin degradation, including the work presented in this thesis.

The second intervention studied in this work related to augmentation therapy which intends to slow down the basal rate of elastin degradation and limit the progression of emphysema. For the reasons discussed in Chapter 5, conventional outcomes, such as mortality and QOL, are not easily assessed in a rare disease with a relatively slow disease progression. This fact has led to worldwide discrepancies in the provision of augmentation therapy, particularly in the UK where the NHS does not currently fund augmentation therapy for lung disease following advice from NICE.

Chapter 5 attempted to address this gap in knowledge by comparing two large AATD cohorts in western healthcare infrastructures. Despite attempts to adjust for covariates between cohorts with variable matching, the results regarding mortality were inconclusive. They were limited by differences in data collection methods, issues with sharing certain data types and particularly robustness of data relating to disease severity in the US cohort. In addition, low mortality rates in more recently diagnosed patients meant a lack of power for the sensitivity analysis after 2008 examining survival. QOL as measured by SGRQ was shown to decline more slowly in patients on augmentation therapy. The data collection around SGRQ was more accurate in the US cohort compared to survival data and therefore the results are more promising.

There was also no useable data for comparison of exacerbation rates between cohorts which could have been linked in with results from the earlier chapters. CT density data could have also strengthened any mortality signal seen given the evidence that augmentation therapy slows rate of emphysema decline in randomised control trials vs

placebo. Similarly, respiratory related mortality may have better represented emphysema decline. Overall, the third hypothesis set out in the introduction was partly answered but remains an important research question.

Going forward, large cohort studies with similar methods and prespecified protocols for data collection and sharing on a worldwide scale would enable a more robust approach to this important research question. Simultaneous collection of CT density data would be desirable. Until then, the only proven disease modifying therapy for AATD related lung disease will remain unavailable to AATD patients in the UK.

6.2 Publication plans

From the submitted works I plan three research papers.

The first paper will incorporate the majority of Chapter 5 with emphasis on the mortality and QOL parts of the analyses.

The second paper will present the main characteristics of exacerbations of AATD related lung disease from Chapter 3. There may also be scope for a separate paper looking at factors that influence patient adherence to symptom diaries in a specialised journal relating to digital health care technologies.

The third paper, pending approval from Mologic, will present a combination of biomarkers results from the lateral flow assays and predictive models with both exacerbations and the eDiary.

6.3 Future work

The work conducted during the writing of this thesis has led me to further interesting research questions that I would like to pursue which are presented in the following sections.

6.3.1 Associations of exacerbations in AATD

In Chapter 2, I set out the methods for the ‘Elastin degradation in exacerbations of AATD related lung disease’ study which recruited patients enrolled into the UK AATD registry, based in Birmingham. This large cohort, formerly called ADAPT, has collected prospective data over the last 25 years on a wide variety of measures including but not limited to symptoms, lung function, medication, transplants, sputum microbiology and blood tests. This database also contains data related to exacerbations. To date, exacerbations have only been looked at in subsets of patients that took part in specific clinical trials and has not been examined as a whole. My plan is to collate all the data relating to exacerbations from this cohort and assess the natural history and progression of pulmonary exacerbations in individual AATD patients and the entire cohort over time.

6.3.2 Bronkotest eDiary analysis in Kamada cohort

Results presented in Chapter 3 have shown that the Bronkotest eDiary with an overall symptom score could be used measure the occurrence of an exacerbation including intensity. Oversimplification of exacerbation outcomes in COPD clinical trials to annualised exacerbation rate or duration to first exacerbation does not take into account symptom intensity, thus may have masked otherwise efficacious treatments of exacerbations. A reduction in intensity of an exacerbation is likely to improve QOL and may reduce hospital admissions. A randomised control trial of inhaled AAT augmentation therapy vs placebo by Kamada(321) which also used the Bronkotest eDiary showed that, despite no overall difference in median duration to first exacerbation, there was a reduction in the intensity of symptoms. There were fewer type 1 Anthonisen defined exacerbations and fewer treated episodes. I would ideally like to perform a post-hoc analysis of the Kamada dataset using the same analysis techniques employed in this thesis to assess the intensity of symptoms

during exacerbations and if inhaled AAT augmentation therapy aids to reduce the intensity of exacerbation symptoms. This may reveal further study approaches that can determine the efficacy of inhaled AAT augmentation therapy. Agreement in principle to conduct the analysis has been obtained from the study sponsor.

6.3.3 Cardiovascular outcomes in exacerbations of AATD lung disease

There is considerable overlap of patients with COPD and cardiovascular disease, largely due to the common risk factor of cigarette smoking. Less overlap exists in patients with AATD since they are younger and have more modest smoking histories by comparison. However, studies propose a link independent of common risk factors and have shown that the cardiovascular risk associated with COPD may be driven by chronic inflammation (322) and acute infective exacerbations that feature in both COPD and AATD related lung disease. For example, COPD exacerbations have been shown to correlate with rises in biomarkers of cardiovascular stress. One study showed a subclinical increase in biomarkers of myocardial injury and dysfunction in COPD patients presenting with moderate exacerbations (use of antibiotics $\pm$ steroids but not hospitalised) and an overall increase in arterial stiffness.(287) Arterial stiffness is a marker of cardiovascular risk and is increased in patients with COPD(323) independent of systemic inflammation or airflow limitation. These increases in cardiovascular biomarkers and arterial stiffness were more marked in cases with confirmed respiratory viral or bacterial infections and suggests that inflammatory spill over from the lungs could contribute to systemic inflammation and myocardial injury.(322, 324) Physiological changes appear to translate to clinical outcomes in COPD with higher incidences of myocardial infarction and stroke in the 28 days following severe COPD exacerbations.(325) Exacerbations in AATD tend to be more inflammatory than usual COPD,(62) hence it is possible the effects, at least acutely, will be even more important. It is

plausible that the impact on the cardiovascular system is an underrecognised and unmet need for this group of patients.

It is evident that there is a need for research into the impact of pulmonary exacerbations on cardiovascular disease in patients with AATD related COPD and emphysema and whether cardiovascular disease contributes to their aetiology. My plan is to extend the work already done in the 'Elastin degradation in exacerbations of AATD lung disease' study and test for cardiovascular biomarkers during the stable state and during exacerbations. I will also aim to gather more data on future risk of major adverse cardiovascular events (MACE) via the UK AATD registry and Hospital Episode Statistics (HES) data. Funding for this work has been obtained from the Chest Foundation, and work will begin during my term as a clinical lecturer.

6.3.4 Effect of intravenous augmentation therapy on AECOPD

Due limitations on data exchange related to exacerbations in the US cohort during Chapter 5, I was unable to analyse the impact of augmentation therapy on exacerbation episodes in AATD related lung disease. Data from the RAPID study (53) showed that there was a higher annualised rate of exacerbations in those receiving augmentation therapy (1.70, 95% CI 1.51 to 1.89) compared to placebo. The duration of episodes was recorded as being on average 2 days longer. It is not clear from the study publication how exacerbation episodes were defined or measured and therefore uncertainty remains regarding how intravenous augmentation therapy effects exacerbation frequency and intensity.

So far, the main focus of augmentation therapy research has focused on emphysema progression. My aim will be to further transatlantic collaboration with my colleagues at AlphaNet and access data related to annualised rates of exacerbations between those receiving augmentation therapy and those that are not. I will also aim to look at the

difference in exacerbation frequency before and after augmentation therapy initiation.

Agreement from colleagues at AlphaNet has been obtained for this analysis, and a German group who also have data on this are interested in the same comparison. A funding application has been submitted for this work to Grifols Biotherapeutics.

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7 APPENDIX

7.1 Introduction

There are no figures or tables for this section.

7.2 Enhanced monitoring in exacerbations of AATD related lung disease - Methods

There are no figures or tables for this section.

7.3 Enhanced monitoring in exacerbations of AATD related lung disease - Results **(i) – eDiary**

There are no figures or tables for this section.

7.4 Enhanced monitoring in exacerbations of AATD related lung disease - Results (ii) – Biomarkers of AECOPD

7.4.1 End of study questionnaire

Your name:	Today's date:

Elastin degradation in exacerbations of Alpha 1 antitrypsin deficiency (AATD) lung disease – End of Study Feedback Questionnaire

Thank you for your participation in the study. Your feedback is very much appreciated.

Section 1: Symptom Diary (Kindle)

1) How easy was the symptom diary to use? (Circle answer)

1	2	3	4	5	6	7	8	9	10
Very Easy					Very Difficult				

2) A. How often did you forget to complete the symptom diary on average?

1	2	3	4	5	6	7	8	9	10
Almost Daily		Once a week		Once a month		Rarely		Never	

B. Why do you think this was?

3) Were there any other barriers to completing the symptom diary?

Section 2: Urine Monitor

1) How easy was the urine sticks/ monitor to use?

1	2	3	4	5	6	7	8	9	10
Very Easy					Very Difficult				

2) A. How often did you forget to measure your urine?

1	2	3	4	5	6	7	8	9	10
Almost weekly		Once a month				Rarely		Never	

B. Why do you think this was?

3) Were there any other barriers to testing urine at home?

4) Long term, how often would you be prepared to use the urine test? (please circle)

Daily	Alternate Days	Twice a week	Weekly

5) Would you like to see a result/output on the urine monitor? (please circle)

Yes	No

6) Would you be able to use a smart phone (provided if required) to send data electronically?

Yes	No

7) Did you collect your urine in a pot or did you dip the test in the stream of urine?

Collected in a pot	Dipped directly into urine stream

8) Where did you run the test? e.g. bathroom/bedroom

9) How long did it take between starting urine measurement and placing it into the holder? (seconds approximately)

Section 3: Other feedback

Please share any other improvements that could have been made to the study

Figure 7.1: Example of end of study questionnaire

7.4.2 Concentration curves for urine cassettes

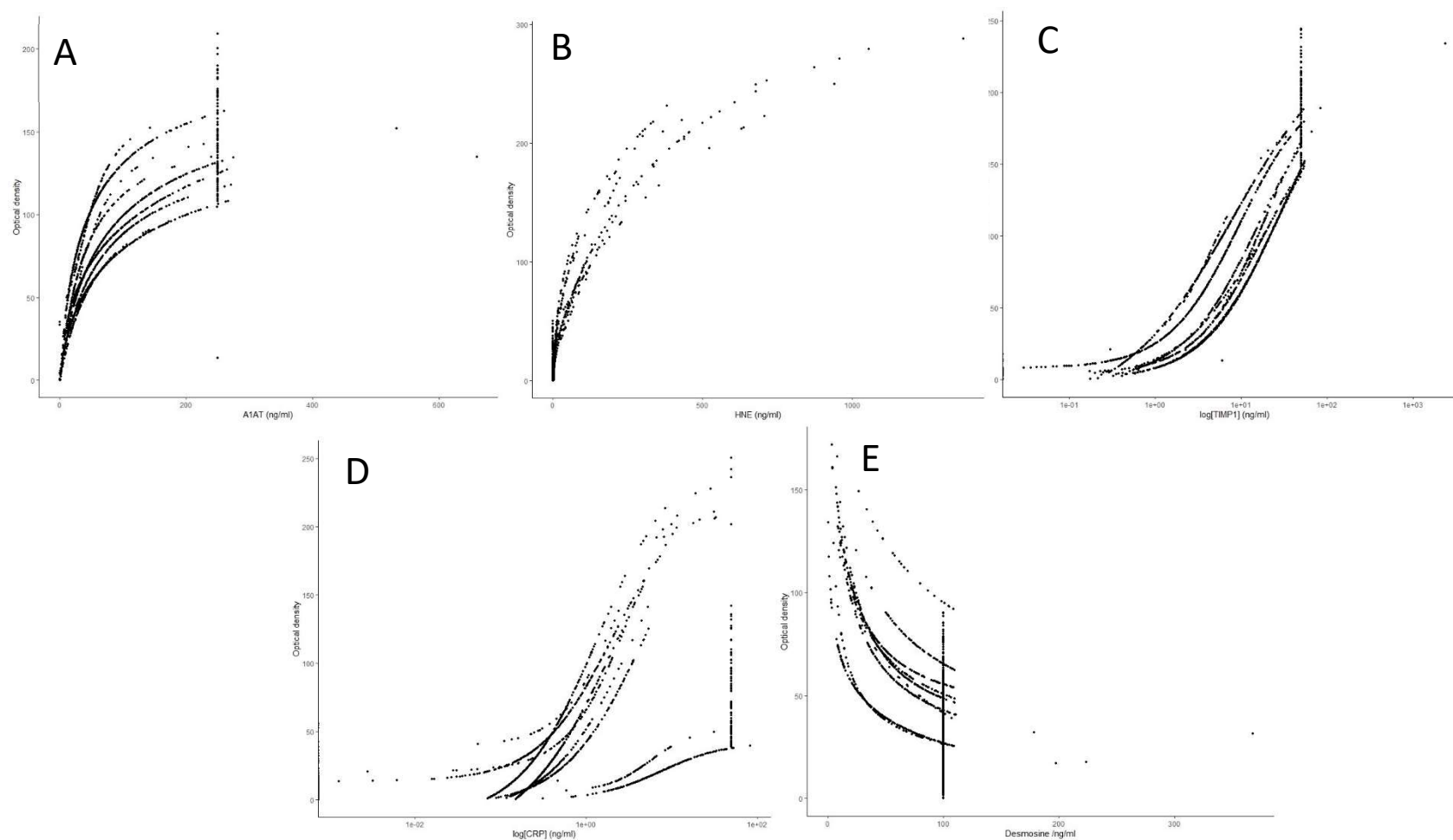


Figure 7.2: Concentration curves for urine cassettes for A) AAT, B) NE, C) TIMP1, D) CRP and E) Desmosine.

7.4.3 Stability of urinary biomarkers for individual patients

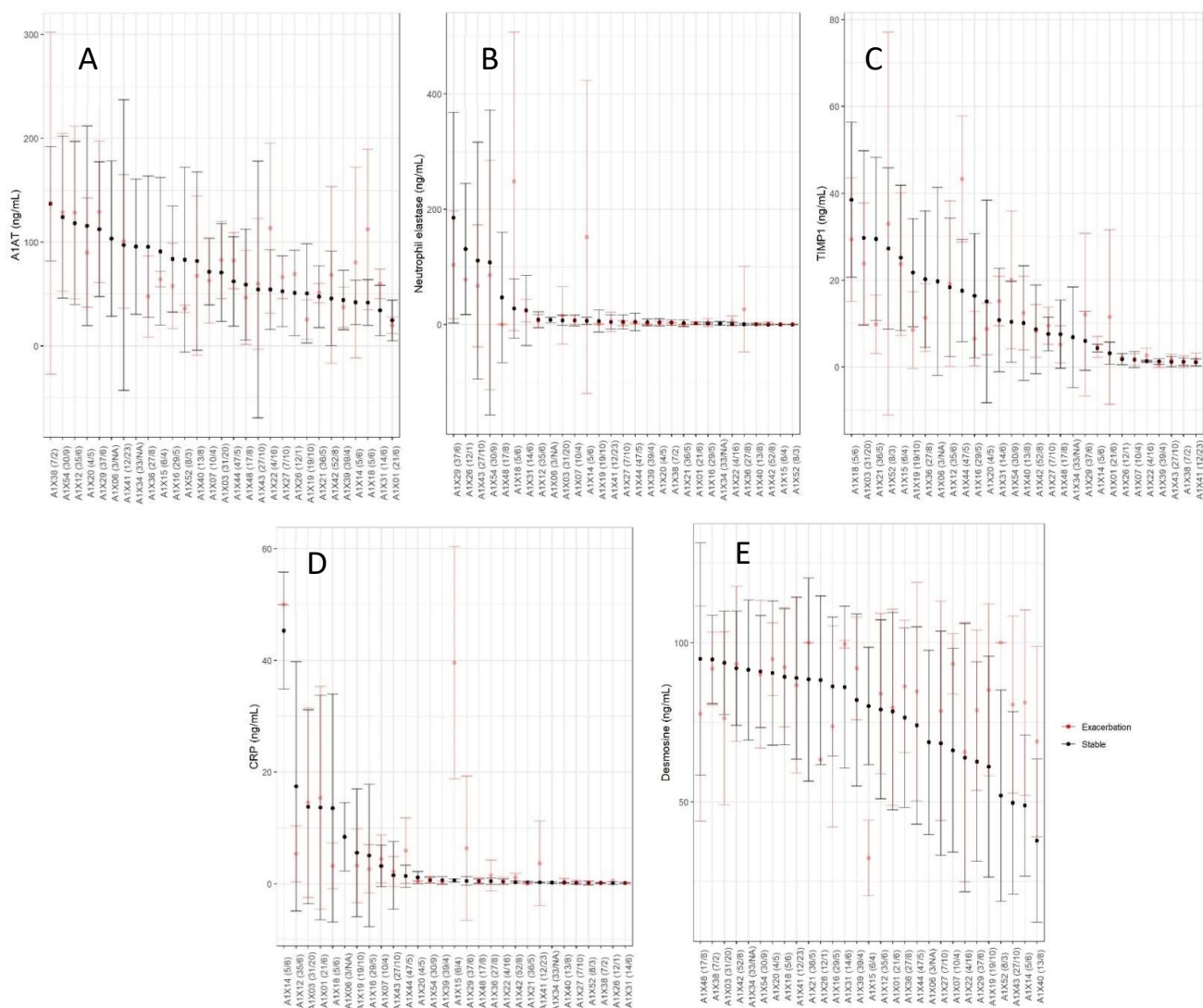


Figure 7.3: Stable (black) vs exacerbation (red) urinary levels for A) AAT, B) NE, C) TIMP1, D) CRP and E) Desmosine for individual patients. X axis - brackets represent (N samples stable/N samples exacerbation).

7.4.4 Threshold levels for CRP in lateral flow assay

Table 7.1: Effect of threshold CRP level on sensitivity and specificity values.

CRP threshold (ng/mL)	Sensitivity	True +ve	False -ve	Specificity	True -ve	False +ve	PPV	NPV
0	1.000	135	0	0.000	0	1545	8.04	-
1	0.444	60	75	0.710	1098	447	11.83	93.6
2	0.278	38	98	0.767	1185	360	9.43	92.4
3	0.222	30	105	0.808	1249	296	9.20	92.2
4	0.222	30	105	0.839	1296	249	10.75	92.5
5	0.111	15	120	0.858	1325	220	6.38	91.7
6	0.111	15	120	0.869	1342	203	6.88	91.8
7	0.111	15	120	0.881	1361	184	7.54	91.9
8	0.111	15	120	0.886	1369	176	7.85	91.9
9	0.056	8	128	0.895	1382	163	4.40	91.5
10	0.056	8	128	0.907	1401	144	4.95	91.6

7.4.5 Unpublished data on urinary biomarkers in AECOPD

Table 7.2: Unpublished data on hospitalised AECOPD vs stable state in usual COPD

Biomarker	Exacerbation Median (IQR)	Stable state Median (IQR)	Raw values	Normalised to Cr
			P value	
Desmosine (ng/mg Cr/ml)	4.13 (0.93-5.34)	3.85 (0.48-6.79)	0.963	0.544
NE (ng/ml)	17.27 (1.90-82.70)	15.62 (3.62-249.95)	0.461	0.914
AAT (ng/ml)	240.0 (81.5-794.7)	90.0 (23.0-260.0)	0.038	0.059
MMP substrate (ng/ml)	5.79 (0-18.92)	3.37 (0-10.69)	0.083	0.139
MMP8 (ng/ml)	0.57 (0-8.11)	0.26 (0-6.75)	0.407	0.753
MMP9 (ng/ml)	1.00 (0.13-4.39)	0.58 (0.26-9.43)	0.104	0.484
MMP activity	0.48 (0.39-1.00)	0.49 (0.31-1.20)	0.718	0.200
TIMP1 (ng/ml)	3.50 (1.61-9.85)	2.35 (0.62-5.21)	0.012	<0.001
TIMP2 (ng/ml)	4.19 (2.74-9.17)	3.77 (1.42-7.92)	0.131	0.029
NGAL (ng/ml)	41.05 (9.26-88.85)	32.57 (13.06-59.65)	0.969	0.753
IL6 (pg/ml)	2.29 (0-7.86)	3.19 (0-10.00)	0.492	0.913
IL8 (pg/ml)	3.44 (0-54.96)	7.97 (0-59.36)	0.075	0.555
Calprotectin (ng/ml)	34.00 (16.00-59.01)	37.60 (11.50-61.63)	0.151	0.196
IL1 β (pg/ml)	1.14 (0-4.97)	2.77 (0-6.57)	0.156	0.255
Cystatin C (ng/ml)	98.3 (48.3-217.3)	91.6 (24.7-153.1)	0.010	0.002
Fibrinogen (ng/ml)	11.94 (4.92-35.91)	15.04 (6.38-57.14)	0.085	0.148
RBP4 (ng/ml)	202.7 (135.1-461.2)	151.9 (83.0-289.7)	0.005	0.016
β 2 microglobulin (ng/ml)	375.4 (154.8-1522)	183.2 (89.4-545.1)	0.023	0.101
Albumin (ng/ml)	4005 (1349-13679)	1710 (636-8026)	0.010	0.033
Creatinine (mg/dl)	72.17 (43.86-105.5)	57.08 (33.54-111.4)	0.333	NA

7.5 Comparison of outcomes in augmentation naïve and augmented patients with AATD

7.5.1 SGRQ regression analyses

Table 7.3: Univariate and multivariate analysis for annual change in SGRQ total and symptom scores

	Estimate	95% CI	P
Univariate Analysis			
Change in SGRQ total/year			
Sex (Female)*	0.65	-0.12 to +1.41	0.098
Age*	0.03	-0.00 to +0.07	0.087
Smoking status (Ex-smoker)	-0.54	-1.39 to +0.30	0.21
Smoking status (Current smoker)	-0.80	-2.91 to +1.31	0.46
Control group*	1.19	0.26 to 2.12	0.013
FEV ₁ % predicted	-0.01	-0.03 to +0.01	0.28
Baseline SGRQ total*	-0.07	-0.09 to -0.05	<0.001
Change in SGRQ† symptom/year			
Female	0.46	-0.66 to +1.58	0.42
Age	0.04	-0.02 to +0.09	0.17
Smoking status (Ex-smoker)	-0.04	-1.28 to +1.21	0.95
Smoking status (Current smoker)	-0.99	-4.08 to +2.10	0.53
Control Group*	1.21	-0.15 to +2.58	0.081
FEV ₁ % predicted	-0.00	-0.03 to +0.02	0.78
Baseline SGRQ symptom*	-0.10	-0.12 to -0.07	<0.001
Multivariate Analysis			
Change in SGRQ total/year			
Control	1.30	0.41 to +2.19	0.004
Age (years)	-0.01	-0.05 to +0.03	0.65
Female	0.45	-0.29 to +1.19	0.23
Baseline SGRQ total	-0.07	-0.09 to -0.05	<0.001
Change in SGRQ symptom/year			
Control Group	2.01	0.73 to 3.30	0.002
Baseline SGRQ symptom	-0.10	-0.13 to +0.08	<0.001

*Variables chosen to be included in multivariate model.

†St. Georges Respiratory Questionnaire (SGRQ) total score is determined by the SGRQ-COPD questionnaire; scores range from 0 to 100 points, with higher scores indicating worse health status (minimally clinically important difference, 4-points(223)). SGRQ total score is calculated from three separate health status domains; symptom, impact and activity scores.

